Understanding Polygenic Risk Testing and Risk Communication for Glaucoma

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SUMMARY

Glaucoma remains the leading cause of irreversible vision loss globally. The term describes a group of progressive optic neuropathies with retinal nerve fibre layer thinning and characteristic visual field changes. This thesis focuses on primary open angle glaucoma (POAG), as the most common subtype, which is recognised to be highly heritable. Raised intraocular pressure (IOP) is often, but not always associated with glaucoma. IOP is the only known modifiable risk factor, and IOP-lowering therapies are very effective in slowing or preventing progression. Disease progression and severity exists on a spectrum, and early diagnosis often presents a diagnostic challenge given its asymptomatic nature in the initial stage of disease. The ability to predict those who are most likely to develop glaucoma, or progress more quickly to severe disease, would vastly improve timely implementation of treatment and prevent loss of vision.

Glaucoma heritability is both Mendelian and complex. More recently, there has been significant progress in the understanding of the complex heritability of POAG, mainly through the identification of associated single-nucleotide polymorphisms from large genome wide association studies. From this, polygenic risk scores (PRS) have been developed to incorporate this knowledge into a clinically meaningful individual risk score. This tool will help to provide more objective guidance to glaucoma risk stratification in the community, especially given there are currently no established screening guidelines for glaucoma in Australia. However, important details about clinical implementation are not yet known. These gaps in knowledge include the perspectives of the community and healthcare professionals, the barriers to the uptake of the test and reporting of results, and the clinical validity of current PRS's which have been generated in research settings. Cross-sectional questionnaire-based studies, multivariate analyses and prospective cohort studies were utilised to address these gaps.

This thesis provided original contribution to knowledge by demonstrating the first data on positive attitudes towards polygenic risk testing for POAG from key stakeholders who will be critical in the success of PRS implementation into clinical practice. This thesis further identified key contributors and barriers for individuals undertaking the test. Finally, this thesis provided novel insight into the utility of PRS in addition to family history and its association with early glaucoma treatment. The delivery of PRS testing will be an evolving journey, however this translational research will bring glaucoma PRS significantly closer to clinical implementation, and therefore, in reducing global vision loss by improving timely diagnosis and treatment.

DECLARATION

I certify that this thesis:

1. does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any university

2. and the research within will not be submitted for any other future degree or diploma without the permission of Flinders University; and

3. to the best of my knowledge and belief, does not contain any material previously published or written by another person except where due reference is made in the text.

Signed: Georgina Louise Hollitt

Date: 17/12/2024

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THESIS OUTCOMES

Peer-reviewed manuscripts published during Doctoral candidature

Hollitt GL, Siggs OM, Ridge B, Keane MC, Mackey DA, MacGregor S, Hewitt AW, Craig JE, Souzeau E. Attitudes towards polygenic risk testing in individuals with glaucoma. *Ophthalmology Glaucoma*. November 2021. doi:10.1016/j.ogla.2021.11.002

Qassim A, Souzeau E, **Hollitt G,** Hassall MM, Siggs OM, Craig JE. Risk Stratification and Clinical Utility of Polygenic Risk Scores in Ophthalmology. *Transl Vis Sci Technol*. 2021;10(6):14.

Hollitt GL, Siggs OM, Ridge B, Keane MC, Mackey DA, MacGregor S, Hewitt AW, Craig JE, Souzeau E. Attitudes towards glaucoma genetic risk assessment in unaffected individuals, *Translational Vision Science & Technology*, accepted August 2022.

Mullany S, Marshall H, Zhou T, Thomson D, Schmidt J, Qassim A, Knight L, **Hollitt G**, Berry E, Nguyen T, To MS, Dimasi D, Kuot A, Dubowsky J, Fogarty R, Sun MT, Chehade L, Kuruvilla S, Supramaniam D, Craig Je. RNA Sequencing of Lens Capsular Epithelium Implicates Novel Pathways in Pseudoexfoliation Syndrome. *Investigative Ophthalmology & Visual Science*. March 2022; 63(3):26

H Marshall, S Mullany, X Han, EC Berry, MM Hassall, A Qassim, T Nguyen, **GL Hollitt**, LSW Knight, B Ridge, J Schmidt, C Crowley, A Schulz, RA Mills, A Agar, A Galanopoulos, J Landers, PR Healey, SL Graham, AW Hewitt, RJ Casson, S MacGregor, OM Siggs, JE Craig. Genetic Risk of Cardiovascular Disease Is Associated with Macular Ganglion Cell–Inner Plexiform Layer Thinning in an Early Glaucoma Cohort, *Ophthalmology Science*, March 2022; 2(1)

Mullany S, Marshall H, Diaz-Torres S, Berry EC, Schmidt JM, Thomson D, Qassim A, To MS, Dimasi D, Kuot A, Knight LSW, **Hollitt G**, Kolovos A, Schulz A, Lake S, Mills RA, Agar A, Galanopoulos A, Landers J, Mitchell P, Healey PR, Graham SL, Hewitt AW, Souzeau E, Hassall MM, Klebe S, MacGregor S, Gharahkhani P, Casson RJ, Siggs OM, Craig JE. The *APOE* E4 Allele Is Associated with Faster Rates of Neuroretinal Thinning in a Prospective Cohort Study of Suspect and Early Glaucoma. *Ophthalmol Sci*. 2022 Apr 19;2(2):100159

Marshall HN*, Hollitt GL*, Wilckens K, Mullany S, Kuruvilla S, Souzeau E, Landers J, Han X, MacGregor S, Craig JE, Siggs OM. High polygenic risk is associated with earlier trabeculectomy in primary open-angle glaucoma. Ophthalmol Glaucoma. 2022 Jul 13:S2589-4196(22)00119-3. doi: 10.1016/j.ogla.2022.06.009. Epub ahead of print. PMID: 35842105.

Marshall H, Berry EC, Torres SD, Mullany S, Schmidt J, Thomson D, Nguyen TT, Knight LS, **Hollitt G**, Qassim A, Kolovos A, Ridge B, Schulz A, Lake S, Mills RA, Agar A, Galanopoulos A, Landers J, Healey PR, Graham SL, Hewitt AW, Casson RJ, MacGregor S, Siggs OM, Craig JE. Association Between Body Mass Index and Primary Open Angle Glaucoma in Three Cohorts.

Am J Ophthalmol. 2023 Jan;245:126-133. doi: 10.1016/j.ajo.2022.08.006. Epub 2022 Aug 13.

Knight LSW, Mullany S, Taranath DA, Ruddle JB, Barnett CP, Sallevelt SCEH, Berry EC, Marshall HN, **Hollitt GL**, Souzeau E, Craig JE, Siggs OM. The phenotypic spectrum of *ADAMTSL4*-associated ectopia lentis: Additional cases, complications, and review of literature. *Mol Vis*. 2022 Sep 4;28:257-268.

Berry EC, Marshall HN, Mullany S, Torres SD, Schmidt J, Thomson D, Knight LSW, **Hollitt GL**, Qassim A, Ridge B, Schulz A, Hassall MM, Nguyen TT, Lake S, Mills RA, Agar A, Galanopoulos A, Landers J, Healey PR, Graham SL, Hewitt AW, MacGregor S, Casson RJ, Siggs OM, Craig JE. Physical activity is associated with macular thickness: a multi-cohort observational study. *Invest Ophthalmol Vis Sci.* 2023; 64(3):11

Marshall HN, Mullany S, Han X, Qassim A, He W, Hassall MM, Schmidt J, Thomson D, Nguyen T, Berry EC, Knight LSW, **Hollitt GL**, Ridge B, Schulz A, Mills RA, Healey PR, Agar A, Galanopoulos A, Landers J, Graham SL, Hewitt AW, Casson RF, MacGregor S, Siggs OM, Craig JE. High polygenic risk is associated with earlier initiation and escalation of treatment in early primary open-angle glaucoma. *Ophthalmology*. August 2023; 130(8):830-836

Hollitt GL, Qassim A, Thomson D, Schmidt JM, Nguyen TT, Landers J, MacGregor S, Siggs OM, Souzeau E, Craig JE. Genetic Risk Assessment of Degenerative Eye Disease (GRADE): study protocol of a prospective assessment of polygenic risk scores to predict diagnosis of glaucoma and age-related macular degeneration. *BMC Ophthalmol*. 2023 Oct 24;23(1):431.

<u>Conference presentations</u>

2023
RANZCO Congress, Perth, Australia, 20-23 October 2023
Poster: ‘High polygenic risk is associated with earlier trabeculectomy in primary open-angle glaucoma’
Poster: ‘Uptake of polygenic risk testing for glaucoma among unaffected individuals’
Poster: ‘Healthcare Professionals’ knowledge and attitudes towards polygenic risk testing for glaucoma’

ANZGS Congress, Queenstown, New Zealand, 16-19 February 2023
Presentation: ‘A glaucoma polygenic risk score is strongly associated with glaucomatous family history, and disease severity amongst affected siblings’

2022
ANZGS Congress, Virtual Congress, 12-13 February 2022
Presentation: ‘Attitudes toward glaucoma genetic risk assessment among affected and unaffected individuals in an Australian population’

RANZCO Congress, Brisbane, Australia, 28 October - 1 November 2022
Poster: ‘A glaucoma polygenic risk score is strongly associated with glaucomatous family history, and disease severity amongst affected siblings’

2021
RANZCO Congress, Virtual, 26 Feb - 1 March, 2022 *(delayed due to COVID)*
Poster: ‘Attitudes toward glaucoma genetic risk assessment among affected and unaffected individuals in an Australian population’

American Society of Human Genetics (ASHG) Annual Meeting, Virtual, 18-22 March 2021
Poster: ‘Attitudes towards polygenic risk testing for glaucoma in an Australian population’

The Association for Research in Vision and Ophthalmology (ARVO) Annual Meeting, Virtual, 2-6 May 2021
Poster: ‘Attitudes towards polygenic risk testing in individuals with glaucoma’

Australian Polygenic Risk Symposium (APRS) Meeting, Virtual, 20-22 April 2021
Presentation: ‘Attitudes towards polygenic risk testing in individuals with glaucoma’

Awards

1. Clinician Special Purpose Fund PhD Scholarship 2021
2. Australian and New Zealand Glaucoma Society (ANZGS) Virtual Congress, Kath Holmes scholarship for best presentation, 2021
3. Shortlisted Best E-Poster RANZCO Virtual Congress 2021
4. SA Health Young Professionals Group Professional Development Scholarship 2022

Other media relating to research during Doctoral candidature

1. Rhiannon Bowman. The future of glaucoma detection and monitoring. Insight Magazine. Published March 11, 2022. https://www.insightnews.com.au/the-future-of-glaucoma-detection-and-monitoring/

ABBREVIATIONS

AMD	Age-related macular degeneration
ANZGS	Australian and New Zealand Glaucoma Society
ANZRAG	Australian and New Zealand Registry of Advanced Glaucoma
AUC	Area under the curve
AUD	Australian dollar
BCVA	Best-corrected visual acuity
CCT	Central corneal thickness
CI	Confidence interval
EMGT	Early Manifest Glaucoma Trial
FDR	First-degree relative
GINA	Genetic Information Nondiscrimination Act
GP	General practitioner
GRADE	Genetic Risk Assessment of Degenerative Eye disease
GWAS	Genome-wide association studies
HGSA	Human Genetics Society of Australasia
HTG	High tension glaucoma
HVF	Humphrey Visual Field
IOP	Intraocular pressure
IQR	Interquartile range
POAG	Primary open-angle glaucoma
MD	Mean deviation
MTAG	Multitrait analysis of GWAS
NHMRC	National Health and Medical Research Council
NTG	Normal tension glaucoma
OCT	Optical coherence tomography
OHTS	Ocular Hypertension Treatment Study
OR	Odds Ratio
PRS	Polygenic risk score
RACGP	Royal Australian College of General Practitioners
RACP	Royal Australian College of Physicians
RANZCO	Royal Australian and New Zealand College of Ophthalmologists
RANZCOG	Royal Australian College of Obstetricians and Gynaecologists

RNFL	Retinal nerve fibre layer
SAC HREC	Southern Adelaide Clinical Human Research Ethics Committee
SD	Standard deviation
SNP	Single nucleotide polymorphism
TARRGET	Targeting at risk Relatives of Glaucoma patients for Early diagnosis and Treatment
UKB	United Kingdom Biobank
USD	United States dollar
VCDR	Vertical cup-to-disc ratio
VEGF	Vascular endothelial growth factor
1KG	1000 Genomes Project

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INTRODUCTION

A: GLAUCOMA

A.1 Definition

Glaucoma refers to a group of progressive optic neuropathies which can result in irreversible vision loss and blindness. The term refers to a spectrum of disorders with multifactorial aetiology rather than a single disease entity. Glaucoma is characterised by neuroretinal rim thinning and retinal nerve fibre degeneration causing characteristic visual field changes, and is associated with, but not dependent on raised intraocular pressure (IOP).[1] Disease severity and progression exists on a clinical spectrum. Glaucoma suspect is a term used to describe individuals where the appearance of the optic nerve head is suspicious for glaucoma without accompanying visual field loss.[1] Similarly, in the early stage of disease, retinal ganglion cell death may not be measurable in the thickness of the retinal nerve fibre layer (RNFL) or ganglion cell layer.[2] Later, imaging techniques such as optical coherence tomography (OCT) allow for the detection of early RNFL and ganglion cell layer loss, before any visual field loss is demonstrated.[3] This is sometimes referred to as preperimetric glaucoma.

While multiple subtypes are encompassed under the umbrella of glaucoma, each are united by a clinically characteristic optic neuropathy.[1] Glaucoma subtypes are defined by the morphology of the anterior chamber angle and the presence or absence of secondary causes of elevated IOP. Traditionally, the normal range of IOP is 10-21mmHg, defined as the mean IOP within two standard deviations.[4] Elevated IOP therefore means an IOP that exceeds the 97.5th percentile for the population under consideration.[1] Broadly, glaucoma subtypes are classified as either open-angle or angle-closure glaucoma. The anterior chamber angle, also known as the iridocorneal angle, is formed by the position of the lens and iris relative to the trabecular meshwork (Figure 1). Primary open-angle glaucoma (POAG), the most common form, accounts for about 80% of all glaucoma in Australia.[5] POAG is defined by an open and normal appearing iridocorneal angle, and where no secondary cause of aqueous humour outflow resistance or elevated IOP is evident. Normal tension glaucoma (NTG) is a further subtype of POAG, whereby the IOP is never observed to be elevated, whereas high tension glaucoma (HTG) refers to POAG with elevated IOP. Meanwhile, ocular hypertension refers to individuals where IOP is elevated in isolation, without any additional glaucomatous features. Secondary open-angle glaucoma refers to glaucomas with an open iridocorneal angle and a cause of elevated IOP has been identified. Pigment dispersion syndrome and pseudoexfoliation syndrome are two of the most common

causes of secondary open-angle glaucoma. Pigment dispersion syndrome refers to the release of pigment granules from the iris.[6] This occurs when the posterior surface of a concave iris rubs against the anterior lens. The pigment may occlude outflow through the trabecular meshwork, causing increased IOP and eventually pigmentary glaucoma. The aetiology of pseudoexfoliation syndrome is unclear, but is characterised by exfoliative material in the anterior chamber.[7] This may also obstruct aqueous humour outflow through the trabecular meshwork. Angle-closure glaucoma occurs when the iridocorneal angle is closed and aqueous humour outflow is obstructed either with anatomic predisposition (primary angle-closure glaucoma) or without (secondary angle-closure glaucoma).

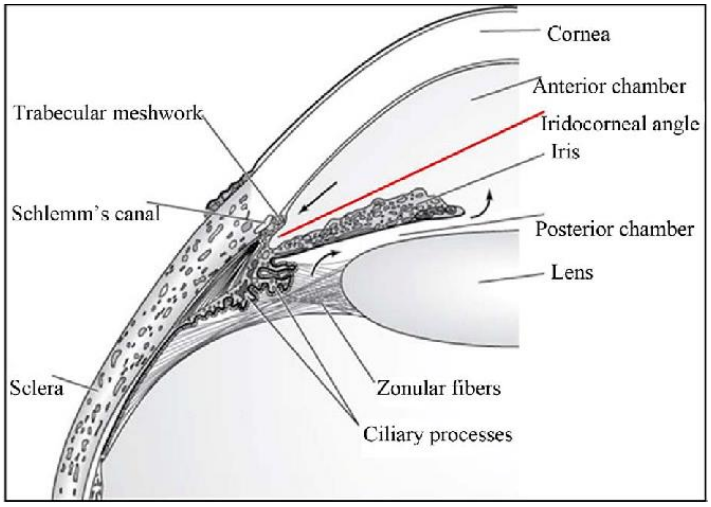

Figure 1: The iridocorneal angle[8]

A.2 Epidemiology and Disease Burden

Glaucoma is the most common cause of irreversible vision loss worldwide, predicted to affect up to 111.8 million people by 2040.[5,9] The global prevalence has been estimated to be 3.54% in those over the age of 40 years.[5] In Australia, prevalence among non-indigenous individuals over 50 years and indigenous Australians over 40 years was estimated to be 3.4% and 1.5%, respectively.[10] POAG is the most common subtype in countries with predominantly European ancestry, accounting for 74-80% of glaucoma cases.[5,9]

The prevalence of the disease is strongly positively correlated with age. A meta-analysis and systematic review of glaucoma prevalence over the last 20 years found prevalence increased from ~1.1% in those aged 40-49 years to ~9.2% among those over 80 years of age.[11] The global population of individuals aged over 60 years and 80 years is predicted to double and triple by

2050, respectively.[12] This means POAG will pose an increasingly significant burden. Sight is generally considered to be the most valued sense by the general public, so identifying cost-effective screening methods to facilitate early diagnosis and timely intervention is important.[13] In Australia, vision impairment results in significant direct and indirect health care costs, ranking as the seventh most costly health condition.[14] It is important to consider the impact of vision loss on an individual, which has been shown to result in poorer wellbeing outcomes through the impact on quality of life, lost income, and personal healthcare costs.[14]

Without treatment, progression from normal vision to blindness would occur in approximately 25 years.[15] Furthermore, 50-68% will experience progression of vision loss despite medical or surgical intervention although at slower rates, and 9-19% will progress to blindness.[16,17] The significant burden on individuals and the health care system may be amplified by the current standard of care where patients with suspected or established glaucoma are subjected to close lifelong monitoring, and sometimes prescribed lifelong treatment that may not always be required.

A.3 Pathophysiology

The exact aetiology of POAG is not yet fully understood, however a number of theories have been proposed to explain the optic nerve degeneration. While elevated IOP is not diagnostic, it remains an important factor of glaucoma pathogenesis, being the only known modifiable risk factor. IOP is the internal pressure produced by the aqueous humour within the anterior chamber of the eye and is determined by the balance of aqueous humour secretion by the ciliary body and outflow through two pathways. Aqueous humour is produced by the non-pigmented epithelial cells of the ciliary body into the posterior chamber and flows around the lens and through the pupil into the anterior chamber.[18,19] Outflow of aqueous humour from the eye occurs through the trabecular meshwork and uveoscleral outflow pathway, termed the conventional and unconventional outflow pathways, respectively.[20] Interruption in either of these processes will disturb this homeostasis. Resistance within the trabecular meshwork contributes to the majority of aqueous humour outflow resistance, while the uveoscleral pathway is relatively independent of IOP.[19,21] Aqueous humour exits the eye via a pressure-dependent pathway through the trabecular meshwork at the iridocorneal angle (Figure 1) into Schlemm's canal and finally into the uveoscleral venous system.[19] The obstruction of the structures responsible for aqueous humour outflow at the iridocorneal angle ultimately determines the classification of glaucoma.

The mechanical theory suggests that increased resistance to aqueous outflow through the trabecular meshwork results in elevated IOP, retinal ganglion cell death and ultimately corresponding vision loss. The level of IOP elevation correlates to the rate of retinal ganglion cell death.[20] The optic disc is composed of neural, vascular, and connective tissues. The retinal ganglion cell axons converge at the optic disc to create the neuroretinal rim. This surrounds the optic disc cup which is a central depression in the disc. Retinal ganglion cell axons then exit the eye through the lamina cribrosa to form the optic nerve. The lamina cribrosa is a mesh-like structure at the optic nerve head that surrounds and supports the retinal ganglion cell axons as they form the optic nerve. In glaucoma, the progressive retinal ganglion cell axonal loss results in loss of the rim and cup enlargement. IOP-induced stress results in thinning and posterior displacement of the lamina cribrosa. This causes mechanical axonal damage, leading to further narrowing of the rim and deepening of the cup. Elevated IOP can cause neuronal axonal injury at the optic nerve head and accelerated retinal ganglion cell death.[1,20]

However, the mechanical theory does not explain those who develop glaucoma despite IOP being within the normal range, those who do not progress to glaucoma despite consistently elevated IOP, and those who continue to progress despite adequate IOP control. Therefore, vascular, biomechanical and genetic theories propose alternate mechanisms for progressive retinal ganglion cell damage and eventual optic neuropathy.[22–24] The vascular theory remains controversial, however suggests alterations in ocular blood flow and vascular dysregulation contribute to axonal injury and loss.[23,25] Ocular perfusion pressure and ocular blood flow remain unproven, with studies failing to demonstrate consistent results.[26,27] In addition, these vascular changes are seen in systemic disorders where individuals do not develop glaucoma, such as multiple sclerosis.[23,28] Emerging biomechanical theories suggest that excitatory amino acids, caspases, protein kinases, oxygen free radicals, nitric oxide, TNF-alpha, neurotrophins and metalloproteins may contribute to glaucomatous optic neurodegeneration.[22] Although the biomechanical theory also remains to be proven, it may offer a new direction and targets for treatment. It is likely that a combination of these and potentially undiscovered mechanisms collectively contribute to glaucoma pathogenesis.

A.4 Risk factors

The early asymptomatic nature of glaucoma presents a diagnostic challenge and clinical presentations can be varied, depending on the extent to which vision has been impacted at the time of review. Furthermore, the rate of disease progression can be highly variable, although only

a minority of those with glaucoma will have rapidly progressing disease. Heijl et al. demonstrated that the average rate of change in the visual field loss through mean deviation was approximately -0.80 decibels per year and only 5.6% of patients progressed at a rapid rate of more than -2.5 decibels per year.[29] An individuals' disease course may be influenced by the interplay of demographic and clinical factors. While the understanding of the genetic underpinnings of glaucoma are gradually deepening, the traditional risk factors discussed below remain an important part of individual risk assessment and clinical decision making.

Glaucoma, and more precisely POAG, can be considered as a degenerative condition of ageing. The Early Manifest Glaucoma Trial (EMGT) showed that participants aged 68 years or older were at 51% increased risk of progression relative to those younger than 68 years.[30] Glaucoma prevalence and incidence increases with age and POAG is not commonly seen in individuals below the age of 50 years.[5,31–35] Therefore, prevalence rates are usually reported for age over 50 years. Individuals aged 70 years or older are at roughly three-times greater risk of developing glaucoma compared to those aged 40 years.[31] Furthermore, ageing per decade is consistently associated with higher IOP and thinner central corneal thickness (CCT) which likely contributes to the higher prevalence of glaucoma in older populations.[34,36–40]

Family history remains one of the most important indicators of glaucoma genetic risk. Recognition of the high heritability of glaucoma was an important trigger for the ongoing research attempting to crack the complex genetics of glaucoma. A population-based familial aggregation study found that first-degree relatives of patients with glaucoma had a 9.2 fold increased risk of developing glaucoma compared to relatives of controls.[41] The population-based Baltimore Eye Survey also identified family history to be an important risk factor for POAG, with associations strongest between siblings.[42] Later, Wang et al. (2017) found that glaucoma was one of the most heritable common conditions in an extensive study of insurance claims in the US.[43] Of 149 studied diseases, glaucoma was ranked third, and the genetic heritability of glaucoma was estimated to be about 70%.[43] Although family history is a recognised and important risk factor for glaucoma, its reliability can be variable due to incomplete or erroneous understanding of family health history. Furthermore, diagnostic bias may exist amongst individuals with a family member with glaucoma, who may be more likely to undergo regular screening than those in the general population. Mathematical modelling has previously estimated that 72% of people with glaucoma would have a family history of glaucoma. However, other studies have reported much lower rates.[42,44–48]

Although, even without complete and precise information about family history of glaucoma, it is still a strong risk factor when assessing an individual's risk.

Ancestry is another recognised risk factor for glaucoma, with studies consistently reporting the highest prevalence of POAG in individuals of African ancestry.[5,49,50] More specifically, the highest reported prevalence of POAG is in sub-Saharan Africa (Figure 2).[51] Thinner average CCT has also been reported in these populations which may contribute to a higher overall prevalence of glaucoma.[52,53]

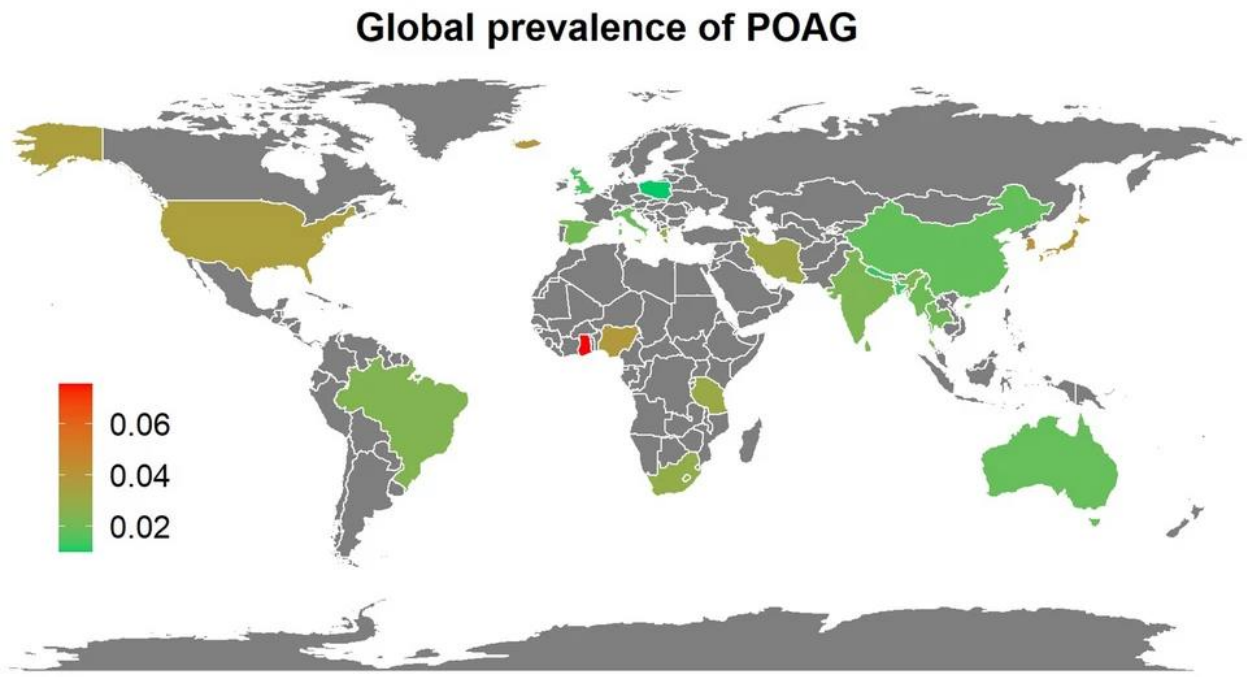

Figure 2: Global prevalence of primary open-angle glaucoma. [11]

Although the presence of increased IOP is not required to diagnose glaucoma, it is the only known modifiable risk factor for glaucoma.[1] IOP is the internal pressure produced by the aqueous humour within the anterior chamber of the eye.[19] The balance of aqueous humour production and outflow mediates the IOP. Interruption in either of these processes will disturb this homeostasis. Aqueous humour is produced by the ciliary process of the ciliary body, a muscular structure located behind the iris which is also responsible for altering the shape of the lens when the eye focuses.[19] The aqueous humour flows through the pupil into the anterior chamber where it is then drained by the trabecular meshwork at the iridocorneal angle (Figure 1).[19] While IOP elevation is an important risk factor for POAG, up to one-third of POAG patients with optic nerve degeneration have IOP within the normal range.[42] Several landmark trials have investigated progression and treatment

response across the POAG disease spectrum. These studies have sought to develop an understanding as to why some will develop POAG despite having a normal IOP, and others do not progress to POAG even with high IOP. The Ocular Hypertension Treatment Study (OHTS) determined that some patients were at very low risk of developing glaucoma despite having high IOP, while others were at much higher risk. Risk of progression to POAG from OHT ranged from 1-35% over 5 years.[54] The EMGT was the first randomised controlled trial to evaluate the outcomes of treatment compared to no treatment in early stage glaucoma.[55] The study showed the effectiveness of IOP-reducing therapy in all patient groups, including those with low and high IOP. Risk of progression was reduced by 10% with each 1 mmHg decrease in IOP.[55]

Myopia, or short-sightedness, has long been identified as a risk factor for POAG with population-based studies indicating that the risk of glaucoma increases with the degree of myopia.[56–60] However the association remains poorly understood and other studies have demonstrated conflicting association results.[61–63] One theory suggests that the underlying structural weaknesses of the nerve fibres, lamina cribrosa and choroid in individuals with myopia may contribute to increased susceptibility of the optic disc to fluctuations in IOP.[64] Several studies have supported moderate-to-high myopia to be a risk factor for POAG. The Blue Mountains Eye Study suggested a dose response relationship, demonstrating correlation between POAG and low myopia (OR 2.3; 95%CI 1.3-4.1), and an even stronger correlation between POAG and moderate-to-high myopia (OR 3.3; 95%CI 1.7-6.4).[60] As the prevalence of myopia increases, the understanding of the role of myopia to POAG may be refined. High myopia, defined by spherical equivalent of -6 diopters or worse is associated with visual field progression which may occur due to ocular complications such as myopic macular degeneration.[65] Abnormal optic disc appearances which can be seen with myopia can complicate the diagnosis of glaucoma, which is also characterised by visible optic disc damage. The variability in optic disc morphology and visual field changes which may be seen in myopic patients can cause confusion when diagnosing glaucoma, either resulting in over or under diagnosis.

Optic disc haemorrhages are a recognised risk factor for glaucoma progression.[66,67] and are thought to result from an ischaemic microinfarction injury.[68] These haemorrhages appear as linear, splinter-like haemorrhages on the outer margin of and perpendicular to the optic nerve head.[69] The presence of an optic disc haemorrhage is not diagnostic of glaucoma, however should warrant thorough investigation for glaucoma and may signal the need to initiate or escalate IOP-lowering therapy. The EMGT showed that a higher percentage of disc haemorrhages across

follow-up reviews was associated with glaucoma progression.[30] Later, the United Kingdom Glaucoma Treatment Study demonstrated that the presence of a disc haemorrhage at the initial visit was predictive of visual field progression.[70] These findings may be explained by evidence of localised RNFL thinning adjacent to disc haemorrhages.[71] OCT of the optic disc has allowed the effects of disc haemorrhages to be examined at a finer level.

CCT is a more controversial clinical risk factor, and the relationship between corneal biomechanics and glaucoma progression is still under investigation. The OHTS demonstrated that a lower CCT was associated with an increased likelihood to develop glaucoma in the future.[53,72] However, this may be due to the confounding influence of corneal biomechanics on IOP measurements. Goldmann applanation tonometry is the gold standard technique for measuring IOP, however can be significantly influenced by corneal biomechanics including CCT.[73] For example, a thin CCT may lead to an overestimation of IOP, and similarly, an increased CCT may result in underestimation of IOP. The clinical interpretation of measurements in these circumstances may therefore result in over- or under-diagnosis of glaucoma, respectively. Because of this, it is unclear whether CCT is a true independent risk factor for glaucoma progression. Furthermore, corneal stiffness parameters may be an additional parameter contributing to progression. Qassim et al. showed that stiffer and thinner corneas had a higher likelihood of progression as evidenced by a faster rate of RNFL thinning and visual field progression.[74]

The association between glaucoma progression and cardiovascular disease and its risk factors has attracted growing interest. Several large population studies have demonstrated cardiovascular disease to be a risk factor for glaucoma diagnosis and rapid progression.[75–78] A prospective, longitudinal study of preperimetric and perimetric glaucoma showed that hypertension was associated with an increased risk of both OCT and Humphrey visual fields (HVF) progression.[79] These studies have presented a number of hypotheses to explain this association, including microvascular damage, ocular perfusion pressure abnormalities, and vascular dysfunction mechanisms.

Overall glaucoma risk is currently calculated by evaluating each of these clinical risk factors together with genetic risk, primarily based on ancestry and family history. The accuracy in assessment and weighting of these risk factors is likely clinician dependent however, so consistent and uniform risk assessment is unlikely.

A.5 Current screening and treatment options

Current screening guidelines for POAG are limited and lack specific guidance. Current screening methods are clearly inadequate as approximately half of those with glaucoma are undiagnosed.[32] Screening for glaucoma is largely opportunistic, and broad community screening has not been demonstrated to be cost-effective.[80,81] For this reason, identifying cost-effective screening methods to facilitate early diagnosis and timely intervention is important. The National Health and Medical Research Council (NHMRC) in Australia currently recommends screening with a clinical examination for first-degree relatives of patients with glaucoma, commencing 5-10 years earlier than the age of glaucoma onset in their affected relative. Additionally, screening from the age of 40 years is recommended in people of African ancestry, compared to from 50 years of age in people of European ancestry.[80]

Globally, glaucoma screening guidelines vary significantly. The variability is likely multifactorial, in part due to lack of specific evidence, as well as differences in access to resources, population demographics, and differences in healthcare structures. Some argue that screening cannot be justified due to the overall low prevalence of glaucoma. In developing countries, limited access to ophthalmic care is a major barrier to optimising all ophthalmic conditions, including glaucoma. A 'Toolkit for Glaucoma Management in Sub-Saharan Africa' was created by a group of ophthalmologists in conjunction with the International Council of Ophthalmology, recommending glaucoma screening for the general population over the age of 35 years.[82] The American Academy of Ophthalmology currently recommends glaucoma screening at age 40 years, or earlier for those with a family history.[83] However, the United States Preventative Services Task Force did not recommend general population glaucoma screening due to the lack of evidence for the clinical utility of screening strategies.[84] Similarly, the United Kingdom's National Screening Committee and the European Glaucoma Society have also concluded that no screening guidelines are currently recommended due to lack of evidence.[85,86] The World Glaucoma Society assessed all current screening methodologies for glaucoma, but concluded there is insufficient evidence supporting any guideline, and offered no formal consensus on who, when, or how screening should be performed.[87]

IOP lowering therapies are currently the only effective treatment available for glaucoma. These include topical medications, laser trabeculoplasty, or incisional surgery. Evidence supporting the efficacy of IOP-lowering treatment was demonstrated by the OHTS and EMGT studies.[53,55] The

OHTS randomised 1,636 individuals with ocular hypertension and no glaucomatous damage to receive topical IOP-lowering medication or observation.[53] The study demonstrated that ocular hypotensive medication was safe and effective in delaying or preventing the onset of POAG in individuals with ocular hypertension.[54] The EMGT randomised 255 individuals with early, untreated POAG to treatment or no treatment to compare the effect of immediate IOP-lowering therapy to late or no treatment on disease progression. Treatment involved a combination of laser trabeculoplasty and topical ocular hypotensive medication.[55] The trial demonstrated that the risk of progression decreased 10% with each 1mmHg IOP reduction from baseline, and that a 25% decrease of IOP from baseline reduced the risk of progression by 50%.[88]

A.6 Genetics of POAG

The genetic contribution to glaucoma risk is well recognised, particularly for POAG, and is underpinned by both Mendelian and complex inheritance patterns. Variants in genes causing glaucoma with high penetrance such as *MYOC, OPTN* and *TBK1* account for less than 5% of adult onset glaucoma.[89] Variants in these disease-causing genes are inherited in an autosomal-dominant manner with incomplete age-related penetrance. Although rare, these variants are highly significant to the patients and their families given variants in these genes have a high likelihood of resulting in glaucoma.

MYOC was the first gene found to be associated with POAG, identified through linkage studies of large pedigrees with open-angle glaucoma.[90] Myocilin is expressed in the trabecular meshwork, an anatomical structure which plays an integral role in glaucoma pathogenesis. Despite this breakthrough in 1997, the function of the myocilin protein is still incompletely understood. Studies examining the function of normal and mutant myocilin expressed in ocular cells and tissue of individuals with and without glaucoma suggest that abnormal protein produced by myocilin variants is retained within the trabecular meshwork and lead to dysfunction of this aqueous outflow pathway.[91–95] Pathogenic variants in the *MYOC* gene contribute to most Mendelian POAG, and are commonly associated with a younger age of diagnosis, high IOP, more advanced disease, and a strong family history of glaucoma.[96] Variants in the *MYOC* gene are associated with up to 8-36% of early-onset POAG (before age 40 years).[97–99] Souzeau et al. showed that the prevalence of *MYOC* mutations in those with advanced POAG compared with those with non-advanced disease was 4.2% and 1.5%, respectively.[100]

The *OPTN* gene codes for the protein optineurin, which is involved in several cellular functions, including autophagy, signal transduction and vesicle trafficking.[101] Optineurin is expressed in trabecular meshwork, nonpigmented ciliary epithelium, retina, and brain.[101] It is speculated that it plays a neuroprotective role, particularly given its association with other neurodegenerative conditions including amyotrophic lateral sclerosis, Alzheimer's disease, and Parkinson's disease.[101] The association of *OPTN* and glaucoma was discovered from a study of 54 families with adult-onset POAG and autosomal dominant inheritance, identifying *OPTN* sequence variants in 16.7% of families.[102] The variants in *OPTN* which are associated with glaucoma are known to result in glaucomatous neurodegeneration in the absence of raised IOP (NTG). Although different variants were initially reported, the only variant that has been replicated in further studies and is supported by functional evidence is the E50K variant..[101,103] In a study of 95 control subjects and 315 patients with POAG, including 132 patients with NTG and 183 with HTG, this variant was seen in 1.5% of those with NTG and none in controls or those with HTG.[104] The exact mechanism by which *OPTN* E50K causes glaucoma is still incompletely understood. However, studies suggest that retinal ganglion cell death associated with E50K involves autophagy.[101,103]

A genetic linkage study found that duplication of the *TBK1* gene was associated with familial cases of NTG.[105] *TBK1* has been shown to be expressed in retinal ganglion cells, the retinal nerve fibre layer and retinal microvasculature.[105] *TBK1* encodes for a protein that regulates the expression of genes in a stress-response signalling pathway. The function and location of expression of this gene has led to the hypothesis that copy number variations of *TBK1* result in dysregulation of stress-response pathways, ultimately contributing to retinal ganglion cell apoptosis and the development of NTG. Previous studies have shown that OPTN interacts with TBK1 and that the OPTN E50K mutant leads to enhanced binding to TBK1 and insoluble aggregates.[106,107]

In summary, disease-causing variants in these genes can lead to early-onset and severe glaucoma. Discovery of these genes has facilitated genetic screening and early identification of at-risk family members[96] who can benefit from closer surveillance and timely intervention.

B: GLAUCOMA GENETIC TESTING

B.1 Polygenic risk scores

Given Mendelian variants account for only a minority of glaucoma cases, the majority of the genetic contribution to disease is thought to be complex. Complete understanding of this complex inheritance has not yet been achieved and is an ongoing area of research.

Genome-wide association studies (GWAS) are an approach that has been particularly successful in identifying genetic variations associated with a specific disease. This method involves scanning genomes of large populations of individuals with and without a particular disease or phenotype to identify genetic variations that are significantly more frequent in people with the disease compared to people without the disease. These single nucleotide polymorphisms (SNPs) associated with the trait studied then act in an additive manner.[108–112] Each SNP confers a different effect on disease risk, with the effect size of each SNP derived from its strength of association with a disease or trait.

The number of identified SNPs associated with POAG is continuing to increase, derived from large multi-ethnic meta-analyses of GWAS studies on cases and controls. The earliest glaucoma risk variant identified in GWAS was a locus near the *Caveolin 1* and *2* (*CAV1* and *CAV2)* genes. This was followed by the identification of loci at or near *TMCO1, CDKN2B-AS1*, *SIX6, ABCA1, AFAP1, GMDS, TXNRD2, ATXN2, FOXC1, ARHGEF,* and *CDKN1A* genes.[111,113–118] A multivariate analysis of GWAS by Craig et al identified 114 statistically independent SNPs associated with glaucoma, confirming all previously identified loci and 49 novel loci.[108] Gharahkhani et al. also conducted a large multi-ethnic meta-analysis of GWAS, identifying 44 novel loci and confirming 83 previously known loci.[119] Most recently, Han et al performed a large-scale multitrait POAG GWAS, identifying 263 loci in a European cohort, and 312 loci in additional cross-ancestry studies.[120]

Advances in technology, reduced costs and larger datasets have allowed for genetic association studies of POAG and its endophenotypes to be performed, including IOP and vertical cup-to-disc ratio (VCDR). These are recognised clinical risk factors, with VCDR being a morphological indicator of optic nerve damage, often as a result of raised IOP. POAG endophenotypes are also highly heritable. Several studies have identified a number of novel variants associated with IOP and optic nerve morphology, including VCDR.[109,111,121]

Polygenic risk scores (PRS) summarise the genetic information identified from GWAS into an accessible tool to quantify the genetic risk for complex diseases. A PRS represents the additive effect of independent risk alleles an individual carries weighted by the effect size of each variant.[122] The PRS is usually presented as a percentile risk relative to the normal population or study cohort to allow for easy assessment of where an individual lies on the population distribution. Ultimately, this score is not a diagnostic tool, rather, it is best utilised in addition to conventional risk factors to estimate overall disease risk.

More recent research utilising improved GWAS, has evaluated the genetic origin of glaucoma endophenotypes, including VCDR and IOP, and their overlap with POAG. Several studies reported evidence of genetic overlap between VCDR and POAG,[123–126] and between IOP and POAG.[110,112,124,125,127] However, the genetic underpinnings of IOP and VCDR appear to be independent, with little demonstrated evidence of genetic overlap.[123] Participants with both IOP PRS and VCDR PRS in the top tertile were 7.77 (95% CI, 2.02-19.93, $p=2.0x10^{-5}$) times more likely to have POAG compared with those with both in the bottom tertiles.[124] PRS has been used to enhance understanding of the differences in genetic origins of glaucoma phenotypes, including HTG or NTG. Mabuchi et al. calculated a genetic risk score based on the number of IOP-related genetic variants and assessed its association with maximum IOP, mean VCDR and phenotype (HTG or NTG).[125] A higher genetic risk score was associated with a higher maximum IOP (p=0.012) and larger VCDR (p=0.010).[125] A high IOP genetic risk score was associated with HTG but not NTG, providing evidence of differences in the genetic origins of these POAG phenotypes.[125] PRS has strengthened the known relationship between IOP and POAG, providing evidence of genetic association. Gao et al. demonstrated that those in the top quintile of their IOP PRS were 6.34 (95% CI 4.82-8.33, $p=2.1x10^{-57}$) times more likely to have POAG compared with those in the bottom quintile.[112] Similar results were found in a study by Qassim et al. which derived a PRS from IOP-associated genetic variants and examined its association with POAG.[110] A dose–response relationship was found between the IOP PRS and the maximum recorded IOP, with the high genetic risk group having a higher maximum IOP by 1.7 mmHg (standard deviation [SD], 0.62 mmHg) than the low genetic risk group (p=0.006).[110] Compared with the low genetic risk group, the high genetic risk group had a younger age of diagnosis by 3.7 years (SD, 1.0 years; p<0.001), more family members affected by 0.46 members (SD, 0.11 members; p<0.001), and higher rates of incisional surgery (odds ratio, 1.5; 95%CI, 1.1–2.0; p=0.007).[110] PRS combining all known glaucoma SNPs has shown positive results in stratifying risk for glaucoma. Craig et al.

demonstrated the utility of a glaucoma PRS to stratify individuals across the risk spectrum for developing glaucoma and likelihood of progression. This PRS was associated with higher glaucoma risk (top 10% PRS compared to remaining 90% glaucoma OR=4.2, 95%CI 3.43-5.17, p= $1.4x10^{-42}$) as well as more rapid disease progression, and higher treatment intensity.[108] Individuals in the top PRS decile were at 15-fold increased risk of developing advanced glaucoma compared to the bottom decile.[108] Furthermore, Siggs et al. found that high polygenic risk conferred a comparable risk to monogenic variants, while being over 15 times more prevalent in the general population, as well as influencing the penetrance and age at diagnosis.[128] Siggs et al. found that individuals in the top 5% of glaucoma PRS risk were at a higher risk of visual field progression compared with the remaining 95% after 5 years (hazard ratio, 1.5; 95%CI 1.13-1.97; p=0.005).[129] These studies highlight the predictive ability of PRS for glaucoma and its endophenotypes for risk stratification of the development, progression and treatment of glaucoma.

In comparing the strength of association for identified genetic variants and disease between glaucoma and other conditions, there are indications that the PRS for glaucoma performs better than PRS's for other conditions. From the recent paper on glaucoma PRS, the OR for the top 1% versus the remaining cohort was 8.5.[108] Comparatively, the OR for the top 1% of distribution for coronary artery disease , type 2 diabetes mellitus , inflammatory bowel disease and breast cancer were 4.83, 3.30, 3.87 and 3.83, respectively.[130] However, direct comparison cannot be made given the significant differences in the populations from which these results were derived. For the glaucoma PRS, a registry comprising participants across a spectrum of glaucoma was used, while the UK Biobank (UKB) comprised a more genetically diverse population. This was addressed by a more recent study which benchmarked the performance of different PRSs for the same disease in the UKB, reporting a lower OR (~2.0).[131]

The ever-expanding understanding of the genetic underpinnings of glaucoma present an exciting opportunity to tailor glaucoma care based on an individual's personal genetic risk. Personalised medicine utilises knowledge of disease-contributing genetic variants to predict individual disease risk, severity, and response to treatment. Theoretically, this allows for screening and management to be tailored for an individual based on their calculated underlying genetic predisposition. PRSs are an emerging clinical tool which offer a unique opportunity to improve disease risk prediction for complex heterogeneous diseases such as glaucoma.

B.2 Clinical utility - benefits

PRSs have the potential to enhance risk prediction, improve population screening, refine clinical diagnosis and disease classification, predict severity and prognosis, and allow for more precise treatment. Given our genetic make-up is largely stable from birth, risk stratification through a PRS presents an opportunity for early identification of high disease risk for many common conditions and has the potential for broad-based applications to population health. The potential clinical utility of PRS has been demonstrated in several common diseases with complex heritability including cardiovascular disease, diabetes, breast cancer, colorectal cancer, prostate cancer, and psychiatric disorders.[130,132,133]

PRS can be utilised and acted upon earlier than can many lifestyle, age and non-genetic factors. By identifying baseline genetic risk early in life, before many environmental risk factors are present, appropriate risk-reducing strategies can be put in place. For some conditions, such as coronary artery disease, increased genetic risk may be mitigated by limiting or reducing the impact of lifestyle risk factors.[134] It can also capture a level of risk that is independent of non-genetic risk factors, and therefore used in conjunction with traditional risk factors to estimate overall risk. The early stages of many common diseases, particularly chronic conditions, are often insidious, meaning PRS has the potential to improve early detection for example by more regular monitoring of those who are identified to be at high risk. Despite age being a non-modifiable risk factor, genetic screening for POAG may be useful in those over the age of 50 where heritability is usually complex. Recent GWAS have shown an area under the receiver operating characteristic curve score of 0.76.[121,135] In this age group, genetic testing may therefore be useful in discriminating cases from controls. Finally, PRSs provide the opportunity to estimate risk trajectories across a lifetime, rather than within a particular time frame.

In the future, PRS may be utilised as a screening tool to identify those who are most at risk of developing a particular condition and facilitate early diagnosis. This will also allow for a more personalised approach to longer term screening and management. This could reduce the number needed to have further potentially invasive screening investigations such as colonoscopies, as well as guide follow-up and timing of treatment. For glaucoma, PRS stratification may help to triage patients and guide surveillance timeframes for glaucoma suspects. For example, those at higher risk of progression may benefit from more frequent review, and earlier or more aggressive intervention.

B.3 Clinical utility - gaps in knowledge

There are a number of knowledge gaps that must first be addressed before PRS can be integrated into clinical practice. Several of these have been addressed in this thesis as original contributions to knowledge.

Firstly, relevant stakeholders must be committed to managing the aims and outcomes of a risk-based test such as a glaucoma PRS. Consumer engagement is an integral aspect which must be addressed before this form of testing can be implemented. This is a significant focus of this thesis. Consumer engagement leads to improved health outcomes and service delivery, and subsequently, ensures health services are being delivered effectively and are targeted to people's needs. We have sought to address this by assessing the attitudes of various groups including patients, family members of individuals with glaucoma, general members of the community and healthcare professionals. We have also investigated what factors may influence their attitudes.

Secondly, benefit should be clearly evident before implementation can occur. A glaucoma PRS has been shown to improve prediction in combination with other risk factors including IOP and VCDR.[110,136] However, the correlation between a glaucoma PRS and other risk factors has not been fully explained. In this thesis, we explore the interplay between PRS and risk factors, including family history and treatment. A model integrating PRS with other risk factors is needed to accurately assess individual risk. Furthermore, a glaucoma PRS has not yet been tested in a prospective cohort. This thesis begins to address this need for evidence of clinical validity by assessing a glaucoma PRS in a general Australian population.

Thirdly, results should lead to actionable and cost-effective measures. Clear guidelines will be needed to clarify which PRS classifications warrant intervention. Ideally, this requires specialist consensus, however realistically, it will likely be highly variable between countries and jurisdictions, driven by cost and differences in funding. Cost-effectiveness analyses are needed for public health frameworks to be developed.

Fourthly, frameworks will need to be developed to ensure results are communicated in a transparent and meaningful manner. In particular, to ensure PRS results promote positive changes in health behaviour, it may be necessary to emphasise that high-risk scores are not diagnostic for disease, or that low-risk scores do not guarantee being disease-free in the future. In this thesis, we sought to develop and assess PRS reports for patients. The complexities of

simplifying PRS results for individuals across a spectrum of numeracy levels was illustrated, however, provided an early indication of several key elements for clear communication. In particular, participants felt their risk response and behaviour would be influenced by clear recommendations guiding the most appropriate action to their risk result.

Finally, a significant concern surrounding the clinical implementation of PRS is that the majority of PRS are developed from predominantly European populations. Comprehensive inclusion of other ancestries and validation of single pan-ancestry PRS, or ancestry-specific scores covering all ancestries, are essential to avoid future health disparities. This thesis will compare risk prediction outcomes between European and non-European groups, depending on the distribution of ancestries within the cohort.

C: CONCLUSION

The ability to identify at-risk individuals will allow for closer monitoring and timely intervention, and ultimately reduce irreversible vision loss. This thesis aimed to address some of the key issues including gaps in understanding the attitudes of various groups towards testing, risk communication and the clinical utility of PRS testing for glaucoma. The outcomes from this thesis will form the basis for future interventional studies to further enable a shift in the detection, treatment and prevention of disease with complex inheritance. PRS may provide the opportunity for individuals to limit the impact of their genetic predisposition for many common conditions.

PART 1: ATTITUDES TOWARDS GLAUCOMA GENETIC RISK ASSESSMENT

INTRODUCTION AND AIMS

Clinical implementation of a glaucoma PRS will rely on general acceptance of genetic predictive testing by the community and healthcare professionals. Studies have assessed the perspective of patients and potential testing target groups[137–140] and clinicians[141–143] in the context of common diseases, however no studies have investigated this for glaucoma.

This chapter addresses three main aims. Firstly, to investigate understanding of genetic risk and the attitude of different groups of individuals toward polygenic risk testing for glaucoma, including individuals with glaucoma, first-degree relatives of individuals with glaucoma, members of the general community and healthcare professionals. Secondly, to assess what variables may influence interest in glaucoma PRS testing and how these factors differ between each group. Finally, to determine whether knowledge about genetic risk would lead to a change in behaviour or clinical practice.

Together with general acceptance, clinicians must have a sound understanding of polygenic risk testing and in interpreting the significance of results. Given the potential for broad population screening, the ordering of PRS testing, interpretation of results, and communication of their significance to patients will likely extend beyond the healthcare professionals directly involved in the management of individuals with glaucoma to those involved in any aspect of PRS testing in the future. Thus, specialist groups including ophthalmologists, general practitioners, clinical geneticists, genetic counsellors, optometrists, orthoptists, and laboratory scientists, have been included.

The results from the studies included in this chapter will help to identify potential target testing populations and guide implementation strategies.

METHODS

Description of study cohorts

The studies in this chapter have utilised a number of databases. These include the Australian and New Zealand Registry of Advanced Glaucoma (ANZRAG) and the Targeting at risk Relatives of Glaucoma patients for Early diagnosis and Treatment (TARRGET) database.

The Australian & New Zealand Registry of Advanced Glaucoma (ANZRAG)

Established in 2007, ANZRAG is a comprehensive registry of clinical and genetic data of glaucoma cases from Australia and New Zealand to identify genetic risk variants of severe or familial glaucoma. Aiming to identify glaucoma genetic risk variants, the registry includes participants across the whole spectrum of glaucoma, ranging from glaucoma suspects to end-stage glaucoma, and includes both open and closed angle glaucoma, as well as primary and secondary cases. However, there are a disproportionate number of advanced glaucoma cases owing to early phase focused recruitment of participants with advanced open-angle glaucoma. Recruitment methodology and cohort description have been described previously, [144] but is summarised here for its relevance to this thesis, and to provide an update on the methodology since the previous publication. Recruitment for ANZRAG is currently ongoing at the time of writing.

Referral to ANZRAG is initiated either through the participants' treating clinicians or via self-referral with subsequent verification of the clinical details through participants' ophthalmologist. Referral was initiated via a paper- or web-based submission, and details verified by registry staff. Clinical diagnosis of glaucoma was made based on optic nerve head appearance, IOP, and visual field testing, and was made by the referring ophthalmologist.[1] Open-angle glaucoma was defined by a glaucomatous optic neuropathy with a CDR of ≥0.7, with neuroretinal rim thinning and corresponding visual field loss in a pattern typical of glaucoma. Glaucoma suspect cases were defined as ocular hypertension (intraocular pressure >22mmHg) alone, CDR of 0.5-0.65 with corresponding field loss on 24-2 field test, or CDR of 0.7-0.75 without field loss. Participants with the following glaucoma risk factors or glaucoma subtypes were recruited irrespective of the presence of field loss, ocular hypertension or neuroretinal rim thinning: pseudoexfoliation, pigment

dispersion, primary congenital glaucoma, angle closure, anterior segment dysgenesis, steroid responders, and nanophthalmos.

Details of clinical assessments were collected at the time of recruitment by the referring clinician. Family history of glaucoma was recorded as the number of family members affected by glaucoma, and the relationship to the closest relative with glaucoma. Additionally, the age of glaucoma diagnosis, self-reported ethnicity, best corrected visual acuity, maximum recorded pre-treatment IOP, refraction, CCT, vertical cup-to-disc ratio, and previous glaucoma surgeries were recorded. Blood or saliva samples were collected from the participants at enrollment for genotyping. Genotyping in ANZRAG participants was performed over several stages through the course of recruitment and was performed using Illumina Omni1M, OmniExpress or HumanCoreExome arrays (Illumina, San Diego, CA, USA). Genotyping quality control, imputation, and association analyses were conducted separately for each phase before being meta-analysed for association studies. Human research ethics approval was obtained from the relevant committees of the Southern Adelaide Clinical Human Research Ethics Committee (SAC HREC)/Flinders University, the University of Tasmania, QIMR Berghofer Institute of Medical Research and the Royal Victorian Eye and Ear Hospital. Written informed consent was obtained from all participants in accordance with the Declaration of Helsinki.

Targeting at risk Relatives of Glaucoma patients for Early diagnosis and Treatment (TARRGET)

The TARRGET study is a prospective, randomised study aiming to measure knowledge of familial glaucoma, perceived risk, self-reported glaucoma status, and prevalence of diagnosed and undetected glaucoma. The study also aims to evaluate a targeted education and screening program for first-degree relatives (FDR) of people with advanced POAG, drawn from the ANZRAG database. Participants were contacted by telephone and a detailed pedigree of FDRs was constructed. FDR contact details were provided by the index participant who were then contacted to record self-reported glaucoma status and recruit into the study. FDRs were included if aged over 40 years or within 10 years of their relatives age of diagnosis if prior to age 40. All participants were aged 18 years and above, and written consent was obtained.

Clinical examination details were collected from FDRs to confirm glaucoma status. Prior to undergoing examination, participants completed a questionnaire to assess self-reported glaucoma status, knowledge of glaucoma family history, perception of personal glaucoma risk,

understanding of the results of their most recent eye examination, and motivation for participation. South Australian participants were invited to attend a complimentary, comprehensive glaucoma assessment performed by a study doctor at Flinders Medical Centre or the Repatriation General Hospital in Adelaide, or by the participants chosen optometrist or ophthalmologist at an external clinic. Examination included visual acuity, refraction, automated perimetry (Humphrey Field Analysis 24-2, SITA Standard), slit lamp microscopy, Goldmann applanation tonometry, central corneal thickness, dilated fundus examination (including VCDR), OCT, and disc stereo photography.

Examination data were reviewed by two glaucoma specialists and a study doctor to determine the glaucoma status of each FDR. Individuals were classified as normal, glaucoma suspect or glaucoma based on the results from visual field testing, disc appearance and imaging. Following the OHTS criteria, a reliable visual field was defined as abnormal if the Glaucoma Hemifield Test was outside of normal limits and/or the Corrected Pattern Standard Deviation was $P < 5\%$.[145]

Optic disc appearances were graded as normal, suspicious or glaucomatous according to the Glaucoma Inheritance Study in Tasmania protocol.[146] Participants were classified as normal if they displayed normal optic disc, OCT, visual field and IOP. Glaucoma suspects were defined as those with normal visual fields, and equivocal glaucomatous optic disc appearance with no evidence of neural rim thinning or notch. Glaucoma was diagnosed by CDR of ≥0.7 and evidence glaucomatous optic neuropathy including focal neuroretinal rim thinning, retinal nerve fibre layer defects, disc haemorrhages, right/left asymmetry, and bared circumpapillary vessels, with corresponding field defects.[5] For further confirmation, any participants who presented with abnormal glaucoma hemifield test on HVF with corresponding optic nerve head changes were asked to repeat the visual field test within 1 to 3 months to confirm the reproducibility of the defect.

Participants received standardised feedback regarding their glaucoma status at the visit with a study doctor or via a mailed feedback letter. Feedback included findings of the screening and recommendations for future action including; 2-yearly eye checks, ongoing monitoring of suspicious signs, enrolment in a longitudinal monitoring project (South Australian residents only) or ongoing ophthalmic care for glaucoma.

AFFECTED INDIVIDUALS

Study Sample

This was a cross-sectional, questionnaire-based study approved by the SAC HREC (2020/HRE00680) and it adhered to the Revised Declaration of Helsinki. The study sample included participants with diagnosed glaucoma, drawn from the ANZRAG.[144] A pilot questionnaire was tested with a sample of ten randomly selected individuals from the community and modifications were made based on the feedback received. A letter of invitation was sent to eligible participants providing them information about a new genetic test to predict a person's risk of developing glaucoma by providing them a genetic risk score. The invitation letter is provided in Appendix A1. The questionnaire was mailed to 2369 of the living ANZRAG participants who met the inclusion criteria of adults with a diagnosis of POAG, had not received genetic results that explain their condition (i.e. not *MYCO* positive), resided in Australia and had agreed to receive correspondence. Consent to participation was implied by completion of the survey. The questionnaire is provided in Appendix A1.

Independent variables

Sociodemographic, health, perception, and emotional factors were examined to assess associations with interest in genetic testing. Perception and emotional variables were assessed in a retrospective sense, with participants asked to consider their possible perspective prior to being diagnosed with glaucoma.

Sociodemographic

Age, gender, ethnicity, education, and urban/rural residence were collected. Family history was acquired from the ANZRAG database and was self-reported by respondents at the time of recruitment. Family history of glaucoma, the number of family members affected, and their degree of relation was collected. Ethnicity was self-identified by respondents and defined in parallel to the ANZRAG classification.[144] Urban/rural status was based on the Australian Bureau of Statistics census data using the participants' postcodes. Urban status was classified as postcodes with over 50,000 residents.

Health factors

Eye health factors included history of myopia, time since last eye check (by an optometrist or ophthalmologist) and frequency of eye examinations. In addition to the information obtained from

the questionnaires, clinical data related to glaucoma was acquired from the ANZRAG database. This included classification as advanced or non-advanced glaucoma, age at diagnosis, and specific indicators of glaucoma severity including best-corrected visual acuity (BCVA) and VCDR. In the ANZRAG database, advanced glaucoma was defined as central visual field loss related to glaucoma with at least two of the four central fixation squares having a pattern standard deviation probability less than 0.5% on a reliable Humphrey 24-2 field analysis (Carl Zeiss Meditec, Dublin, CA), or a mean deviation (MD) worse than -15dB or, in the absence of visual field testing, BCVA worse than 20/200 due to glaucoma.[144] Additionally, evidence of glaucoma was required to be present in the less severely affected eye, demonstrated through glaucomatous visual field defects with corresponding optic disc rim thinning, including an enlarged cup-to-disc (≥0.7) or cup-to-disc asymmetry (≥0.2) between both eyes.[144] BCVA was converted to a decimal equivalent for ease of analysis and interpretation. Legal blindness was defined by a visual acuity of 20/200 or worse. The poorest recorded result between the right and left eye of the clinical indicators of severity were used for analysis.

Perception and emotional factors

Perceptive factors were assessed through single-item measures with Likert-like scale response options. Variables included perceived knowledge regarding glaucoma, perceived severity of disease, and perceived glaucoma susceptibility prior to diagnosis. To assess the influence of emotion on interest in testing, participants were asked about their anxiety related to the possibility of developing glaucoma prior to diagnosis.

Outcome variable

Interest in genetic testing was evaluated through assessing likelihood to take the test to predict personal risk of disease and disease severity, and whether the individual would recommend the test to family or non-family members. A Likert-like scale was used to assess personal interest and attitude towards testing for others. Participants were given the opportunity to comment on their selected responses regarding their interest in genetic testing and how they might have changed their health-seeking intentions toward glaucoma screening and management.

Additional factors

Other factors relating to the test itself and communication of results were assessed. Aspects of

the test that were considered important to know about prior to undergoing genetic testing and preferred method of receiving genetic test results were assessed. Participants were given the opportunity to comment on any additional aspects of concern or interest regarding the test itself.

Statistical analysis

Prior to the distribution, the survey was trialled with 10 volunteers at Flinders Medical Centre and members of the community to ensure ease of completion and that questions were comprehensible. In addition, the survey was trialled with clinicians however, was not validated for its effectiveness by an expert panel. Data were analysed using the Statistics Package for the Social Sciences (Version 25.0, SPSS Inc., Chicago, USA). Descriptive statistics were used to characterise the study sample. Responses were combined into bivariate outcomes; 'highly unlikely' and 'unlikely' responses were merged into a single 'uninterested' group, and 'likely' and 'highly likely' were merged into a single 'interested' group. 'Unsure' responses for all questions were excluded. Univariate logistic regression was performed between level of interest and covariables (sociodemographic, emotional and perception variables). Variables that had significance levels of $p < 0.1$ in the univariate analysis were included in the multivariate regression model. Multivariate logistic regression modelling was used to identify factors independently associated with interest in testing ($p<0.05$) using a backward stepwise approach. Where multiple comparisons were made on the same data, Bonferroni correction was applied.

Non-respondents

Demographic and clinical data were obtained for those who did not complete the survey (obtained at referral to the ANZRAG) and analysed for comparison. These demographic data included age, gender, and urban/rural status, and clinical data included family history of glaucoma, age at diagnosis, classification of severity (advanced/non-advanced), VCDR and BCVA.

UNAFFECTED INDIVIDUALS

Study Sample

This was a cross-sectional, questionnaire-based study approved by the SSAC HREC (2020/HRE00680) that adhered to the Revised Declaration of Helsinki. The study sample included three different groups of individuals who may be target populations for polygenic risk testing for glaucoma and who were recruited between March 2020 and March 2021. We aimed to recruit 100 participants in each group. Using a one-sided test with multiple test correction (alpha 0.01), 100 participants in each group will yield 100% power to detect a difference in levels of interest of 20% or more. The first group included unaffected first-degree relatives of individuals with a known glaucoma diagnosis, with participants drawn from the ANZRAG and the TARRGET study. The second group included people attending an optometrist for an eye assessment for conditions other than glaucoma, or those with no ocular health history who had undergone an eye assessment within the last six months. This group is referred to as the 'optometry' group. These participants were recruited from private (Specsavers) and public (Flinders University) optometry clinics. The third group comprised members of the general community without an ocular health history, who had not undergone a recent eye examination. Recruitment occurred at Flinders Medical Centre (including the Flinders Volunteer service) and Noarlunga Hospital in Adelaide, Australia and included Flinders volunteer members, patients, and their relatives in outpatient hospital clinics. Individuals for the first two groups were also recruited from these clinics if they had a first-degree relative with glaucoma or had a recent eye examination. Recruitment from public hospital settings as well as public and private clinics was opportunistic. Participants were included if they had capacity to complete the questionnaire without assistance (except if needing an interpreter). Participants were excluded if they were <18 years old or did not have cognitive capacity to complete the questionnaire. Similar to the affected cohort, a letter of invitation was sent to eligible participants providing them information about a new genetic test to predict a person's risk of developing glaucoma by providing them a genetic risk score. The invitation letter is provided in Appendix A2.

Data Collection

The questionnaire was adapted from previously published surveys[147] and used Likert-like scale items. The questionnaire was first tested with ten individuals from Flinders Medical Centre and modifications were made based on the feedback received. Socio-demographic, health, cognitive,

emotional and influencing factors were used to assess association with interest in genetic testing. The questionnaire is provided in Appendix A2.

Socio-demographic

Age, gender, ethnicity, highest level of education, and urban/rural residency were collected. Ethnicity was self-reported and classified into 10 ethnic groupings, then into categories of "European" and "non-European" ethnicity. Those recorded as "unknown" were excluded from analyses involving ethnicity. Residency was based on the Australian Bureau of Statistics census data using the participants' postcodes. Urban residency was classified as postcodes with populations greater than 50,000 persons. Rural residency included regional, rural and remote areas of populations less than 50,000 persons.

Health factors

Family history, including the number of family members affected by any form of glaucoma and their degree of relation, was self-reported by participants. Eye health factors assessed included a history of myopia, most recent eye check, and the frequency of eye checks.

Cognitive factors

Cognitive factors were assessed through single-item measures with Likert-like scale response options. We assessed participants' understanding of the heritability of glaucoma, perception of the severity of glaucoma and perceived likelihood of developing glaucoma.

Emotional factors

To assess the influence of emotion on interest in genetic testing for glaucoma, we asked participants to indicate their level of worry related to the possibility of developing glaucoma in the future using Likert-like scale response options.

Factors affecting decision to be tested and concerns

We assessed several factors which could affect participants' decision to be tested related to their own risk, their family's risk and advice from others. We assessed factors which would concern participants about testing, including personal anxiety, cost, future requirements, and issues relating to confidentiality and implications of results. Participants could also include additional factors or comments.

Outcome variable

Interest in genetic testing for glaucoma was evaluated by assessing likelihood to undergo genetic testing to predict personal glaucoma risk with Likert-like scale response options.

Additional factors

Participants were asked about aspects of the test that would be considered important to know prior to undergoing genetic testing, the cost participants would be willing to pay, and their preferred method of receiving results. Participants were asked to indicate how their behaviour towards their eye health might change based on theoretical results of higher and lower risk of developing glaucoma, and the frequency of eye checks which they would be willing to undergo.

Statistical analysis

Data were analysed using Statistics Package for the Social Sciences (Version 27.0, SPSS Inc., Chicago, USA). Descriptive statistics were used to characterise the study sample. Responses from the three groups were combined for the statistical analysis. Responses were combined into bivariate outcomes; for example ‘highly unlikely and unlikely’ were merged into an ‘uninterested’ group, and ‘likely’ and ‘highly likely’ were merged as an ‘interested' group. Unsure or missing responses for all questions were excluded. Associations of different variables between the three groups were analysed using one-way ANOVA and Chi-square test for association for continuous and categorical variables, respectively. The association between level of interest and covariables (sociodemographic, emotional, and cognitive variables) was performed using a univariate logistic regression model. Variables that had significance levels of $p<0.1$ in the univariate analysis were initially included in the multivariate regression model. Multivariate logistic regression models were performed to identify factors independently associated with interest in testing ($p<0.05$) using a backward stepwise approach.

HEALTHCARE PROFESSIONALS

Study Sample

The study was approved by the SAC HREC (2020/HRE00680). An anonymous cross-sectional online questionnaire was developed using the software Qualtrics. The study sample included seven groups of healthcare professionals who may be involved in interpreting and/or communicating PRS results for glaucoma to patients in the future: Ophthalmologists, optometrists, orthoptists, general practitioners (GPs), clinical geneticists, genetic counsellors, and laboratory scientists were invited to participate. Participants were eligible if they self-identified to one of these groups and had completed training. Participants were grouped by specialty into Ophthalmic (ophthalmologists, optometrists and orthoptists), Genetics (geneticists and genetic counsellors) and GPs. Participants who self-identified as laboratory scientists were not included in the statistical comparisons due to the small number. Consent was implied by completion of the questionnaire.

Data Collection

The questionnaire was generated based on the existing literature on healthcare professionals attitudes and was refined by the authors. A pilot was conducted on a sample of 14 healthcare professionals and modifications were made based on the feedback received. Participants were recruited between November 2021 and October 2022. There was no set sample size based on the exploratory nature of the study. A non-probability sampling approach was used to distribute the questionnaire to healthcare professionals via relevant professional governing bodies in Australia. This included the Royal Australian and New Zealand College of Ophthalmologists (RANZCO), the Royal Australian College of Physicians (RACP), the Royal Australian College of General Practitioners (RACGP), the Human Genetics Society of Australasia (HGSA), Optometry Australia, and Orthoptics Australia. The questionnaire was also emailed to an in-house mailing list of ophthalmologists. The questionnaire is provided in Appendix A3.

Demographic characteristics: Age, gender and ethnicity were collected. Ethnicity was self-reported, classified into ethnic groupings, then into categories of “European” and “non-European” ethnicity. Those with more than one self-reported ethnicity were categorised as “Mixed” ethnicity. Those recorded as “unknown” were excluded from analyses involving ethnicity. Family history of glaucoma, including the number of family members affected and their degree of relation, was self-reported by participants. To characterise the professional characteristics of the cohort, we asked the number of years since completing training and practising in their occupation, the type and

structure of the workplace, exposure to genetics training, and whether participants held an academic position. We further assessed ophthalmologists' professional exposure and experience with glaucoma by asking the percentage of patients seen with glaucoma.

Glaucoma knowledge: Knowledge of glaucoma heritability was measured with 3 items on the perceived importance of assessing family history of glaucoma (first and second degree) and the age at glaucoma diagnosis, using a 5-point Likert-style response scale. Self-reported glaucoma knowledge was assessed with 5 items using scaled responses (1 to 10) to the condition in general, risk factors, diagnosis, genetics and current screening recommendations.

Experience with genetic testing: Participants were asked whether they had counselled a patient about a genetic issue, had requests from patients about genetic tests, referred patients for a genetic test or ordered a genetic test for glaucoma, an eye condition or any genetic condition. The methods that results were received and communicated were also assessed.

Confidence with genetic testing: Participants were asked to rate their level of knowledge in genetics using scaled responses (1 to 10). Their level of confidence to assess genetic risk through family history, counsel patients on genetic testing, order genetic tests, interpret results and refer to genetic services was assessed with 8 items using a 5-point Likert-style response scale.

Familiarity with polygenic risk: We assessed participants' familiarity with polygenic risk for any condition, an eye condition and glaucoma using a 5-point Likert-style response scale.

Attitudes towards polygenic risk testing: Participants were asked about their likelihood to recommend the test to 6 different groups of individuals using a 5-point Likert-style response scale based on the known risk factors for glaucoma (positive family history, older age, African ancestry): individuals with first-degree relatives, second-degree relatives, over the age of 50 years, over the age of 70 years, Asian ancestry and African ancestry.

Factors affecting the decision to recommend and order genetic tests: The importance of 12 different factors in recommending the test and the importance of 4 different factors in ordering the test were assessed using a 5-point Likert-style response scale.

Preferences for ordering and communicating polygenic risk results: We asked participants who would be the most appropriate healthcare professionals to order the test, to communicate low vs high PRS risk and what would be their preferred method of communicating results.

Training requirements: Participants reported whether they would benefit from additional training on genetic testing, interpreting genetic test results and polygenic risk scores, using a 5-point Likert-style response scale, and their preferred method for education delivery.

Statistical analysis

Data were analysed using Statistics Package for the Social Sciences (Version 27.0, SPSS Inc., Chicago, USA). Descriptive statistics were used to characterise the study sample. Due to the small sample size of some of the groups, Fisher's exact test was used to explore differences between the 3 different specialty groups. Bonferroni corrections were made for multiple comparisons, and $p<0.017$ was considered significant when comparing differences between the 3 specialty groups.

CHAPTER 1: ATTITUDES TOWARDS POLYGENIC RISK TESTING IN INDIVIDUALS WITH GLAUCOMA

PUBLISHED MANUSCRIPT

The contents of this chapter have been published in a peer-reviewed manuscript of which I am the first author:

Hollitt GL, Siggs OM, Ridge B, Keane MC, Mackey DA, MacGregor S, Hewitt AW, Craig JE, Souzeau E. Attitudes towards polygenic risk testing in individuals with glaucoma. *Ophthalmology Glaucoma*. November 2021. doi:10.1016/j.ogla.2021.11.002

My contributions to the manuscript involved the research conception and design (60%), data collection including participant recruitment and data entry (90%), generation of a dataset (100%), data analysis including statistical analysis (80%), interpretation of the data (60%), and drafting the manuscript (100%). Jamie Craig, Emmanuelle Souzeau and Owen Siggs contributed equally to the manuscript including study concept and research design (40%), data analysis (10%), interpretation of the data (30%), critically revising the contents of the manuscript, project funding and supervision. Emmanuelle Souzeau and Bronwyn Ridge contributed equally to data collection (10%). Brownyn Ridge, Miriam Keane, David Mackey, Stuart MacGregor and Alex Hewitt contributed equally to data analysis (10%), data interpretation (10%), and critically revising the contents of the manuscript. The introduction and methods of this manuscript have been edited to fit the structure of this thesis.

1.1 RESULTS

1.1.1 Demographic Characteristics

In total, 2369 ANZRAG participants were invited to participate in the study, with 1169 completing the questionnaire, yielding a response rate of 49.3%. The demographic and personal characteristics of respondents are shown in Table 1.1. In summary, 53.5% of respondents were female, 92.9% were European, and 51.7% had an education level above secondary school. The mean age of the cohort was 75.7 ± 10.3 years, with 93.8% being over the age of 60 years. A positive family history of glaucoma was reported by 63.3% of respondents, with 87.2% of those with a positive family history having at least one affected first-degree relative. Of the 1200 who did not respond, limited demographic and clinical data were obtained from the ANZRAG database. In summary, 55.2% of non-respondents were female and 83.1% were European. The mean age of non-respondents was 77.7 years ± 14.5 years. Respondents and non-respondents did not differ by gender, age at diagnosis, family history or residency. However, respondents were more likely to be younger (p <0.001), of European ethnicity (p <0.001), and have less severe glaucoma reflected by non-advanced disease classification (p <0.001) and rate of legal blindness (p <0.001) compared to non-respondents (Table 1.1).

Variable	**Respondents** n = 1169 (49.3%)	**Non-Respondents** n = 1200 (50.7%)	**p Value**
Age (years) Range Mean (standard deviation) Median	 22.7 – 101.8 75.7 (10.3) 76.1	 20.4 – 108.5 77.7 (14.5) 79.7	**p <0.001***
Age at diagnosis (years) Range Mean (standard deviation) Median Unknown (excluded from analysis)	 20.0 – 89.0 59.1 (12.8) 60.0 n = 80	 17.0 – 94.0 59.2 (14.4) 60.0 n =93	p = 0.58*
Gender, n (%) Female Male	 625 (53.5) 544 (46.5)	 662 (55.2) 538 (44.8)	p = 0.43**

Ethnicity, n (%) European Non-European - Asian - Mixed Ethnicity - Middle Eastern - African - Australian Aboriginal - Hispanic Unknown (excluded from analysis)	 1086 (92.9) 76 (6.5) 46 (3.9) 19 (1.6) 5 (0.4) 4 (0.3) 1 (0.1) 1 (0.1) 7 (0.6)	 971 (83.1) 123 (10.5) 81 (6.9) 19 (1.6) 8 (0.7) 10 (0.9) 0 (0.0) 5 (0.4) 106 (8.8)	**p <0.001****
Residency, n (%) Urban Rural	 881 (75.4) 288 (24.6)	 932 (77.7) 268 (22.3)	p = 0.20**
Highest level of education, n (%) Primary School Secondary School Vocational Training University Unknown (excluded from analysis)	 73 (6.3) 487 (42.0) 285 (24.6) 314 (27.1) 10 (0.9)	-	-
Family history of glaucoma Positive Negative Unknown (excluded from analysis) Positive: - First-degree - Second-degree - Third-degree - Fourth-degree Unknown (excluded from analysis)	 768 (63.3) 389 (33.6) 12 (1.0) 674 (87.7) 86 (11.2) 7 (0.9) 3 (0.4) 2 (0.3)	 740 (63.2) 431 (36.7) 29 (2.4) 647 (87.4) 74 (10.0) 12 (1.6) 3 (0.4) 4 (0.5)	p = 0.12** p = 0.26**

Glaucoma severity, n (%) Advanced Non-Advanced <u>BCVA</u> >20/200 ≤20/200 Unknown (excluded from analysis) <u>VCDR</u> Range Mean (SD) Median <0.9 ≥0.9 Unknown (excluded from analysis)	 534 (45.7) 635 (54.3) 1063 (90.9) 72 (6.2) 34 (2.9) 0.10 - 1.0 0.81 (0.13) 0.80 728 (62.3) 409 (35.0) 32 (2.7)	 735 (61.2) 465 (38.8) 987 (82.3) 179 (14.9) 34 (2.8) 0.2 - 1.0 0.84 (0.12) 0.90 570 (47.5) 592 (49.3) 38 (3.2)	**p <0.001**^{**} **p <0.001**^{**} **p < 0.001**[*] **p <0.001**^{**}
Last ophthalmic review, n (%) Within 6 months 6-12 months 1-2 years More than 2 years Unknown (excluded from analysis)	 886 (77.5) 182 (15.9) 60 (5.3) 15 (1.3) 26 (2.2)	-	-
Frequency of clinical reviews, n (%) 3 monthly 6 monthly Annually Every 2 years More than every 2 years Unknown (excluded from analysis)	 172 (15.1) 678 (59.3) 229 (20.0) 47 (4.1) 11 (1.0) 32 (2.7)	-	-

Table 1.1: Characteristics of the study respondents and non-respondents.
BCVA: best-corrected visual acuity, VCDR: vertical cup-to-disc ratio. *denotes values calculated using paired Mann-Whitney U test for differences in median rank. ** denotes values calculated using Chi-square test for Association. Differences in ethnicity were assessed between Europeans and non-Europeans, family history between positive and negative history and VCDR between <0.9 and ≥0.9 groups.

1.1.2 Understanding of glaucoma and perception of severity and risk.

Prior to being diagnosed with glaucoma, only 16.9% of respondents felt they knew a fair amount or a lot about glaucoma (Supplementary Table 1). This was significantly associated with family

history, with those having a family history of glaucoma being more likely to have a better understanding of the condition prior to diagnosis (68.3% vs 50.2%, p <0.001). Furthermore, having a higher number of affected family members was associated with increased awareness of glaucoma (OR 1.43, 95%CI (1.29-1.59), p <0.001). The majority of participants (86.3%) considered glaucoma to be a moderately severe or very severe medical condition. About one third believed that they were either likely or highly likely to develop glaucoma in their lifetime (29.2%) and were either slightly, moderately worried, or very worried about developing glaucoma (29.1%) prior to receiving their diagnosis. A belief of being at risk and being worried about developing glaucoma were both associated with the presence of a family history (self-reported) (OR 6.01, 95%CI (4.18-8.62), p < 0.001; OR 3.0 95%CI (2.23-4.10), p < 0.001) and increasing number of affected family members (OR 1.88, 95%CI (1.65-2.15), p <0.001; OR 1.42, 95%CI (1.30-1.57), p <0.001).

1.1.3 Interest in genetic risk testing for glaucoma

Responses to survey questions are summarised in Supplementary Table 1. Overall, participants were in favour of glaucoma PRS testing. Over two-thirds (69.4%) of individuals were likely or highly likely to have taken a genetic test to predict their risk of developing glaucoma if it had been offered to them before they were diagnosed (Figure 1.1). Additionally, 96.2% of participants would possibly, probably, or definitely take a test to predict their risk of rapid progression or developing severe disease if stronger treatments could prevent blindness.

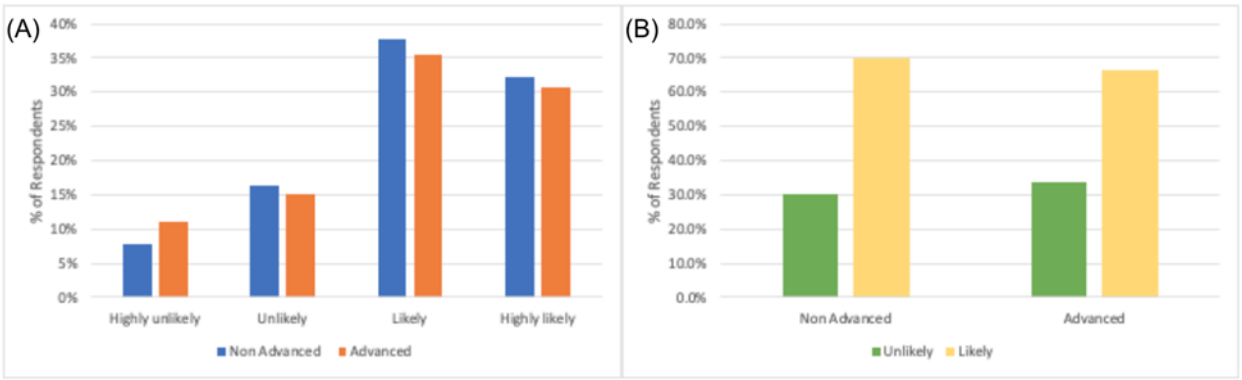

Figure 1.1: Level of interest in polygenic risk testing for glaucoma according to disease severity (A) and positive versus negative attitude (B). Responses to the question 'How likely would you have been to take a genetic test to predict your risk of developing glaucoma if it had been offered to you before you were diagnosed?'. Responses were grouped by disease severity (advanced or non-advanced) and by individual response (highly unlikely, unlikely, likely, or

highly likely) (A), or grouped into a positive (likely or highly likely) or negative (highly unlikely or unlikely) expressed interest (B). 60 respondents indicated being 'unsure' (5.2%).

1.1.4 Factors affecting interest in genetic risk testing for glaucoma

The association between demographic, perception and emotional predictor variables and interest in genetic risk testing for glaucoma was analysed (Table 1.2). Age, age at glaucoma diagnosis, gender, ethnicity, level of education, BCVA, VCDR, timing of last eye check, and frequency of eye checks were not associated with interest in glaucoma genetic risk testing in the univariate logistic regression. Variables that reached a significance level of $p \leq 0.1$ were included in a multivariate logistic regression to identify the impact of these variables on a positive interest in genetic risk testing for glaucoma (either likely or highly likely to have undergone testing if it were available). After adjusting for other predictor variables, urban residency was associated with increased interest in testing (OR 1.70, 95%CI (1.15-2.49), p 0.007).

Level of knowledge of glaucoma prior to diagnosis and perceived severity of glaucoma were not associated with increased interest in testing. Level of pre-diagnosis glaucoma awareness, perceived risk of glaucoma, and pre-diagnosis concern of developing glaucoma were significantly associated with interest in genetic risk testing for glaucoma in univariate analysis. Increased interest in testing was associated with an increased perceived risk of glaucoma (OR 2.05, 95%CI (1.28-3.29), p = 0.003) and pre-diagnosis worry about developing glaucoma (OR 2.07, 95%CI (1.27-3.37), p = 0.004) in the multivariate logistic regression model (Table 1.2).

Variable	**Univariate logistic regression**		**Multivariate logistic regression**	
	OR (95%CI)	**P value**	**OR (95%CI)**	**P value**
Age (per increasing year)	1.00 (0.99-1.01)	0.871		
Age at diagnosis (per increasing year)	1.00 (0.99-1.02)	0.487		

Gender - Male - Female	 1.00 1.15 (0.88-1.50)	 0.312		
European ethnicity - Yes - No	 1.00 1.05 (0.61-1.83)	 0.857		
Residency - Rural - Urban	 **1.00** **1.44 (1.06-1.83)**	 **0.018**	 **1.70 (1.15-2.49)**	 **0.007**
Level of education - Primary School - Secondary School - TAFE/Vocational Education - University	 1.00 0.88 (0.48-1.63) 0.80 (0.43-0.78) 0.78 (0.41-1.46)	0.797 0.682 0.502 0.428		
Family history - Unaffected - First-degree relative - Other relative	 **1.00** **1.66 (1.25-2.22)** **1.28 (0.77-2.12)**	**0.002** **<0.001** **0.338**	 1.06 (0.67-1.68) 1.39 (0.70-2.73)	0.638 0.802 0.348
Number of family members affected (per extra)	**1.16 (1.04-1.29)**	**0.009**	1.06 (0.92-1.13)	0.429
Glaucoma severity - Advanced - Non-advanced	 1.00 1.14 (0.87-1.49)	 0.341		
Best-Corrected Visual Acuity (Per improvement of 0.1 on decimal scale)	1.09 (0.70-1.71)	0.698		
Best-Corrected Visual Acuity - ≤ 20/200 - > 20/200	 1.00 1.13 (0.65-1.95)	 0.676		
Vertical Cup-to-Disc Ratio (Per increase of 0.1)	0.67 (0.23-1.92)	0.455		
Vertical Cup-to-Disc Ratio - ≥ 0.9 - <0.9	 1.00 1.19 (0.90-1.57)	 0.236		

Last eye check - > 2 years - 1 to 2 years - 6 to 12 months - Within 6 months	 1.00 0.60 (0.14-2.49) 1.12 (0.28-4.40) 1.08 (0.28-4.10)	0.229 0.479 0.875 0.913		
Frequency of eye checks - > 2 years - 1 to 2 years - Annually - Every 6 months - Every 3 months	 1.00 1.38 (0.30-6.38) 1.46 (0.35-6.01) 1.47 (0.36-5.93) 1.14 (0.28-4.76)	0.754 0.684 0.605 0.592 0.854		
Pre-diagnosis glaucoma awareness - Not aware - Aware	 **1.00** **1.65 (1.26-2.16)**	 **<0.001**	1.03 (0.67-1.06)	0.883
Knowledge of glaucoma - No knowledge - Good knowledge	 1.00 1.19 (0.66-2.12)	 0.565		
Perceived severity - Severe - Not severe	 1.00 1.33 (0.62-2.87)	 0.462		
Perceived risk - Not at risk - At risk	 **1.00** **3.14 (2.15-4.59)**	 **<0.001**	**2.05 (1.28-3.29)**	**0.003**
Pre-diagnosis worry[e] - Not worried - Worried	 **1.00** **3.24 (2.28-4.62)**	 **<0.001**	**2.07 (1.27-3.37)**	**0.004**
Likeliness to change health-seeking intentions - Not interested in PRS - Interested in PRS	 1.00 1.53 (1.11-2.11)	 **0.009**		
Interest in test for prognosis - Not interested in PRS - Interested in PRS	 1.00 4.97 (2.47-10.00)	 **<0.001**		

Interest in non-family test recommendation - Not interested in PRS - Interested in PRS	 1.00 3.67 (2.66-5.06)	 **<0.001**		
Interest in family test recommendation - Not interested in PRS - Interested in PRS	 1.00 12.83 (6.33-25.99)	 **<0.001**		

Table 1.2: Univariate and multivariate logistic regression assessing predictors of a positive interest in genetic risk testing and the impact of interest in testing on health-seeking intentions. An OR greater than 1 indicates that participants were more likely to be interested in testing.

1.1.5 Health-seeking intentions

We assessed whether interest in glaucoma genetic risk testing was associated with an individual's eye health-seeking intentions or their likelihood to recommend genetic testing to others (Table 1.2). Interest in testing was significantly associated with an intention to change health-seeking intentions relating to eye health (OR 1.53, 95%CI (1.11–2.11), p=0.009). In addition, interest was positively associated with increased likelihood of recommending testing to family (OR 12.83, 95%CI (6.33-25.99), p <0.001) and non-family members (OR 3.67, 95%CI (2.66-5.06), p <0.001), and the likelihood of undergoing testing for the purpose of predicting prognosis and disease severity (OR 4.97, 95%CI (2.47-10.00), p <0.001) using univariate logistic regression.

1.1.6 Factors about testing and follow-up

We assessed the aspects of glaucoma genetic risk testing and follow-up that respondents would like to know prior to undergoing analysis, regardless of their indicated interest in testing. All four options provided were deemed important by more than 70% of respondents (cost, process involved, meaning of results and follow-up). We assessed the preferred method of receiving results, identifying that most participants would prefer to receive results in person, in a letter, or via email, rather than via a telephone call. The factors about testing and follow-up are summarised in Supplementary Figure 1A and the preferred method of receiving results are summarised in Supplementary Figure 1B. Several participants commented that their preferred method would depend on the result; if at high risk, face-to-face would be preferred, and if low risk, other methods would be sufficient. It was also noted that if results were received non-verbally or via telephone,

an option to speak with someone in person would be appreciated to discuss implications and answer questions. Regarding cost, approximately 80% of participants would be willing to pay for testing, with over half of those willing to pay indicating that a cost of AU$50-AU$100 would be appropriate (Figure 1.2). Others commented that they would expect that the test be covered by Medicare (Australia's universal health insurance system), particularly if they themselves were a senior citizen/pensioner.

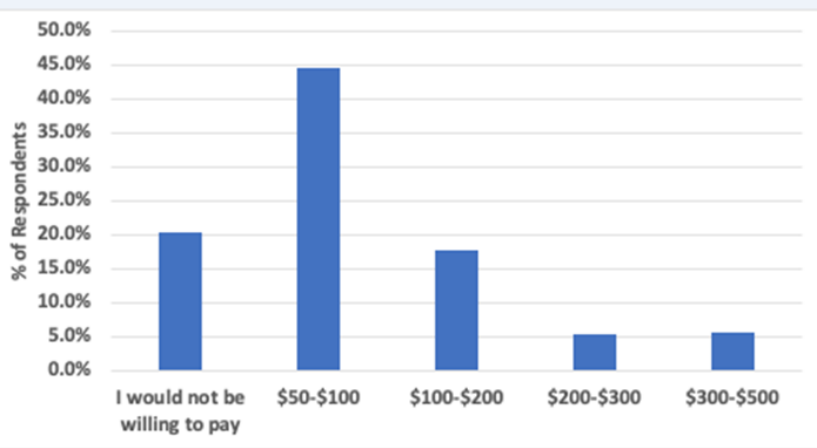

Figure 1.2: Cost participants would be willing to pay for a glaucoma genetic risk test. Responses to the question 'If a cost were involved, how much would you be willing to pay for the test?'.

1.1.7 Additional results

Some respondents made comments in addition to answering from provided response options, with several noting that developing glaucoma was somewhat expected given their family history of glaucoma, and therefore felt that a genetic test was not necessary given they were already undergoing regular eye examinations. Participants were given the opportunity to make additional comments on aspects of the test they would like to know more about, and how such testing would change their behaviour regarding their eye health. Some noted the accuracy of the test would be important to know prior to undergoing testing, with respect to false positive and false negative rates, and the specificity and sensitivity of the test. Privacy was also highlighted as a concern, given the need to provide genetic material and the implications results may have on employment or insurance. Some were interested in whether any additional risks (other than glaucoma risk) could be identified from the test, and whether the test would be recommended to family members automatically based on their results. Recommended age to undergo the test, treatment options, adverse effects of the test and available treatments, and short- and long-term prognosis were

also identified as information of interest.

1.2. DISCUSSION

Recent studies on PRS have shown that implementing of PRS for clinical use in ophthalmology (and other fields of medicine) is becoming increasingly realistic.[89,108–110,122,137,148–153] For conditions such as glaucoma, PRS testing has strong clinical utility given the complex nature and heritability of the disease, its treatability, as well as the difficulties associated with diagnosis.[122] Polygenic risk testing has the potential to improve disease prediction, diagnosis and management of vision loss - from reactive and responsive to predictive and preventative. For this adaptation to be successful, thorough understanding of stakeholders' attitude toward such testing is required first to develop implementation frameworks and successful uptake.

To our knowledge, no studies have assessed the attitude of individuals with glaucoma toward PRS testing for the condition, and critical gaps in understanding barriers to implementing such testing persist. This study provides useful insights into the potential uptake of PRS testing for glaucoma. Our results supported the hypothesis that individuals with glaucoma have a positive attitude towards genetic risk testing for glaucoma (69.4% being likely or highly likely to have undergone testing), as well as testing to predict risk of severe disease or rapid progression. The reported interest was similar to a previous study investigating attitudes toward single gene testing for glaucoma reporting 61.8% interest, among a large glaucoma pedigree.[154,155] In addition, our results are comparable to studies on predictive genetic testing in other conditions including inherited breast and colorectal cancer.[137,148–151,156–158] In particular, studies assessing interest in predictive genetic testing for breast cancer among individuals affected by the disease reported similar levels of interest, ranging from 57.0%-61.8%.[137,150] Further research is needed to validate the effectiveness of PRS testing in an unaffected population.

Increasing interest in glaucoma genetic risk testing was associated with a positive family history of glaucoma and a higher number of affected family members. However, it was shown that the affected relative must be at least a second-degree relative or closer. These results are in line with other studies that found interest in genetic testing was particularly supported if there was a family history of the condition.[148,149,151,156–158] However, while significant in the univariate logistic regression, these variables were not statistically significant when controlling for other associated variables. This may be attributable to some respondents recognizing their predisposition from having an affected family member. Several respondents commented that developing glaucoma

was somewhat expected given their family history of glaucoma, and therefore felt that a genetic test was not necessary given they were already undergoing regular eye examinations. These responses suggest that genetic determinism plays into risk perception. However, it has been shown that glaucoma risk can vary significantly even in individuals with high penetrant variants, ranging from very high to average population risk depending on their PRS.[108,159] This indicates a need for community education regarding genetic risk. Studies in inherited breast and colorectal cancer reported positive interest particularly in those with a positive family history of the condition.[149,151,156–158,160–165] It should be recognized however, that screening for these conditions via colonoscopy or mammogram is generally more invasive than an eye examination, so genetic testing may be preferable by those at an increased risk to avoid this type of investigation.

Previous studies have shown that individuals who have a higher perceived risk of glaucoma are the most motivated to reduce their risk of vision loss.[147] This is consistent with our results showing that those who had a higher perceived risk of developing glaucoma were more interested in genetic risk testing for glaucoma. Similarly, those who had been worried about developing glaucoma prior to being diagnosed were more interested in testing. Interest in testing was associated with having an intention to change behaviour towards eye health. This is not in keeping with other genetic studies that have shown that knowledge of risk has little effect on risk-reducing behaviours.[166] However, this may be less relevant to glaucoma as, unlike most common conditions, there are no established environmental risk factors that could be modified through risk-reducing lifestyle changes.

Positive attitude towards genetic risk testing for glaucoma appears to extend beyond personal interest. Increasing interest was associated with increased likelihood to recommend testing to family and non-family members. Interestingly, a positive family history of glaucoma was not associated with an increased likelihood to recommend testing to family members. However, although not significant, having an affected first-degree relative was associated with an increased interest in testing, suggesting that close affected relatives might still influence interest in testing.

Non-respondents were significantly older, more likely to be of non-European ethnicity and more likely to have advanced disease and legal blindness. Those who are of an older age may feel a genetic test regarding risk and prognosis is not relevant at their stage of life. These individuals may also have added difficulty completing a questionnaire that requires reading and comprehension, and the dexterity to record their responses. However, it is possible that the

disparity in age seen between groups is due to ANZRAG participants who have died remaining on the database. While the database is regularly updated, if not notified of a participant's death, it may not be recorded. Similarly, individuals of non-European ethnicity may not speak English as their primary language and may have difficulty completing the questionnaire which was delivered only in English. In addition, it is not yet clear how a glaucoma PRS may perform across non-European populations and participants of non-European ethnicity may not be aware of this. Finally, those with advanced disease may have had more difficulty completing the questionnaire due to their impaired vision. Moreover, they may not have felt that genetic risk testing would be personally relevant given their severe disease. These individuals may have expected to develop glaucoma regardless of the potential calculated genetic risk, based on a strong family history of the disease. However, there was no significant association between advanced glaucoma and the presence of a family history of glaucoma ($p = 0.245$).

We asked participants about components of the test which they would like to know about prior to undergoing the test. The cost of the test, process involved in taking the test, implications of the results, and likely follow-up were each roughly of equal importance to respondents, with over 70% indicating this would be important to know. Respondents indicated mail, in person, and email to be the most preferred methods of receiving results. In addition, the largest proportion of participants (~45%) indicated a cost of AU$50-AU$100 for the test would be reasonable. Some who indicated an unwillingness to pay for the test commented on the challenges of affording additional health care costs whilst on a pension. This is important to consider given the older age of those most commonly affected by glaucoma.

The findings from this study should be interpreted in light of the following limitations. PRS involves complex genetic concepts which may not be easily or fully understood by general members of the community with limited genetics knowledge. While the questionnaires simplified the concept of PRS to aid understanding, complexities which may influence attitudes may not have been captured. Questions assessing glaucoma knowledge, risk and interest in genetic testing for the study population were framed as a retrospective concept given that individuals in this group had already been diagnosed. This may be difficult for some to interpret and answer without the bias of hindsight influencing their response. The questionnaires were not easily accessible to those with advanced glaucoma with poor vision. Although it was possible to verbally discuss the questionnaires with those with visual impairment, this was not actively offered and likely, not identified as a reason for not responding amongst those who received a questionnaire via mail or

email. Offering audio versions of the questionnaire could help to address this, as well as contacting all non-respondents to ascertain whether poor vision impaired their ability to complete the questionnaire and offering to verbally complete it. Our study participants were drawn from an existing glaucoma research registry which may have introduced a selection bias. By participating in the ANZRAG (a study in which participants must consent for genetic testing in a research context), participants may be more interested in genetic research and therefore more likely to be interested in such testing in a clinical context. Participants were asked to indicate their level of interest in such testing from a retrospective point of view, which may reduce this bias. Almost 95% of our study sample was of European ethnicity, highlighting the need for further validation across other ancestral backgrounds prior to implementation, which is also pertinent to the predominantly European-derived PRS instruments themselves.

Additional challenges to clinical implementation of PRS testing for glaucoma remain. One challenge of conveying PRS results is to ensure that these results are communicated as absolute and relative risk values in conjunction with other established and validated clinical risk factors, and not as predictive or prognostic risk.[152] Clinical implementation of PRS will require that clinicians and the public receive education about the significance and limitations of the results. Furthermore, additional issues will arise in public health infrastructure and policy including economically balancing the cost of screening with the cost of management, identifying the most appropriate target screening population, and ensuring adequate access to testing and follow-up treatment.[167] These findings represent a valuable assessment of interest in glaucoma polygenic risk testing among potential target populations, which will be integral to the implementation and uptake of novel PRS-based tests into clinical practice. Further research should assess attitudes amongst those who are offered testing, once PRS tests are clinically available.

CHAPTER 2: ATTITUDES TOWARD GLAUCOMA GENETIC RISK ASSESSMENT IN UNAFFECTED INDIVIDUALS

PUBLISHED MANUSCRIPT

The contents of this chapter have been published in a peer-reviewed manuscript of which I am the first author:

Hollitt GL, Siggs OM, Ridge B, Keane MC, Mackey DA, MacGregor S, Hewitt AW, Craig JE, Souzeau E. Attitudes towards glaucoma genetic risk assessment in unaffected individuals, *Translational Vision Science & Technology*, accepted August 2022.

My contributions to the manuscript involved the research conception and design (60%), data collection including participant recruitment and data entry (90%), generation of a dataset (100%), data analysis including statistical analysis (80%), interpretation of the data (60%), and drafting the manuscript (100%). Jamie Craig, Emmanuelle Souzeau and Owen Siggs contributed equally to the manuscript including study concept and research design (40%), data analysis (10%), interpretation of the data (30%), critically revising the contents of the manuscript, project funding and supervision. Emmanuelle Souzeau and Bronwyn Ridge contributed equally to data collection (10%). Brownyn Ridge, Miriam Keane, David Mackey, Stuart MacGregor and Alex Hewitt contributed equally to data analysis (10%), data interpretation (10%), and critically revising the contents of the manuscript. The introduction and methods of this manuscript have been edited to fit the structure of this thesis.

2.1 RESULTS

2.1.1 Demographic and Personal Characteristics

In total, 418 participants completed the questionnaire; 193 had at least one affected first-degree relative, 117 had had a recent eye review and 108 were from the community. In total, 243 unaffected family members in ANZRAG and TARRGET were invited to participate in the study, and 143 completed the questionnaire, yielding a response rate of 58.8%. The other 50 participants with a first-degree relative were recruited from outpatient clinics and hospital settings. The demographic and personal characteristics of each group and the whole study sample are shown in Table 2.1. In summary, 66.5% were female, 95.0% were of European ethncitiy, 75.4% were from an urban area, and 63.8% had an education level above secondary school. The mean age of the total cohort was 62.1 years ± 13.3 years, with 28 individuals being under the age of 40 years. There was a significant difference in residency, family history, timing of last eye check and frequency of eye checks between groups (Table 2.1 - significant results in bold). Participants with affected first-degree relatives, those who had a recent eye check, and members of the general community did not differ by age, gender, and level of education (Table 2.1). The majority (74.9%) of participants had undergone an eye check within at least the last year and over half (55.0%) reported undergoing eye checks at least annually.

Variable	**First-degree relative** n = 193	**Optometry** n = 117	**Community** n = 108	**TOTAL** n = 418	**p Value**
Age (years) Range Mean (standard deviation) Median Missing	 33.0 - 89.8 61.7 (11.2) 62.1	 21.0 - 89.3 63.2 (15.4) 65.4 n=1	 19.4 - 94.6 61.5 (14.3) 66.3 n=1	 19.4 - 94.6 62.1 (13.3) 63.3 n=2	p = 0.573*
Gender, n (%) Female Male	 134 (69.4) 59 (30.6)	 73 (62.4) 44 (37.6)	 71 (65.7) 37 (34.3)	 278 (66.5) 140 (33.5)	p = 0.437†

Ethnicity, n (%)					**p = 0.028†**
European ethnicity	185 (95.9)	114 (97.4)	98 (90.7)	397 (95.0)	
Non-European ethnicity	6 (3.1)	2 (1.7)	9 (8.3)	17 (4.1)	
- African	2 (1.0)	0	0	2 (0.5)	
- Asian	3 (1.6)	1 (0.8)	3 (2.8)	7 (1.7)	
- Hispanic	0	1 (0.8)	1 (0.9)	2 (0.5)	
- Middle Eastern	0	0	2 (1.9)	2 (0.5)	
- Mixed	1 (0.5)	0	3 (2.8)	4 (1.0)	
Unknown	2 (1.0)	1 (0.9)	1 (0.9)	4 (1.0)	
Residency, n (%)					**p = 0.019†**
Urban	137 (71.0)	90 (76.9)	88 (81.5)	315 (75.4)	
Rural	55 (28.5)	21 (17.9)	16 (14.8)	92 (22.0)	
Unknown	1 (0.5)	6 (5.1)	4 (3.7)	11 (2.6)	
Highest level of education, n (%)					p = 0.056†
Primary School	2 (1.0)	4 (3.2)	1 (1.0)	7 (1.7)	
Secondary School	60 (31.1)	43 (36.8)	39 (36.1)	142 (34.1)	
Vocational Training	52 (26.9)	39 (33.3)	40 (37.0)	131 (31.3)	
University	77 (39.9)	31 (26.5)	28 (5.9)	136 (32.5)	
Unknown	2 (1.0)	0	0	2 (0.5)	
Family History, n (%)					**p <0.001†**
Positive	193 (100.0)	9 (7.7)	7 (7.4)	209 (50.0)	
Negative	0	107 (95.1)	87 (80.6)	209 (50.0)	
Unknown	0	1 (0.9)	14 (13.0)	0	
Positive (closest affected relative):					
- First-degree	193 (100.0)	0	0	193 (92.3)	
- Second-degree	0	7 (6.0)	5 (4.6)	12 (5.7)	
- Third-degree	0	1 (0.9)	1 (0.9)	2 (1.0)	
- Unknown	0	1 (0.9)	1 (0.9)	2 (1.0)	
Last eye check, n (%)					**p <0.001†**
Within 6 months	63 (32.6)	117 (100.0)	0	180 (43.1)	
6-12 months	82 (42.5)	0	51 (47.2)	133 (31.8)	
1-2 years	41 (21.2)	0	30 (27.8)	71 (17.0)	
More than 2 years	4 (2.1)	0	25 (23.1)	29 (6.9)	
Never	1 (0.5)	0	0	1 (0.2)	
Missing	2 (1.0)		2 (1.9)	4 (1.0)	

Frequency of eye checks, n (%)					**p = 0.003†**
3 monthly	2 (1.0)	1 (0.9)	0	3 (0.7)	
6 monthly	9 (4.7)	5 (4.3)	2 (1.9)	16 (3.8)	
Annually	107 (55.4)	61 (52.1)	43 (39.8)	211 (50.5)	
Every 2 years	61 (31.6)	32 (27.4)	34 (31.5)	127 (30.4)	
More than every 2 years	10 (5.2)	13 (11.1)	22 (20.4)	45 (10.8)	
Never	2 (1.0)	3 (2.6)	6 (5.6)	11 (2.6)	
Missing	2 (1.0)	2 (1.7)	1 (0.9)	5 (1.2)	

Table 2.1: Characteristics of the study sample (including individuals with a first-degree relative with glaucoma [First-degree relative], those who had undergone a recent eye check [Optometry] and general members of the community [Community]).

*denotes p-value calculated using one-way ANOVA. †denotes p-value calculated using Chi-square test for Association. Differences in ethnicity were assessed between European and non-European ethnicity.

2.1.2 Understanding of glaucoma and perception of severity and risk

In the overall cohort, 57.7% believed glaucoma was at least somewhat hereditary, with 57.7% of those having an affected first-degree relative. A large proportion (39.5%) of the total cohort were unsure about the hereditary nature of glaucoma. The majority (91.9%) of respondents considered glaucoma to be a severe medical condition, with an approximately equivalent proportion with (47.9%) and without (52.1%) an affected first-degree relative. Perception of glaucoma as a severe condition was associated with being likely to increase the frequency of eye checks if found to be at high risk (OR 7.36, 95%CI (1.32-40.89), p=0.023). Almost a third (31.8%) of participants believed they were likely or highly likely to develop glaucoma in their lifetime, and 89.1% of these expressed worry about this belief. Those with at least one first-degree relative with glaucoma were more likely to believe they were at risk of developing glaucoma (OR 5.06, 95%CI (2.99-8.58), p<0.001), and were worried about this (OR 3.75, 95%CI (2.33 - 6.06), p <0.001). Being worried about the possibility of developing glaucoma was associated with a preference to know glaucoma risk (OR 2.19, 95%CI (1.40-3.43), p <0.001). Responses to survey questions relating to understanding of glaucoma and perception of severity and risk are summarised in Supplementary Table 2.

2.1.3 Interest in genetic risk prediction testing for glaucoma

Overall, the majority of individuals expressed an interest in genetic risk prediction testing for glaucoma, with 71.3% of respondents indicating they would be either likely or highly likely to take a test if it were available. The attitudes of each group are shown in Figure 2.1. Over half of those

who were interested in testing (62.2%) also reported they would probably or definitely like to know more about glaucoma before being tested. Individuals with at least one affected first-degree relative were more likely to be interested in genetic testing for glaucoma than those without (OR 2.90, 95% CI 1.65-5.09, p <0.001) (Table 2.2). There was no significant difference between the level of interest between those aged below and above the age of 40 years (75.0% vs 81.2% respectively, p = 0.459). Responses to survey questions relating to interest in glaucoma PRS testing are summarised in Supplementary Table 2.

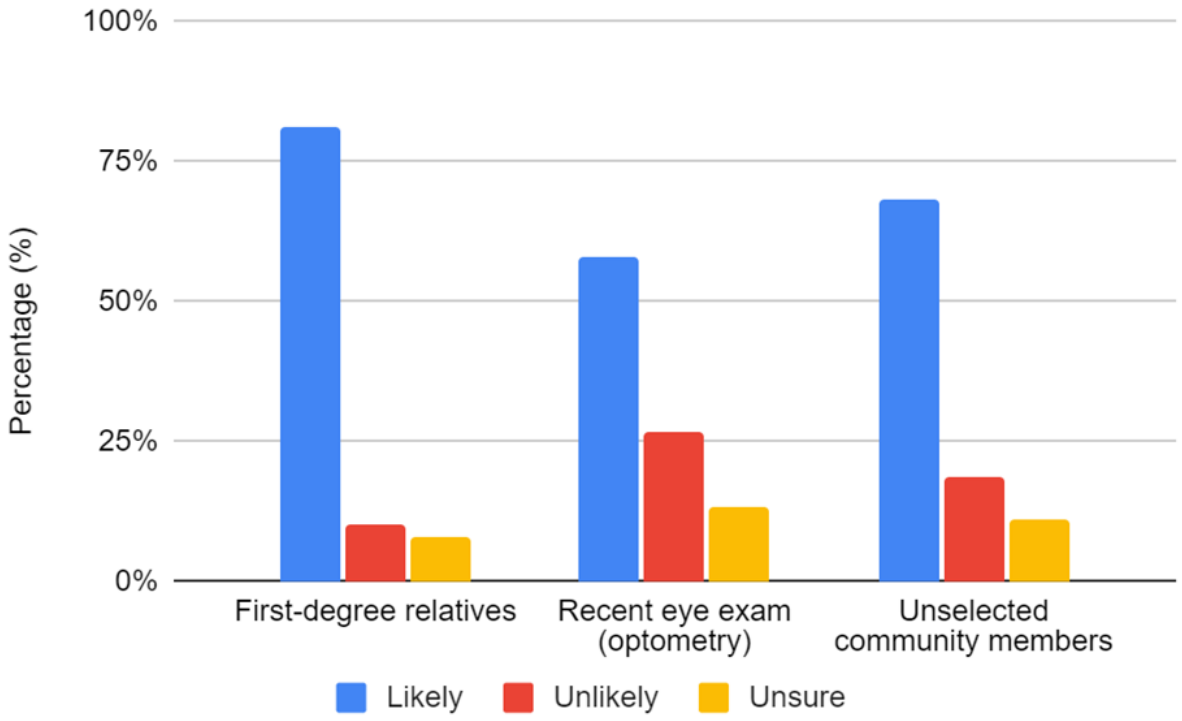

Figure 2.1: Level of interest in polygenic risk testing for glaucoma (positive versus negative) according to group classification. Responses to the question 'How likely would you be to take a genetic test which could predict your risk of developing glaucoma?'. Responses were grouped by group classification (first-degree relatives, recent eye exam [optometry], and general members of the community [community]), and grouped into interested (likely or highly likely) or uninterested (highly unlikely or unlikely) expressed interest. Forty-two respondents indicated being 'unsure' (10.0%).

2.1.4 Factors affecting interest in genetic risk prediction testing for glaucoma

We assessed the factors that may affect participants' decision to be tested (Figure 2.2) and factors that may concern participants about genetic risk prediction testing (Figure 2.3). After adjusting for all variables that were significant in univariate regression, interest in glaucoma genetic risk

prediction testing was more common in those who believed glaucoma to be a severe medical condition (OR 14.58, 95%CI (1.15-185.50), p = 0.039), were concerned about developing glaucoma (OR 4.37, 95%CI (2.32-8.25), p <0.001), had an intention to take appropriate measures regarding eye health (OR 2.39, 95%CI (1.16-4.95), p=0.019), or who preferred to know if they were at risk of glaucoma or not (OR 4.52, 95%CI (2.32-8.83), p<0.001) (Table 2.2). The average number of factors which may affect participants' decision to be tested was 3.7. Responses to survey questions relating to factors affecting interest in genetic risk prediction for glaucoma are summarised in Supplementary Table 2.

The majority (75.8%) of individuals had at least one concern about genetic risk prediction testing for glaucoma, with cost the most frequent (42.3%), followed by personal anxiety about the possibility of the test showing increased glaucoma risk (29.7%) (Figure 2.3). The average number of concerns per individual was 1.4. We assessed the factors concerning individuals about undergoing genetic risk assessment for glaucoma and why participants may be less likely to take the test. These are summarised in Supplementary Figure 3. Of those who indicated being uninterested in testing, 24.6% had no concerns about the test. Having to attend follow-up appointments was the most concerning factor (37.7%), followed by the cost of the test (23.6%), potential anxiety caused by results (20.8%), concern about how results would affect employment (11.1%) and insurance (8.3%), confidentiality concerns (6.9%) and rather not knowing their risk (4.2%).

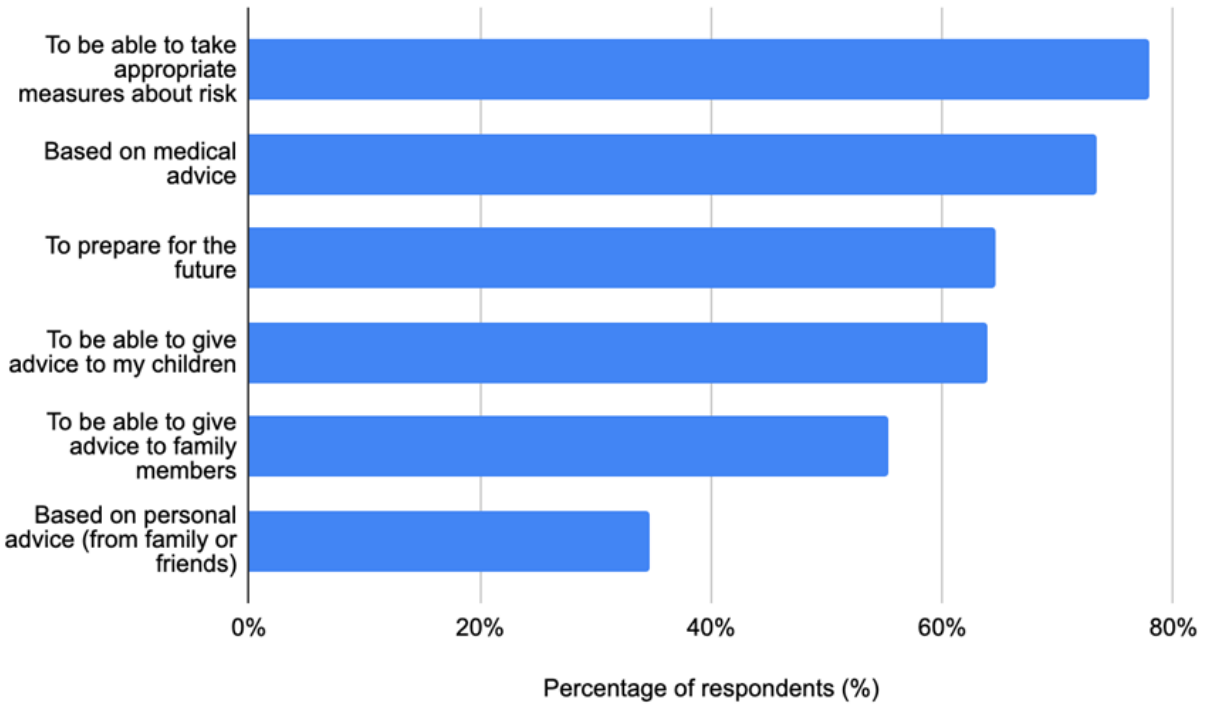

Figure 2.2: Factors affecting participants' decision to be tested. Responses to the question 'Which of the following factors would affect your decision to be tested? (Choose as many as appropriate)'.

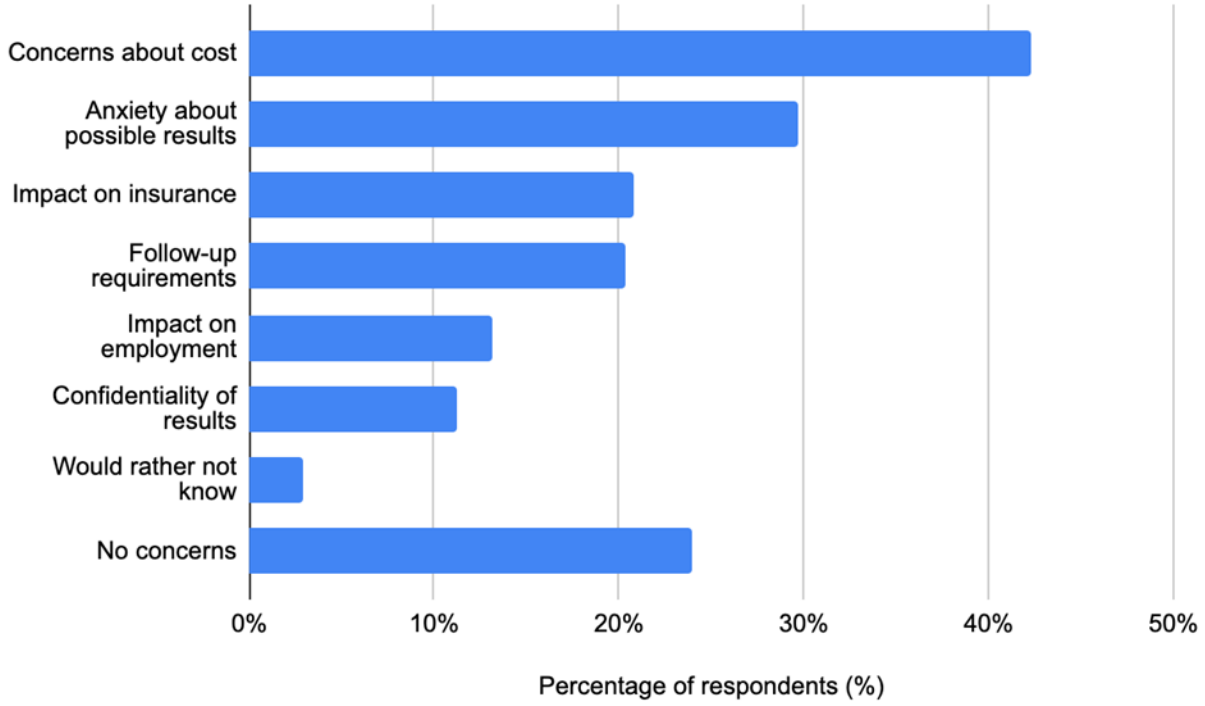

Figure 2.3: Factors concerning participants about having the test. Responses to the question 'Which of the following factors would concern you about having the test? (Choose as many as appropriate)'.

2.1.5 Behaviour

In addition to assessing which factors may influence the decision to undergo genetic risk prediction testing, we assessed whether the potential result would influence attitudes towards the frequency of future eye checks. If testing were to indicate a low risk of developing glaucoma, 91.6% of individuals indicated they would not change the frequency of their eye checks. However, if testing were to indicate a high risk of developing glaucoma, 76.6% of individuals indicated they would have more frequent eye examinations. Those with an affected first-degree relative were not likely to change the current frequency of their eye examinations, regardless of whether a test indicated they were at either low risk (p=0.344) or high risk (p=0.092). Individuals indicated that their decision to undergo testing would be influenced more by medical advice compared to advice from family or friends (74.6% vs 35.1%, p <0.001).

Variable (demographic)	**Univariate logistic regression**		**Multivariate logistic regression**	
	OR (95% CI)	**P value**	**OR (95% CI)**	**P value**
Age (years)	0.99 (0.96-1.01)	0.165		
Gender - Male - Female	 **1.00** **1.71 (1.01-2.89)**	**0.045**	1.67 (0.85-3.30)	0.138
Ethnicity - Non-European - European	 1.00 1.86 (0.56-6.23)	0.312		
Residency - Urban - Rural	 1.00 1.05 (0.56-1.98)	 0.877		
Education - School (primary or secondary) - Tertiary (vocational training or university	 **1.00** **1.67 (0.99-2.84)**	 **0.056**	1.28 (0.51-3.24)	0.593
Family History - Negative - First-degree relative - Other relative	 **1.00** **2.89 (1.64-5.11)** 0.98 (0.25-3.85)	**<0.001** **<0.001** 0.980	 2.05 (0.76-2.17) 0.24 (0.03-2.17)	0.111 0.156 0.205
Last eye check - <1 year (0) - >1 year (1)	 1.00 1.15 (0.62-2.12)	 0.665		
Frequency of eye checks - At least annually (0) - Every 2 years or more (1)	 1.00 1.02 (0.60-1.71)	 0.954		
Perceived glaucoma heredity - Non hereditary - Hereditary	 **1.00** **9.14 (1.47-56.86)**	 **0.018**	1.9 (0.02-193.52)	0.779

Perceived severity - Not severe - Severe	 **1.00** **18.69 (2.05-170.14)**	 **0.009**	**14.58 (1.15-185.50)**	**0.039**
Perceived Risk - Not at risk - At risk	 **1.00** **2.47 (1.32-4.63)**	 **0.005**	1.88 (0.81-4.35)	0.139
Concern of developing glaucoma - Not worried - Worried	 **1.00** **5.00 (2.87-8.72)**	 **<0.001**	**4.37 (2.32-8.25)**	**<0.001**
Interest in obtaining more information about the test - Not interested - Interested	 **1.00** **2.04 (1.16-3.59)**	 **0.013**	1.71 (0.71-4.11)	0.233
Intention to take appropriate measures - Would not change behaviour - Would change behaviour	 **1.00** **5.00 (2.83-8.83)**	 **<0.001**	**2.39 (1.16-4.95)**	**0.019**
Advice to children - No - Yes	 **1.00** **3.00 (1.77-5.08)**	 **<0.001**	1.15 (0.25-5.39)	0.860
Advice to family members - No - Yes	 **1.00** **2.92 (1.71-4.99)**	 **<0.001**	0.49 (0.18-1.32)	0.160
Personal advice - No - Yes	 1.00 1.34 (0.77-2.33)	 0.304		
Medical advice - No - Yes	 1.00 1.17 (0.64-2.13)	 0.614		
Would rather know - No - Yes	 **1.00** **6.78 (3.86-11.90)**	 **<0.001**	**4.52 (2.32-8.83)**	**<0.001**

Would rather not know - No - Yes	 1.00 0.378 (0.09-1.62)	 0.190		
Anxiety - No - Yes	 1.00 1.55 (0.83-2.89)	 0.170		
Cost - No - Yes	 1.00 1.37 (0.80-2.34)	 0.254		
Follow-up - Yes - No	 1.00 1.29 (0.69-2.38)	 0.424		
Insurance - No - Yes	 **1.00** **3.44 (1.43-8.27)**	 **0.006**	3.11 (0.99-9.79)	0.052
Employment - No - Yes	 1.00 1.26 (0.56-2.82)	 0.573		
Confidentiality - No - Yes	 1.00 1.89 (0.71-4.98)	 0.201		
Concerns - No concerns - At least 1 concern	 1.00 1.23 (0.69-2.20)	 0.485		

Table 2.2: Univariate and multivariate logistic regression assessing predictors for interest in polygenic risk testing.
Bold text in the Multivariate Logistic Regression indicates variables which were retained in the final model. Where a variable was excluded, the listed values given related to the point at which the variable was removed from the model. Results reflect questionnaire answers provided by participants, although the authors acknowledge that some responses are not logical.

2.1.6 Factors about testing and follow-up

Finally, we surveyed aspects of genetic risk prediction testing that participants wanted to know prior to undergoing testing. These are summarised in Supplementary Figure 3. Over 77.0% of participants deemed cost, the test process, possible implications of results, and follow-up to be important factors to understand prior to undergoing testing. Email was the most preferred method

to receive results (56.5%), followed by face to face (38.3%) and letter (35.2%), with telephone call being the least preferred (21.5%). Several individuals commented that their preference would depend on the result, with face to face being preferred if results showed high glaucoma risk, and other methods, particularly email, being preferred if results showed low risk. A majority of participants (64.6%) indicated they would be willing to pay at least $50 for a glaucoma genetic test if required, with AUD $50 - $100 (approximately USD $40-$70 at the time of writing) being the most acceptable range (Figure 2.4). Those who were willing to pay, were more likely to be interested in testing (OR 1.81, 95% CI (1.07-3.07), p = 0.028) and to have completed tertiary education (OR 1.95, 95% CI (1.28 - 2.98), p = 0.002). Regarding the possible frequency of eye checks, 88.8% of all participants indicated they would be willing to have either biannual or annual eye examinations if required (Figure 2.5).

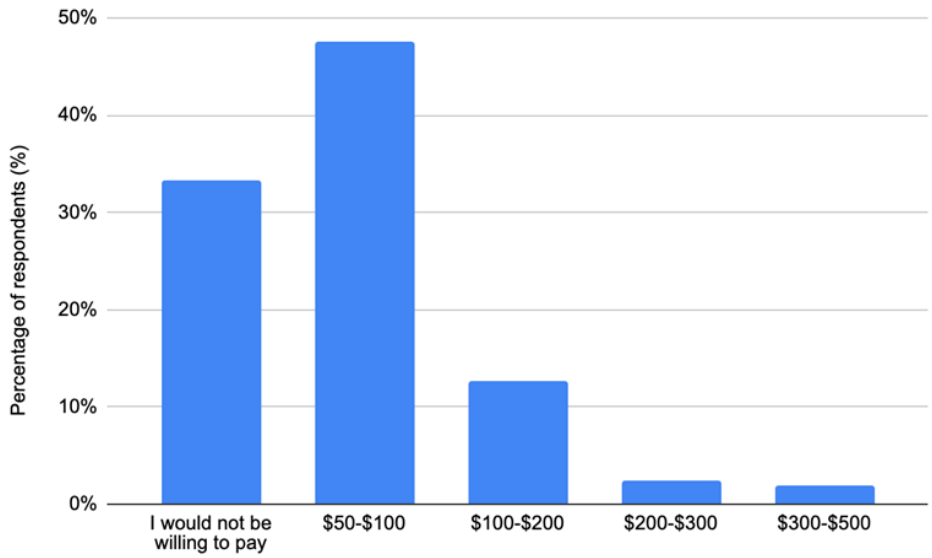

Figure 2.4: Cost participants would be willing to pay for a glaucoma genetic risk test. Responses to the question 'If a cost were involved, how much would you be willing to pay for the test?'

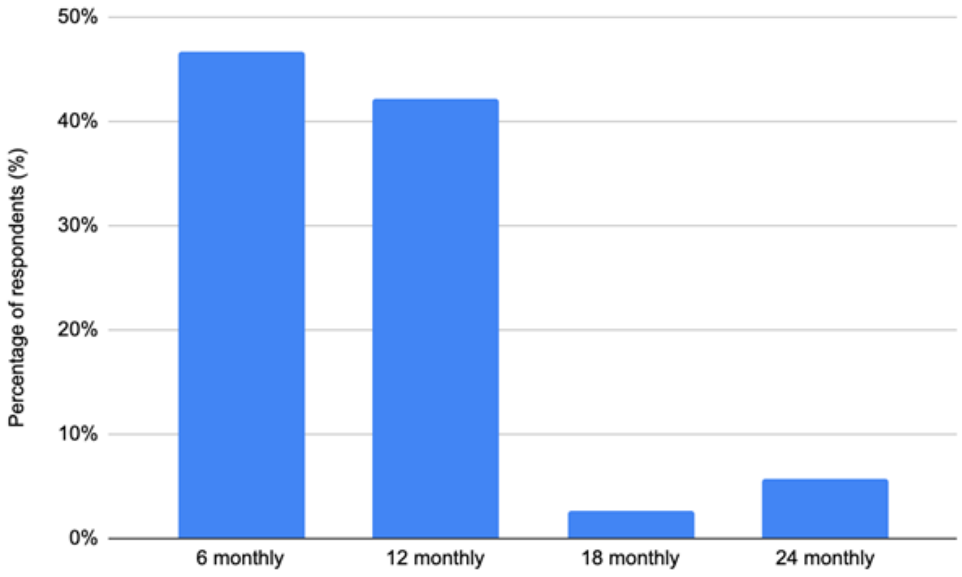

Figure 2.5: Frequency of eye checks participants would be willing to undergo. Responses to the question 'How frequently would you be willing to have an eye check?'.

2.2 DISCUSSION

Genetic risk stratification for diseases with complex inheritance will become increasingly accessible with the development of PRS. Studies have previously assessed interest and attitudes toward such testing in affected and high-risk individuals for breast and colorectal cancer.[137,151] To the best of our knowledge, the attitudes of those outside of an already identified at-risk population have not been investigated for any condition. Given one of the greatest potential advantages of PRS testing is population-scale risk stratification, it is crucial to understand the attitudes of the broader population toward this form of testing. Our findings provide useful insights into the attitude of unaffected individuals toward glaucoma genetic risk testing, and demonstrated a similar level of interest towards PRS testing for glaucoma among unaffected individuals (71.3%) compared to individuals with diagnosed glaucoma (69.4%).[168]

Although glaucoma is the most common cause of irreversible vision loss, current screening methods are insufficient and not cost-effective at the population level.[169,170] Evidence of the benefit of PRS testing was demonstrated by a previous study showing that individuals in the top decile of a glaucoma PRS distribution reach the same absolute risk of developing the disease 10 years earlier than those in the bottom decile.[108] Glaucoma PRS testing could improve current screening strategies given the disease's high and complex heritability, lack of environmental risk factors,

asymptomatic nature of early disease, and effectiveness of early treatment options to slow disease progression.[122] Risk stratification may help to guide monitoring and treatment of high-risk individuals, as well as potentially avoiding unnecessarily regular follow-up or over-treatment of low-risk individuals. While hesitancy for reduced screening for those at low risk has been reported,[171,172] PRS may assist in deciding on monitoring frequency or context, such as by an ophthalmologist or optometrist, particularly given the difficulty in diagnosing glaucoma in the early stage of disease and the large number of individuals who are diagnosed as glaucoma suspects.[89]

Interest in the test was not significantly associated with having a family history in the multivariate analysis, even though individuals with a family history were more likely to be interested in polygenic risk testing than those without. Previous studies have reported increased interest in PRS testing among first-degree relatives of individuals with breast cancer or colorectal cancer.[137,148,149,151,161,173,174] These discrepancies may be due to an assumed predisposition to glaucoma and frequent monitoring already in place in this cohort. The majority of those with an affected first-degree relative (74.1%) were drawn from existing glaucoma research databases. As part of their participation in these registries, individuals will have received information about the purpose of the research being to investigate the genetic nature of glaucoma as well as targeted glaucoma educational material, and may be more aware of the risk associated with having a family history. This is supported by our results which showed that those with an affected first-degree relative were more likely to believe they were at risk of developing glaucoma. Previous studies have shown that risk perception is often influenced by lived experience[175–179] and that PRS may not alter perceived risk in these cases.[175] Interestingly, in this study individuals with an affected first-degree relative were not more likely to change the current frequency of eye examinations, regardless of whether a test indicated they were at either low or high risk. However, this cohort was also the one reporting the highest frequency of eye examination and may therefore feel that additional testing is not necessary.

These issues may represent a potential barrier to the uptake of PRS testing for glaucoma in this high-risk group and will need to be further investigated for successful implementation of the test and combination with existing screening methods. This is highly relevant in the context of a prediction model which showed that approximately one quarter of people will have a PRS counteracting their risk due to their family history.[108] These individuals may be unaware of any underlying risk and will not be identified early through current screening guidelines given earlier age at screening is only recommended for those with a family history.[80]

Individuals who believed glaucoma to be a severe condition were more likely to be interested in PRS testing for glaucoma, and were more likely to increase the frequency of their eye examinations if shown to be at high risk. Furthermore, being worried about the possibility of developing glaucoma in the future appears to be a strong motivating factor to undergo testing. However, despite 76.7% of participants indicating being likely to have more frequent eye checks if results showed increased glaucoma risk, increased frequency of eye checks was not associated with interest in PRS testing for glaucoma. This is in keeping with other studies which have shown that knowledge of risk does not correspond to a change in risk-reducing behaviours.[165,166] Previous studies have shown that motivation for undergoing genetic testing commonly stems from a conviction to altruism and desire to understand more about personal health, rather than to make preventative lifestyle behaviour changes or change screening behaviours.[140,150,180–182] The option to choose to know of a genetic susceptibility to disease may seem to be valued more than the results and their possible implications.[182] Future research should examine whether knowledge of risk from the actual uptake of the test leads to change in glaucoma screening behaviours.

We asked participants which components of the test they would like to know more about prior to undergoing the test. The cost of the test, process involved in taking the test, implications of the results, and likely follow-up were each equally important to respondents with over 75% indicating they would want to know. Respondents indicated email as the preferred method of receiving results, with face to face, letter and telephone call being approximately equally preferred. The majority of those who expressed willingness to pay for the test indicated $50 - $100 to be an appropriate cost for the test. While early indications of the likely cost of PRS testing are above $100, public preference is relevant in order to consider future cost subsidisation and possible impact on uptake of the test. Moreover, concerns about insurance were significantly associated with testing in the univariate regression analysis and close to significance in the multivariate analysis. Insurance concerns may be particularly important in an older population who are more likely to be at risk. Our results may reflect the study population, with many being recruited from public hospitals where the provision of health services, including investigations and treatments for glaucoma are not associated with any out-of-pocket costs for patients in Australia. Furthermore, Medicare (Australia's universal health insurance system) subsidises the cost of most pathology tests, thus the Australian population are generally not accustomed to paying for such tests. However, genetic tests are currently not widely subsidised. It will be important to address concerns associated with costs in the future, especially given some respondents

commented that they would expect that the test would be subsidised by Medicare and cost was one of the main reasons for not being interested in testing.

Given the potential for broad population screening, ordering PRS testing, interpreting results and communication of their significance to patients will extend beyond the clinicians directly involved in glaucoma diagnosis and management. Clinical implementation of PRS will rely on sound clinician understanding of the test and its results. It will be important to emphasise that PRS results represent a probability of individual disease risk and are therefore not diagnostic, and results will need to be interpreted in conjunction with other established clinical risk factors, in particular age.[152] Integrated risk models that incorporate established clinical and demographic risk factors will need to be developed. Genetic counsellors have the skill set to assist individuals in making informed decisions about their results and the implications for their family members. However, their role may be most necessary for those who receive high-risk results, as the current workforce will not be able to carry the entire burden of a population-based screening test. Further research will need to evaluate the views and the needs of clinicians and healthcare professionals who may be involved in ordering PRS testing, interpreting results, and communicating their significance to patients. Adequate resources will need to be available to upskill all clinicians and healthcare professionals who may be involved in glaucoma PRS testing.

Results should be interpreted in light of the study's strengths and limitations. Of the total participants, 34.4% were drawn from existing glaucoma research registries (ANZRAG and TARRGET). These participants have previously demonstrated interest in glaucoma research, particularly regarding genetic studies and family history, and may therefore be more likely to report interest in glaucoma genetic testing. However, the interest toward PRS testing was still strong among individuals who were not part of existing research projects (65.6%). The majority of our study sample (95.0%) was of self-reported European ethnicity, highlighting the need for further validation across other ancestral backgrounds prior to implementation. It will also be pertinent to ensure the utility of predominantly European-derived PRS instruments themselves in non-European ancestries. Furthermore, the attitudes of individuals of European ethnicity may vary depending on cultural and geographic differences, such as between individuals in Australia, Northern America and Europe. Although we have included unaffected individuals from three different groups, the study cohort may not be representative of a broader population of unaffected individuals. Additional studies would be needed to extrapolate these results to the general population. Opportunistic recruitment may also have introduced a sample bias as the sampled

population may not equally represent the total population. Finally, the methodology of this study relates to anticipated behaviours and future intentions and is not a representation of actual behaviour. Further research should compare the uptake of PRS testing for glaucoma in those with reported interest.

PRS has the potential to stratify individual risk across a broad population for many common conditions with complex inheritance, including glaucoma. We found positive interest towards glaucoma PRS testing among three different groups of unaffected individuals and have identified possible target populations for initial clinical implementation. We have also identified factors affecting interest toward the test and potential barriers to address. Acceptability of genetic risk testing by the general population is crucial for clinical implementation to be successful.

CHAPTER 3: HEALTHCARE PROFESSIONALS' KNOWLEDGE AND ATTITUDES TOWARDS POLYGENIC RISK TESTING FOR GLAUCOMA

3.1 RESULTS

3.1.1 Demographic Characteristics

In total, 105 participants completed the questionnaire. The demographic characteristics of the study sample are shown in Table 3.1. In summary, 60.0% were female, 72.4% were of European ethnicity and 73.3% were under the age of 50 years. Ophthalmologists made up the largest group who completed the questionnaire (34.3%), with on average 43.4% of their patients having glaucoma. Only one participant had a personal history of glaucoma, however, 27.6% participants reported having at least one family member with glaucoma. The professional characteristics of the study sample are shown in Table 3.2. Overall, the average time since completing training was 12.6 years and the average number of years practising was 12.3 years. Two-thirds (68.1%) of the non-Genetics participants had little or no exposure to genetics during training, and 91.2% had not undertaken postgraduate genetics training.

Variable	Number (%)	Variable	Number (%)
Primary Occupation		Age (years)	
- Ophthalmologist	36 (34.3)	- <30	17 (16.2)
- Optometrist	22 (21.0)	- 30-39	31 (29.5)
- Orthoptist	17 (16.2)	- 40-49	29 (27.6)
- GP	16 (15.2)	- 50-59	18 (17.1)
- Clinical Geneticist	4 (3.8)	- 60-69	7 (6.7)
- Genetic Counsellor	6 (5.7)	- ≥70	3 (2.9)
- Laboratory scientist	1 (1.0)		
- Other	3 (2.9)		
Gender		Personal history of glaucoma	
- Female	63 (60.0)	- No	100 (95.2)
- Male	41 (39.0)	- Yes	1 (1.0)
- Missing	1 (1.0)	- Missing	4 (3.8)
Ethnicity		Family history of glaucoma	
- European	76 (72.4)	- No	72 (68.6)
- Non-European	25 (23.8)	- Yes	29 (27.6)
- African	1 (1.0)	- Missing	4 (3.8)
- Asian	20 (19.0)	Closest affected relative	
- Middle Eastern	4 (3.8)	- First-degree	15 (55.6)
- Mixed	4 (3.8)	- Second-degree	11 (40.7)
		- Third-degree	1 (3.7)

Table 3.1: Demographic characteristics of the study sample.

Variable	**Number (%)**	**Variable**	**Number (%)**	
Years since completing training - Range - Mean (SD) - Median	 1-45 13.8 (11.5) 12	Years practising in profession - Range - Mean (standard deviation) - Median	 0-44 13.3 (10.2) 12	
Amount of training completed in Australia - All - Most - Some - None - Missing	 60 (57.1) 23 (21.9) 9 (8.6) 7 (6.7) 6 (5.7)	Amount of genetics education during training - None - A little - A moderate amount - A lot - A great deal - *Response missing*	Non-genetics^ 6 (6.6) 56 (61.5) 15 (16.5) 6 (6.6) 4 (4.4) 4 (4.4)	Genetics 0 1 (10.0) 1 (10.0) 0 6 (60.0) 2 (20.0)
Percentage of patients with glaucoma* - Range - Mean (SD) - Median	 5-99 43.4 (32.7) 35.0	Post-graduate training in genetics - No - Yes - Missing	 83 (91.2) 5 (5.5) 3 (3.3)	 1 (10.0) 8 (80.0) 1 (10.0)
Structure of primary practice - Solo practice - Single speciality group - Multi-speciality group - Not applicable/other - Missing	 11 (10.5) 29 (27.6) 46 (43.8) 14 (13.3) 5 (4.8)	Primary workplace - Private hospital - Public hospital - Private clinic/practice - Public clinic/practice - Corporate practice - Academic institution/University - Laboratory - Other - Missing	 2 (1.9) 18 (17.1) 53 (50.5) 3 (2.9) 6 (5.7) 12 (11.4) 1 (1.0) 5 (4.8) 5 (4.8)	

Table 3.2: Professional characteristics of the study sample.
**Answered only by ophthalmologists, ^Non-genetics included Ophthalmic & GPs groups.*

3.1.2 Glaucoma knowledge

Among those who answered, 79.3% of participants believed it was 'very' or 'extremely' important to assess for a family history of glaucoma among first-degree relatives, while only 42.9% indicated a similar level of importance to assess this in second-degree relatives. Similarly, 75.8% of participants who answered believed it is 'very' or 'extremely' important to elicit the age of diagnosis of an affected family member. Healthcare professionals who indicated that assessing a family history of glaucoma in first-degree relatives was 'very' or 'extremely' important were mainly Ophthalmic and Genetic (82.6% and 80.0%, respectively), followed by GPs (25.1%) ($p<0.001$ and $p=0.005$ compared to Ophthalmic and Genetic groups respectively) (Figure 3.1). Similarly, 90.0% of Genetic and 69.3% of Ophthalmic professionals indicated that asking about the age at

glaucoma diagnosis in the family was ‘very’ or ‘extremely’ important, followed by 50.1% of GPs (p=0.061) (Figure 3.1).

Self-reported confidence in knowledge of glaucoma in general, its risk factors, diagnosis, genetics, and current screening recommendations was assessed across all groups. Responses for each healthcare group are summarised in Table 3.3. Overall, Ophthalmic professionals were the most confident in their knowledge of glaucoma, risk factors, diagnosis, genetics and screening. GPs reported the lowest confidence across all 5 questions. The lowest overall self-reported level of knowledge was in glaucoma genetics with a median of 5.5 out of 10 in the Genetic group, 5.0 in the Ophthalmic group, and 2.0 among GPs.

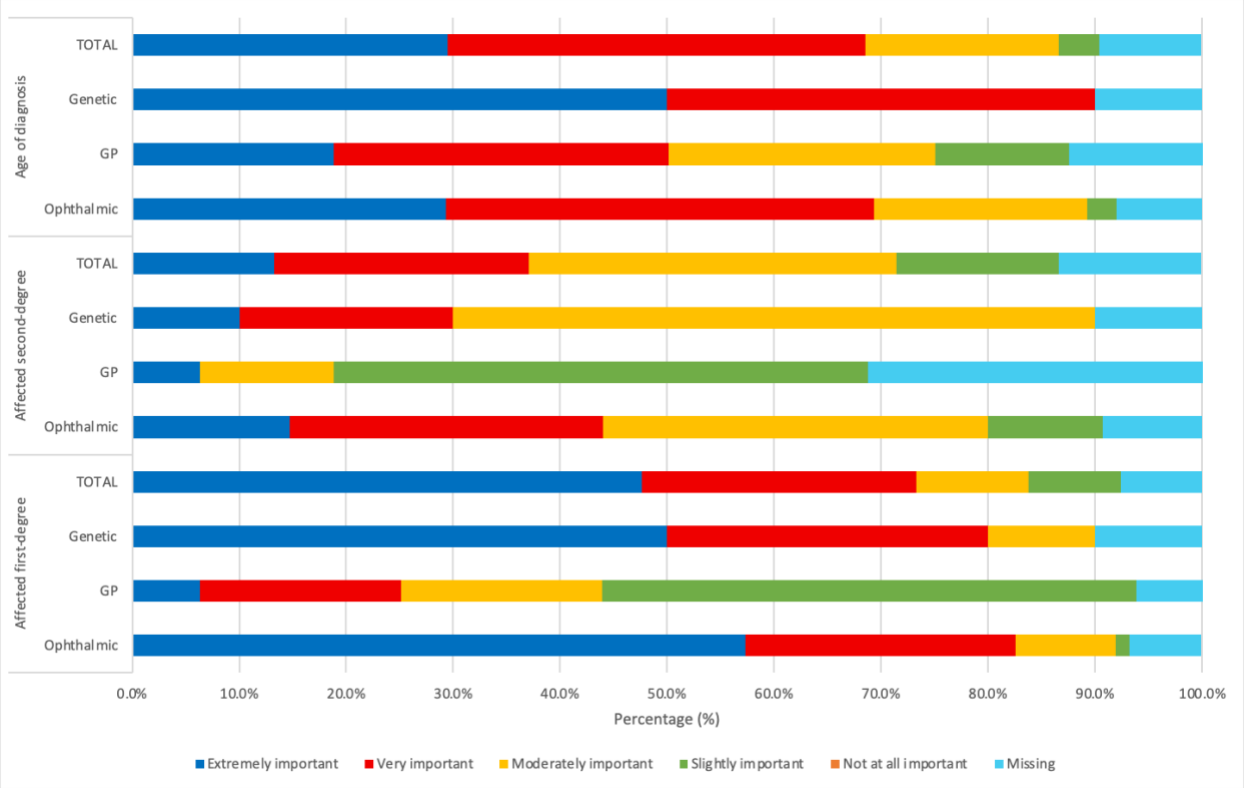

Figure 3.1: Importance of assessing glaucoma genetic risk. Responses to the question ‘When seeing a new patient, how important do you think it is to assess their family history of glaucoma in consideration of: affected first-degree relatives? (eg. mother, father, sibling); affected second-degree relatives? (grandparents, aunt, uncle, cousins)’ and, ‘If a patient reported a family history of glaucoma, how important do you think it is to ask about the age of diagnosis of their affected family member(s)?’.

Clinician group	Ophthalmologists n=33	Optometrists n=19	Orthoptists n=16	**Ophthalmic** n=68	**GP** n=15	Clinical Geneticists n=4	Genetic Counsellors n=4	**Genetics** n=10	**TOTAL** n=94
Glaucoma									
Range	5-10	6-10	5-10	**5-10**	**1-6**	5-7	3-6	**3-7**	**1-10**
Mean (SD)	8.4 (1.1)	7.8 (1.1)	7.3 (1.4)	**8.0 (1.3)**	**3.9 (1.9)**	6.3 (1.0)	4.5 (1.7)	**5.4 (1.6)**	**7.0 (2.1)**
Median	9.0	8.0	7.0	**8.0**	**5.0**	6.5	4.5	**6.0**	**7**
Risk factors									
Range	4-10	6-10	0-10	**0-10**	**0-6**	5-6	2-6	**2-6**	**0-10**
Mean (SD)	8.2 (1.5)	8.1 (1.0)	6.3 (2.3)	**7.7 (1.8)**	**3.7 (1.8)**	5.8 (0.5)	3.5 (1.9)	**4.6 (1.8)**	**6.8 (2.4)**
Median	8.0	8.0	6.5	**8.0**	**4.0**	6.0	3.0	**5.5**	**7**
Diagnosis									
Range	6-10	6-10	4-10	**4-10**	**0-7**	3-6	1-3	**1-6**	**0-10**
Mean (SD)	8.6 (1.0)	8.0 (1.0)	7.0 (1.7)	**8.1 (1.3)**	**3.3 (2.2)**	5.0 (1.4)	2.3 (1.0)	**3.6 (1.8)**	**6.8 (2.6)**
Median	9.0	8.0	7.0	**8.0**	**3.0**	5.5	2.5	**3.0**	**8**
Genetics									
Range	2-9	1-9	1-7	**1-9**	**0-6**	5-6	2-6	**2-6**	**0-9**
Mean (SD)	5.7 (2.3)	5.2 (1.9)	4.0 (2.2)	**5.2 (2.2)**	**1.6 (1.7)**	5.8 (0.5)	4.3 (1.7)	**5 (1.4)**	**4.5 (2.5)**
Median	6.0	5.0	4.0	**5.0**	**2.0**	6.0	4.5	**5.5**	**5**
Screening									
Range	1-10	5-10	3-10	**1-10**	**0-10**	3-7	0-4	**0-7**	**0-10**
Mean (SD)	7.4 (2.0)	8.0 (1.5)	6.5 (2.0)	**7.4 (1.9)**	**2.7 (2.6)**	4.5 (1.9)	2.5 (1.7)	**3.5 (2.0)**	**6.2 (2.8)**
Median	8.0	8.0	7.0	**8.0**	**2.0**	4.0	3.0	**3.0**	**7**

Table 3.3: Self-reported level of knowledge of glaucoma, risk factors for glaucoma, diagnosis, genetics of glaucoma, and current glaucoma screening recommendations amongst various healthcare professionals. Responses to the question 'How would you rate your knowledge of the following on a scale of 0 to 10? (with 0 being no knowledge): Glaucoma? Risk factors for open-angle glaucoma? Diagnosing glaucoma? The genetics of glaucoma? Current screening recommendations for glaucoma?'

3.1.3 Experience with genetic testing

Recent experience with genetic testing for any condition, an eye condition or glaucoma was assessed. Results are summarised in Table 3.4. Among those who answered, counselling for a genetic condition (61.4%) or an eye condition (62.9%) over the last 12 months was overall more common than counselling for glaucoma (49.5%). Counselling for glaucoma has been performed by 61.4% of ophthalmic professions and 28.6% of genetics professionals. Although requests from patients for genetic testing were overall low, they were lower for glaucoma (13.4%) than for eye conditions (28.7%) or other genetic conditions (39.1%). Ophthalmic professionals were less likely to refer a patient for a genetic test for glaucoma (25.8%) than for other eye conditions (44.6%). Few among Ophthalmic professionals (26.0%) and none among Genetics professionals had ordered a genetic test for glaucoma. None of the GPs had requests for glaucoma genetic testing from patients or referred a patient for a genetic test for glaucoma, and only one (7.1%) reported having ordered a genetic test for glaucoma.

Of those who had received genetic results for a patient, a written report (74.5%) was the most common method the results were received, followed by email (25.5%), electronic results (23.6%), and electronic medical records (18.2%). When a genetic report had been received, the results were most commonly reported using words only (76.1%), compared to with words and graphics (21.7%).

Clinician group n(%)	Ophthalmologists n=36	Optometrists n=22	Orthoptists n=17	**Ophthalmic** n=75	**GP** n=16	Clinical Geneticists n=4	Genetic Counsellors n=6	**Genetics** n=10	**TOTAL** n=105
A: Counselled a patient on a genetic issue during the past 12 months for:									
Any genetic condition									
Yes	24 (66.7)	7 (31.8)	5 (29.4)	**36 (48.0)**	**12 (75.0)**	3 (75.0)	3 (50.0)	**6 (60.0)**	**54 (51.4)**
No	9 (25.0)	11 (50.0)	11 (64.7)	**31 (41.3)**	**2 (12.5)**	0	1 (16.7)	**1 (10.0)**	**34 (32.4)**
Missing/Not applicable	3 (8.3)	4 (18.2)	1 (5.9)	**8 (10.7)**	**2 (12.5)**	1 (25.0)	2 (33.3)	**3 (30.0)**	**17(16.2)**
Eye condition									
Yes	28 (77.8)	14 (63.6)	9 (52.9)	**51 (68.0)**	**0**	3 (75.0)	2 (33.3)	**5 (50.0)**	**56 (53.3)**
No	5 (13.9)	5 (22.7)	7 (41.2)	**17 (22.7)**	**14 (87.5)**	0	2 (33.3)	**2 (20.0)**	**33 (31.4)**
Missing/Not applicable	3 (8.3)	3 (13.6)	1 (5.9)	**7 (9.3)**	**2 (12.5)**	1 (25.0)	2 (33.3)	**3 (30.0)**	**16 (15.2)**
Glaucoma									
Yes	25 (69.4)	11 (50.0)	7 (41.2)	**43 (57.3)**	**0**	1 (25.0)	1 (16.7)	**2 (20.0)**	**45 (42.9)**
No	9 (25.0)	9 (40.9)	9 (52.9)	**27 (36.0)**	**14 (87.5)**	2 (50.0)	3 (50.0)	**5 (50.0)**	**46 (43.8)**
Missing/Not applicable	2 (5.6)	2 (9.1)	1 (5.9)	**5 (6.7)**	**2 (12.5)**	1 (25.0)	2 (33.3)	**3 (30.0)**	**14 (13.3)**
B: Had a patient request a genetic test for:									
Any genetic condition									
Yes	12 (33.3)	2 (9.1)	4 (23.5)	**18 (24.0)**	**10 (62.5)**	3 (75.0)	3 (50.0)	**6 (60.0)**	**34 (32.3)**
No	18 (50.0)	18 (81.8)	12 (70.6)	**48 (64.0)**	**4 (25.0)**	0	1 (16.7)	**1 (10.0)**	**53 (50.5)**
Missing/Not applicable	6 (16.7)	2 (9.1)	1 (5.9)	**9 (12.0)**	**2 (12.5)**	1 (25.0)	2 (33.3)	**3 (30.0)**	**18 (17.1)**
Eye condition									
Yes	14 (38.9)	2 (9.1)	4 (23.5)	**20 (26.7)**	**0**	3 (75.0)	2 (33.3)	**5 (50.0)**	**25 (23.8)**
No	18 (50.0)	17 (77.3)	11 (64.7)	**46 (61.3)**	**14 (87.5)**	0	2 (33.3)	**2 (20.0)**	**62 (59.0)**
Missing/not applicable	4 (11.1)	3 (13.6)	2 (11.8)	**9 (12.0)**	**2 (12.5)**	1 (25.0)	2 (33.3)	**3 (30.0)**	**18 (17.1)**
Glaucoma									
Yes	8 (22.2)	1 (4.5)	1 (5.9)	**10 (13.3)**	**0**	1 (25.0)	1 (16.7)	**2 (20.0)**	**12 (11.4)**
No	25 (69.4)	18 (81.8)	15 (88.2)	**58 (77.3)**	**14 (87.5)**	2 (50.0)	3 (50.0)	**5 (50.0)**	**77 (73.3)**
Missing/Not applicable	3 (8.3)	3 (13.6)	1 (5.9)	**7 (9.3)**	**2 (12.5)**	1 (25.0)	2 (33.3)	**3 (30.0)**	**16 (15.2)**

C: Referred a patient for a genetic test for:									
Any genetic condition									
Yes	16 (44.4)	2 (9.1)	2 (11.8)	**20 (26.7)**	**12 (75.0)**	2 (50.0)	2 (33.3)	**4 (40.0)**	**36 (34.3)**
No	14 (38.9)	17 (77.3)	11 (64.7)	**42 (56.0)**	**2 (12.5)**	0	1 (16.7)	**1 (10.0)**	**45 (42.9)**
Missing/Not applicable	6 (16.7)	3 (13.6)	4 (23.5)	**13 (17.3)**	**2 (12.5)**	2 (50.0)	3 (50.0)	**5 (50.0)**	**24 (22.9)**
Eye condition									
Yes	22 (61.1)	4 (18.2)	3 (17.6)	**29 (38.7)**	**0**	2 (50.0)	1 (16.7)	**3 (30.0)**	**32 (30.5)**
No	11 (30.6)	15 (68.2)	10 (58.8)	**36 (48.0)**	**13 (81.3)**	0	2 (33.3)	**2 (20.0)**	**51 (48.6)**
Missing/Not applicable	3 (8.3)	3 (13.6)	4 (23.5)	**10 (13.3)**	**3 (18.8)**	2 (50.0)	3 (50.0)	**5 (50.0)**	**12 (21.0)**
Glaucoma									
Yes	16 (44.4)	0	1 (5.9)	**17 (22.7)**	**0**	1 (25.0)	0	**1 (10.0)**	**18 (17.1)**
No	18 (50.0)	19 (86.4)	12 (70.6)	**49 (65.3)**	**13 (81.3)**	1 (25.0)	3 (50.0)	**4 (40.0)**	**66 (62.9)**
Missing/Not applicable	2 (5.6)	3 (13.6)	4 (23.5)	**9 (12.0)**	**3 (18.8)**	2 (50.0)	3 (50.0)	**5 (50.0)**	**21 (20.0)**
D: Ordered a genetic test for:									
Any genetic condition									
Yes	8 (22.2)	3 (13.6)	2 (11.8)	**13 (17.3)**	**10 (62.5)**	3 (75.0)	2 (33.3)	**5 (50.0)**	**28 (26.7)**
No	22 (61.1)	13 (59.1)	10 (58.8)	**45 (60.0)**	**4 (25.0)**	1 (25.0)	1 (16.7)	**2 (20.0)**	**51 (48.6)**
Missing/Not applicable	6 (16.7)	6 (27.3)	5 (29.4)	**17 (22.7)**	**2 (12.5)**	0	3 (50.0)	**3 (30.0)**	**26 (24.8)**
Eye condition									
Yes	11 (30.6)	3 (13.6)	2 (11.8)	**16 (21.3)**	**2 (12.5)**	3 (75.0)	2 (33.3)	**5 (50.0)**	**23 (21.9)**
No	21 (58.3)	12 (54.5)	10 (58.8)	**43 (57.3)**	**12 (75.0)**	1 (25.0)	1 (16.7)	**2 (20.0)**	**57 (54.3)**
Missing/Not applicable	4 (11.1)	7 (31.8)	5 (29.4)	**16 (21.3)**	**2 (12.5)**	0	3 (50.0)	**3 (30.0)**	**25 (23.8)**
Glaucoma									
Yes	8 (22.2)	3 (13.6)	2 (11.8)	**13 (17.3)**	**1 (6.3)**	0	0	**0**	**14 (13.3)**
No	25 (69.4)	12 (54.5)	10 (58.8)	**47 (62.7)**	**13 (81.3)**	4 (100.0)	3 (50.0)	**7 (70.0)**	**67 (63.8)**
Missing/Not applicable	3 (8.3)	7 (31.8)	5 (29.4)	**15 (20.0)**	**2 (12.5)**	0	3 (50.0)	**3 (30.0)**	**24 (22.9)**

Table 3.4: Experience with genetic testing. Responses to the questions A: ‘ During the past 12 months, have you counselled a patient on a genetic issue for any of the following: for any genetic condition? For an eye condition? For glaucoma?’; B: ‘During the

past 12 months, have any of your patients asked you if they can get a genetic test: for any genetic condition? For an eye condition? For glaucoma?'; C: 'During the past 12 months, have you referred a patient for a genetic test: for any genetic condition? For an eye condition? For glaucoma?'; D: 'During the past 12 months, have you ordered a genetic test for a patient: for any genetic condition? For an eye condition? For glaucoma?'

3.1.4 Confidence in understanding genetic concepts and interpreting genetic test results

Self-reported confidence in knowledge of genetics and disease susceptibility is summarised in Table 3.5. When asked to score their confidence on a scale of 0 to 10, not surprisingly the highest level of knowledge was reported by clinical geneticists and genetic counsellors, with a median score of 9.0. In comparison, the Ophthalmic group gave a median score of 5.0 followed by GPs with a median score of 4.0. Approximately two thirds of the cohort (63.8%) felt 'not at all' or only 'slightly' qualified to order genetic tests. This group comprised non-genetic professionals, with orthoptists feeling least qualified (93.8%), followed by optometrists (89.5%), ophthalmologists (87.9%), and GPs (66.7%).

Self-reported understanding of genetics was reflected in assessing healthcare professionals' confidence to perform various genetic risk assessments. A summary of responses is shown in Figure 3.2. Overall, a majority of participants felt 'very' or 'extremely' confident to take a family history (84.2%), identify genetic services (56.7%), and identify family history of a potentially inherited condition (56.3%). In contrast, only a minority of participants felt 'very' or 'extremely' confident in determining the mode of inheritance (38.4%), estimating the risk of a patient having or developing a genetic condition based on their family or medical history (23.2%), counselling patients on genetic testing (30.7%) and interpreting the results of a genetic test (29.2%). There was a significant difference between the three main professional groups in: Confidence to take a family history ($p=0.026$), identify a family history ($p=0.028$), determine the mode of inheritance from a pedigree ($p<0.001$), estimate risk based on family and medical history ($p<0.001$), counsel patients on genetic testing ($p<0.001$) and interpret genetic test results ($p=0.004$) and were significantly higher amongst Genetics professionals than GPs ($p<0.017$ for all).

Clinician group	Ophthalmologists n=34	Optometrists n=20	Orthoptists n=16	**Ophthalmic** n=70	**GP** n=15	Clinical Geneticists n=4	Genetic Counsellors n=5	**Genetics** n=9	**TOTAL** n=97
Range Mean (SD) Median	2-10 5.4 (2.1) 5.0	2-8 4.1 (1.7) 4.0	1-8 4.1 (2.2) 4.5	**1-10** **4.7 (2.1)** **5.0**	**2-7** **4.1 (1.7)** **4.0**	9-10 9.5 (0.6) 9.5	7-9 8.0 (0.7) 8.0	7-10 8.7 (1.0) 9.0	1-10 5.0 (2.3) 5.0

Table 3.5: Self-reported level of knowledge of genetics and disease susceptibility amongst various healthcare. professionals. Responses to the question 'How would you rate your level of knowledge on genetics and disease susceptibility? (scale from lowest to highest)'.

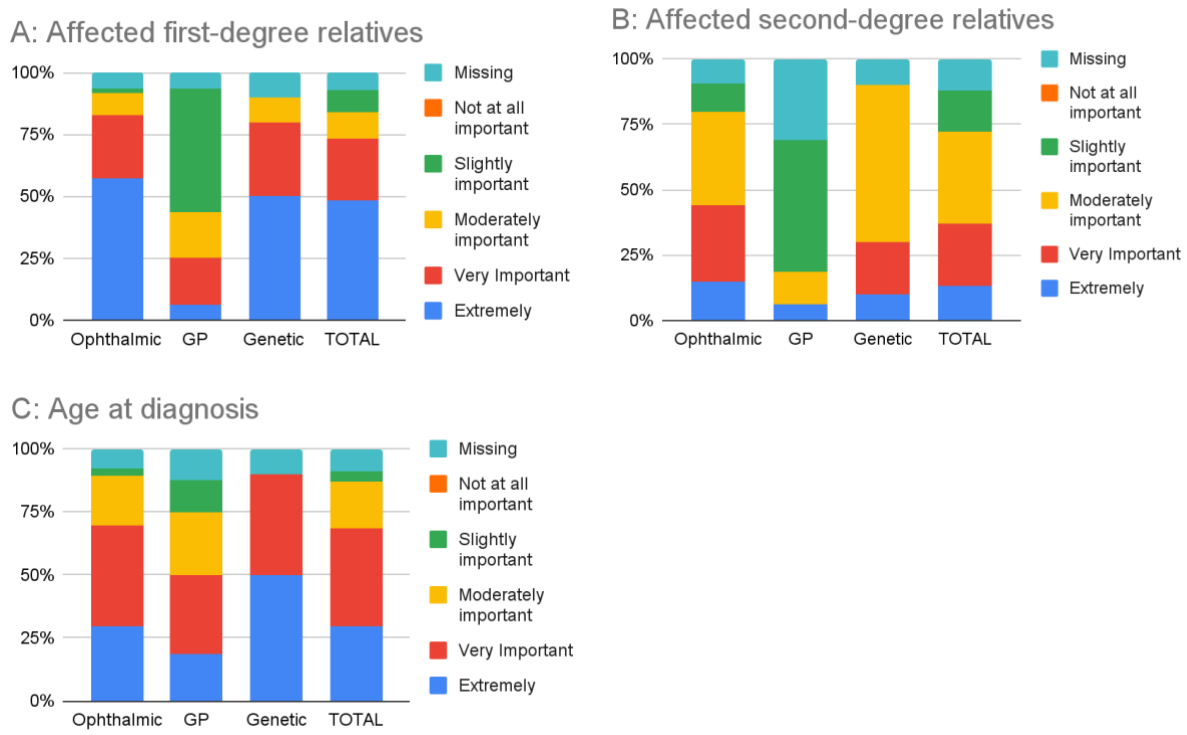

Figure 3.2: Reported importance of assessing family history and age of diagnosis. Responses to the questions A: 'When seeing a new patient, how important do you think it is to assess their family history of glaucoma in consideration of affected first-degree relatives?'; B: 'When seeing a new patient, how important do you think it is to assess their family history of glaucoma in consideration of affected second-degree relatives?'; C: 'If a patient reported a family history of glaucoma, how important do you think it is to ask about the age of diagnosis of their affected family member(s

3.1.5 Familiarity with polygenic risk

Familiarity with PRS for any condition, for an eye condition, and for glaucoma was low amongst the study cohort (Figure 3.3). There was a significant difference between the three profession groups for the familiarity with PRS for any condition (p=0.002), for an eye condition (p=0.003) and for glaucoma (p=0.027). Those from a Genetics background were most comfortable with PRS for any condition with 75.0% reporting being 'very' or 'extremely' familiar, compared to 9.2% of Ophthalmic professionals (p<0.001) and 6.7% of GPs (p=0.003). However, this familiarity was not reflected for PRS for an eye condition or glaucoma, with only 12.5% of Genetic professionals reporting feeling 'very' or 'extremely' familiar with both concepts. Over half of Ophthalmic professionals were 'not at all' or 'slightly' familiar with PRS for any eye condition (60.0%) or for glaucoma (63.1%), while the majority of GPs reported being 'not at all' or only 'slightly' familiar with PRS for eye conditions (93.3%) and glaucoma (93.3%) (p=0.004 and p=0.063 respectively with the Ophthalmic group).

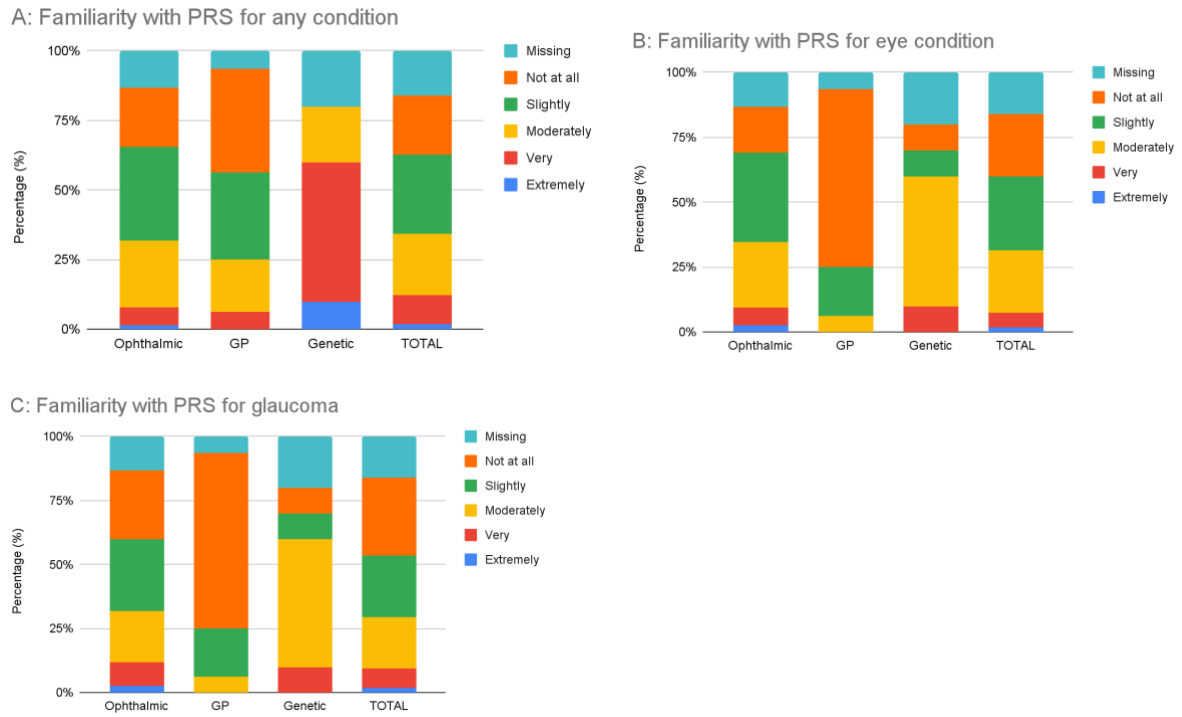

Figure 3.3: Familiarity with polygenic risk. Responses to the question 'How familiar are you with the concept of polygenic risk for: A: any condition, B: for eye conditions, and C: for glaucoma?'.

3.1.6 Attitudes towards genetic risk prediction testing for glaucoma

Likelihood to recommend testing depending on risk factors is summarised in Table 6 and Figure 3.4. Overall, participants who answered were more likely to recommend PRS testing for first-degree relatives (90.9%) compared to second-degree relatives (54.5%), and individuals aged over 50 years (56.6%) compared to over 70 years of age (44.7%). Likelihood to recommend testing for individuals of Asian (58.1%) and African (64.0%) ethnicity was similar. The likelihood to recommend testing for first-degree relatives was significantly different among the whole cohort ($p=0.032$). Although not significantly different with Bonferroni correction, GPs were less likely to recommend testing to those with first-degree relatives than Ophthalmic professionals ($p=0.019$). This is consistent with the results in Figure 3.2A, showing the GPs place less emphasis on first-degree relatives during history taking for glaucoma.

Clinician group	Ophthalmologists n=36	Optometrists n=22	Orthoptists n=17	**Ophthalmic n=75**	**GP n=16**	Clinical Geneticists n=4	Genetic Counsellors n=6	**Genetics n=10**	**TOTAL n=105**
First-degree relatives									
- Unlikely	2 (5.6)	1 (4.5)	0	**3 (4.0)**	**4 (25.0)**	0	0	**0**	**7 (6.7)**
- Likely	26 (72.2)	17 (77.3)	11 (64.7)	**54 (72.0)**	**9 (56.3)**	3 (75.0)	4 (66.7)	**7 (70.0)**	**70 (66.7)**
- N/A/missing	8 (22.2)	4 (18.2)	6 (35.3)	**18 (24.0)**	**3 (18.8)**	1 (25.0)	2 (33.3)	**3 (30.0)**	**28 (26.7)**
Second-degree relatives									
- Unlikely	10 (27.8)	11 (50.0)	4 (23.5)	**25 (33.3)**	**7 (43.8)**	2 (50.0)	1 (16.7)	**3 (30.0)**	**35 (33.3)**
- Likely	18 (50.0)	7 (31.8)	7 (41.2)	**32 (42.7)**	**6 (37.5)**	1 (25.0)	3 (50.0)	**4 (40.0)**	**42 (40.0)**
- N/A/missing	8 (22.2)	4 (18.2)	6 (35.3)	**18 (24.0)**	**3 (18.8)**	1 (25.0)	2 (33.3)	**3 (30.0)**	**28 (26.7)**
Age >50 years									
- Unlikely	13 (36.1)	11 (50.0)	2 (11.8)	**26 (34.7)**	**4 (25.0)**	2 (50.0)	1 (16.7)	**3 (30.0)**	**33 (31.4)**
- Likely	14 (38.9)	7 (31.8)	9 (52.9)	**30 (40.0)**	**9 (56.3)**	1 (25.0)	3 (50.0)	**4 (40.0)**	**43 (41.0)**
- N/A/missing	9 (25.0)	4 (18.2)	6 (35.3)	**19 (25.3)**	**3 (18.8)**	1 (25.0)	2 (33.3)	**3 (30.0)**	**29 27.6)**
Age >70 years									
- Unlikely	17 (47.2)	12 (54.5)	4 (23.5)	**33 (44.0)**	**5 (31.3)**	3 (75.0)	1 (16.7)	**4 (40.0)**	**42 (40.0)**
- Likely	10 (27.8)	6 (27.3)	7 (41.2)	**23 (30.7)**	**8 (50.0)**	0	3 (50.0)	**3 (30.0)**	**34 (32.4)**
- N/A/missing	9 (25.0)	4 (18.2)	6 (35.3)	**19 (25.3)**	**3 (18.8)**	1 (25.0)	2 (33.3)	**3 (30.0)**	**29 (27.6)**
Asian ancestry									
- Unlikely	11 (30.6)	10 (45.5)	3 (17.6)	**24 (32.0)**	**5 (31.3)**	1 (25.0)	1 (16.7)	**2 (20.0)**	**31 (29.5)**
- Likely	15 (41.7)	8 (36.4)	8 (47.1)	**31 (41.3)**	**8 (50.0)**	2 (50.0)	2 (33.3)	**4 (40.0)**	**43 (41.0)**
- N/A/missing	10 (27.8)	4 (18.2)	6 (35.3)	**20 (26.7)**	**3 (18.8)**	1 (25.0)	3 (50.0)	**4 (40.0)**	**31 (29.5)**
African ancestry									
- Unlikely	8 (22.2)	10 (45.5)	2 (11.8)	**20 (26.7)**	**5 (31.3)**	1 (25.0)	1 (16.7)	**2 (20.0)**	**27 (25.7)**
- Likely	19 (52.8)	8 (36.4)	9 (52.9)	**36 (48.0)**	**8 (50.0)**	2 (50.0)	2 (33.3)	**4 (40.0)**	**48 (45.7)**
- N/A/missing	9 (25.0)	4 (18.2)	6 (35.3)	**19 (25.3)**	**3 (18.8)**	1 (25.0)	3 (50.0)	**4 (40.0)**	**30 (28.6)**

Table 3.6: Likelihood to recommend PRS testing for different groups. Responses to the question ‘If polygenic risk testing were to become available, how likely would you be to recommend a polygenic risk test for glaucoma in: first degree relatives of patients with glaucoma? People aged >50 years? Individuals of Asian ancestry? Individuals of African ancestry? Second degree relatives of patients with glaucoma? Individuals aged >70 years?’. ‘Highly unlikely’ and ‘unlikely’ responses, and ‘highly likely’ and ‘likely’ were grouped as ‘unlikely’ and ‘likely’, respectively.

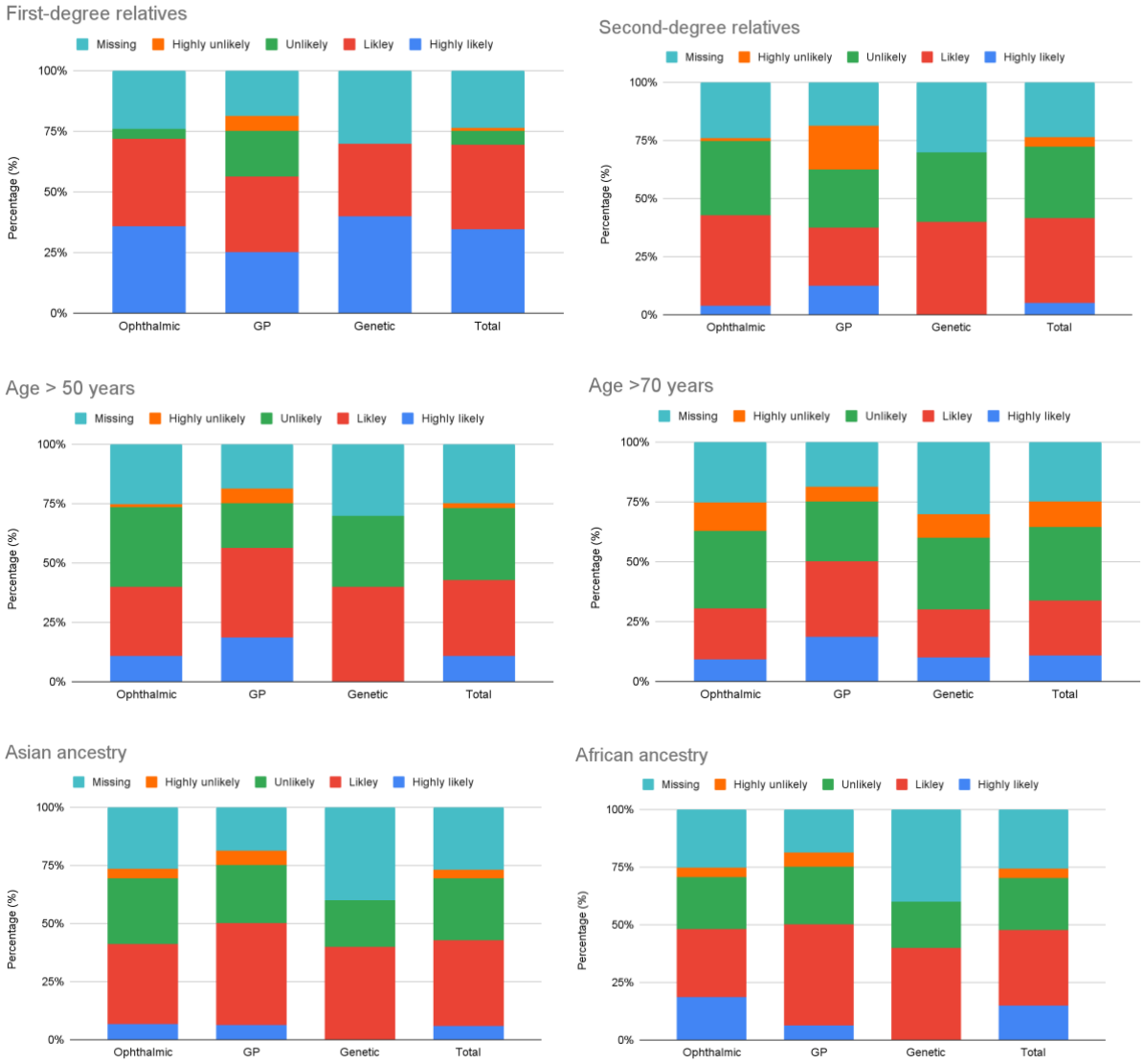

Figure 3.4: Likelihood to recommend PRS testing for different groups. Responses to the question 'If polygenic risk testing were to become available, how likely would you be to recommend a polygenic risk test for glaucoma in: A: first degree relatives of patients with glaucoma? B: Second degree relatives of patients with glaucoma? C: People aged >50 years? D: Individuals aged >70 years? E: Individuals of Asian ancestry?F: Individuals of African ancestry?'.

3.1.7 Factors affecting decision to recommend and order genetic tests

Important test characteristics when ordering a test are summarised in Figure 3.5. Among participants who answered, the performance characteristics of the test, including the positive/negative predictive value and sensitivity/specificity was the most important recorded factor with 93.2% who scored it 'very' or 'extremely' important. This was followed by the ability of

the test to provide prognostic information (86.4%), the ability of the test to predict clinical benefit from specific treatments or need for surgery (75.0%), and the ability to provide information about family members' risk (72.7%).

A summary of the importance of various factors in healthcare professionals' decision to recommend PRS testing for glaucoma is summarised in Figure 3.6. Recommendations or guidelines from medical societies, published clinical data and the availability of genetic testing services were the most important factors which would affect healthcare professionals' decision to recommend polygenic risk testing for glaucoma with 87.8%, 81.4% and 80.3% of respondents who thought these were 'very' or 'extremely' important factors respectively. Similarly, confidence to interpret test results (76.8%), information obtained through continuing medical education (76.9%), the individual's attitude towards genetic testing (74.4%) and recommendations from their institution or practice (74.4%) were each of approximately equal importance.

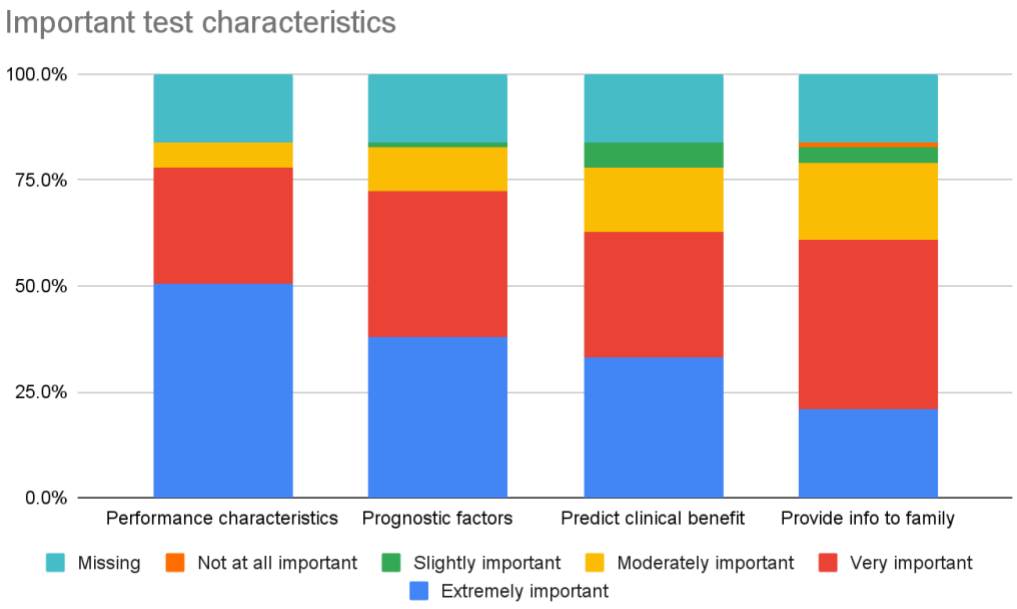

Figure 3.5: Important test characteristics to consider when ordering a test. Responses to the questions: 'When ordering a test, how important are the following factors? A: Performance characteristics of the test (positive/negative predictive value, sensitivity/specificity)? B: Ability of the test to provide prognostic information? C: Ability of the test to predict clinical benefit of specific treatments or need for surgery? D: Ability to provide information about family members' risk?'

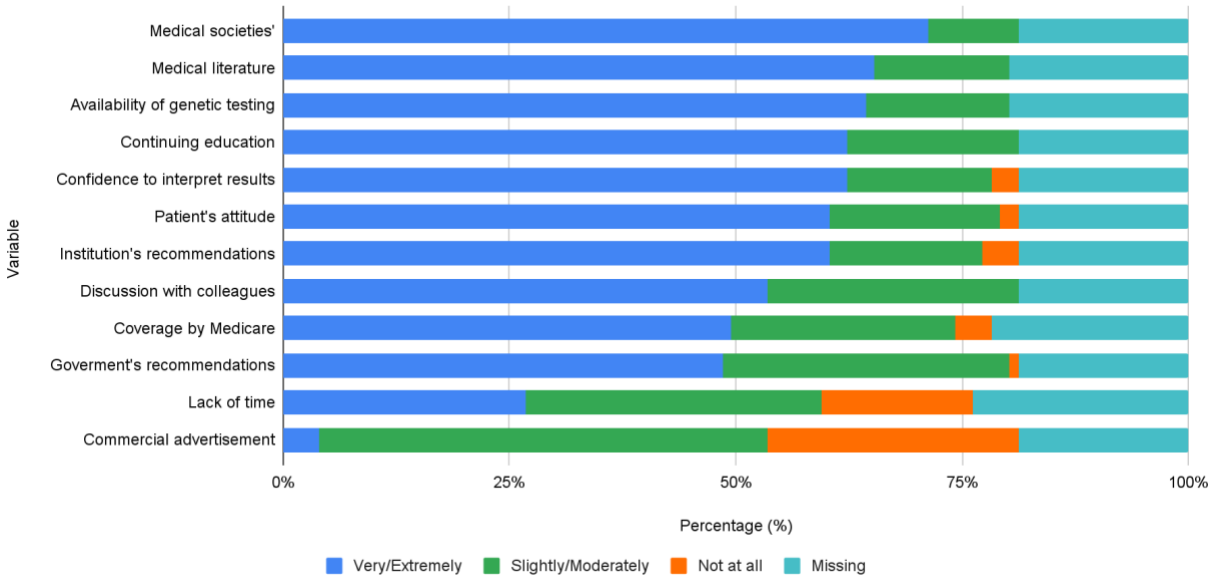

Figure 3.6: Factors affecting decision to recommend polygenic risk testing for glaucoma. Responses to the question 'How important are each of the following in your decision whether or not to recommend polygenic risk testing for glaucoma?'. Responses of 'extremely' and 'very' were grouped and presented as 'very/extremely', responses of 'slightly' and 'moderately' were grouped as 'slightly/moderately'.

3.1.8 Preferences for ordering and communicating polygenic risk results

Preferences for most appropriate healthcare professionals to deliver PRS testing are summarised in Table 3.7. Overall, the majority of participants (96.6%) indicated that ophthalmologists would be the most appropriate group to order PRS testing and communicate low (93.0%) or high (91.9%) risk results. This was followed by medical geneticists, genetic counsellors, optometrists, general practitioners, and orthoptists. Despite over half of ophthalmologists being 'not at all' or only 'slightly' familiar with the concept of PRS for glaucoma, 96.8% felt they were the most appropriate group to order glaucoma PRS testing. Similarly, although the majority of GPs reported being 'not at all' or only 'slightly' familiar with the concept of PRS for glaucoma, 92.3% felt they would be appropriate to deliver low risk PRS results.

The most preferred method of communicating PRS results to patients differed depending on the result, however direct verbal communication was most preferred overall (Figure 3.7). Communicating high risk results in-person was felt to be most appropriate, followed by via telephone conversation, and mail or email. For delivering low risk results, telephone was the most preferred method, followed by in-person, email and mail.

Healthcare professional	Most appropriate healthcare professional to ORDER PRS testing n(%)					
	Ophthalmologist	Optometrist	Orthoptist	GP	Clinical Geneticist	Genetic Counsellor
Ophthalmologist	30 (96.8)	5 (16.1)	0	4 (12.9)	24 (77.4)	19 (61.3)
Optometrist	19 (86.4)	13 (65.0)	4 (20.0)	11 (55.0)	18 (90.0)	16 (80.0)
Orthoptist	14 (100.0)	7 (50.0)	8 (57.1)	4 (28.6)	11 (78.6)	10 (71.4)
GP	14 (100.0)	6 (42.9)	1 (7.1)	7 (50.0)	13 (92.9)	9 (64.3)
Clinical Geneticist	3 (100.0)	2 (66.7)	0	1 (33.3)	3 (100.0)	3 (100.0)
Genetic Counsellor	3 (75.0)	1 (25.0)	0	1 (25.0)	4 (100.0)	3 (75.0)
Total	85 (96.6)	35 (39.8)	13 (14.8)	29 (33.0)	75 (85.2)	62 (70.5)

Healthcare professional	Most appropriate healthcare professional to communicate LOW risk results n(%)					
	Ophthalmologist	Optometrist	Orthoptist	GP	Clinical Geneticist	Genetic Counsellor
Ophthalmologist	30 (96.8)	9 (29.0)	1 (3.2)	8 (25.8)	23 (74.2)	24 (77.4)
Optometrist	17 (89.5)	14 (73.7)	6 (31.6)	11 (57.9)	17 (89.5)	16 (84.2)
Orthoptist	14 (100.0)	9 (64.3)	10 (71.4)	8 (57.1)	10 (71.4)	10 (71.4)
GP	12 (92.3)	7 (53.8)	1 (7.7)	12 (92.3)	11 (84.6)	10 (76.9)
Clinical Geneticist	3 (100.0)	3 (100.0)	0	2 (66.7)	3 (100.0)	3 (100.0)
Genetic Counsellor	3 (75.0)	2 (50.0)	1 (25.0)	3 (75.0)	4 (100.0)	4 (100.0)
Total	80 (93.0)	45 (52.3)	19 (22.1)	44 (51.2)	70 (81.4)	69 (80.2)

Healthcare professional	Most appropriate healthcare professional to communicate HIGH risk results n(%)					
	Ophthalmologist	Optometrist	Orthoptist	GP	Clinical Geneticist	Genetic Counsellor
Ophthalmologist	29 (93.5)	5 (16.1)	0	4 (12.9)	22 (71.0)	18 (58.1)
Optometrist	18 (94.7)	14 (73.7)	6 (31.6)	8 (42.1)	16 (84.2)	15 (78.9)
Orthoptist	12 (85.7)	7 (50.0)	6 (42.9)	5 (35.7)	11 (78.6)	8 (57.1)
GP	13 (100.0)	4 (30.8)	0	6 (46.2)	12 (92.3)	7 (53.8)

Clinical Geneticist	3 (100.0)	1 (33.3)	0	0	3 (100.0)	3 (100.0)
Genetic Counsellor	3 (75.0)	1 (25.0)	0	1 (25.0)	4 (100.0)	4 (100.0)
Total	79 (91.9)	32 (37.2)	12 (14.0)	25 (29.1)	70 (81.4)	57 (66.3)

Table 3.7: Most appropriate healthcare professional to deliver PRS testing. Responses to the questions: Which of the following healthcare provider(s) would you consider are appropriate to do the following? (tick as many as appropriate): a) Order polygenic risk testing for glaucoma? b) Communicate polygenic risk test results showing LOW individual risk? c) Communicate polygenic risk test results showing HIGH individual risk?' Missing values have been excluded.

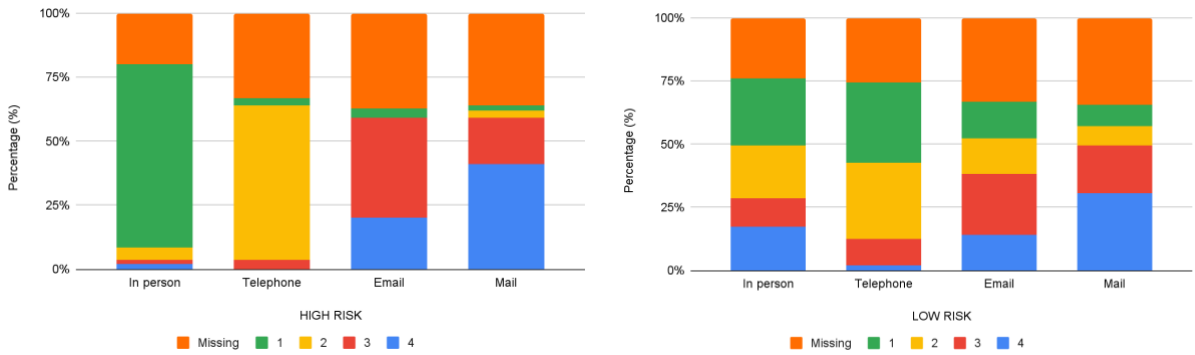

Figure 3.7: Preferred method of communicating results to patients. Responses to the question 'What is your preferred method of communicating results to patients' for the following? (Number as many as appropriate in order from 1 to 4, with 1 being most preferred) A: if results were shown to be high risk. B: if results were shown to be low risk.'

3.1.9 Training needs

The majority of the Ophthalmic professionals and GPs who answered felt they would 'probably' or 'definitely' benefit from more training in genetic testing (92.6% and 100% respectively), interpretation of genetic test results (92.6% and 100% respectively) and PRS (92.8% and 100% respectively) (Table 3.8). Comparatively, 33.3% and 44.4% of the Genetic professionals who answered felt they would 'probably' or 'definitely' benefit from more training in genetic testing and interpretation of results, however, 77.8% felt more training on PRS was needed. The most preferred methods to undergo further training was either an online course or a day-long conference or workshop (Figure 3.8). Training through a department meeting, grand round, accessing online information, or receiving mailed information were less preferred options.

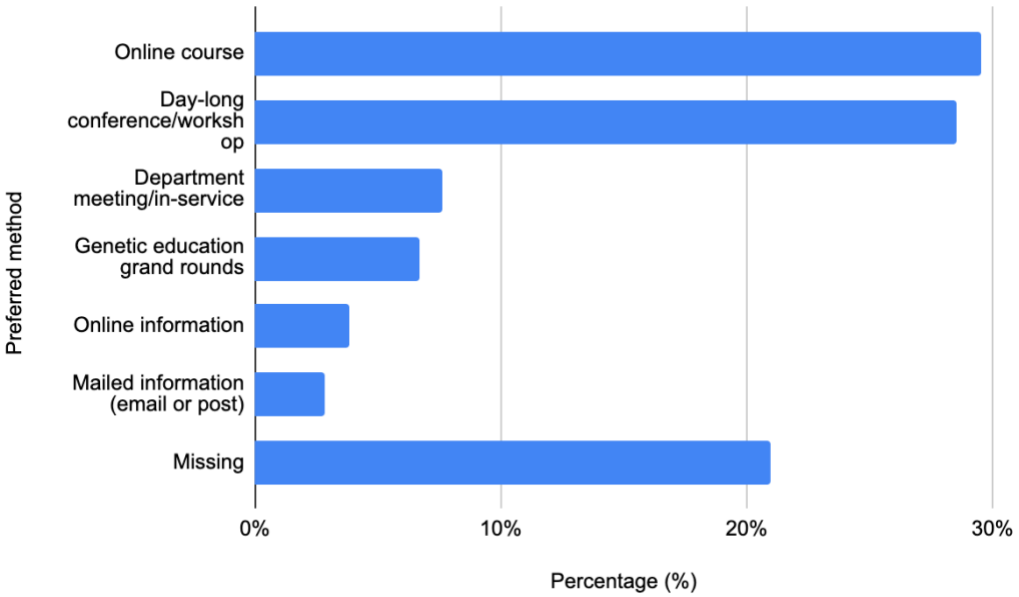

Figure 3.8: Preferred method of undergoing further genetics training. Responses to the question 'What would your preferred method of training be? (Please rank in order of preference - with 1 being most preferred)'.

Clinician group	Ophthalmologists n=36	Optometrists n=22	Orthoptists n=17	**Ophthalmic** n=75	**GP** n=16	Clinical Geneticists n=4	Genetic Counsellors n=6	**Genetics** n=10	**TOTAL** n=105
Genetic testing									
No	4 (11.1)	1 (4.5)	0	**5 (6.7)**	**0**	4 (100.0)	2 (33.3)	**6 (60.0)**	**11 (10.5)**
Yes	29 (80.6)	18 (81.8)	16 (94.1)	**63 (84.0)**	**15 (93.8)**	0	3 (50.0)	**3 (30.0)**	**83 (79.0)**
Missing/Not applicable	3 (8.3)	3 (13.6)	1 (5.9)	**7 (9.3)**	**1 (6.3)**	0	1 (16.7)	**1 (10.0)**	**11 (10.5)**
Interpreting results									
No	3 (8.3)	2 (9.1)	0	**5 (6.7)**	**0**	4 (100.0)	1 (16.7)	**5 (50.0)**	**11 (10.5)**
Yes	30 (83.3)	17 (77.3)	16 (94.1)	**63 (84.0)**	**15 (93.8)**	0	4 (66.7)	**4 (40.0)**	**83 (79.0)**
Missing/Not applicable	3 (8.3)	3 (13.6)	1 (5.9)	**7 (9.3)**	**1 (6.3)**	0	1 (16.7)	**1 (10.0)**	**11 (10.5)**
PRS									
No	5 (13.9)	0	0	**5 (6.7)**	**0**	1 (25.0)	1 (16.7)	**2 (20.0)**	**8 (7.6)**
Yes	29 (80.6)	19 (86.4)	16 (94.1)	**64 (85.3)**	**14 (87.5)**	3 (75.0)	4 (66.7)	**7 (70.0)**	**86 (81.9)**
Missing/Not applicable	2 (5.6)	3 (13.6)	1 (5.9)	**6 (8.0)**	**2 (12.5)**	0	1 (16.7)	**1 (10.0)**	**11 (10.5)**

Table 3.8: Preferences for further training in genetic testing, interpreting results of genetic tests, and polygenic risk scores. Responses of 'Definitely not' and 'Not really', and 'Probably' and 'Definitely' were grouped

3.2 DISCUSSION

Despite being the most common cause of irreversible vision loss worldwide, current screening methods for glaucoma are insufficient and not cost-effective at the population level.[169,170] Genetic factors play a significant role in an individual's risk of developing glaucoma, and glaucoma PRS has shown predictability of glaucoma status, severity and progression.[108] We have previously demonstrated strong interest in polygenic risk testing for glaucoma amongst affected and unaffected individuals,[168,183] however less is known about the attitudes of healthcare professionals towards PRS testing. Acceptance and understanding of such testing from all key stakeholders is crucial for the implementation into clinical practice to be successful. To our knowledge, this is the first study to assess the attitudes of various healthcare professionals towards PRS testing for glaucoma.

Genetic testing has traditionally been directed by specialist medical practitioners in the context of monogenic disease. In our cohort, ordering genetic tests for eye conditions has mainly been done by geneticists and ophthalmologists. Referring for or ordering genetic testing for glaucoma was overall low and may be explained by the lower monogenic contribution compared to other eye conditions such as inherited retinal diseases. The delivery of genetic testing will likely change as polygenic risk testing for glaucoma will capture a broader population, and will necessitate a range of healthcare professionals to show competence in PRS concepts. We therefore aimed to explore the knowledge, confidence and attitude of all healthcare professionals who may be involved in order, interpreting and/or communicating results from polygenic risk testing for glaucoma. These included professionals from genetics (geneticists, genetic counsellors), ophthalmology (ophthalmologists, optometrists and orthoptists) and general practitioners.

Current evidence indicates that healthcare professionals lack knowledge and confidence in dealing with genetic risk and genetic testing.[184,185] Our findings showed that non-genetics professionals felt less confident in their knowledge of genetic concepts, and performing tasks such as estimating risk based on family and/or medical history and interpreting genetic results. However, all healthcare professionals reported an overall low knowledge of glaucoma genetics. Recent studies have also shown low knowledge and confidence levels with the concept of polygenic testing.[143,186,187] Similarly, in our study familiarity with polygenic risk in general was lower in non-genetic compared to genetics professionals. However, none of the healthcare professionals felt familiar with polygenic risk in the context of glaucoma, highlighting the need to

improve healthcare professionals' knowledge of genetic concepts including polygenic risk testing for glaucoma.

Knowledge and experience with glaucoma diagnosis and management was relatively low amongst non-ophthalmic professionals. Having a family history of glaucoma is currently the strongest risk predictor, however only a quarter of GPs felt it was important to assess for a family history of glaucoma in first-degree relatives of patients with glaucoma, compared to the majority of Ophthalmic and Genetic professionals. This may be explained by the fact that glaucoma is an ophthalmic condition almost exclusively diagnosed and managed by ophthalmic professionals, particularly ophthalmologists and optometrists. Exposure and experience in managing patients with glaucoma is unlikely to become a regular part of clinical practice in primary care, however GPs may still be required to refer patients for glaucoma PRS testing and interpret the results. These results highlight an urgent need to provide education and support to all healthcare professionals on the use and interpretation of PRS in the context of glaucoma risk assessment. Our results were consistent with other studies,[143,188] indicating healthcare professionals are interested and motivated to improve their knowledge with additional training, preferably through online resources.

Healthcare professionals reported a generally positive attitude toward recommending polygenic testing for glaucoma based on known risk factors, with the majority of the cohort indicating that they would recommend polygenic testing for those with first-degree relatives and in age groups at higher risk. A number of key factors were reported as significant in the decision to recommend the test, including the performance characteristics of the tests and its clinical utility. This is in line with a metasynthesis of healthcare professionals' perceptions of predictive genetic testing for chronic disease that expressed reservations about the clinical validity and utility of genetic risk information.[189] A recent study assessing health professionals' views in the context of polygenic testing for cancer reported concerns regarding equity, clinical utility and a lack of clinical guidelines.[186] Similarly, our results showed that clear evidence and guidelines from published data and governing medical bodies were of most importance to healthcare professionals. While studies assessing the equity and clinical utility of glaucoma PRS are being undertaken, decision aids to guide healthcare professionals and clinical guidelines will need to be developed to guide management. For example, the Royal Australian College of Obstetricians and Gynaecologists (RANZCOG) recently produced consensus guidelines around genetic carrier screening.[190] So too, RANZCO recently produced consensus guidelines around the role of targeted genetic testing in

the clinical management of inherited retinal diseases.[191] As evidence of the clinical utility of glaucoma PRS testing grows, consensus based guidelines can be produced by RANZCO, Glaucoma Australia and the Australian and New Zealand Glaucoma Society (ANZGS).

Polygenic testing for glaucoma will require healthcare professionals from non-genetic areas to interpret and communicate results. We assessed healthcare professionals' opinions as to which healthcare professional groups would be appropriate to order glaucoma PRS testing, and communicate results of high and low glaucoma PRS. Ophthalmologists were deemed as being the most appropriate to order and to communicate both high and low risk, followed by medical geneticists and genetic counsellors. Over half of the cohort, including the majority of GPs, indicated that GPs would be appropriate to communicate low risk results. This is reflected by a previous study expecting GPs to triage polygenic testing at a population level, with referral to genetic professionals for high-risk individuals.[192] The engagement of different healthcare professionals in the provision of the test and communication of results is important to establish in order to tailor education and support needs to the groups involved.

Affected and unaffected individuals have previously indicated that their preferred method of receiving PRS results for glaucoma would depend on the result.[168,183] Face to face being preferred if results showed high glaucoma risk, and other methods, particularly email, being preferred if results showed low risk. Our study showed similar findings from healthcare professionals, with face to face being the preferred method of communication for high risk results, and other methods, particularly telephone being considered acceptable for low risk results.

The attitudes found in this study represent the views of healthcare professionals in Australia. There may be important cultural differences in general perspectives towards genetic testing and previous experience with genetic testing. As such, the findings may not be translatable to other healthcare systems. The small sample size of this study restricted the comparisons between groups and ability to control for confounding factors, and may also limit the generalisability of the findings. Finally, there may have been a recruitment bias towards those familiar with polygenic risk. Larger scale studies assessing attitudes towards PRS testing will be required, as well as assessing how attitudes change in response to interventions such as targeted education.

In conclusion, healthcare professionals were generally positive toward recommending polygenic testing for glaucoma in certain situations. However, they did not feel familiar or confident with

polygenic risk testing for glaucoma and felt more education is required. Strong evidence of the clinical validity and utility of the test, as well as clear recommendations to guide decision making are needed for the spectrum of risk levels. While it is clear that knowledge gaps in genetics and PRS testing currently exist, resolving this is likely to be a gradual process. Our findings indicate a critical need for training strategies to prepare and support healthcare professionals who will be involved in the different steps of the test.

PART 2: CLINICAL IMPLEMENTATION OF GLAUCOMA POLYGENIC RISK TESTING

INTRODUCTION AND AIMS

Part two of this thesis aims to address the current gap in understanding of appropriate approaches to the clinical delivery of PRS testing. Whilst studies support the clinical utility of a glaucoma PRS, there are several practical questions which must first be considered before such testing can be implemented into practice, including accessibility to testing and strategies for communicating results.

Identifying potential barriers to implementation and uptake will facilitate streamlined delivery. Being largely restricted to research contexts, the current literature on PRS uptake is currently limited. There is no data on the uptake of polygenic risk testing for glaucoma in the general population, and whether interest is a good predictor of uptake. Previous studies reporting uptake of PRS testing for other conditions including breast, colorectal and prostate cancers are difficult to compare due to differences in methodologies.[137,193–196] These studies identified a number of patient- and testing-related factors affecting uptake of PRS testing including higher level of education, efficacy of testing, and the influence of discussion with relevant providers. While only demonstrated within research settings at this stage, these findings will help to streamline the delivery of PRS testing in the future.

With genetic testing becoming an increasingly likely tool for disease screening and diagnosis, the ability to report and communicate results in an effective and efficient manner is essential. Given PRS testing has the potential to be implemented as a risk stratifying tool for a broad population, results must be communicated in a clear, efficient and meaningful manner. Interpretation of results will likely involve consumers/patients and non-specialist healthcare providers, whereby this complex data must be communicated with great care. While results are highly individual due to the complexity of genetic information, reports must still be standardised, accurate, meaningful, and transparent. The goal of PRS reports is to help individuals understand their personal risk status, recognise their management options and their potential prognosis.

The results from this section identify factors contributing to the uptake of the test and strategies to efficiently communicate PRS results based on patient's preferences, to facilitate clinical implementation.

CHAPTER 4: UPTAKE OF POLYGENIC RISK TESTING FOR GLAUCOMA AMONG UNAFFECTED INDIVIDUALS

4.1 INTRODUCTION

With recognised complex heritability, low diagnostic rate with current screening methods, and high treatability to prevent blindness, polygenic risk testing for glaucoma has the potential to facilitate risk stratification across a broad population. Clinical genetic testing for POAG is largely not currently supported, apart from cascade testing or for early-onset disease for Mendelian genes, and better testing strategies are needed to identify those at high risk. We reported a strong positive attitude (70%) among both affected (Chapter 1) and unaffected individuals (Chapter 2) toward polygenic risk testing for glaucoma.[89,197] Being largely restricted to research contexts, however, there is currently no data on the uptake of polygenic risk testing for glaucoma in the general population, and whether interest is a good predictor of uptake.

Here we report the uptake, and assessed factors influencing uptake, of polygenic risk testing for glaucoma among individuals who do not have diagnosed disease. This study was conducted as part of a prospective cohort study, which aims to assess the effectiveness of a glaucoma PRS to stratify risk amongst an Australian population.

4.2 METHODS

This is a translational study approved by the SAC HREC (2020/HRE00968) and adheres to the Revised Declaration of Helsinki. All participants provided written informed consent.

4.2.1 Study design and participants

An existing study cohort was used to assess uptake of polygenic risk testing for glaucoma. A cohort of 417 individuals without glaucoma who participated in a questionnaire-based study (Chapter 2) [183] assessing attitudes towards polygenic risk testing for glaucoma were contacted and invited to participate in a prospective cohort study (Chapter 8)[198] aiming to assess the clinical validity of polygenic risk testing for glaucoma and macular degeneration. All individuals who had previously completed the questionnaire were invited to participate via a combination of either mail, phone, and/or email, based on the contact details provided in the survey. Individuals were contacted preferentially via email, then mail, then phone, depending on the contact details previously provided. If no response was received, individuals were contacted up to three times

via the same or alternate contact methods. Individuals had to be over the age of 50 years and live in South Australia to be eligible to enrol in the cohort study. Individuals without cognitive capacity or with no contact details were excluded. In total, 293 individuals who completed the questionnaire-based study were eligible. Participants were recruited between April and November 2021 and provided a blood or saliva sample to be used for genotyping and to derive an individual PRS for glaucoma and macular degeneration. Blood collection was performed either in person at Flinders Medical Centre, or a blood kit was sent to participants to be done at their most convenient pathology centre. Participation in this study was used as a proxy for the uptake of polygenic risk testing, as it required provision of a sample upon which genetic analysis would occur. Those who enrolled in the study were assigned “Enrolled” while those who declined participation or did not respond to the invitation letter were assigned “Declined”.

4.2.2 Data collection

Sociodemographic data was collected in a previous study[183] and included age, gender, ethnicity, highest level of education, and urban/rural residency. Ethnicity was self-reported and classified into 10 ethnic groupings, then into categories of “European” and “non-European” ethnicity. Those recorded as “unknown” were excluded from analyses involving ethnicity. Residency was based on the Australian Bureau of Statistics census data using the participants' postcodes. Urban residency was classified as postcodes with populations greater than 50,000 persons. Rural residency included regional, rural and remote areas of populations less than 50,000 persons. Family history, including the number of family members affected by any type of glaucoma and their degree of relation, was self-reported by participants. A positive family history was recorded if a participant reported any family history of glaucoma.

4.2.3 Outcome Measures

Intention to undergo PRS for glaucoma was evaluated from a previous questionnaire-based study which assessed the likelihood to undergo genetic testing to predict personal glaucoma risk.[183] Positive intention included those who indicated being either likely or highly likely to take a genetic test which could predict risk for developing glaucoma. Uptake of glaucoma PRS testing was determined by consent to provide a blood or saliva sample for the purpose of genomic analysis. Other factors that may affect individuals’ decision to undergo testing have been previously collected.[183]

4.2.4 Statistical analysis

Data were analysed using SPSS 27. Descriptive statistics were used to describe the sociodemographic characteristics of the sample. The association between each variable and uptake of PRS testing for glaucoma was performed using a univariate logistic regression model. Variables that had significance levels of $p<0.1$ in the univariate analysis were initially included in the multivariate regression model. Multivariate logistic regression models were performed to identify factors independently associated with uptake of testing ($p<0.05$) using a backward stepwise approach. Missing data points were excluded from analyses.

4.3 RESULTS

4.3.1 Recruitment and response rate

Recruitment is shown in Figure 4.1. From the original cohort of 417 individuals who had completed the survey, 299 individuals were over the age of 50 years with a listed South Australian residential address and were eligible to participate in the prospective cohort study assessing the clinical validity of polygenic risk testing for glaucoma. Of these, four individuals did not have any contact details listed and one individual had died since completing the questionnaire. Adjusting for these exclusions, 294 individuals were eligible. Among those, 152 did not enrol, including 104 who were unable to be contacted or did not respond, 31 who declined to enrol, and 17 who had indicated being interested but failed to attend their enrolment appointment. Overall, 142 individuals enrolled, yielding an uptake rate of 48.3%. It is unknown whether the remaining 104 individuals received the study invitation letter, or if they were actively not interested in enrolling in the study. Adjusting for this, the true uptake of testing in this cohort may lie between 48.3% and 74.7%.

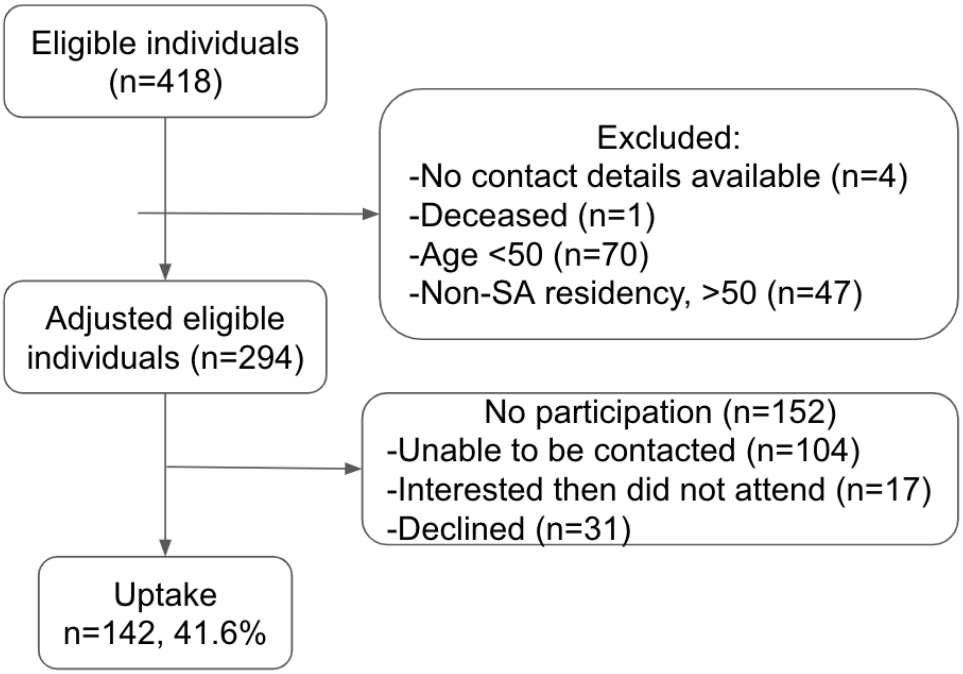

Figure 4.1: Participant recruitment and uptake of polygenic risk testing for glaucoma.

4.3.2 Socio-demographic characteristics

The demographic and personal characteristics of the study sample, collected from the questionnaire study data, are shown in Table 4.1. In the eligible cohort, 66.2% were female, 98.0% were of self-reported European ethnicity, 77.8% were from an urban area, and 44.4% had an education level above secondary school. The mean age of the cohort was 66.7 ± 8.6 years. The differences in characteristics between those who enrolled and those who did not are shown in Table 4.1.

Variable n (%)	**Enrolled** n = 142 (48.3)	**Declined** n = 152 (51.7)	**Total** n = 294	**P value**
Age (years) Mean (standard deviation) Median (range)	 67.6 (7.7) 66.6 (51.0-87.1)	 67.6 (9.6) 67.2 (50.9-95.5)	 67.6 (8.7) 67.1 (50.9-95.5)	 0.954
Gender (Female)	94 (66.2)	101 (66.4)	195 (66.3)	1.000
Self-reported ethnicity - European - Non-European - Asian - Hispanic - Middle Eastern - Mixed	 138 (97.2) 4 (2.8) 1 (0.7) 1 (0.7) 1 (0.7) 1 (0.7)	 149 (98.0) 3 (2.0) 1 (0.7) 0 1 (0.7) 1 (0.7)	 287 (97.6) 7 (2.4) 2 (0.7) 1 (0.3) 2 (0.7) 2 (0.7)	 0.935
Residency (Urban)	118 (83.1)	113 (74.3)	231 (78.6)	0.087
Family history (Positive) Number affected relatives	85 (59.9) 1.02 (1.2)	45 (29.6) 0.47 (0.9)	130 (44.2) 0.73 (1.1)	**<0.001** **<0.001**
Highest level of education - Primary School - Secondary School - Vocational Training - University - Unknown	 1 (0.7) 44 (31.0) 47 (33.1) 50 (35.2) 0	 2 (1.3) 74 (48.7) 44 (28.9) 30 (19.7) 2 (1.3)	 3 (1.0) 118 (40.1) 91 (31.0) 80 (27.2) 2 (0.7)	 **0.002**
PRS testing intention - Likely/Highly likely - Unlikely/Highly unlikely - Unsure	 106 (74.6) 23 (16.2) 13 (9.2)	 98 (64.5) 30 (19.7) 24 (15.8)	 204 (69.4) 53 (18.0) 37 (12.6)	 0.129
Eye exam frequency - Annually or more - Less than once a year	 90 (63.4) 51 (35.9)	 82 (53.9) 69 (45.4)	 172 (58.5) 120 (40.8)	 0.122

Table 4.1: Characteristics of the study sample

4.3.3 Intention to undergo testing

Previously reported intention to undergo testing and actual uptake of PRS testing is summarised in Figure 4.2. Of those who indicated being interested in PRS testing for glaucoma, 52.0% (106/204) enrolled as participants. In comparison, 43.4% (23/53) of those who indicated being uninterested enrolled as participants. Furthermore, 35.1% (13/37) of those who indicated being unsure if they would undergo PRS testing for glaucoma enrolled as participants. Overall, participation did not differ significantly (OR 1.622 95%CI (0.981-2.684), p=0.059) between the groups who had or had not previously shown interest in PRS testing (Figure 4.2).

Because participation required an eye examination in a metropolitan clinic, we further looked at the interest among those from an urban area only. Of those from an urban area who had originally indicated being interested in undergoing PRS testing for glaucoma, 55.0% (88/160) enrolled as participants (OR 1.670 95%CI (0.950-2.938), p=0.075). In comparison, of those from an urban area who indicated being interested in undergoing PRS testing for glaucoma, 40.9% (18/44) enrolled as participants (OR 1.500 95%CI (0.480-4.685), p=0.485)

4.3.4 Factors associated with uptake

After adjusting for all variables that were significant in univariate regression, uptake of glaucoma PRS testing was associated with having a positive family history (OR 4.033 95%CI (2.376-6.845), p<0.001) and having a tertiary education (vocational training or university) (OR 1.999 95%CI (1.171-3.412), p=0.011). Among those with a positive family history, 65.4% enrolled whereas only 34.8% of those with no family history enrolled. Age, gender, ethnicity, and residency were not associated with participation.

We previously assessed factors which may affect individuals' interest in PRS testing for glaucoma, and assessed which of these factors may affect uptake of glaucoma PRS testing and in this study. Those who were more certain about whether they wanted to know more about glaucoma or not before having the test were more likely to participate (OR 1.807 95%CI (1.001-3.264), p=0.05).

Variable (demographic)	**Univariate logistic regression**		**Multivariate logistic regression**	
	OR (95% CI)	**P value**	**OR (95% CI)**	**P value**
Age (years)	0.999 (0.973-1.026)	0.954		
Gender (ref. Female) Male	1.011 (0.623-1.641)	0.964		
Ethnicity (ref. European) Non-European	1.440 (0.317-6.548)	0.637		
Residency (ref. Rural) Urban	1.697 (0.959-3.001)	0.069		
Education (ref. Primary/Secondary) Tertiary	**2.214 (1.374-3.567)**	**0.001**	**2.072 (1.200-3.579)**	**0.009**
Family history (ref. Negative)				

Positive	**3.546 (2.186-5.751)**	**<0.001**	**5.165 (2.898-9.205)**	**<0.001**
PRS testing intention* (ref. No/Unsure) Yes	1.622 (0.981-2.684)	0.059		
Eye exam frequency (ref. >Annually) ≤ Annually	1.485 (0.928-2.375)	0.099		
Perceived glaucoma severity (ref. Not/Slightly) Moderately/Very	2.793 (0.867-8.997)	0.085		
Perceived glaucoma risk* (ref. No) Yes	1.657 (0.920-2.984)	0.092		
Concern of developing glaucoma (ref. No/Slightly) Moderately/Very	1.002 (0.601-1.670)	0.994		
Interest in obtaining more information about the test^ ref. No Yes ref. Possibly No/Yes	0.967 (0.547-1.709) **1.982 (1.146-3.427)**	0.909 **0.014**	 **1.949 (1.054-3.605)**	 **0.033**
Factors affecting decision to be tested Take appropriate measures (ref. No) Yes To provide advice to children (ref. No) Yes To provide advice to relatives (ref. No) Yes Personal advice (ref. No) Yes Medical advice (ref. no) Yes Would rather know (ref. No) Yes	 **2.409 (1.335-4.348)** **1.622 (1.003-2.623)** 1.164 (0.734-1.847) 1.073 (0.657-1.751) 0.880 (0.518-1.495) **1.734 (1.068-2.814)**	 **0.004** **0.049** 0.519 0.778 0.636 **0.026**	 1.585 (0.732-3.431) 0.801 (0.431-1.488) 1.837 (0.967-3.489)	 0.242 0.483 0.063
Concerns about having the test Anxiety (ref No) Yes Would rather not know (ref. No) Yes Cost (ref. No)	 0.709 (0.431-1.166) 0.549 (0.135-2.238)	 0.176 0.403		

Yes	1.253 (0.785-1.999)	0.344		
Attending follow-ups (ref. No) Yes	0.798 (0.454-1.402)	0.433		
Insurance (ref. No) Yes	1.445 (0.794-2.630)	0.228		
Employment (ref. No) Yes	0.849 (0.359-2.003)	0.708		
Confidentiality (ref. No) Yes	1.131 (0.519-2.467)	0.757		

Table 4.2: Univariate and multivariate logistic regression assessing predictors for uptake of polygenic risk testing for glaucoma.
Bold text in the Univariate Logistic Regression indicates variables which were retained in the Multivariate model. * “Highly unlikely” and “Unlikely” were combined into “No” while “Likely” and “Highly likely” into “Yes” answers. ^ “Definitely Not” and “Probably Not” were combined into “No” while “Probably” and “definitely” were combined into “Yes” answers.

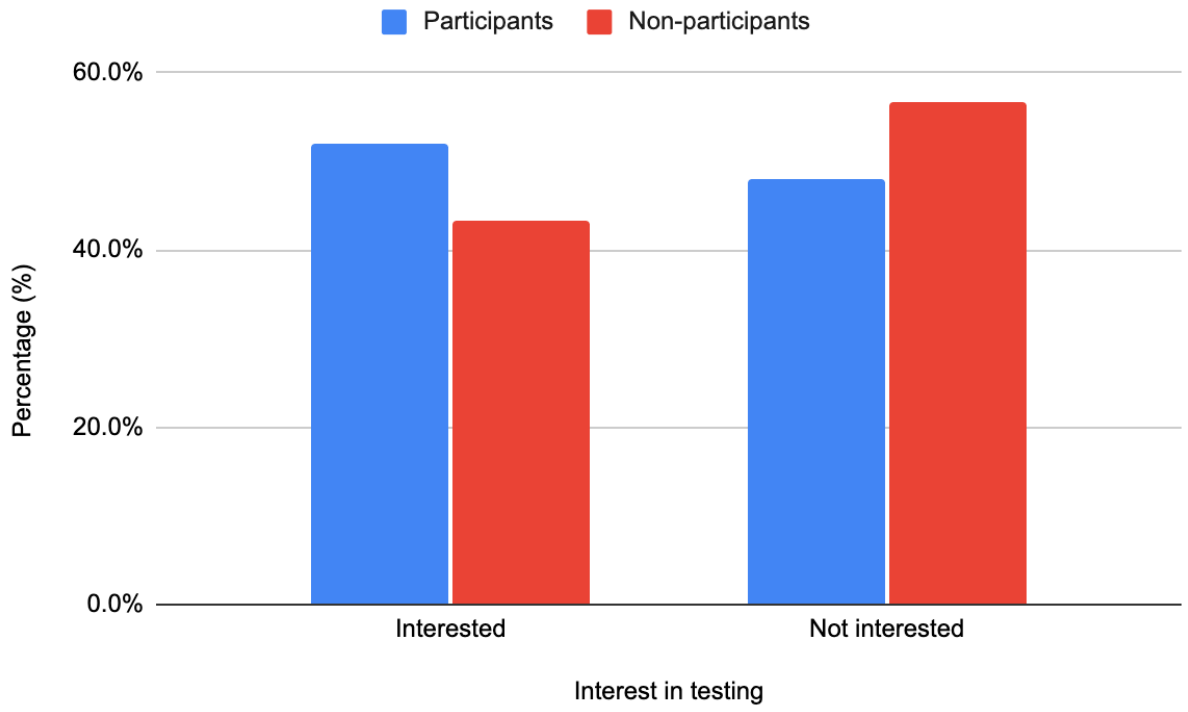

Figure 4.2: Participation between those who had previously indicated being interested or not interested in PRS testing.

4.4 DISCUSSION

To our knowledge, this is the first study to investigate the uptake of PRS testing for glaucoma and assess factors affecting uptake. We have previously demonstrated strong interest in polygenic risk testing for glaucoma (70%) and identified factors that affected interest.[168,183] Here, in a cohort of individuals who previously completed a questionnaire on their attitude toward polygenic risk testing for glaucoma, 48.3% enrolled in a research study to assess their PRS and provided a sample for the test.

Previous studies have demonstrated the utility of a glaucoma PRS to stratify individual risk, predict disease severity, and the level of required treatment intensity.[108,110,199,200] PRS testing may be used to guide individual screening and management: by identifying those most at risk of developing POAG, screening can be performed regularly to ensure early diagnosis and timely treatment. The prevalence of glaucoma increases after 50 years of age, affecting 3% in this age group.[201] Current Australian guidelines recommend screening to commence in those of European and Asian ancestry over the age of 50 years, and over the age of 40 years in those of African descent.[80] Our study sample consisted of individuals over 50 years, predominantly of European ethnicity, and is representative of the population that would benefit from genetic risk stratification in Australia.

The current literature on PRS uptake is limited. Previous studies have reported an uptake between 26% and 96% for breast, colorectal and prostate cancers.[137,193–196] These studies assessed similar cohorts of adults mostly over the age of 40 years and of predominantly European ancestry. However, differences in methodologies makes it difficult to compare results and may explain the variability in results between studies.

The uptake of a breast cancer PRS was similar at 42.1% in a cohort of affected women and their unaffected relatives.[137] However, given roughly half of the participants had a personal history of breast cancer and half previously had genetic testing for the condition (e.g. *BRCA1/2*), this may not be comparable. Individuals may feel PRS testing to assess risk was not relevant if the condition has already been diagnosed, or alternatively may have been more keen to undergo additional genetic testing. Our results showed a higher uptake among those with a family history of glaucoma (65.4%) compared to those with a family history of breast cancer (49.0%).[137] Family history was positively associated with participation in the multivariate analysis. Previous studies

have shown that having a family history may influence the perception of risk and the decision to undergo testing.[202,203]

Variability in research methodology and recruitment strategies can also play a role in inconsistencies of uptake levels.[204,205] Hypothetical scenario methodology to inform potential uptake may poorly predict actual uptake, relating to evidence, communication and psychological burden. A quantitative review of 38 articles identified a number of factors affecting uptake of genetic testing. Most consistently, temporal proximity of the genetic susceptibility test was implicated. In simple terms, this means giving consideration to the extent to which a decision is portrayed as being immediate or having immediate consequences.[206] A study on PRS testing for colorectal cancer reported an overall uptake of 48%.[193] However, among participants who were approached directly and invited to participate after an explanation and discussion about the test, the uptake was 84%. Similarly, a small study that invited individuals directly from a primary care setting for colorectal cancer PRS testing reported an uptake of 96%.[196] In comparison, the BARCODE1 study recruited participants for PRS testing for prostate cancer by sending invitation letters and recorded an uptake of 26%.[194] Our results showed that individuals who were unsure about obtaining more information about the test before deciding were less likely to enrol. Previous research suggests that the perceived benefits and the uptake of a test can be influenced by the service provider and information provided,[195] and indicates that discussion and education about testing is more likely to increase uptake. A targeted approach to testing, involving discussion with healthcare providers, may therefore increase uptake.

We previously assessed factors which may affect individuals' interest in PRS testing for glaucoma and found that the perceived severity of the condition, concerns about developing the condition, the intention to take preventative measures and the intention to learn results were significantly associated with interest in testing. In this study, none of these factors were significant in the multivariate analysis. However, the intention to take appropriate measures and to know if at increased risk were significant in the univariate analysis. We previously found that perceived benefits and risk were associated with intention to test, however this was not replicated in this study with actual uptake of the test. A study on breast cancer PRS similarly reported greater benefits, but not perceived severity or perceived risk, as a predictor of uptake for the test.[137] Response efficacy (the perceived effectiveness in an intervention to reduce risk) was previously reported as more important in predicting uptake of genetic testing for susceptibility to common diseases than perceived severity and worry.[207] Future research should assess whether

communication of genetic risk leads to behaviour changes to reduce disease risk, as well as changes in perceived efficacies, risk, and threat.

Our findings showed that interest in testing did not correlate with actual uptake. Just under half (48.0%) of those who indicated being interested in PRS testing for glaucoma did not undergo testing when they were invited, while 43.4% of those who had indicated that they were uninterested in testing did actually undergo testing. Previous studies have shown that interest in genetic testing is usually higher than actual uptake of testing.[162,204,208] Although attitude toward testing was previously reported as a strong predictor of test uptake in common conditions broadly,[207] this is not supported here. Other reasons might affect the decision. Although many did not provide a reason for not participating, not having enough time or being too busy were reported. A large number could not be contacted so we cannot exclude whether they were not interested or did not receive the information. Individuals were invited to participate in the study approximately 12 months after completing the survey assessing attitudes towards a glaucoma PRS. Many had little recollection of the survey, so their decision to participate may be a more accurate reflection of their likelihood to undergo testing if it were generally available.

Our results showed that there was no significant difference in enrolment in those from an urban area compared to those from a rural area. We hypothesised that individuals from a rural area would be less likely to enrol, driven by limitations to accessing health services in non-urban areas. It should be acknowledged that participation in the PRS study requires an eye examination, performed at an urban clinic or a single remote location. Potentially, some may have declined if it would be difficult to travel from their home. To reduce the chance of potential selection bias towards those from a rural area, we offered for a blood kit to be sent to participants, allowing blood sampling to be done at their nearest pathology centre. Whilst not demonstrated in this study, access to pathology centres for sample collection and inaccessibility of eye health services for follow-up may still be a barrier to uptake of testing in the future. Future research should assess additional potential barriers to accessing testing through either surveys or interviews of individuals who express interest in testing but do not complete the test.

Fundamental themes and inconsistencies within the literature were highlighted in a comprehensive systematic review.[205] The review included 115 studies that had provided quantitative analysis of subjective and/or objective predictors of genetic testing interest, intentions, or uptake.[205] There was a broad variety of genetic testing in the studies included in the

review, ranging from testing for highly penetrant Mendelian genes, to direct to consumer tests. The analysis highlighted several important insights into the current literature, and aspects requiring attention in future studies to better delineate factors affecting genetic testing decision. The study suggests that qualitative, rather than quantitative, methodology yields the most useful information.[205] Although providing less precise information, qualitative outcomes promote informed decision making about genetic testing. This is important to consider when comparing studies within the current literature, and for planning of future studies.

These results should be interpreted in light of the study's strengths and limitations. Our study cohort included unaffected individuals over the age of 50 years, assessing potential uptake of the test if it was offered to the general population. It remains to be investigated if the uptake of PRS testing would be different in a younger cohort, although this is a less relevant population to test given prevalence increases with age.[5] A significant proportion of those who did not enrol were unable to be contacted (68.4%). As mentioned, it is not known what proportion of this cohort did not receive the study invitation letter, or if they were actively not interested in enrolling in the study. Little is understood about the reasons for declining to participate in genetic risk testing,[209] however, future studies could reduce the unknown aspect of non-responders by incorporating a mandatory confirmation of receipt, options for individuals to notify investigators of their reason not to participate, or allowing more time and resources for investigators to re-contact individuals. Over 97% of our study sample was of self-reported European ethnicity, highlighting the need for further validation across other ancestral backgrounds prior to implementation. It is also worth noting that a majority of the cohort was from an urban area, with urbanicity and lack of diversity being frequent criticisms in studies assessing genetic testing uptake.[210] Participants were drawn from a cohort that previously completed a survey on the attitude toward polygenic testing, which may have introduced bias through increased awareness about glaucoma and PRS testing, as well as potentially introducing selection bias in those that participated originally being willing to answer such questions.

In summary, this study explored the uptake of PRS testing for glaucoma among unaffected individuals, and showed a lack of association between intention to test and actual uptake, while education and family history were predictors of uptake. Further work is needed to develop strategies to effectively implement glaucoma PRS testing into clinical practice and provide equity in access to the test, especially for those living in rural areas. Developing effective educational material will be important for patients at all levels of education and literacy as well as for specialist

clinicians who may be less involved in delivering pre-test counselling and communicating results to increase access to the test and uptake.

CHAPTER 5: PREFERENCES FOR REPORTING A GLAUCOMA POLYGENIC RISK SCORE

5.1 INTRODUCTION

Polygenic risk testing is an emerging concept with the capacity to stratify risk for common conditions with complex heritability for a broad population. A polygenic risk score (PRS) summarises overall genetic risk for a condition through a single value which is easy to interpret. While PRS tests have mainly been described in research settings, several commercial groups have developed early models. Despite advances in this technology, standardised best practice guidelines for reporting results have not yet been established.

Currently, genetic tests in Australia are available for a range of monogenic conditions.[211] Genetic test results in these circumstances have traditionally been delivered in person, largely by genetic counsellors.[212] However, this is unlikely to be feasible for PRS testing where widespread testing could be possible. Fundamental differences between polygenic risk testing and testing for monogenic variants necessitates important adaptations in reporting results. The most effective approach of reporting PRS results is yet to be identified and there has been high variability in the reports trialled thus far.[213] Risk communication of PRS results is a new concept which requires novel reporting strategies.

Given PRS testing has the potential to be implemented as a risk stratifying tool for a broad population, results must be communicated in a clear, efficient and meaningful manner. Interpretation of results will likely involve consumers/patients and non-specialist healthcare providers, whereby this complex data must be simplified with great care. Careful consideration must be given to the range of education levels, as well as health literacy and numeracy levels. Variability in health literacy and genetics knowledge may pose significant challenges to engaging the general public. Individual numeracy levels have previously been shown to be the strongest independently associated variable with genetic test comprehension.[214]

While results are highly individual due to the complexity of genetic information, reports must still be standardised, accurate, meaningful, and transparent. The goal of PRS reports is to help individuals understand their personal risk status, recognise their management options and appreciate their potential prognosis. Adequate communication of PRS results is critical to the success of this testing and promotion of consumer engagement. Despite growing evidence of the

clinical utility of a glaucoma PRS, there is a lack of evidence of effective communication tools which will facilitate its implementation into clinical practice. This is the first study to develop PRS reports for glaucoma and investigate individuals' understanding and perspectives.

5.2 METHODS

5.2.1 Literature review and development of draft PRS reports

We first performed a review of the literature to understand the existing knowledge around reporting methods and preferences. The reports discussed are provided in Appendix E. Patient risk stratification using PRS is an emerging technique which is not yet in routine clinical use. Therefore, there is limited data reporting the most effective methods of reporting results and the perspectives of patients to different report styles. In light of this, we reviewed some of the existing literature to understand the fundamental principles of reporting genetic results, including understanding of health risk presented as absolute versus relative risk, and the utility of various graphs to aid understanding. The literature reviewed is summarised in Table 5.1.

Risk framing can have a significant impact on risk perception, where identical risk information can be presented in different ways and result in bias.[215] It has been reported that absolute risk is usually preferred and more accurately understood by the general population than relative risk.[216–218] However, different ways of communicating risk can result in bias and misinterpretation.[219] For example, relative risk can exaggerate the perception of difference, especially when the absolute risk is small. Conversely, absolute risk can be misleading if not given with more information about population averages. Because of this, we assessed understanding of the same PRS result presented in both absolute and relative terms. Visual aids are a recognised, critical tool for risk communication, particularly for those with low literacy levels. A Cochrane review found that visual decision aids helped patients feel more informed and knowledgeable, and improve accuracy in interpreting results, allowing them to participate actively in decision making.[220] Pictographs have been reported to be especially effective to convey risk information, as well as bar graphs or pie charts.[221–224]

PRS reports currently available in academic and commercial settings were reviewed, including consideration of graphs/charts used to convey risk, colour formats, information included, and general formatting.[213,225–227] Given reporting PRS results is relatively new area, there was variability in design amongst many of the reports viewed.

Brockman et al reviewed nine publicly available polygenic score reports. Reports were highly variable in terms of colour, numeric risk estimate provided, categories used to describe amount of risk, and availability of additional resources and recommendations based on the test result.[228] From this review, a two-page draft polygenic score report for CAD was developed that was designed to be understandable by both prospective patients and clinicians based on existing reports. The design principles that they used to highlight important concepts and optimise understandability of the report were repetition and emphasis. They also aimed to maximise accessibility and understanding of the information included in the report by minimising technical language and using simple sentence structure.[228]

Direct to consumer (DTC) genetic tests are commercially available tests allowing consumers to access information about their genetics without involvement of a healthcare professional. Although few are approved by national authorising bodies, DTC genetic test reports are some of the first to communicate PRS results. Each company uses their own format to report results, with most using a combination of text, numbers and figures. Sample reports are available on some company websites.

23andme provides examples of genetic risk for various conditions for a fictional consumer, 'Jamie'.[229] These reports communicate an absolute risk percentage and pictograph together with a short explanation of the risk and description of the condition using easily understood language. Each report includes a qualitative summary of the customer's genetic predisposition, an estimate of their remaining lifetime risk (based on genetics, age, and self-reported ethnicity), a 10-year risk estimate, a "prevalence explorer" tool illustrating the impact of risk factors on risk, information about behavioural changes, general information about the condition, limitations of the test, and scientific details of the methodology used to generate the report. The reports were said to be developed from reviewing clinical literature about the condition, qualitative sessions, and with input from professionals in the condition. The method of reporting quantitative risk was informed by one-on-one interviews where individuals were presented with a variety of statistical tools conveying risk. Remaining lifetime risk was preferred to total lifetime risk, as well as being seen to be more beneficial than 10-year estimates. The "prevalence explorer" tool is an interactive means allowing consumers to appreciate the impact of genetic and environmental elements on overall disease risk.[230]

The P5 study, conducted in Finland, created a web portal as a method of communicating genetic risk information to a large number of individuals and their physicians.[231] Absolute risk was presented as a thermometer, with risk categorised as being low, increased, high or very high. PRS was presented as a single value in relation to the whole population on a normal distribution curve.[231] MyGeneRank offers consumers additional risk testing on existing samples provided to 23andMe by giving the company access to their data. Genetic risk scores for conditions are presented as single values on a graded scale. Risk scores are accompanied by information on how behavioural changes may offset risk and an option to connect to a genetic counsellor.[232,233] My Toolbox Genetics is a testing service allowing consumers to access their results through a mobile application.[234] The service presents a risk summary with a coloured scale. Other features of the app include health insights, a genetic action plan, epigenetic results, meal guide, personalised fitness program and lifestyle tracking.[234]

Several breast cancer PRS tests are commercially available. Ambry Genetics and Myriad Genetics both reported PRS as an absolute lifetime risk (percentage), classifying risk into categories of average or increased risk.[235,236] AmbryScore breast cancer PRS, which combined a PRS with a clinical risk estimate, removed their model in May 2021 partly due to limited data across ethnic populations.[228] CanRisk is an online tool which combines an individual's PRS with personal and family history of breast cancer.[237] This tool is widely used in assessing risk in individuals with a clinically elevated risk but without a cancer susceptibility gene. Formats contributing to the development of the design were not specified, however were reported to be developed from a range of popular output formats and informed by frequent input from target healthcare professionals. Ambry Genetics also offers PRS testing for individuals affected and unaffected by prostate cancer, without a known history of Mendelian inheritance, with PRS results reported as an absolute lifetime risk (percentage) and as an odds ratio, respectively.[238,239]

PRS reports developed in research settings have also shown significant variability. A report developed to communicate a child's PRS results for asthma and diabetes to parents did not include a visual aid.[240] The report categorised risk as either ‘high risk’ or ‘NOT at high risk’. Forrest et al invited individuals with a PRS in the highest quartile of breast cancer risk to receive their results and provide feedback using two visual risk communication tools.[213] One visual aid consisted of a skewed normal distribution curve with an indication of the individuals’ relative risk, while the second consisted of a pictograph representing the individuals absolute breast cancer

risk. The study showed that participants were able to understand their risk from the pictography, however fewer were able to confidently understand their relative risk.[213]

Study (research)	**Risk format**	**Colour format**	**Included information**	**General formatting/other**
	Bell curve - relative risk Pictograph - lifetime (absolute) risk	Bell curve - Red line indicating example of high risk - Green line representing average risk Pictograph - Pink - empty and solid figures	Interpretation of risk result Method PRS ranges Common variants	Black text Simple layout
	Table and scale	Scale yellow, orange, red	Test results and explanation Results related to the reason for testing Explanation in text and scale format Explanation of inheritance pattern Next steps Letter to share with relatives	13 page report Detailed
	Verbal - phone call Information booklet	Information booklet not provided to view	Information booklet not provided to view	Information booklet not provided to view
	Pie chart Pictograph Bar graph Scale diagram Box plot Relative risk: pie chart, pictograph, bar graph, box plot Absolute risk: scale diaphragm	Blue/red - pie Blue/red - pictograph Yellow/red - pictograph Bar graph - red Scale diagram - orange/red gradient Box plot - red	- Accompanying text describing risk in relative and absolute terms	Not described
	Bell curve	Green to red gradient	Participant information Participant score Explanation of polygenic risk scores Explanation of coronary artery disease How to reduce risk	Two page report with mix of text and graphical elements

	Thermometer Bell/pictograph Bar graph	Thermometer - absolute risk scale green (low) to red (high) Bell/pictograph - single risk value in relation to the whole population on a normal distribution curve Bar graph - 10 year risk	Explanation of result presented in each graphical format	Risk calculator
	Scale diagram - relative risk Bar chart - 10 year risk	Scale diagram - blue/orange/red gradient from low to high risk Bar chart - orange base risk, blue genetic risk, red combined risk	How to reduce risk Find a genetic counsellor Activities News	Phone application
	Text only	N/A	PRS result High risk PRS recommendation	
Company	**Risk format**	**Colour format**	**Included information**	**General formatting/other**
	Pictograph Absolute risk Remaining lifetime risk 10-year risk	Multi	Pictograph with written explanation of result Description of condition Behavioural change recommendations General information about test Scientific details of PRS methodology	Tool illustrating the impact of risk factors on risk
	Text Line graph - breast cancer risk (%) vs age for individual and population Pictograph - 5/10/lifetime risk	Line graph - blue/black Pictograph - pink/black	Risk category by guidelines Mutations Inputs Extra information	Multiple tabs within web-based tool
	Scale diagram	Blue (low) to red (high) gradient of risk	Risk change with behaviour modification tool	Phone application
	Scale diagram	Red (poor) to green	Health insights	Phone application

		(good)	Genetic action plan, Epigenetic results Meal guide Personalised fitness program Lifestyle tracking	
	Percentage remaining lifetime risk Bar chart - population risk vs individual risk	Pink/grey/orange	Explanation of risk Cancer and clinical history summary	4 page report, mostly text
Ambry Genetics - AmbryScore[236,238,239]	Absolute lifetime risk (percentage) - average or increased risk	Pink/grey		

Table 5.1: Summarised literature of PRS reports.

Three versions of a two-page draft polygenic risk score report for POAG were developed. The mock reports developed presented the same risk result in three different formats. Each report included identical general information, including an explanation of polygenic risk scores and glaucoma. We aimed to maximise understanding through the level of detail included in the report, while minimising the amount of technical language used. Reports were developed representing both relative and absolute risk. Based on the literature, a bell curve, pictograph and pie chart were chosen to each convey a glaucoma PRS result in the 95th percentile.

The information included in the report aimed to optimise understanding by minimising scientific/medical language and balancing text with corresponding images. The reports included six sections, each intended to support the explanation of a glaucoma PRS and give context to the importance of the result. The sections included were 1) PRS Result - Glaucoma, 2) Polygenic Risk Scores (PRS) explained, 3) What does my test result mean for me?, 4) Your Polygenic Risk in Detail, 5) Frequently Asked Questions, and 6) Resources - for more information and where to get help. A blue-orange colour scale was chosen in order to avoid confusion in individuals with red-green colorblindness, the most common form of colour blindness.[241]

In light of the limitations in the review of the literature, and the lack of specific official guidance, this study was undertaken with an objective of providing recommendations for making the content

and structure of a genetic test report more accessible to patient and non-specialist clinicians. This is the first study to assess individuals' attitudes towards three novel PRS reports for glaucoma.

5.2.2 Study Sample

This is a qualitative-descriptive study approved by the Southern Adelaide Clinical Human Research Ethics Committee (SAC HREC 2023/HRE0085) that adhered to the Revised Declaration of Helsinki. Participants in the GRADE study (Chapter 8) were randomly selected and invited to participate to represent individuals from the general population. Inclusion criteria for this study were individuals over 50 years of age. A target of 10-14 participants was set, subject to data saturation and the responses provided.

5.2.3 Data collection and survey

Participants who consented to the study were sent a short survey to complete online to collect demographic information (age, gender, education, ethnicity, colour blindness) and assess numeracy, graph and genetic literacy using validated tests (Appendix B). Numeracy was assessed using the Objective Numeracy Scale,[242] graph literacy using the Short Graph Literacy Scale and genetic literacy using both the Genetic Literacy Fast Test[243] and 8 true/false statements based on existing measures and adapted to glaucoma. Finally, participants were asked what information they would like to see included in a report for glaucoma genetic risk with 8 options and an open question for additional comments. Once the online survey was completed, participants were sent the three mock reports developed to have time to review them before being invited to do an online interview. The mock reports are provided in Appendix C.

5.2.3 Interviews

Semi-structured interviews were performed via telephone. The interview guide was developed and modified from limited existing literature,[228] and is available as a supplementary document (Appendix D). There was no time limit applied and participants could take breaks whenever necessary. Participants had the opportunity to ask questions throughout. There were no incorrect responses, rather the opinions and thoughts of participants were sought to better understand the most effective method of communicating risk. Prompts were used to elicit insightful and productive information. Field notes were taken throughout and the interviews were audio-recorded for the purpose of validating notes at a later stage if necessary.

The interviews were structured to cover five main themes: preference of visual risk communication aids, understanding of risk, influence of reports on risk perception and behaviour, usefulness of report content, and general report format and layout. Participants were first asked about their previous experience with receiving genetic reports or medical results, and any aspects of reports they had received in the past they did or did not find useful. This gave an insight into their baseline experience as a starting point for the interviews. The remaining themes were then discussed for each figure to allow for clearer comparison between formats and participant reflection on their preferences. The content, layout and structure of the remaining aspects of the report were then assessed, including the balance of text and visual elements, font and colours used. In assessing the most preferred reports, participants were given the opportunity to give an opinion as to how it could be improved.

Each interview was audio recorded and transcribed by a professional and approved transcription service. Analysis was performed based on the notes taken and by reviewing the transcripts. Interviews were performed until no new themes or feedback were reported. Interviews and analysis were conducted by G.H.

5.3 RESULTS

Twelve interviews were performed. The characteristics of the study sample are shown in Table 5.1. Half of the participants were female, all were of European ethnicity, and 50% had a university education. The mean duration of the interviews was 25 minutes and 50 seconds. Participants had a range of numeracy, genetic and graph literacy as shown in Table 5.2. No participant had received a genetic report before, however some had received medical results such as x-ray reports before. Most participants commented that routinely, test results would be sent to the requesting healthcare provider, or would be sent to another relevant practitioner, rather than to the patient themselves.

Age	Gender	Ethnicity	Education Level	Colour blindness	Interview time	Preference
≥70	Male	European	University	No	39:22	1. Pictograph 2. Bell curve 3. Pie chart
60-69	Male	European	Vocational training	No	21:34	1. Pictograph 2. Pie chart 3. Bell curve

60-69	Female	European	Secondary School	No	23:10	1. Pie chart 2. Pictograph 3. Bell curve
≥70	Female	European	University	No	28:43	1. Pictograph 2. Pie chart 3. Bell curve
≥70	Male	European	Vocational training	No	28:43	1. Pictograph 2. Pie chart 3. Bell curve
60-69	Female	European	University	No	15:15	1. Pie chart 2. Bell curve 3. Pictograph
≥70	Female	European	Vocational training	No	33:16	1. Pictograph 2. Pie chart 3. Bell curve
60-69	Female	European	University	No	23:38	1. Pie chart 2. Pictograph 3. Bell curve
60-69	Male	European	University	No	23:38	1. Pie chart 2. Pictograph 3. Bell curve
60-69	Male	European	Secondary School	No	18:10	1. Pictograph 2. Pie chart 3. Bell curve
60-69	Male	European	Vocational training	No	28:34	1. Pictograph 2. Pie chart 3. Bell curve
60-69	Female	European	Secondary School	No	26:02	1. Pictograph 2. Pie chart 3. Bell curve

Table 5.2: Characteristics of the study sample

Survey	
Numeracy score Mean (SD) Range (0-3)	 2.1 (0.9) 0-3
Genetic literacy score Mean (SD) Range (-20 to 46)	 24.2 (6.2) 14-39
Genetic knowledge	

Mean (SD) Range (0-8)	7.0 (1.0) 5-8
Graph literacy score Mean (SD) Range (0-4)	 2.8 (0.8) 2-4

Table 5.3: Participants' numeracy, graph, and genetic literacy

A summary of the participants' preferences for the graph format reporting results is summarised in Table 5.3. Participants first preference was the pictograph format, followed by the pie chart and lastly the bell curve. In analysing preferences for the format of representing risk, three main themes were identified to contribute to overall understanding of the mock reports. Firstly, preferences towards the figure used to visually represent the risk, which included a bell curve, pictograph, and pie chart. The format of this figure was discussed, presenting either absolute or relative risk. Secondly, accuracy of understanding and confidence in interpreting the graph together with the corresponding text explaining the result. Thirdly, the informative text providing more detail about the test and glaucoma, together with the overall format and layout of the reports.

Graph format	First preference	Second preference	Third preference
Pictograph	8	3	10
Pie	4	67	21
Bell	0	32	910

Table 5.4: participants' preferences for graph format

5.3.1 Theme One: Preferences towards visual risk communication aid

Overall, absolute risk was preferred, either in the format of a pictograph or pie chart, with the bell curve being the least preferred option. The two absolute risk figures helped participants understand their risk by visually comparing personal risk to the general population.

Most participants felt the pictograph was visually clear and could be interpreted quickly, without needing the corresponding text to help interpret the result.

'Clear and simple.'

'I don't think you have to think about it, it's [the result] is there in front of you…it's very definitive in its message.'

Participants were more readily able to correctly interpret the result.

'That I'm more than twice as likely to get glaucoma than the general population.'

'You can see that you're at higher risk than the average (population), but you're not 95% like the other one gives the impression of.'

Similarly, participants felt the pie chart was easy to interpret, mainly because of its clear comparison to the average population.

'Very clear, you don't have to think about it.'

'I didn't need the (corresponding) text as much.'

The bell curve was less effective in helping participants conceptualise their risk. Most commented that this figure gave the impression of extremely high risk, or almost certainty, of developing glaucoma and would therefore cause significant worry. The relative risk, presented as a percentile with the bell curve, was difficult to understand for some.

'I don't really get it. Don't even go there.'

'It's hard to get it all in the head…and work it out.'

'I don't think [other people would understand]…you have to look at it.'

The bell curve was the most preferred visual aid for only one participant, who prefaced their feedback by noting having had quite a lot of experience with interpreting bell curves in the past and therefore being very comfortable with this format.

'The picture matches the words underneath..I think it's easy to understand.'

However, despite the bell curve being their most preferred, this participant felt most people would not be able to understand this figure.

> *'I think that a lot of people are probably not familiar with looking at themselves within a population.'*

5.3.2 Theme Two: Understanding risk

The visual graph had a significant impact in understanding and assisting in translating visual risk to numerical risk. Participants generally agreed with the statement that the absolute risk figure would give more understanding without making the individual feel more worried. The bell curve was felt to be most confusing, and was generally misinterpreted as a percentage risk of developing glaucoma. Most participants did not fully understand the concept of a percentile to represent risk within a population, compared to a percentage.

> *'I looked at the graph first and went, oh 95%, and then I read it…and realised it was 2.3 (times) higher, not 95% chance.'*

Confusion and misinterpretation of the risk presented also influenced the degree of worry participants felt from the reports and the potential influence on risk perception and behaviour. Participants felt that the absolute risk, such as represented by the pictograph and pie charts, were reassuring. Participants generally felt that, while each figure was reporting the same high-risk result, the pictograph and pie chart represented a much lower risk compared to the bell curve. Some felt that this may negatively affect risk-reducing behaviour.

> *'It still…can indicate, compared to the rest of the population, at a relatively low risk of developing glaucoma. Whether or not people would act on that.'*

All participants felt the corresponding written content underneath the graph, explaining the result, was useful and necessary to aid understanding.

> *'It explains it, so I thought that it was useful.'*

Although participants often felt their understanding of the report was sound, interest in obtaining guidance or recommendations with the risk results was expressed.

'It doesn't tell the ophthalmologist anything, other than I'm at high risk. You don't need the report to generate any action from anybody else. The ophthalmologist doesn't need it, it's not going to help them.'

In addition, a timeframe to the recommendation was considered important. Many felt an indication of timeframe would significantly contribute to their behaviour by indicating urgency and in terms of wanting to review the report with a healthcare professional, such as their optometrist or GP, undergoing an eye examination, or discussing their result with their family.

'The main thing I want to know is what to do with my result. What do I need to do next and when?'

'I was happy with the content - people want to know what it means for them and where to go next.'

5.3.3 Theme Three: Report format and visual elements

The visual and design elements played an important role in facilitating understanding and risk perception. In Particular, they contributed most to a user's first impression.

'The first thing you look at is the visual, and then you read.'

1. Colour

Colour was a predominantly discussed design element, which contributed to confusion for some participants with the bell curve. Participants felt that the blue-orange colour scheme did not make sense initially, and negatively influenced their overall understanding and experience with this graph.

'The colours too…didn't make a statement.'

'I think that the shading probably makes it a little more confusion…the shading make it less definitive.'

'I can see how you've faded the colours, gone from the caution colour to the cool colour, but I didn't pick up on that immediately.'

2. Font

All participants felt the font used was appropriate and of adequate size, particularly given reports may be read by individuals with visual impairment. There was little feedback on this aspect.

3. Layout

Participants generally felt the layout of the report was simple and easy to follow. Bullet points were useful in communicating relevant information without too much detail, using simple language. Participants felt there was an appropriate balance of text and visual elements. One participant suggested pictures could be numbered and then referenced with the corresponding text, to more clearly identify the relevant information.

Most participants felt the content of the report was appropriate, however all wanted further detail and emphasis on follow-up or treatment recommendations. One participant felt there was too much information included.

'The section I thought was over the top was those second and third sections, that's a lot of text. People are just not going to read it and frankly they're not going to care.'

A suggested modification to improve and synthesise the information presented was to include more detailed information as smaller text at the end of the report.

'You could have, in a lot smaller print, on the back of the pamphlet the limitations of the test and all of those sorts of stuff that you need to perhaps tell people, but it's not the primary objective of the result.'

5.3.4 Potential modifications based on feedback

Based on the feedback received from these interviews, a number of modifications to our reports could be made. While the colour scheme of orange and blue was chosen to aid interpretation of those with red-green colour blindness, all participants felt another colour scheme would add to the visual interpretation. Improving understanding for a larger majority may be more useful in achieving greater understanding, although it would come at the sacrifice of the smaller number of

those with colour blindness. A red-green colour scheme was suggested, which is familiar to most people in settings such as traffic lights and temperature gradients.The blue colour, used to convey low risk, could be altered to green. Similarly, the orange colour, used to convey high risk, could be altered to red. Section three in the report draft could be moved to follow the reported PRS result, to further improve and support their understanding of the results.

5.4 DISCUSSION

As PRS testing is progressing towards clinical implementation for prediction of disease risk and prognosis, the ability to effectively report and communicate results is essential. Given PRS testing has many potential clinical applications,[244] results must be communicated in a clear, efficient and meaningful manner. Interpretation of results will likely involve consumers/patients and non-specialist healthcare providers, whereby this complex data must be communicated with great care. While results are highly individual due to the complexity of genetic information, reports must still be standardised, accurate, meaningful, and transparent.

Preliminary data from our surveys conducted on affected and unaffected individuals toward polygenic risk testing attitude highlighted that individuals were interested to learn about the implications of results and the significance for follow up. We have also previously demonstrated that the preferred method of receiving results may depend on the result itself, so variance in report content and structure may be necessary depending on risk classification.[168,183]

The approach to reporting and communicating polygenic risk results will require a strategy that supports their potential for screening of large populations and differs from the current delivery of Mendelian testing. This is due to several notable differences between polygenic risk testing and Mendelian genetic testing. Firstly, a PRS is not diagnostic but rather denotes an estimate of risk derived from many DNA variants. Therefore, a PRS represents a result within a spectrum of risk, rather than a binary result. This means that results are not binary, and must be reported and interpreted in the context of a population reference distribution. Secondly, as PRS is a risk estimate, clinical recommendations will need to be developed for the different risk groups to provide appropriate advice to patients based on their results. As defined by Bunnick (2015), ‘genomic information has personal utility if and only if it can reasonably be used for decisions, actions or self-understanding which are personal in nature.[245] Studies commonly report lack of clinical guidelines as a barrier to PRS implementation.[186,246] Although there are currently no guidelines for glaucoma which clearly identify follow-up or intervention for each PRS classification,

the development of clinical guidelines is a current focus of research. The importance of clear guidelines and recommendations based on genetic risk was also highlighted by participants in our study, especially for guiding an individual's behaviour in response to their genetic risk. Finally, PRS have mainly been tested in research settings with clear definitions of disease. There may be significant variance in the definitions used in research from those used in clinical practice.[247] ,

While the content of PRS reports is crucial to ensure accuracy and understanding, the design of the report may be equally as impactful in achieving this. Generally, graphic design aims to achieve a common denominator between its readers. In this study, participants reported that the visual components were important for understanding results. Although risk is often communicated verbally to patients, design has a significant impact on first impressions, and can influence how an individual perceives credibility, relevance, and overall experience.[248,249]

One of the most significant challenges to consider in designing PRS reports is the significant variation in literacy and numeracy levels within the general population. Whilst varied, public familiarity with genomic risk information is generally low.[250–252] Our study included a sample of individuals with average genetic and graph literacy, as well as numeracy levels. Participants reported that relative risk, as illustrated by the bell curve in our reports, was more difficult to understand across a spectrum of education levels. Similar results were shown in a study assessing patients and primary care providers responses to mock PRS clinical reports. This showed that individuals, including some with high numeracy, confused percentile with percentage.[246] Participants in this study had difficulty conceptualising their risk and tended to overestimate their risk when relative risk was presented. Previous studies have shown that absolute risk presentations are usually the preferred format over relative risk.[221,253–255] This is consistent with our findings demonstrating absolute risk was the preferred risk format. While reporting risk in a simplified, easily understood format is important, it is also important that reports accurately depict the risk. As a result, relative risk may be best reported in the context of absolute risk to improve comprehension of results. Presenting risk in multiple formats, such as numerical, graphical, and written, to account for differing learning styles may further enhance comprehension.

Genomic results communication will rely on healthcare professionals beyond genetic specialists given there will be insufficient geneticists and genetic counsellors to meet expected demand.[256] However, several studies have demonstrated low confidence in interpreting genomic results

among non-specialists.[185,257–262] A study assessing clinician acceptability of a prototype breast cancer risk tool highlighted the challenges of developing a tool reporting complex information. Whilst the tool was generally accepted by the primary care and genetics specialists included in the study, there was concern about the time needed to interpret the reported results and to adequately communicate these to patients.[263] This is the next step for our study. Although only prospective patients were included in our study, the reports could also be relevant to clinicians and healthcare professionals involved in PRS testing and results communication. Further research is needed to assess clinicians' perspective towards PRS reports.

This study aimed to elicit key elements for the clear communication of PRS results. Our findings provided an early framework for the disclosure of glaucoma PRS, and illustrated the challenges in simplifying complex information to be accurately understood by the wider community. Ultimately, PRS testing for glaucoma will rely on end-user engagement and further work should adapt the feedback from this study into the reports to then deliver PRS results to patients in a clinical setting.

PART 3: APPLICATION OF POLYGENIC RISK TESTING FOR GLAUCOMA

INTRODUCTION AND AIMS

Part three of this thesis aims to assess the validity of glaucoma PRS testing, and explore the interplay of the PRS with key risk factors and treatment pathways. This section includes three interconnected studies involving known glaucoma risk factors and a glaucoma PRS, however each has a distinct study design, study sample, methodology and analysis. The methodology of each study within this section will be outlined in detail in the appropriate chapter.

This section addressed the application and validity of a glaucoma PRS, focusing on three main aims. Firstly, I aimed to investigate the relationship between glaucoma PRS and family history of glaucoma in a disease registry. Secondly, to investigate the utility of PRS testing to predict disease severity and likelihood to require surgical intervention. And thirdly, to assess the clinical validity of a PRS for glaucoma and AMD in the general Australian population.

To date, results from validity studies for glaucoma PRS testing have been positive, however there are several gaps in knowledge which I aimed to address in this part of the thesis. A glaucoma PRS has not yet been prospectively applied to a general population. With the ability to identify those at highest risk of disease, as well as estimating disease severity and treatment response, there is potential to offer personalised care for glaucoma patients as well as change in disease screening and treatment. Previous PRS studies have so far been retrospective. Here we present a prospective population-based study which will assess the prevalence of glaucoma across its relative PRS spectrum. This will be the first study to assess the clinical validity of a PRS for glaucoma for clinical implementation in a real world setting.

The results from the studies included in this chapter solidify the clinical utility of glaucoma PRS testing for use in guiding treatment escalation, as well as in stratifying risk amongst those at clinically increased risk, for example first-degree relatives, as well as those at unknown clinical risk.

CHAPTER 6: HIGH POLYGENIC RISK IS ASSOCIATED WITH EARLIER TRABECULECTOMY IN PATIENTS WITH PRIMARY OPEN-ANGLE GLAUCOMA

PUBLISHED MANUSCRIPT

The contents of this chapter have been published in a peer-reviewed manuscript of which I am a co-first author:

Marshall HN*, Hollitt GL*, Wilckens K, Mullany S, Kuruvilla S, Souzeau E, Landers J, Han X, MacGregor S, Craig JE, Siggs OM. High polygenic risk is associated with earlier trabeculectomy in primary open-angle glaucoma. Ophthalmol Glaucoma. 2022 Jul 13:S2589-4196(22)00119-3. doi: 10.1016/j.ogla.2022.06.009. Epub ahead of print. PMID: 35842105.

I contributed to the study concept and design (25%), data collection (10%), data analysis and interpretation (30%), and drafting the manuscript (50%). I contributed equally to Henry Marshall. Henry Marshall contributed to the study concept and design (25%), data collection (10%), data analysis and interpretation (30%), and drafting the manuscript (50%). Kristopher Wilckens, Sean Mullany, Shilpa Kuruvilla, John Landers, Xikun Han, Stuart MacGregor, Jamie Craig and Owen Siggs contributed equally to data collection (80%). Kristopher Wilckens and Shilpa Kuruvilla contributed to the study conception and design (10%). Jamie Craig and Owen Siggs contributed to the study conception and design (40%), data analysis and interpretation (20%), and critically revising the contents of the manuscript. Emmanuelle Souzeau, Xikun Han and Stuart MacGregor contributed to data analysis and interpretation (20%). Jamie Craig contributed to project funding.

6.1 INTRODUCTION AND METHODS

As mentioned previously, POAG is a highly heritable progressive optic neuropathy and[108,110] a higher glaucoma PRS has been associated with a greater risk of glaucoma diagnosis, an earlier age of diagnosis, and a greater need for surgery.[108,128]

Trabeculectomy is a therapeutic incisional procedure that aims to lower IOP, and is usually considered only in advanced cases which are refractory to topical medical or laser treatments.[264] Predicting which patients may require this procedure remains a clinical challenge. This study aimed to assess if genetic risk scoring aids predicting which patients will need earlier surgery.

The ocular surgical history was reviewed for all participants of the ANZRAG[144] with POAG, recruited at clinics within the state of South Australia with self-reported European ethnicity. Participants with secondary forms of glaucoma (e.g. pseudoexfoliation glaucoma), or a documented Mendelian form of POAG, were excluded. Age at trabeculectomy, and laterality were recorded. The following covariates were also recorded: age at glaucoma diagnosis, self-reported sex, highest recorded IOP, and family history of glaucoma. Research was approved by local human research ethics committees (2021/HRE00032), and all research adhered to the tenets of the Declaration of Helsinki.

A glaucoma PRS was calculated for each individual using a previously-described multi-trait glaucoma PRS.[108] Genotyping was performed on DNA extracted from a peripheral blood sample using Illumina Omni1M, OmniExpress or HumanCoreExome arrays (Illumina, San Diego, CA). PRSs were calculated using PLINK (version 1.90 beta) and normalised as z-scores using 17,642 normative individuals from the QSkin Sun and Health Study (QSkin).[265]

Multivariate linear regression analyses assessed the correlation between glaucoma PRS and age at trabeculectomy. Covariates included: self-reported sex, and family history of glaucoma. IOP was not included in the model due to correlation between PRS and IOP.[1,2] Based on their PRS values, participants were stratified into the top decile, bottom decile and intermediate group (10th-89th percentile). Stratifications were performed using internal normalisation due to the skewed distribution of this dataset. Secondary analysis correlated glaucoma PRS with length of time from diagnosis to trabeculectomy. Covariates included age at diagnosis, self-reported sex, and family history of glaucoma. The p-value for statistical significance was set at 0.05.

6.2 RESULTS

Surgical data for 903 genotyped participants with POAG was reviewed, from which 187 had undergone at least one trabeculectomy with a recorded date of surgery. The mean age at glaucoma diagnosis was 64.1±9.91 years, 57.3% were female, and the mean highest recorded IOP was 28.0±8.66mmHg (Table 6.1).

Participants in the top decile were diagnosed with glaucoma at a younger age (mean difference: 5.19 years 95% CI [3.03, 7.36] P<0.001 Table 6.1) and had a highest recorded IOP than participants in the bottom decile, although this did not reach statistical significance (P=0.052; Table 6.1)

Characteristics	Whole Cohort (n = 187)	Top Decile (n = 19)	Intermediate Group (n = 149)	Bottom Decile (n = 19)	P-value
Age at diagnosis (years)	62.64±11.41	59.28±10.79	62.76±11.43	64.48±11.16	**P<0.001**
Bilateral Trabeculectomy (%)	39.0	52.6	40.2	15.7	**P=0.021**
Self-reported sex (% Female)	57.3	54.8	57.8	66.7	P=0.872
Glaucoma Family History (% True)	62.0	74.4	64.3	57.3	**P<0.001**
Highest Recorded IOP (mmHg)	25.39±9.33	26.93±8.26	25.16±8.46	25.29±8.52	P=0.052

Table 6.1: Summary of study cohort. Summary demographic and clinical characteristics of the study population, with further stratification into top decile, intermediate group (10th-89th percentile) and bottom decile. P-values are derived from univariate regression analyses between top and bottom deciles. The P-value threshold for statistical significance (bold values) was 0.05. IOP: Intraocular Pressure; PRS: Polygenic Risk Score; IQR: Interquartile range.

Linear regression correlated a higher PRS with a younger age at first trabeculectomy (beta: -1.94 years/SD 95% CI: [-0.41, -3.47] P=0.014). Participants in the top decile underwent their first trabeculectomy approximately 7 years earlier than participants in the lowest decile (mean difference: -7.04 years [2.82, 11.26] multivariate P=0.002; Figure 6.1 Panel A).

A secondary multivariate analysis assessed the correlation between glaucoma PRS and time

from diagnosis to first trabeculectomy. Participants in the top decile underwent trabeculectomy 5.8 years earlier than participants in the bottom decile (Beta: -5.84 years/SD [-1.14, -10.55] P=0.022). This association persisted after inclusion of highest IOP in the model (Beta: 5.85 years 95% CI: [0.96 10.73] P=0.024). Time from diagnosis to trabeculectomy was not associated with glaucoma PRS in a univariate analysis (P=0.143)

Finally, participants in the top decile were observed to be 1.41 fold more likely to require bilateral trabeculectomy than participants in the bottom decile (OR: 1.41 [1.06, 1.91] P=0.021 Figure 6.1 Panel B).

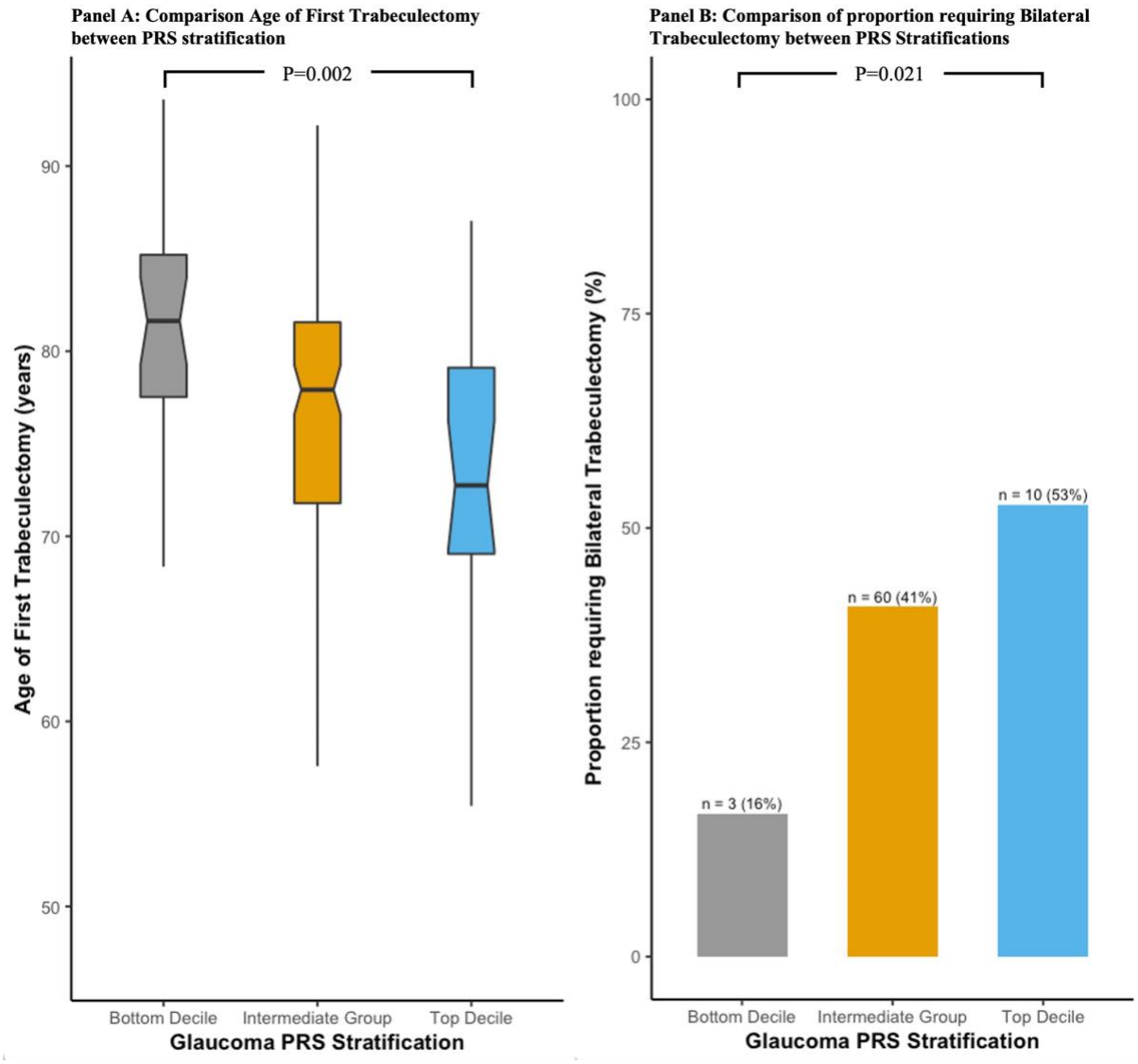

Figure 6.1: Comparison of Glaucoma PRS with age of trabeculectomy and with need for bilateral trabeculectomy. Panel A: Comparison Age of First Trabeculectomy between PRS stratification; **Panel B:** Comparison of proportion requiring Bilateral Trabeculectomy between PRS Stratifications. **Grey box:** Bottom decile; **Orange box:** Intermediate group (10th to 98th percentile); **Blue box:** top decile. Participants in the top decile underwent their first trabeculectomy approximately 7 years earlier than the bottom decile (mean age at first trabeculectomy: 73.45±7.82 years versus 80.9±6.44 years; multivariate P=0.002). Participants in the top decile were also 1.41 fold more likely to require bilateral trabeculectomy following multivariate analysis (OR: 1.41 95% CI [1.06, 1.92] P=0.021; Panel B).

6.3 DISCUSSION

This report correlated a higher glaucoma PRS with a younger age at first trabeculectomy, a shorter duration between diagnosis and first trabeculectomy, and greater need for bilateral trabeculectomy in POAG.

Our findings extend previous studies linking glaucoma PRS with glaucoma treatment outcomes.[108] For those with disease that may ultimately require surgery, this could mean that trabeculectomy is considered earlier in higher-risk individuals, potentially avoiding vision loss resulting from failed trials of more conservative options. It may also help prevent unnecessary surgery, or delay surgery, in those who are deemed to be low risk. The results of this work, combined with the observation that a majority of individuals with POAG are interested in PRS testing,[168] highlights the potential utility of genomic risk stratification in this disease.

There are several limitations to our study design. It only included participants who had undergone at least one trabeculectomy, and did not include individuals who had not undergone this procedure. The absence of a univariate association between time to trabeculectomy and glaucoma PRS is potentially of reflection of survivorship bias, where patients diagnosed later in life are more likely to have a lower glaucoma PRS, and possibly also less likely to survive long enough, or be fit enough, to require surgery. Furthermore, the small number in both the bottom and top decile groups may prevent extrapolation of the findings. The exclusion of participants of non-European ethnicity limits the application of these findings to other populations. The focus on trabeculectomies as a treatment intervention means the association between PRS and other incisional procedures (i.e. glaucoma drainage devices), or the timing of initiation or escalation of other interventions, remains unknown. Since these treatment decisions have significant quality of life and health economic implications, there is a clear need for further investigation in this area.

CHAPTER 7: A GLAUCOMA POLYGENIC RISK SCORE IS STRONGLY ASSOCIATED WITH GLAUCOMATOUS FAMILY HISTORY, AND DISEASE SEVERITY AMONGST AFFECTED SIBLINGS

7.1 INTRODUCTION

Genetic risk of glaucoma is conventionally estimated using family history alone. In a study of the familial aggregation of POAG in a general population, first-degree relatives of individuals with glaucoma had a 9.2-fold increased risk of developing glaucoma, highlighting the critical role of genetic risk in glaucoma development.[41] However, collection of this information can be imprecise as many are unaware of their family members medical history, may have inexact knowledge of their relatives' vision related condition, and are subject to recall and survival biases.[266] These limitations are addressed by the PRS as an objective quantitative risk-stratification tool. Nonetheless, previous studies for other traits have shown only partial overlap between a positive family history and PRS, which suggests a complex and complementary relationship between them [267–270]. Furthermore, family history captures the effect of shared environmental factors, and very rare variants which are not part of the PRS, so will remain an important aspect of individual risk estimation.

We have previously reported an association between glaucoma PRS and family history [108]. In this study, we sought to investigate the interplay between a positive family history and PRS, and how PRS may vary amongst family members. In particular, we investigated the relative distribution of PRS amongst family history status and the degree of relatedness. In affected relatives, we investigated the relation between PRS and key glaucoma severity parameters: highest IOP, age of diagnosis and whether they have had incisional glaucoma surgeries.

7.2 METHODS

7.2.1 Patient cohort

Clinical and documented family history data of individuals with glaucoma in the ANZRAG was used. In brief, participants with glaucoma were recruited from outpatient clinics, with clinical and demographic information collected at the time of recruitment. Family history of glaucoma was specifically documented by questioning on the self-reported details of glaucoma in the family up to the fourth degree by the referring specialist, and reviewed with the participant by members of the ANZRAG team at the time of recruitment. Every effort was made to accurately record this

data, including examining relatives when possible, and follow-up mail-based questionnaires. Affected close relatives were also invited to be recruited to the registry.

In this study, only participants with POAG were included. Those with established diagnosis of 'monogenic' variants associated with glaucoma (such as those carrying pathogenic *MYOC* or *OPTN* variants) were excluded, as to not skew the family history data. Of note, the majority of ANZRAG participants have been previously screened for known 'monogenic' variants.[100,271] Only individuals who self-reported as European ethnicity were included, to maximise the applicability of the PRS, which is derived from European ancestry populations. In families with more than one affected member in the registry, only the proband was included in the primary analysis.

Further analysis of variation of PRS amongst affected relatives was performed, where all participants affected by glaucoma were included. Unaffected relatives have not been genotyped within ANZRAG, and thus PRS data of unaffected relatives was not available for comparative analysis. The relatedness of each pair of participants was recorded based on the available family history information. Additional glaucoma phenotypes were used for analysis, obtained from the registry, including age of glaucoma diagnosis, highest recorded (pre-treatment, where possible) IOP, HVF mean deviation, clinician-graded VCDR, and previous incisional glaucoma surgeries.[144] This study was approved by the SAC HREC (2021/HRE00032) and adhered to the Revised Declaration of Helsinki. Written informed consent was obtained from all participants.

7.2.2 Genotyping and Imputation

Genotyping was performed on DNA extracted from a peripheral blood sample using HumanCoreExome arrays (Illumina, San Diego, CA, USA). A glaucoma PRS was calculated on each individual using a multi-trait glaucoma PRS, the derivation of which is described elsewhere.[108] Briefly, the PRS was calculated for each individual using a weighted allele-sum approach, based on the summary statistics of large genome-wide association studies of glaucoma, intraocular pressure (IOP) and vertical cup-to-disc ratio. These genome-wide association studies were based on primarily European ancestry individuals from the UK BioBank and International Glaucoma Genetics Consortium. PRS scores were then normalised as z-scores in reference to a normative population-cohort of 17,642 individuals aged 40-69 years (QSkin cohort).[265] For clinical translational purposes, the study cohort was stratified into

high risk (upper quintile), low risk (lower quintile), and intermediate risk (remaining sixty percent) based on the normative glaucoma PRS thresholds.

7.2.3 Statistical Analyses

Linear regression modelling was used in analyses of numeric PRS and family history, with adjustment for age at recruitment and gender. Poisson regression was used for the number of affected family members. Where pairwise comparisons were performed, P-values were adjusted using the Holm-Bonferroni method. Amongst related individuals, PRS correlation was calculated using the Pearson correlation coefficient. Analysis of clinical glaucoma parameters amongst related individuals was done using a nested mixed-effect model with a random intercept per family, then a random intercept for each sibling pair.[272] Additional fixed-effects 'covariates' of age and gender were included as applicable. This model statistically accounts for the relatedness amongst individuals, with the latter random intercept accounting for additional variance for families with more than 2 siblings affected.[272] A poisson model was used for the number of incisional surgeries per individual, to better model the count nature of this data. All analyses were performed in R (version 4.1.0). Mixed effect models were fitted using the *lme4* package (version 1.1.28) and statistical tests of significance were performed using the *lmerTest* package (version 3.1.3).

7.3 RESULTS

7.3.1 The correlation between positive family history and PRS

We identified 2,066 unrelated European ethnicity individuals with POAG for whom no 'monogenic' cause of glaucoma has been found. The mean age at recruitment was 72.3 (12.2) years, 45% were male, and 62.6% had a family history of glaucoma, with 52.4% having at least one first-degree relative with glaucoma. A detailed summary of the reported family history, stratified by PRS groups is reported in Table 7.1. Glaucoma PRS of participants enrolled in ANZRAG is skewed towards a higher PRS, due to the enrichment of glaucoma in this cohort.[128]

Polygenic risk groups	Low-risk	Intermediate-risk	High-risk
Number	128	1050	888
Age at recruitment (mean, SD)	75.0 (11.3)	73.1 (12.4)	71.1 (12.0)

Gender, male (%)	66 (51.6)	476 (45.3)	393 (44.3)
Family history of glaucoma (%)	68 (53.1)	625 (59.5)	601 (67.7)
Number of affected family members with glaucoma (%)			
None	60 (49.2)	425 (42.8)	287 (34.6)
1	31 (25.4)	275 (27.7)	237 (28.6)
2	19 (15.6)	129 (13.0)	122 (14.7)
3	6 (4.9)	83 (8.4)	85 (10.2)
4 or more	6 (4.9)	82 (8.2)	99 (11.9)
Closest relative affected with glaucoma (%)			
1st degree	48 (39.7)	491 (49.4)	480 (57.8)
2nd degree	11 (9.1)	62 (6.2)	51 (6.1)
3rd/4th degree	2 (1.7)	15 (1.5)	12 (1.4)

Table 7.1: Summary of the study participants stratified by glaucoma polygenic risk score.

Individuals with a first-degree relative with glaucoma had a higher PRS than those with no family history of glaucoma (P <.001, Figure 7.1A), and than those with a family history of a second degree or more distant relative (P = .025, Figure 7.1A). Additionally, there was an incremental increase in PRS with an increasing number of affected family members (P <.001, Figure 7.1B).

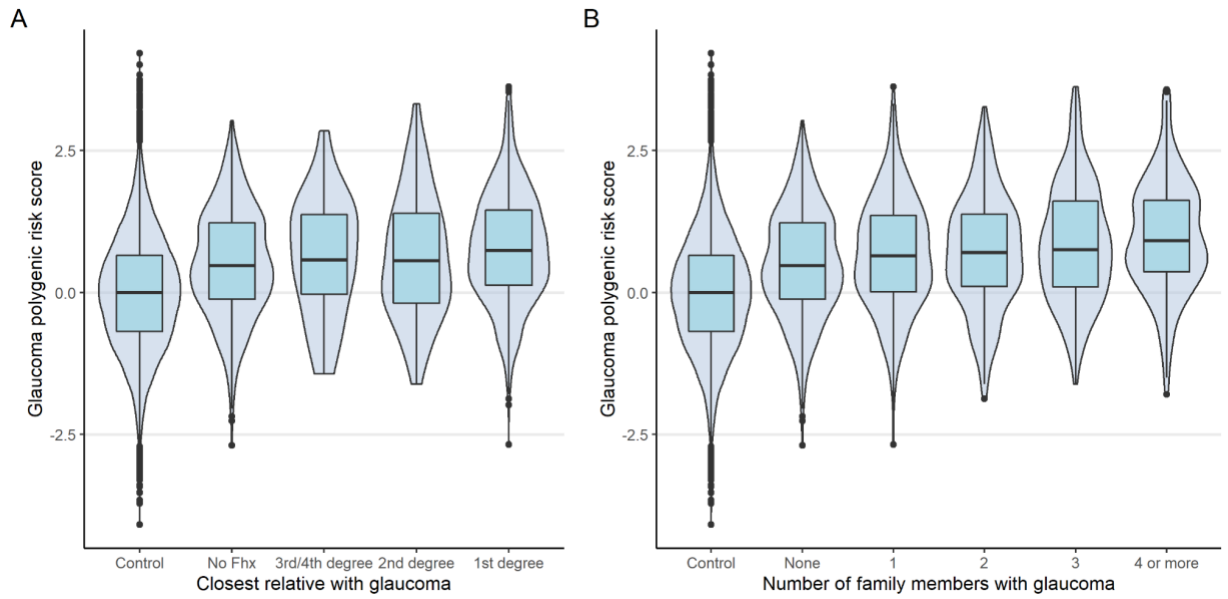

Figure 7.1: Distribution of glaucoma polygenic risk score based on the closest relative with glaucoma (A), or the number of family members with glaucoma (B) in the ANZRAG cohort, and relative to a control cohort. Fhx, family history.

There was an incomplete overlap between a high PRS (top quintile of PRS relative to normative population) and a positive family history of glaucoma. Fourteen percent of the cohort were identified as ‘unsuspecting cases’, defined as high-risk by the PRS but with no known family history of the disease (Figure 7.2A). This represents a subgroup that can be identified as a higher risk of glaucoma in the absence of a known glaucoma family history. Furthermore, the high-risk group reported more family members affected by glaucoma compared to the low-risk and intermediate-risk groups (P<.001, Figure 7.2B). For instance, 21% of the individuals in the high-risk PRS group had at least three family members affected with glaucoma, compared to 10% in the low-risk group (Figure 7.2B).

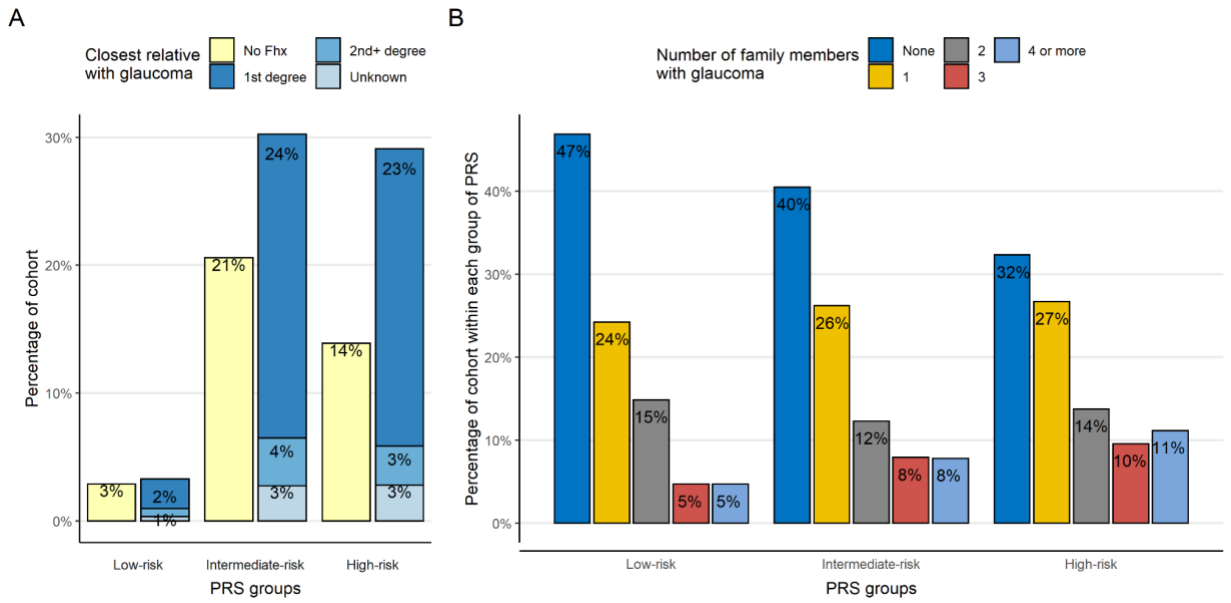

Figure 7.2: A. Overlap between PRS risk stratification groups and a positive self-reported family history of glaucoma, highlighting the utility of PRS in identifying high-risk individuals with a negative family history of glaucoma. B. Relative proportion of the affected number of family members with glaucoma within each glaucoma PRS risk group, highlighting a higher yield in screening families of high-risk PRS individuals.
Fhx, family history. 2nd+ degree, any of second, third, or fourth degree relatives.

7.3.2 Variation of PRS amongst relatives with glaucoma

Variation of PRS amongst the relatives affected with glaucoma was then investigated. 281 related individuals with glaucoma were identified, forming 199 unique relationship pairs, the majority of which were between siblings (96, 48%), and parents-children (62, 31%). PRS was most correlated amongst siblings (Pearson's correlation = 0.46, P<0.001), followed by parents-children (Pearson's correlation = 0.33, P=0.009; Figure 7.3A). For example, amongst sibling pairs where at least one sibling was identified as high-risk, PRS risk groups were concordant (i.e., both siblings were high-risk) in 45% of the cases. The imperfect PRS correlation between first-degree relatives is explained by the laws of allele inheritance during meiosis; siblings are expected to share 50% of their genetic variants since they have an equal chance of inheriting each allele. Thus, PRS can differ significantly amongst family members, including first-degree relatives.

The association between PRS variation amongst affected relatives and their clinical glaucoma parameters was evaluated. This analysis was limited to siblings only, to minimise the

confounding effect of age and follow-up time on the glaucoma phenotype. Using a nested mixed-model of affected siblings which takes into account the relatedness of family members, observing that siblings who were identified as high-risk (top quintile of the PRS) were diagnosed 4.1 years earlier (95% CI 0.16 – 8.05, P = 0.042 after adjusting for gender; Figure 7.3B) compared to the intermediate-risk siblings (middle 60% of the PRS). Additionally, high-risk siblings had a higher maximum-recorded IOP by 3.09 mmHg (95% CI 0.95 – 5.22, P = 0.005; Figure 7.3C), and were more likely to have incisional glaucoma surgeries (incident rate ratio 1.83, 95% CI 1.07 – 3.13, P = 0.027) compared to the intermediate-risk siblings, after adjusting for age and gender.

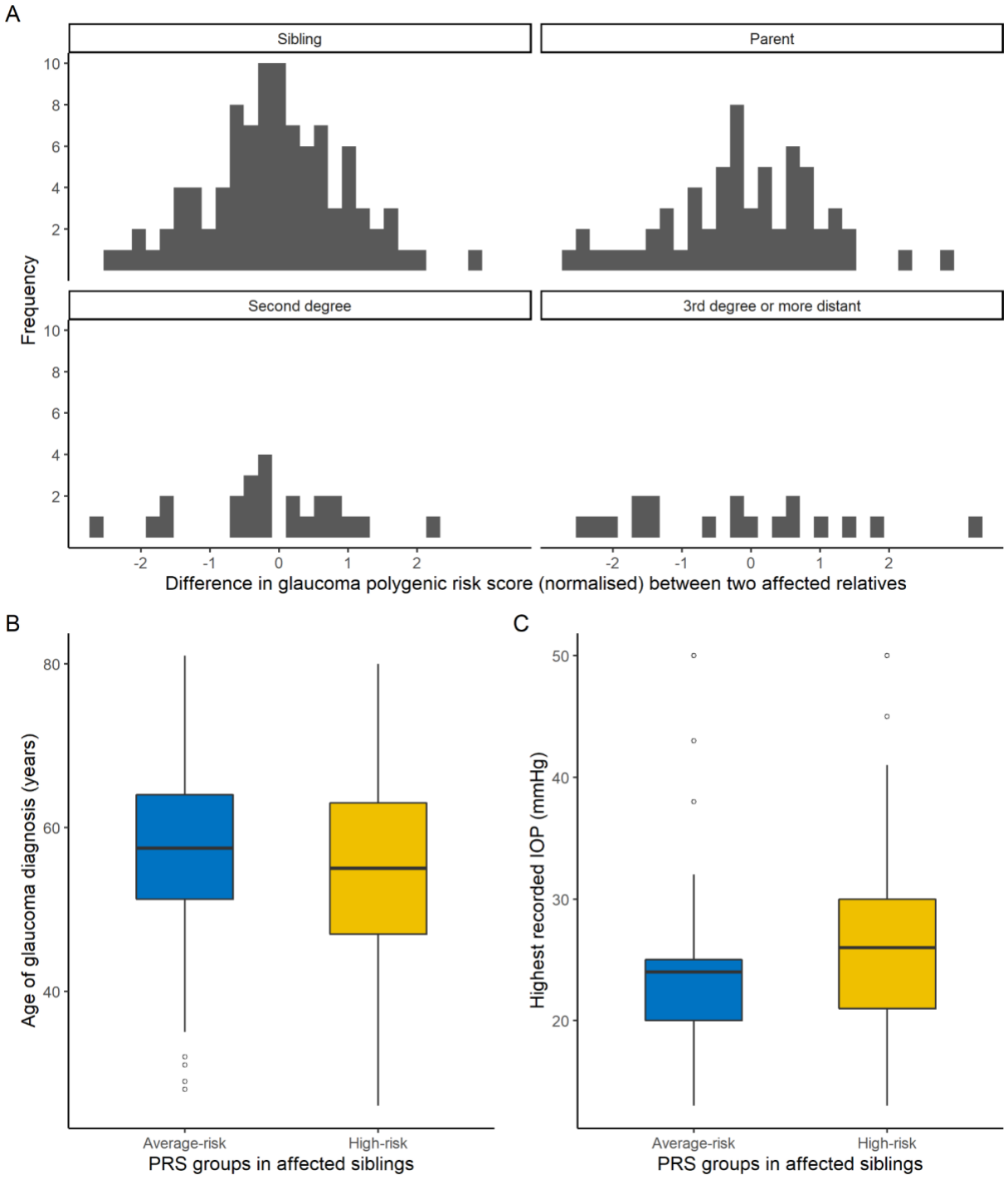

Figure 7.3: Variation of the PRS between relatives, grouped by the degree of relatedness (A). Since the PRS was normalised for analysis, each unit of PRS difference (x-axis) represents a difference of 1 standard deviation of the PRS between two relatives. The difference was calculated with reference to the proband case, where applicable. Differences in the age of glaucoma diagnosis (B) and highest recorded IOP (C) show a more severe glaucoma

phenotype in high-risk PRS siblings compared to their intermediate-risk group siblings (P<0.05 for both).
PRS, polygenic risk score. IOP, intraocular pressure.

7.3.3 Comparative clinical predictability of PRS and a positive family history

The relative performance of a high-risk PRS was examined, and a positive family history on predicting clinical outcomes related to glaucoma severity (Table 7.2). The combination of high-risk PRS and a positive family history appear to correlate with greater disease severity than either risk factor alone. For example, individuals with high-risk PRS and a positive family history had a 46% greater risk of having advanced visual field loss (MD <-15 db, P = 0.001), a risk higher than that observed in those with a high-risk PRS (25%, P = 0.02) or a positive family history (14%, P = 0.14). A similar trend was observed with the risk of incisional glaucoma surgeries, worse visual field MD, and a higher vertical cup-to-disc ratio.

The observed differences in the highest-recorded IOP and age of diagnosis were not step-wise. A high-risk PRS, but not a positive family history, was associated with a greater highest-recorded IOP. This is due to the PRS including all of the known IOP-associated genetic variants,[273] whereas such a correlation with self-reported family history would be less apparent. We also observed that the age of diagnosis is strongly influenced by a positive family history, an effect that may be confounded by ascertainment bias, and informal cascade screening.

Clinical outcome of the models	**Family history model** **Effect of a positive family history on the clinical outcome**	**PRS model** **Effect of high-risk PRS on the clinical outcome**	**Combined risk model** **Effect of the combination of a positive family history and a high-risk PRS on the clinical outcome**
Highest-recorded intraocular pressure (per 1 mmHg)	0.92 [-0.09, 1.92] P = 0.07	1.48 [0.51, 2.46] P = 0.003	1.27 [0.19, 2.36] P = 0.02

Age at diagnosis (per 1 year)	-4.46 [-5.44, -3.48] P < 0.001	-1.48 [-2.44, -0.51] P = 0.003	-3.33 [-4.39, -2.28] P < 0.001
Vertical cup-to-disc ratio	0 [-0.01, 0.02] P = 0.42	0.01 [0, 0.02] P = 0.21	0.01 [0, 0.02] P = 0.04
Mean deviation (per 1 dB)	-0.58 [-1.44, 0.28] P = 0.19	-0.81 [-1.64, 0.03] 0.06	-1.3 [-2.21, -0.39] P = 0.005
Advanced field loss, defined as mean deviation <-15 db (odds ratio)[a]	1.16 [0.95, 1.42] P = 0.14	1.25 [1.03, 1.52] P = 0.02	1.46 [1.17, 1.81] P = 0.001
Incisional surgery (odds ratio)[a]	1.40 [1.23, 1.60] P < 0.001	1.36 [1.2, 1.53] P < 0.001	1.47 [1.29, 1.67] P < 0.001

Table 7.2: Three models examining the relative and combined performances of a positive family history and high-risk PRS in glaucoma clinical variables.
All three models are multivariable regression models adjusting for age and gender. The reference group for "a positive family history" is all individuals with no known family history of glaucoma. The reference group for "high-risk PRS" is all those not in the high-risk (top quintile) group. Linear regression models were used for all clinical variables except those labelled with (a), such as that the effect columns reflect the quantitative difference in the clinical outcome between the risk and reference groups. A binomial general linear regression model was used for binary clinical outcomes (a), such that the effect columns reflect the odds ratio of the risk group to the clinical outcome.
PRS: polygenic risk score

7.4 DISCUSSION

Glaucoma is a highly heritable condition, with recognised mendelian and complex inheritance patterns. Traditionally, family history has been used to estimate genetic risk for glaucoma. However, this can be unreliable as many are unaware of the health history of their family members, or have an erroneous view as to the cause of vision loss in a family member. Here, we demonstrate the utility of a glaucoma PRS to capture those at high risk among both those with and those without a known family history of glaucoma.

The NHMRC guidelines in Australia currently recommends screening with a clinical examination for first-degree relatives of patients with glaucoma, commencing 5-10 years earlier than the age of glaucoma onset in their affected relative.[80] Additionally, screening from the age of 40 years is recommended in people of African ancestry, compared to from 50 years of age in people of European ancestry. However, the guidelines lack sensitivity and specificity, and are mainly relevant to those with a family history of glaucoma. PRS testing for glaucoma may be useful for those who are unsuspecting cases with high PRS but no known family history of glaucoma. This includes those who may be unaware of family members having glaucoma, including in circumstances of adoption or estrangement. These individuals are less likely to be identified early by current screening guidelines given that screening at an earlier age is only recommended when a positive family history is recognised by the individual. In our cohort, 14% of those with established glaucoma who would have been identified by PRS as "high risk" did not have any (known) family history of glaucoma. This group of individuals who are at a higher-risk of more severe glaucoma [108] are currently not captured by screening based on family history. Furthermore, the positive correlation between PRS and the number of affected family members raises the possibility of identifying additional (undiagnosed) relatives at risk of the disease in the high-risk group. A screening strategy incorporating PRS may have a higher yield of identifying at-risk individuals, than a strategy based on family history alone.

In this study, we demonstrated that due to the inheritance laws of complex traits, PRS can vary greatly amongst relatives, including first-degree relatives. Mars et al recently reported the concordance of high PRS amongst 1st degree relatives to be about 33.7% across 24 diseases including glaucoma, and only about 20% in second degree relatives.[270] Thus, PRS is imperfectly concordant amongst relatives, and an individual identified as 'average risk' (in the middle 60% band of a normative population) may have a sibling with a 'high risk' PRS. Whilst this is an expected finding under models of quantitative genetics, this finding has clinical implications for glaucoma care.[153] The advantage of the quantitative risk-prediction ability of the PRS is the added granularity relative to the binary approach used in family history. We reported that even amongst siblings who have already been diagnosed with glaucoma, those with a high PRS tended to have more severe disease, and were more likely to have incisional glaucoma surgery to control their disease. This raises the utility of PRS in informing the individual of additional disease severity risk than that captured by a positive family history alone.

Like POAG, family history is used as an indirect measure of genetic risk for other common conditions such as cardiovascular disease and type 2 diabetes. While risk stratification using PRS continues to evolve in these areas, few studies have assessed the overlap of risk captured by PRS compared to family history.[270] This was explored for 24 common conditions within the FinnGen registry, including 306,418 participants. In this study, we demonstrated that there was an incomplete overlap between high POAG PRS and positive family history.[270] Similarly, this study showed that family history and PRS were independent measures, explaining on average 10% of the effect of first-degree relatives.[270] This supports PRS as a complementary tool to estimate risk when combined with other risk factors.

Additional utility of the PRS includes severity and progression risk-stratification. We have previously reported that a higher glaucoma PRS correlates with glaucoma severity.[108,273] This is possible due to the quantitative probabilistic nature of the PRS, whereby individuals at the highest risk (e.g. those at the top 10%, or even 1%) could be more specifically targeted by clinicians or screening programs.[130] It should be noted however that a positive or a strong family history of disease captures genetic variants that may not be part of the PRS, such unidentified intermediate frequency or rare variants that are particularly relevant to some individuals irregardless of their PRS.[42,47] Recent literature in other diseases supports the integration of PRS and family history in improving risk prediction, highlighting the potential complementary role of these factors in future clinical practice.[270,274,275] In our study, we identified that the combination of high PRS and a positive family history, conferred a greater risk of glaucoma disease severity than either risk factor alone, emphasising the additive nature of these factors in predicting disease severity.

Strengths of our study included using a comprehensive glaucoma PRS derived from the largest datasets available to date, and has been previously reported to strongly correlate with glaucoma risk.[108]We used a well-characterised and large dataset of glaucoma cases enrolled in ANZRAG, and have excluded secondary glaucoma or those with known monogenic variants as a cause of their glaucoma to avoid skewing the results. Importantly, the PRS was calculated from samples independent of our study cohort. The family history data in ANZRAG has been finely curated, which whilst a strength to our analyses, may ‘overestimate’ the number of family members affected compared to routine practice.[266] This compounds the limitation that family history data was self-reported. Another limitation is that the PRS performs best in ancestries matching that from which it was derived. Future genome-wide association studies will need to examine cross-ancestry associations to broaden PRS applicability. Thus, our findings may not be immediately

translatable to other ancestries, despite some evidence that this PRS has some predictive ability in other ancestries.[108] The effect of PRS amongst siblings was partly limited as our study cohort was of individuals with diagnosed glaucoma, thus it is likely that the PRS correlation will be stronger than if unaffected family members were available; this however, does not impact the analyses stratifying glaucoma severity amongst affected siblings. Further research is needed to explore the influence of PRS in unaffected (or unknown glaucoma status) family members. Additionally, a direct comparison between the predictive ability (for screening or glaucoma risk stratification) of a positive family history compared to high PRS was beyond the scope of this work, but is highly relevant in furthering our understanding of translating glaucoma PRS to clinical practice.

Glaucoma PRS is positively associated with a greater number and closer relatedness of family members affected by glaucoma, whilst correlating with a more severe glaucoma phenotype even amongst affected siblings. The quantitative, probabilistic, and objective nature of the PRS supports its use to guide glaucoma screening guidelines, as a complementary tool to family history.

CHAPTER 8: GENETIC RISK ASSESSMENT OF DEGENERATIVE EYE DISEASE (GRADE): STUDY PROTOCOL OF A PROSPECTIVE ASSESSMENT OF POLYGENIC RISK SCORES TO PREDICT DIAGNOSIS OF GLAUCOMA AND AGE-RELATED MACULAR DEGENERATION

PUBLISHED MANUSCRIPT

The contents of this chapter have been published in a peer-reviewed manuscript of which I am a the first author:

Hollitt GL, Qassim A, Thomson D, Schmidt JM, Nguyen TT, Landers J, MacGregor S, Siggs OM, Souzeau E, Craig JE. Genetic Risk Assessment of Degenerative Eye Disease (GRADE): study protocol of a prospective assessment of polygenic risk scores to predict diagnosis of glaucoma and age-related macular degeneration. *BMC Ophthalmol*. 2023 Oct 24;23(1):431.

I contributed to the study concept and design (12.5%), data collection including participant recruitment (100%), data analysis and interpretation (50%), and drafting the manuscript (100%). Ayub Qassim, Thi Thi Nguyen, John Landers, Stuart MacGregor, Owen Siggs, Emmanuelle Souzeau and Jamie Craig contributed equally to the study conception and design (87.5%), and critically revising the manuscript. Daniel Thomson, Joshua Schmidt and Stuart MacGregor developed the data analysis tools (PRS pipeline) and contributed to data analysis and interpretation (50%). Daniel Thomson prepared Figures 8.1 and 8.2.

8.1 INTRODUCTION

Glaucoma and age-related macular degeneration (AMD) are the two most common causes of irreversible vision loss among elderly people worldwide.[5,276] With the ageing population, these diseases will pose an increasingly significant burden. Furthermore, sight is generally considered to be the most valued sense by the general public, so identifying cost-effective screening methods to facilitate early diagnosis, prevention, and timely intervention is important.[13] In Australia, vision impairment results in significant direct and indirect health care costs, ranking as the seventh most costly health condition.[14] It is important to also consider the impact of vision loss on an individual, which can result in poorer wellbeing outcomes through the impact on quality of life, lost income, and personal healthcare costs.[14]

Similar to glaucoma, AMD is a common eye condition, with a reported prevalence of 13% in those aged over 85 years[276] and is predicted to affect 288 million people by 2040.[277] It is a progressive condition that causes degeneration of the macula, leading to central vision loss. AMD is asymptomatic in its early stages, with variable progression to visually significant advanced disease depending on clinical and environmental factors.[278] Recognised risk factors for AMD include increasing age, smoking and genetic predisposition.[276] Advanced AMD is classified as either non-neovascular (dry AMD) or neovascular (wet AMD) based on the presence or absence of choroidal neovascularisation. Currently, dry AMD management relies on lifestyle modifications such as smoking cessation and dietary supplementation,[279] while wet AMD is treated with intravitreal injections of vascular endothelial growth factor (VEGF) inhibitors, a key modulator of neovascularisation.[280] Importantly, treatment with VEGF inhibition must be implemented in a timely fashion from the onset of exudative disease. Although some environmental risk factors are well recognised, research indicates there is a strong genetic basis for AMD.[276] Genetic factors may explain variance in disease severity, with heritability estimated at 45-70%.[281]

Screening for glaucoma and AMD is largely opportunistic, and broad community screening has not been demonstrated to be cost-effective.[80,81] For this reason, identifying cost-effective screening methods to facilitate early diagnosis and timely intervention is important. The NHMRC guidelines in Australia currently recommends screening with a clinical examination for first-degree relatives of patients with glaucoma, commencing 5-10 years earlier than the age of glaucoma onset in their affected relative. Additionally, screening from the age of 40 years is recommended in people of African ancestry, compared to from 50 years of age in people of European ancestry.[80] There are no similar recommendations for AMD.

PRS are an emerging clinical tool which offer a unique opportunity to improve disease risk prediction for complex heterogeneous diseases, such as glaucoma and AMD.[282] A glaucoma PRS has been effective in stratifying risk within the general population, as well as predicting structural progression and the likelihood of requiring surgical intervention in those with already diagnosed glaucoma.[108] Similarly, an AMD PRS using 52 variants showed a 44-fold increased risk of developing AMD for those in the top decile compared to the bottom decile.[283] Furthermore, this PRS was associated with more rapid disease progression.[284,285] The discovery of genetic associations has also helped to reveal underlying pathophysiologic mechanisms of AMD, exposing potential new treatment targets.[286]

With the ability to identify those at highest risk of disease, as well as estimating disease severity and treatment response, there is potential to offer personalised care for glaucoma and AMD patients. This predictive approach could facilitate an exciting change in disease screening and treatment, and ultimately lead to a reduction in vision loss caused by these common conditions. Throughout the earlier chapters of this thesis, we have identified perspectives and aspects of PRS testing which may affect the behaviour of those involved in the testing process. Strong evidence supporting the clinical utility of a glaucoma PRS is an integral next step in validating this testing. The GRADE study aims to address this gap and provide the necessary evidence within a prospective cohort. Here, we present a prospective population-based study which will assess the prevalence of both glaucoma and AMD across their relative PRS spectra. This will be the first study to assess the clinical validity of a PRS for glaucoma and AMD for clinical implementation in a real world setting.

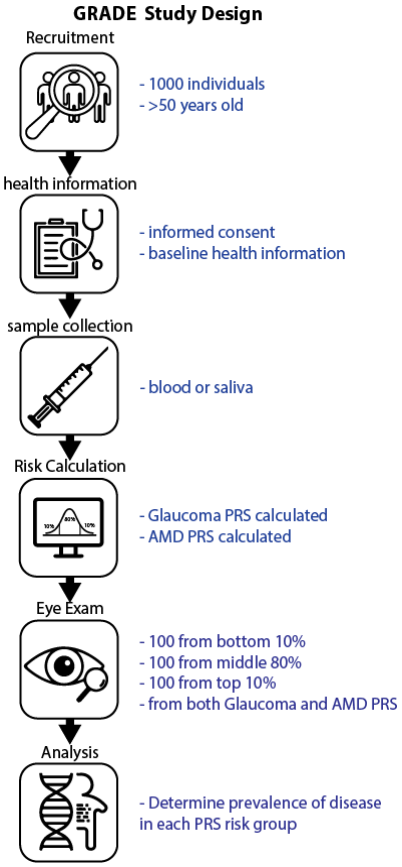

Figure 8.1: Study design

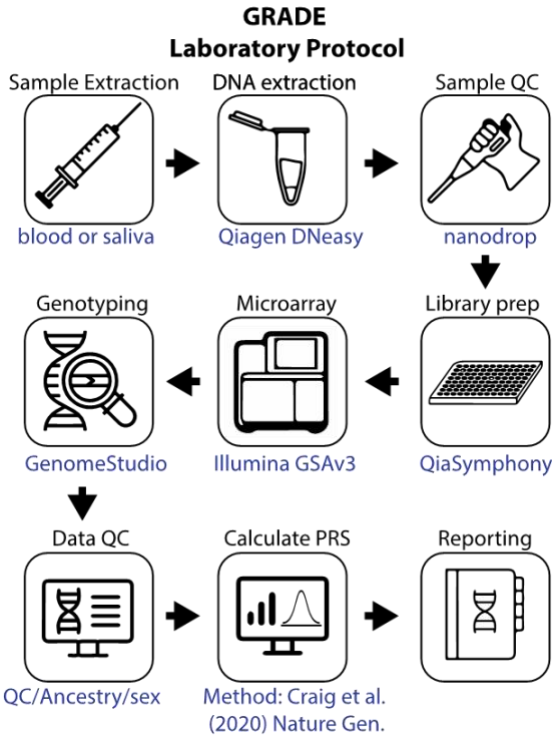

Figure 8.2: Flowchart of PRS calculation framework

8.2 METHODS

8.2.1 Study Design

This prospective cohort study was approved by the SAC HREC (2020/HRE00968) and adheres to the Revised Declaration of Helsinki. The study design is summarised in Figure 8.1. The research is being conducted at the Department of Ophthalmology at Flinders University, and the QIMR Berghofer Medical Research Institute under separate ethics approvals.

8.2.2 Study objectives and hypotheses

The study will apply PRS testing in 1000 individuals over the age of 50 years from the general population, and then examine a subset of individuals across the PRS spectrum with the aim of ascertaining all cases of glaucoma and AMD. We will prospectively assess the clinical validity of a PRS in stratifying high and low risk individuals, and hypothesise that there will be a higher prevalence of glaucoma and AMD in the high risk PRS groups compared to the middle and low risk groups.

8.2.3 Participants

Participant recruitment methods are compliant with the Health Care Act 2008. A minimum of 1000 individuals over the age of 50 years will be invited to participate. Glaucoma and AMD prevalence increases with age, with prevalence rates commonly reported from 50 years of age.[5,276,277] Consequently, identifying early or established disease in individuals across the risk spectrum will be easier for individuals within this age range. Exclusion criteria include age under 50 years, or an inability to provide written informed consent. Individuals already diagnosed with glaucoma and/or AMD will not be excluded, nor will they be targeted. Recruitment will be unselected to include individuals of any ethnicity.

Potential participants will be identified using several approaches. All eligible individuals who participated in a questionnaire-based study of individuals without glaucoma assessing attitudes towards polygenic risk testing for glaucoma will be invited to participate in this study.[168] A flyer advertising the project will be displayed in public and private outpatient clinics, and sporting venues and community clubs, provided to social/community organisations and distributed via email to these groups. Presentations about degenerative eye disease will be given to community organisations to promote interest and stimulate recruitment from the general population. Individuals in outpatient clinics will be approached in person and invited to participate if the inclusion criteria are met. Demographic and health information recorded for each participant will include past medical, ocular and medication history. Individuals with a personal or family history

of glaucoma or AMD may be more likely to respond to advertisements, however selection bias will largely be mitigated by wide and non-selective recruitment from all other avenues.

8.2.4 Participation requirements

Participation requires individuals to provide a blood sample (2 x 9ml EDTA tubes) or a saliva sample (Oragene OG-500 collection tube, DNA Genotek, Ottawa, Ontario, Canada). A subset of participants will be invited to undergo a detailed eye examination for glaucoma and/or AMD. Eye examinations will be performed on 100 individuals each in the bottom 10%, top 10%, and middle 80% of the PRS distributions for glaucoma and AMD. Individuals undergoing eye examinations will be randomly selected within their respective PRS grouping. In total 300 participants will be examined for each disease, with a maximum of 600 participants being examined. In practice, some participants will be selected to be examined for both their glaucoma and AMD PRS results, so the number of participants undergoing eye examinations will be less than 600.

8.2.5 Genetic studies

The laboratory protocol is summarised in Figure 8.2. Genomic DNA will be extracted using column-based DNA purification protocols (Qiagen DNeasy) from either blood or saliva samples. Both blood and saliva will be considered viable alternatives for DNA extraction. De-identified samples of extracted DNA will be provided to a genotyping provider for array-based genotyping. Samples will be genotyped on Illumina GSA v3 arrays, with genotype imputation performed locally with Minimac3 using the 1000 Genomes data as a reference panel. Imputation and derivation of glaucoma PRS values will be performed in the laboratory of S.M. using the multitrait analysis of GWAS (MTAG) glaucoma PRS described in detail elsewhere.[108] All individuals will have their PRS percentile determined from the relevant 1000 Genomes population,[287] with individual ancestry based on estimates from principal components derived from the genome-wide genetic data. Depending on the distribution of ancestries within the cohort, a sub-analysis may then be performed comparing outcomes between European and non-European groups. Imputation and derivation of AMD PRS values will also be performed by S.M. using a MTAG AMD PRS described in detail elsewhere.[283,288]

8.2.6 Eye examinations

Clinical eye examinations will be performed on 100 individuals from each of the bottom decile, top decile, and the middle 80% of the PRS distributions for glaucoma and AMD. Individuals will be selected using random sampling methods. Examinations will include best-corrected visual

acuity, IOP (as measured by Goldmann applanation tonometry), corneal pachymetry, 24-2 Humphrey automated perimetry, spectral domain OCT of the optic disc and macula, fundus autofluorescence, anterior segment OCT, stereo-disc and fundus photography.[74] All clinical investigation results will be reviewed by independent clinicians who will determine their glaucoma or AMD classification by consensus. Examiners and clinicians reviewing results will be blinded to individuals' PRS results. Glaucoma diagnostic classification will follow previous definitions used in the PROGRESSA study.[144] Each eye will be classified as either normal examination, glaucoma suspect, open-angle glaucoma or non-open-angle glaucoma (e.g. primary closed angle glaucoma). For AMD, each eye will be classified as either no AMD or normal ageing changes, early AMD, intermediate AMD, or late AMD.

8.2.7 Sample size and power calculations

Using data from the UKB (age at ICD-10 or self-reported glaucoma diagnosis), we estimate that ~3% of individuals will have a glaucoma diagnosis by the age of 64 years (Figure 3D in reference [108]). Assuming an equal representation of subjects across all age groups, and assuming that 50% of glaucoma is undiagnosed in the community,[32] we expect ~10% of individuals in the top decile will have glaucoma, compared to ~3% in the bottom decile. The proportion of glaucoma suspects is expected to be more than 2 times the glaucoma cases based on the same preliminary analyses.[32] Based on the combined estimated incidence of glaucoma plus glaucoma suspect cases in each group (i.e. 30% in the top decile vs 9% in the bottom decile), the current sample size will yield >95% power (α=0.05) to detect a significant difference between the top and bottom deciles of the PRS distribution (logistic regression of glaucoma status on PRS decile).

Similar analyses for AMD suggest a disease prevalence of 0.7% in the bottom decile, and 22.7% in the top decile,[283] within a general population above 75 years of age and a disease prevalence of 5%. Australian epidemiological studies have estimated an AMD population prevalence of 14.3% in individuals aged 49 years and over,[289] so we expect to be sufficiently powered to detect a significant difference between the top and bottom PRS deciles at >80% power (α=0.05). Based on the same published analyses,[283] we are also sufficiently powered to detect a difference between the top PRS decile (AMD prevalence of 22.7%) and the bottom PRS decile (AMD

prevalence of 0.7%). While these analyses were used for the purpose of a power calculation, we acknowledge that the study population may be younger.

8.2.8 Statistical analyses

For all cases, family history of glaucoma and AMD, gender, and ethnicity will be self-reported. Genetic ancestry and biological sex will also be determined from genotyping array data. Statistical analyses will be performed in *R* (RCore Team, Austria). Missing information will be treated as missing data in analyses. For association analysis, logistic or linear regression will be used, including covariates to account for confounding variables as clinically and statistically appropriate. Appropriate regressions will be performed to investigate the rate of each glaucoma or AMD classification across the risk spectrum of the PRS, and to identify any additional factors which were associated with these outcomes. An individual will be defined as a glaucoma or AMD case regardless of whether one or both eyes meet diagnostic criteria.

8.2.9 Study outcomes

The primary outcome will be assessing the prevalence of glaucoma and AMD between the bottom decile, middle 80% and top decile of both respective PRS spectra. The clinical sensitivity and specificity, as well as the positive and negative predictive values of each of the glaucoma and AMD PRS will be assessed. Secondary outcomes will compare glaucoma suspect cases to their PRS results, compare disease prevalence with the presence or absence of various comorbid conditions, treatment intensity requirements including the number of cases with actionable disease, the rate of diagnosed versus undiagnosed disease, and the prevalence of family history. Additionally, glaucoma and AMD cases may be graded by severity, and compared to their PRS results.

8.3 PRELIMINARY RESULTS

In total, 1062 participants have been recruited (current at the date of submission). Of which, a glaucoma PRS has been calculated for 941 participants. The demographic characteristics of the participants are shown in Table 8.1. In summary, 57.7% of participants were female, 91.4% were of self-reported European ethnicity, 26.8% had a positive family history of glaucoma, and 15.0% had a positive family history of AMD. The mean age of the cohort was 70.9 ± 9.7 years.

Demographic Characteristic	**Number (%)**
Age (years)	
- Range	50 - 99
- Mean (standard deviation)	70.9 (9.7)
- Median	71
Gender	
- Female	613 (57.7)
- Male	449 (42.9)
Ethnicity	
- European	971 (91.4)
- Asian	26 (2.4)
- Hispanic	2 (0.2)
- Middle Eastern	3 (0.3)
- Mixed ethnicity	13 (1.2)
- Unknown	46 (4.3)
Family history of glaucoma	
- Yes	285 (26.8)
- No	708 (66.7)
- Missing	70 (6.6)
Family history of AMD	
- Yes	159 (15.0)
- No	800 (75.3)
- Missing	104 (9.8)

Table 8.1: Demographic characteristics of the study sample.
Current at date of submission. All data self-reported from participants.

Using the 1000 Genomes Project (1KG) samples as a labelled training set,[290] a random forest classifier was trained to assign genetic ancestries in the GRADE cohort utilising the first 10 principal components of ancestry defined by the 1000 Genomes Project.[290] Assigned genetic ancestries in the GRADE cohort are shown in Figure 8.1 and summarised in Table 8.2. The ancestry categories differ from our options for self-reported ethnicity. GRADE samples were projected onto the principal component analysis (PCA) space inferred from 1000 Genomes super-

populations EUR, EAS, SAS and AFR. Figure 8.1 shows the plotted positions of samples on the first two main axes of genetic variation. The topology of the 1000 Genomes Project samples is well known and corresponds to AFR vs Non-AFR (PC1), and the 'Eurasian cline' (PC2).[291] The majority of GRADE samples cluster with EUR samples.

Ancestry	Percentage (%) in GRADE
European	93.96
East Asian	2.25
South Asian	0.24
Americas	0.12
Complex	3.44

Table 8.2: Genetic ancestry in GRADE. Complex ancestry refers to GRADE participants who could not be assigned a major genetic ancestry component (defined as >90% probability).

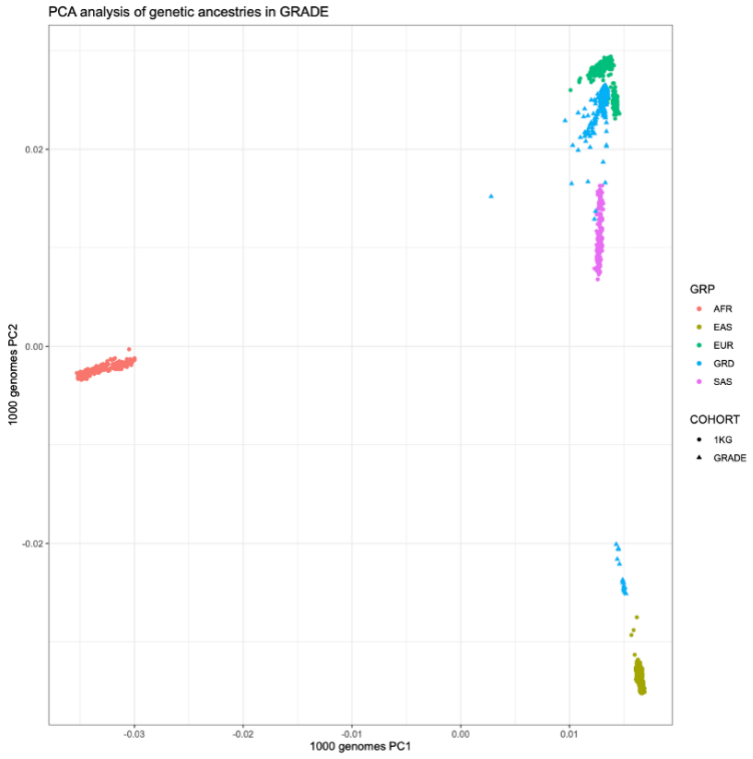

Figure 8.3: Genetic ancestries in GRADE.
Ancestry groups (GRP): African (AFR), East Asian (EAS), European (EUR), GRADE (GRD), South Asian (SAS). 1KG: 1000 Genome Project.

Figure 8.4 demonstrates the distribution (densities) in GRADE of genetic risk (PRS) after normalisation to ancestry matched normative populations from the 1000 Genomes Project. The distribution of genetic risk indicates that GRADE participants are largely a representative sample of the general population, albeit with the possibility of slightly higher than expected density of very high genetic risk individuals.

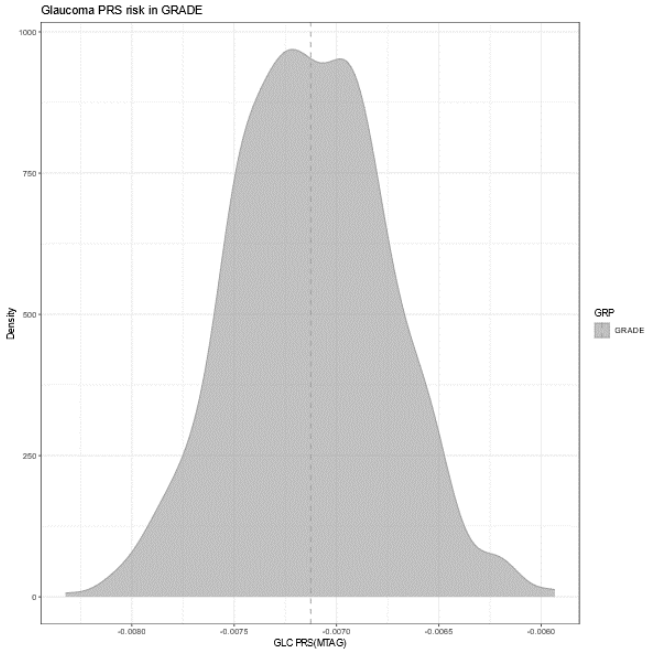

Figure 8.4: Distribution of glaucoma genetic risk (PRS) in GRADE.
Dashed line represents the group mean.

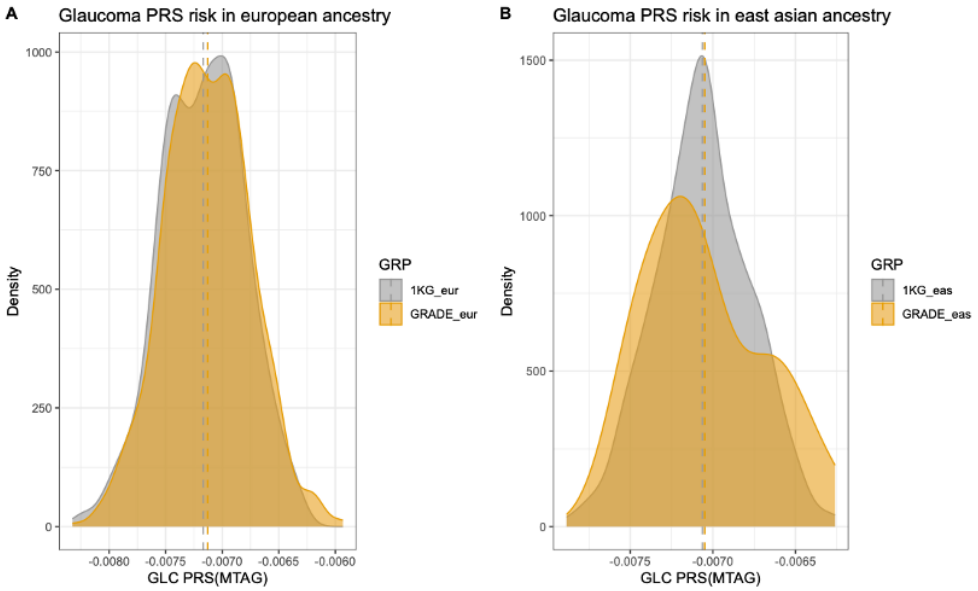

Figure 8.5: Distribution of glaucoma genetic risk (PRS) in GRADE, by genetic ancestry. Distribution (densities) in GRADE versus ancestry matched normative populations of European ancestries (panel A) and east Asian ancestries (panel B) from the 1000 genomes project (1KG). Dashed lines represent the group means.

Distribution of glaucoma genetic risk (PRS) in GRADE, by genetic ancestry, are shown in Figure 8.5. The similarity of both distribution shapes and mean suggests that GRADE participants are largely a representative sample of the general population, albeit with the possibility of slightly higher than expected density of very high genetic risk individuals. Results are plotted separately for the two ancestries and they are normalised separately with respect to their ancestry match normative populations from 1KG, and the MTAG PRS was derived from samples of European genetic ancestries.

Despite a greater representation of female versus male GRADE participants, genetic risk (MTAG) distributions between sex's are largely identical, with perhaps some evidence that males contain a skewed distribution to higher risk individuals (Figure 8.6).

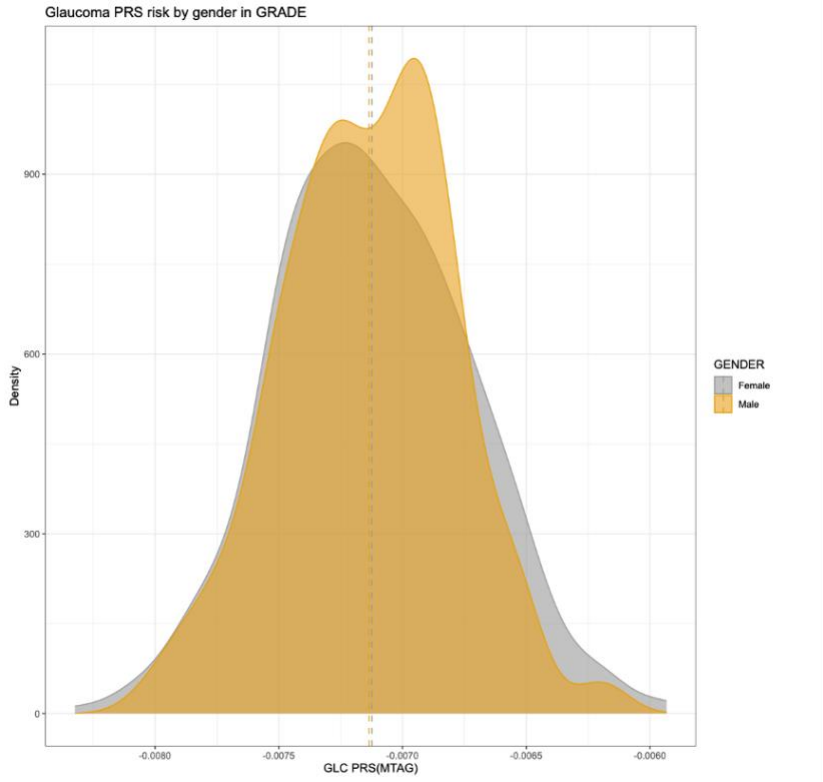

Figure 8.6: Distribution of glaucoma genetic risk (PRS) in GRADE, by biological sex. Dashed lines represent group means.

8.4 DISCUSSION

Glaucoma and AMD are the most common causes of irreversible blindness worldwide.[5] Both conditions are highly heritable, with recognised Mendelian and complex inheritance.[43,292,293] There are a paucity of screening protocols for both diseases and current guidelines are not cost-effective, in part due to poor sensitivity or specificity. To our knowledge this is the first prospective study to apply PRS testing for glaucoma and AMD in individuals from the general population, specifically recruited for this purpose.

The current NHMRC screening guidelines in Australia lack specific guidance, and are mainly relevant to those with a family history of glaucoma.[80] PRS testing for glaucoma is likely to be useful for those who do not have a known family history and have an unrecognised underlying risk. These individuals are less likely to be identified early by current screening guidelines given screening at an earlier age is only recommended for those with a family history and people of African ancestry.[80] There are no current screening guidelines for AMD in Australia. Detection is reliant on an individual experiencing symptoms and seeking ophthalmic review, or opportunistic recognition of disease during a routine assessment. The findings from this study will assist in the development of better screening guidelines for glaucoma and AMD.

Currently, risk estimation for developing glaucoma and AMD are based on a combination of demographic and clinical factors. The predictive ability of polygenic risk models for POAG and AMD are well established, particularly in European populations, and are summarised elsewhere.[136] For glaucoma, risk factors include increasing age, family history of glaucoma, African ancestry, and elevated IOP.[33,53] Genetic risk has been largely estimated through family history alone. A positive family history carried a 9-fold risk for first-degree relatives compared to controls in one study, but this required full examination of all first degree relatives rather than self-report.[41] The accuracy of self-reported family history for glaucoma has been studied and found to be an unreliable measure as many patients are unaware of family members with diagnosed glaucoma, or have erroneous views as to what caused vision loss in relatives.[146] More recent data indicates that PRS provides a more accurate representation of risk with family history in an Australian population based study.[108] Several risk calculators have been developed to aid clinicians in screening and treatment decisions, however there remains no consensus regarding optimal timing and frequency of population screening for glaucoma.[72,294] PRS provides a more accurate estimation of risk than traditional methods alone, with risk prediction optimised when all factors are combined.[108] AMD risk involves an interplay of genetic and environmental factors.

There are several recognised environmental risk factors including age and smoking, with sex, ancestry, cardiovascular disease, and diet also suggested to be implicated.[286] A prediction model incorporating genetic, demographic and environmental risk factors was independently associated with incidence and prevalence of advanced AMD, all with strong predictive power.[295] Effective risk algorithms incorporating environmental, clinical and genetic risk factors will need to be developed. While environmental and clinical risk factors may change over time, the genetic contribution to overall risk will remain constant given genetic disease liability is fixed from conception. Therefore, an important benefit of polygenic risk testing is that PRS can be calculated at any stage of life and may be useful to inform disease prognosis and response to treatment before individuals exhibit vision loss.

Glaucoma genetic testing is currently limited to Mendelian genes (e.g. *MYOC)* which explain less than 5% of adult onset glaucoma.[89,128] PRS testing, however, captures a much larger component of glaucoma genetic risk. Those with high polygenic risk had a comparable glaucoma risk to those with the most common Mendelian variant (OR 2.77 vs OR 4.19), as well as being ~15 times more prevalent.[128] At present, genetic testing for AMD is not recommended and exists predominantly in research contexts.[197,286,296] Direct to consumer tests incorporating various PRS tests for both diseases are available, however these lack prospective evidence demonstrating their effectiveness.[297,298] This study will assess the clinical validity of PRS testing in a sample representative of the general population in Australia in order to determine its application in the community.

We have previously demonstrated strong interest in polygenic risk testing for glaucoma among various groups, including those with diagnosed glaucoma, those with a first-degree relative with glaucoma, and those without any personal or family history of the condition.[168,183] Although PRS testing for glaucoma was theoretically accepted, a number of concerns and potential barriers to implementation were identified, including residing in a rural location and unwillingness to pay for testing. There are a number of additional questions which must first be addressed before PRSs can be integrated into clinical practice.

Firstly, results must lead to actionable and cost-effective measures. Guidelines will be needed to clarify which PRS classifications warrant intervention. Those identified to be at high risk for developing glaucoma or AMD may receive more regular follow-up with an optometrist or ophthalmologist, allowing for timely treatment initiation. Treatment may be commenced before the

disease becomes symptomatic. Early interventions for glaucoma may include topical IOP-lowering medication or laser therapy. Earlier surgical intervention may be considered for those with a PRS indicating a likelihood to progress rapidly or to advanced disease. While treatment options for early AMD are lacking, there are a large number of treatments under research including various pharmaceutical agents, gene therapies and surgical interventions.[299] Antioxidant supplements based on the Age-Related Eye Disease Studies (AREDS) may have benefit in those with intermediate disease in one or both eyes to reduce the risk of progressing to late AMD, or in those with late stage disease in only one eye to reduce the risk of developing it in the other eye.[300] Smoking is the only established modifiable risk factor for AMD, with the risk of progression to neovascular AMD shown to be double for those who had ever smoked.[301] Despite there being few treatment options for AMD, risk factor modification and antioxidant supplementation may still be valuable interventions in high-risk individuals. Progression from early to advanced AMD may occur rapidly and result in severe vision loss if treatment is delayed. Using tools such as an Amsler grid, individuals who are recognised to be at higher risk of this occurring could be educated to self-monitor for progression, with a pathway to access rapid assessment if symptomatic. Conversely, PRS may prevent unnecessary follow-up or treatment in those presumed to be at higher risk based on traditional risk prediction models. This may improve the cost-effectiveness of the PRS.

Secondly, it will be critical to develop frameworks which allow PRS results to be reported and communicated in a meaningful manner. Pilot reports need to be developed and tested to assess communication preferences and understanding of reported results among different stakeholders, including patients and healthcare professionals. We have previously demonstrated that the preferred method of receiving results may depend on the result itself, so report content and structure will likely vary depending on risk classification.[168,183] This study will form the foundations of future research to develop our understanding of the clinical implementation of PRS testing for glaucoma and AMD.

Finally, there are a number of health economic elements which need to be considered before implementing PRS into clinical practice. Population-based screening for glaucoma or AMD is not currently cost-effective, so public health frameworks need to be developed which allow identification of those at increased risk while also ensuring adequate access to further treatment. Disease prevention is at the forefront of public health policy, and polygenic risk stratification has the potential to enhance primary, secondary and tertiary facets of this. Ultimately, enhanced

disease screening will minimise the personal and economic costs of significant vision loss. Improved risk stratification will alleviate workload created by over investigation and treatment of those at high risk calculated using traditional risk factors, but at low genetic risk. However, it will be important to integrate genetic risk with clinical or environmental risk factors. Individuals with a strong family history would still be recommended to have regular clinical testing, even if shown to have a low PRS, due to the influence of Mendelian variants or other factors not covered by the PRS. We have shown that financial implications appear to be important to people and while some are unwilling to pay for testing the majority of individuals would be prepared to pay varying amounts.[168] Subsidisation may improve uptake, however will only be an option if it is cost-effective for the healthcare system which remains to be demonstrated.

Current PRSs for glaucoma or AMD are based on predominantly European populations and have not yet been comprehensively tested across other ancestry. Individuals of non-European ancestry are not excluded from the study, although the accuracy of their risk predictions may be reduced. Better validation of a single pan-ancestry PRS, or ancestry-specific scores covering all ancestries, are a major unmet need to avoid future health disparities.

In conclusion, this prospective study aims to demonstrate the clinical validity of PRS to stratify individuals from the general population and identify those who are at high risk of developing glaucoma or AMD. This will help to move towards the implementation of PRS into clinical practice and provide an objective screening tool for glaucoma and AMD. The ability to identify at-risk individuals will allow for closer monitoring and timely intervention, and ultimately reduce irreversible vision loss. Further studies will need to look into how PRS testing could alleviate some of the socioeconomic burden resulting from vision loss. The outcomes from this study will form the basis for future interventional studies to further enable a shift in the detection, treatment and prevention of diseases with complex inheritance.

DISCUSSION

Polygenic risk scores are an emerging concept allowing underlying genetic disease predisposition to be objectively estimated. However, despite the breadth of literature on this topic, a number of knowledge gaps remain. My thesis presents an original contribution to knowledge by addressing several knowledge gaps at different levels to guide future implementation into clinical practice.

Vision impairment is reported to affect approximately 2.2 billion people worldwide, with almost half of these attributed to potentially preventable or treatable conditions.[302] The causes of vision loss are broad and vary between demographics, particularly between low- and middle-income regions compared to high-income regions.[302] The World Health Organization reports the prevalence of impaired distance vision in low- and middle-income regions to be four times higher than in high-income regions.[302] The most common causes of vision loss worldwide include uncorrected refractive errors, cataract, glaucoma, age-related macular degeneration, and diabetic retinopathy. In Australia, vision impairment affects an estimated 13 million people. The majority (over 93%) of chronic eye conditions affect individuals over 65 years of age, with slightly higher prevalence in females.[303] Of the conditions resulting in vision impairment, glaucoma is the most common cause of irreversible vision loss.[5] A meta-analysis and systematic review reported a worldwide prevalence of primary open-angle glaucoma of 2.4% globally.[11] In Australia, prevalence of POAG is estimated between 1.5-3.4% among non-Indigenous Australians, while prevalence of all glaucomas is estimated between 0.6-1.6% among Indigenous Australians.[10] According to Australian statistics, prevalence has been stable since 2007, reflecting the limited impact of environmental factors on the development of glaucoma.[304]

Visual impairment due to glaucoma translates to a significant public health and economic burden. This includes productivity losses, the cost to health care systems to provide access to treatment, and costs stemming from complications and comorbidity related to vision loss.[305] The medical and non-medical direct costs of all causes of vision impairment in Australia was estimated at USD2.69 billion, with approximately USD340 million being costs related to glaucoma.[305] Governments continually evaluate the cost effectiveness of treatment subsidies within their healthcare structures, however, this is an ongoing challenge given the ageing population and rapidly progressing technology.

The early, asymptomatic state of glaucoma presents a diagnostic challenge, and explains why up to half of those with glaucoma are unaware and undiagnosed.[32] Despite being the most common cause of irreversible vision loss worldwide, glaucoma remains under-recognised.[60] Knowledge of personal risk for glaucoma may allow for earlier action, such as close management or preventative treatment, before vision is lost. This is important given vision impairment has significant personal and economic impacts. Vision loss can have a profound influence on an individuals' well being, through impacting independence, social function, and education and employment opportunities. Those with visual impairment are more likely to experience depression and lower quality of life, particularly due to limiting independence and social interactions.[306,307]

The high heritability of glaucoma provides an opportunity for genetic based tools to be developed to aid diagnosis and monitoring of patients. Evidence supporting the clinical utility of glaucoma PRS instruments has been widely demonstrated in research settings, confirming the association between the scores and disease status. High polygenic risk has been associated with earlier age at glaucoma diagnosis, higher IOP, faster visual field progression, larger cup-to-disc ratio, increased need for trabeculectomy, and earlier initiation or escalation of treatment.[110,112,128,129,273,308,309] Despite strengthening evidence supporting clinical utility, gaps remain in understanding how PRS is associated with family history of glaucoma and disease severity. The increasing likelihood of polygenic risk scores becoming part of future clinical practice is supported by growing evidence of clinical validity. Prospective validation is yet to be demonstrated, which is the primary aim of the GRADE study. Furthermore, general acceptance of individual genetic risk stratification, in the form of PRS testing, is crucial for implementation into clinical practice to be successful.

Multilevel barriers to accessing polygenic risk testing exist on societal, system, provider, and individual levels. Identifying these barriers is the first step towards realising the full potential of polygenic risk testing, and will require significant input from multiple stakeholders. The work presented in this thesis provides an original contribution to knowledge, reporting the first insights into the individual, provider and societal factors involved in delivering glaucoma PRS testing and supporting its use in clinical practice.

Individual factors

Identifying and addressing barriers for PRS testing will help ensure its successful application in clinical practice. Assessing potential individual-level barriers has been a key focus of this thesis,

including the attitudes of patients toward the test (Chapters 1 and 2), the factors affecting uptake of the test (Chapters 1, 2, and 4) and communication strategies for PRS results (Chapter 5). We found that perceived risk, residential location, and affordability may impact an individual's interest in polygenic risk testing. A population-based testing model would address some testing barriers by design, including normalising testing and improving affordability through improved accessibility or incorporation into a government-sponsored program, however other barriers will need more precise attention to overcome. Some studies suggest an underlying fear of genetic testing and the potential for genetic discrimination, which may impact uptake of polygenic risk testing.[139] This may stem from lack of knowledge, fear of potential results, and distrust in the healthcare system.[139] The increased use of PRS testing will potentially help improve these factors.

Studies have captured the attitudes of participants towards PRS testing, all of which may impact uptake of testing.[137,139,140,209] However, none had explored the acceptability of polygenic testing for glaucoma. In Chapters 1 and 2, we showed strong interest toward the test, both among affected (69%) and unaffected individuals (71%). We found that those who perceived their risk of developing glaucoma as higher, and those who were worried about developing glaucoma, were more likely to be interested in PRS testing (Chapter 1), which is consistent with previous findings on predictive genetic testing for Mendelian glaucoma.[147] Attitudes and perspectives can then also impact behaviour. We found that people who were interested in testing were more likely to change their eye health-seeking intentions and recommend testing to family and non-family members, as well as undergo testing for prognostication. Similarly, a study assessing preferences for a cancer PRS found that respondents were more interested in testing if it enabled risk reduction through lifestyle modification, screening, or medication.[310] However, intention to change health behaviours does not always correlate with actual changes. A previous meta-analysis reported that knowledge of genetic risk had little effect on risk-reducing behaviours such as diet, smoking cessation and physical activity.[166] Similarly, a systematic review found variable changes in lifestyle and screening behaviours associated with PRS information.[311] Inconsistency in health behaviour change resulting from genetic testing may be partially attributable to study design and lack of consideration for health behaviour theory. Most studies within this space rely on knowledge of genetic risk as the primary driver for change in health behaviour,[311] however more positive health behaviour is seen in studies where behaviour change theory is considered in communicating polygenic risk.[312] Further research is needed to determine whether PRS communication modifies actual behavioural outcomes using communication methods informed by health behaviour theories.

Genetic risk for glaucoma can also be estimated through family history. Those aware of a relative affected by glaucoma may already have a better understanding of the potential impact on vision loss, the importance of undergoing regular glaucoma screening and the treatment options available. Awareness of the impact glaucoma can have may also act as a motivating factor to undergo PRS testing. In this thesis, increased interest in glaucoma PRS testing was associated with a positive family history of glaucoma and a higher number of affected family members (Chapter 2). Statistical significance was observed only if the affected family member was a first- or second-degree relative. This is in line with other studies identifying that interest in genetic testing was supported if there was a family history of the condition.[148,149,151,156–158]

A higher glaucoma PRS has been associated with a greater risk of glaucoma diagnosis, an earlier age of diagnosis, and a greater need for surgery.[108,120,128] This highlights the utility of PRS testing to not only estimate those who are likely to be diagnosed with glaucoma, but also to predict prognosis and inform treatment decisions. We showed that participants in the top decile were younger at the time of first trabeculectomy, had a shorter duration between diagnosis and first trabeculectomy, and were more likely to require bilateral trabeculectomy than participants in the lowest decile.[308] This could mean that trabeculectomy is considered earlier in higher-risk individuals who are likely to ultimately require incisional surgery, and avoid stress and vision loss potentially resulting from failure of more conservative treatment options. It may also prevent unnecessary surgery in those who are less likely to progress quickly. Currently, treatment is approached in a stepwise manner, where incisional surgery is considered only when other treatments have not adequately controlled disease progression. Changing this paradigm requires consideration of the impact of surgery on quality of life and economic costs.

Our findings extend previous studies linking glaucoma PRS with glaucoma treatment outcomes.[108] For those with disease that may ultimately require surgery, this could mean that trabeculectomy is considered earlier in higher-risk individuals, potentially avoiding vision loss resulting from failed trials of more conservative options. It may also help prevent unnecessary surgery, or delay surgery, in those who are deemed to be low risk. The results of this work, combined with the observation that a majority of individuals with POAG are interested in PRS testing,[168] highlights the potential utility of genomic risk stratification in this disease.

The current literature on polygenic testing uptake is limited, with previous studies reporting uptake of PRS testing for breast, colorectal and prostate cancers between 26% and 96%.[137,193–196] While there were similarities in the cohorts assessed in these studies, including age and ethnicity, differences in methodology means direct comparisons of results is difficult. In Chapter 4, we reported an uptake rate of 54% to enrol in a research study to assess PRS from a cohort of individuals who completed a questionnaire on their attitude toward the test. We found that having a higher level of education (at least tertiary education) and a positive family history were potential indicators of uptake of PRS testing for glaucoma. This may assist in identifying groups where further education is needed, or guide selection of target populations for testing, such as family members of individuals with glaucoma, particularly in the early stages of implementation. Similar to other studies, we identified that interest is not always associated with uptake, with interest being often higher than actual uptake.[162,183,204,208] However, we found that on the other hand, those who indicate being uninterested in testing may in fact undergo testing if the opportunity is available to them. Whilst discorrelation between intention and behaviour is a well recognised theme and should be interpreted with caution, this information will still form the basis of our understanding surrounding PRS decision by identifying barriers to uptake and patterns of behaviour among different populations.

With the potential for PRS testing to be delivered as a broad, population-based screening tool, results may need to be interpretable by consumers themselves. There are important differences between genetic tests for high-penetrance variants in single genes for monogenic conditions, and polygenic risk tests reporting baseline risk for disease or traits across a spectrum. Genetic tests for high-penetrance variants usually report an absolute risk for developing a condition and have significant implications for the patient in terms of interventions to minimise this risk. An example of this is the *BRCA1/BRCA2* genes and their association with breast cancer, whereby patients with pathogenic variants in these genes may undergo radical mastectomy to reduce their risk of breast cancer.[313] In comparison, PRS testing indicates an underlying risk which can be reported as a relative or absolute risk. However, the absolute risk conferred by a PRS is usually lower than for higher-penetrance variants such as *MYOC.* Clinically, these differences also translate to application, where testing for monogenic conditions is usually performed as a diagnostic test, while PRS testing is mostly prognostic and should be considered in the context of other clinical risk factors. The distinction between the diagnostic and prognostic properties of genetic testing can become blurred, for example in scenarios where a high PRS confers a similar or higher risk than a monogenic variant.[128]

A PRS indicates an individuals' risk across a continuum, typically relative to a reference population. In this relative risk scenario, PRSs can help to identify which individuals are more at risk for a certain outcome compared to others. However, this gives no indication of how much the genetic risk, as reported by the PRS, explains an individual's actual risk of developing glaucoma.[314–316] However, absolute risk can be more meaningful in terms of putting an individual's risk into perspective. The relative risk of PRS and translation of results to absolute risk will be useful and consideration should be given to the inclusion of both in PRS reporting.[315] It is also well established that risk is more easily and accurately perceived by non-experts when presented on an absolute scale, meaning, the probability an individual will develop the outcome.[217,317] The absolute risk conferred by a given relative risk can also be determined by the predictive utility of the polygenic risk score and the population prevalence of the phenotype, or calculated directly in a suitable cohort.[316]

We have demonstrated that cost is important to consumers (Chapters 1 and 2). Venning et al reported that the higher price of a PRS test had a significant negative impact on choice.[310] Participants in our studies (most of whom were recruited from public hospital clinics) felt that it would be appropriate for testing to be covered by Medicare, particularly for glaucoma PRS testing given the affected age group includes those supported by a seniors pension. While it is likely that testing will be increasingly affordable with time as technology advances and economies of scale will make it more cost effective, it is important to appreciate the impact of cost at an individual level.

Provider factors

Healthcare professionals will play a key role in the delivery of polygenic risk testing through helping patients overcome individual-level barriers, as well as acting as an important link between the community and the healthcare system. The important role that healthcare professionals play is evident through the higher adherence to risk-reducing interventions by high-risk individuals if recommended by a physician.[318] The current genetic testing model for monogenic conditions requires healthcare professionals to recognise a likely underlying genetic cause and facilitate referrals to specialist clinicians to access testing. This model will clearly not be suitable for polygenic risk testing, particularly for the large number of individuals at risk of common diseases who potentially stand to benefit.

As an emerging tool in genetic risk assessment, many clinicians are not yet widely aware of the concept of polygenic risk and polygenic risk testing, across all specialties. A study surveying 960 child and adolescent psychiatrists' about their experiences, perspectives, and potential uses of psychiatric PRS reported that 23% had never heard of PRS.[141] In a study from the UK, 49% of GPs were not familiar with the concept of PRS.[143] We found a similar general lack of awareness of polygenic risk amongst non-genetic healthcare professionals, and even genetic healthcare professionals regarding familiarity with polygenic risk for glaucoma (Chapter 3). The increased use of PRS testing will improve providers' familiarity and confidence in interpreting results, and allow clinics and healthcare systems to establish clear workflows and guidelines. However, this will rely on investment in infrastructure, integration and extensive education.

We found that there is a widespread lack of confidence among clinicians around genetic concepts in general. This is echoed in other studies, which highlight lack of confidence and knowledge are significant barriers concerning clinicians in regard to genomic medicine.[142,186,259,260,262,319–322] Lack of confidence in this area appears to relate most to post-test counselling. Our results were consistent with another study which indicated clinicians were least confident in interpreting and communicating genetic test results, rather than obtaining a genetic risk history or identifying appropriate genetic services.[142]

Healthcare professionals will be at the forefront of promoting and delivering personalised medicine, incorporating PRS into routine clinical practice. Healthcare professionals indicated that the availability of guidelines from government bodies and medical societies would affect their decision to recommend PRS testing for glaucoma to patients. While evidence for guidelines incorporating traditional risk factors with PRS have not yet been developed, early indications of the additive utility are positive.[323] We demonstrated the ability of a glaucoma PRS to further delineate risk in individuals with a family history of glaucoma (Chapter 7). Guidelines have been developed for other conditions in research settings. Risk models for breast, colorectal and prostate cancer have shown the most promise in improving prediction capabilities with the addition of PRS: several breast cancer studies have compared risk assessment between models with and without the inclusion of PRS, finding improved AUC and risk classification when PRS was included.[324–329] Similar results have been replicated for colorectal cancer, where the addition of PRS to existing risk models as improved AUC and risk classification.[330–335] Again, the addition of PRS to prostate cancer risk models were improved, demonstrating higher AUC and risk classification.[336–339] Due to a larger number of less well-defined environmental risk factors, there

is significant heterogeneity in the definition and selection of risk factors for melanoma risk models, resulting in difficulty in assessing and comparing.[340] Results in cardiovascular studies have been mixed with studies showing improvements, minimal change, and reduced efficacy of risk models with the addition of PRS.[130,341–348] This may be due to the significant impact of environmental risk factors on developing cardiovascular disease, and their evolving nature. The lack of dynamic environmental risk factors will be a strength for developing clear guidelines for glaucoma risk, as risk is highly heritable.

Ophthalmic care is uniquely structured to include optometrists and ophthalmologists as specific primary and secondary eye health providers, respectively. This structure allows for improved access to eye healthcare, more appropriate triaging of patients requiring specialist medical care, and collaboration in ongoing follow-up. As primary eye healthcare providers, optometrists are integral to the detection of undiagnosed glaucoma cases, as well as in long-term surveillance and management of early or stable glaucoma patients. As the primary access to eye healthcare in the community, optometrists may be the most appropriate healthcare providers to deliver glaucoma PRS testing, allowing for stratifying risk in patients with early clinical features of glaucoma, or in those with known family history. This would then help to guide referral to specialists, and conversely, prevent unnecessary review of those with a family history or glaucomatous findings who are actually at low risk of progression to glaucoma. There is little data in the literature documenting the attitudes of optometrists towards PRS testing, probably due to their very specific role in primary healthcare. We assessed the perspectives of optometrists towards PRS testing, finding that 65% of optometrists felt they were an appropriate group to order PRS testing and over 70% felt they would be the most appropriate group to communicate both low and high risk PRS results to patients. However, the majority of optometrists surveyed lacked knowledge and confidence in understanding genetic concepts, especially PRS. There is a clear need to develop strategies for further education to support optometrists in their potentially critical role in delivering glaucoma PRS testing.

In Australia, GPs are usually the first point of access to healthcare for the community, and service the largest number of patients.[349] Over 80% of Australians see a GP for at least one consultation each year.[349] They play an integral role in care coordination of patients and may therefore be an appropriate setting to deliver PRS testing and facilitate tailored preventative measures or referrals based on results. Several studies identified GPs to be the most appropriate healthcare professional to deliver PRS testing.[193,310,350–352] Healthcare professionals in this thesis indicated

specialists would be more appropriate to order PRS testing, including ophthalmologists and clinical geneticists or genetic counsellors (Chapter 3). This is problematic given there are fewer specialists, who are often more expensive and mainly accessible in capital cities. Additionally, offering testing only through specialist practitioners may exacerbate the marginalisation of those in regional and remote areas.

Given GPs are the cornerstone of providing and coordinating care to the broadest population, many feel this will be an appropriate setting to deliver PRS testing (Chapter 3).[142,192] However, there are numerous barriers to the integration of PRS testing into GP-based primary care. For a GP to deliver PRS testing for a condition would require correct identification of high risk patients through thorough risk assessment, ordering tests, being able to correctly interpret results, adequately communicate results to patients, and finally refer patients to appropriate services in a timely manner. In addition to this significant workload across all areas of medicine, PRS may add another unrealistic expectation on GPs. Furthermore, given there is already an undersupply of GPs in Australia, care must be taken to ensure implementation of PRS does not overburden an already struggling primary healthcare system.[353] On the other hand, GPs are frequently patients' first point of contact with the health system and could be an ideal setting to introduce and coordinate PRS testing. We found that many felt that utilising GPs to deliver PRS testing is appropriate and necessary. This is consistent with other studies which have shown that various healthcare professionals, including GPs themselves, agree the primary care setting is likely the most appropriate to incorporate PRS testing.[142,192] A recent statement by the Human Genetics Society of Australasia identifies the likely need to utilise healthcare professionals with limited exposure to genetics, and acknowledges the importance to support and trust these professionals in evolving their genetics skill-set.[244]

GPs do not currently routinely order genetic tests for monogenic conditions, however have a key role in discussing disease risk, screening and prevention, supporting their appropriateness in delivering PRS testing. A high glaucoma PRS has been shown to increase disease risk similar to monogenic disease-causing glaucoma variants, however is 15-times more prevalent in the general population.[128] Therefore, the implications of high-risk PRS results will be just as significant for patients in terms of the requirement for treatment and ongoing follow-up to minimise risk of disease and progression. Given the potential for broader population testing, and therefore increased capture of high-risk individuals, the primary care setting will be important in the delivery of PRS testing.

Time constraints may be a significant restricting factor in the clinical implementation of PRS testing. At one extreme, a different PRS may exist for every common complex disease, raising a number of issues. Firstly, an individual could have access to many separate tests, each requiring a separate test order and reports, as well as the possibility of an appointment with a healthcare professional to discuss the results and direct the next appropriate step. This would lead to a significant increase in workload, and therefore time involved, for one individual. Healthcare professionals are frequently already limited by appointment times, and prioritising such conversations would become increasingly difficult once risk testing becomes widely available. In breaking down the nuances in discussing genetic risk and PRS testing with patients, it is clear this comes with a significant time burden. Clinicians may need to perform a thorough history, including family history, to help identify appropriate individuals, order and discuss how to obtain testing, and then potentially discuss the results and their implications, and facilitate appropriate referrals or screening plans or treatment.

This also highlights the need for additional education of healthcare professionals targeted at genetics and non-specialist level resources. However, primary care providers including optometrists and GPs currently lack knowledge and confidence to deal with this field.[192] The HGSA identified that it is important to avoid ‘genetic exceptionalism’ and that with additional education, guidelines and experience, GPs will adapt to improve their knowledge of PRS testing.[244] Clinician knowledge contributes to patient adherence to screening guidelines, which may vary significantly depending on experience and area of specialisation. For example, an ophthalmologist or optometrist is more likely to be able to recommend appropriate management of a patient with glaucoma than a general practitioner. Patients who do not have private insurance may have less timely access to specialists for non-urgent review or discussion such as for PRS results, so may be less likely to receive appropriate advice.

Implementation of PRS will require input from multiple stakeholders to decide the most appropriate location and personnel to deliver this technology, whilst balancing cost and equitable access. Initial implementation is occurring through private providers until clearer evidence of clinical utility and cost-effectiveness is demonstrated. There may be very limited reimbursement for testing in the early stages of implementation, however with improved technology allowing for increased accessibility, as well as increasing demand and competition between test providers, and economies of scale, costs may decrease. In addition, cost of testing may become subsidised

by public insurers in the future if evidence of clinical utility and cost-effectiveness is clearly demonstrated. However, the process of applying for and achieving a Medicare item number for public reimbursement of services is extremely complex, and is an additional time barrier to implementation.[354] Although administration of PRS testing may be relatively simple, where only a blood or saliva sample is needed, access to follow-up and treatment must be as equitable as possible, regardless of geographic location. Therefore, as the scale of PRS testing evolves, primary care healthcare professionals, such as GPs and optometrists, will likely play a key role in helping patients access PRS testing and then helping to navigate their management and follow-up journey, particularly in non-urban settings.

Societal factors

As highlighted in different chapters from this thesis, societal factors such as healthcare provision in rural and remote areas, cost, and health insurance schemes must be addressed to ensure equitable access to testing and necessary services.

In Australia, publicly-subsidised genetic tests are available for reproductive genetic carrier screening, newborn screening, and for high-risk variants if specific criteria are met. Newer direct-to-consumer genetic testing and private-pay genetic testing, both of which may offer PRS testing, are currently available only through private providers, meaning consumers are responsible for the full cost of the test, unless they are enrolled in research projects covering the costs. Given the strong coverage of public healthcare, most Australians are not accustomed to paying for medical tests, and this may be a barrier to the uptake of testing in the early stages of its availability. Robust cost-effectiveness studies will be needed to inform policy-makers of the cost to individuals for PRS testing. A recent analysis of coronary artery disease PRS indicated an incremental cost effectiveness ratio of approximately $140,000 per quality adjusted life year, pricing the PRS at $70 per person.[355]

Cost transparency and the potential financial burden passed on to consumers is an important factor to consider in ensuring equitable access to this technology. While costs of genomic sequencing have decreased with improved technology and availability, out-of-pocket cost to consumers. Almost 50% of healthcare professionals indicated that coverage of PRS testing under Medicare would be an important factor affecting their decision to recommend PRS testing (Chapter 3). This was echoed by potential consumers, who reported that the cost of the test was indicated as an important factor to know by over 70% of respondents, as well as being the most frequently indicated concern about PRS testing for glaucoma (Chapter 1 and 2). In addition, the

largest proportion of participants (~45%) indicated willingness to pay the lowest cost offered for the test. Some who indicated being unwilling to pay for the test commented on the challenges of affording additional health care costs whilst receiving the pension. This is important to consider given the older age of those most commonly affected by glaucoma. Early indications of the likely cost of PRS testing are above AUD $100, public preference is relevant in order to consider future cost subsidisation and possible impact on uptake of the test. Although willingness to pay has been shown to be influenced by the perceived benefit of the information received,[356] This was reflected in a study of preferences for a cancer PRS that showed that price and interest in testing generally has an inverse relationship.[310] Several private companies now offer polygenic risk testing. One Australian company offers PRS testing for cancer and cardiovascular risk priced at AUD $795.[357]

Approximately one in four Australians live in rural and remote areas.[358] National data has shown that those living in rural and remote areas have poorer health outcomes relating to the unique challenges faced due to their geographic location.[358] These include higher rates of hospitalisations, deaths and injury, as well as poorer access to primary health care services.[358] A glaucoma PRS can identify those at higher risk of developing the disease or of progressing. In the case of glaucoma, this might involve commencing intraocular lowering treatments implemented by specialists as soon as symptoms start or progression is noted. Publicly funded (bulk-billed) specialist clinics are largely accessible through large tertiary centres in urban areas. Fewer specialist services are accessible through private clinics in regional centres with associated additional cost which may not be affordable for some. This highlights the need for other delivery models if PRS was to be delivered at a population level and identify a significant proportion of the rural population necessitating screening and treatment.

Rapid advances in telehealth services and providers was seen during the COVID-19 pandemic to ensure safety of patients and healthcare professionals and continuity of care.[359] Telehealth consultations allow patients to be connected to healthcare professionals via telephone or video instead of in person. Among several benefits, a key advantage of telehealth is allowing patients with limited mobility or those from remote areas to access consultations with healthcare professions. Given PRS kits are routinely mailed to consumers and DNA samples from blood or saliva then returned via post, this could drastically improve access to this technology to those in rural areas. If artificial intelligence technology is also improved, patients could be clinically screened and monitored at a closer location depending on the risk result received. Artificial intelligence involves the development of computer systems and algorithms to perform tasks

replicating human behaviour.[360] Artificial intelligence is well suited to extracting and interpreting the large amount of data required for the detection and monitoring of glaucoma and macular degeneration.[361,362] Several studies are looking developing algorithms for each parameter including IOP,[363] optic disc photography,[364–368] OCT,[369–376] anterior segment OCT,[377,378] visual fields,[379–383], and combined approach.[384] Developing artificial intelligence technology to assist with clinical surveillance and improving telemedicine may help to address poorer health outcomes in rural and remote areas by changing the way medical services can be delivered to rural and remote areas.

Prior to establishing any population-based PRS screening programs, rigorous cost-effectiveness studies will need to be performed. Australia currently has five national population screening programs including for bowel cancer, breast cancer, cervical cancer, newborn bloodspot screening for rare, life threatening conditions, and newborn hearing screening.[385] The Australian government has clear criteria for deciding whether a new population screening program should be introduced.[385] The condition must be an important health problem and have a recognisable latent or early asymptomatic stage. A screening test must be highly sensitive and specific, validated, safe, have a relatively high positive predictive value and negative predictive value.[385] The test must be acceptable to the target population including participants from culturally and linguistically diverse backgrounds, people from disadvantaged groups, and people with a disability.[385] By these criteria, glaucoma would be a good candidate for population screening, with PRS being an appropriate test to deliver this. However, key gaps in knowledge remain to be addressed to fulfil the criteria for population based screening in Australia. These include developing national policy and protocol frameworks, designing of the screening program, developing a quality management plan with clearly defined governance, and ensuring adequate resources for ongoing program re-evaluation and monitoring.

According to the National Health and Medical Research Council in Australia, genetic discrimination describes ‘the different treatment of individuals or their relatives based on their actual or assumed genetic make-up’.[386] Genetic risk may be identified formally through genomic testing and/or results or informally through factors such as family history or ethnicity. In Australia, there are existing national, state and territory laws dealing with genetic discrimination. These laws are applicable to certain social circumstances including employment, insurance, education, and access to publicly available services. More specifically, it is against the law to discriminate against someone because of their genetic information, for example, based on results from a genetic

test.[386] Genetic information can be taken into account by life insurance providers and affect policy applications. Death and income protection policies covered by life insurance providers are determined based on individual risk assessments. Providers have reportedly agreed that consumers will not be required to undergo DNA testing to allow complete genomic risk to be assessed and used to determine policies, however, if consumers have received genetic results, they may be required to report it and the result may be be used by insurers.[386] The Moratorium on Genetic Tests in Life insurance currently protects consumers from being required to provide genetic test results to insurance providers up to set thresholds. In Australia, this is up to the value of $500,000 for death and total permanent disability, $200,000 for trauma and $4,000 per month for income protection.[387] Internationally, other countries have strict anti-discrimination acts to prevent insurance companies from using genetic test results to discriminate against consumers such as by declining an application, restricting cover, or increasing insurance premiums.[388–391] In Canada, the Genetics Nondiscrimination Act (2017) prevents genetic test results from being used in all insurance policy decisions.[389] The UK Code on Genetic Testing and Insurance bans the use of all predictive genetic test results, except for Huntington disease.[392] Similar protection is not provided in other countries. For example, in the United States, the Genetic Information Nondiscrimination Act (GINA) prevents discrimination by health insurance companies based on genetic test results, however this protection does not apply to other insurance policies, such as for long-term care, disability or life insurance.[393] While research has shown that fears of insurance discrimination can negatively impact individuals' decision to undergo genetic testing for Mendelian conditions, less is known whether this is also true for polygenic risk testing.[138,209] Lower levels of consumer protection may amplify the impact insurance concerns have on uptake of PRS testing.

We showed that concerns about insurance were significantly associated with testing (Chapter 1 and 2), in keeping with other studies which have shown this to be a concern of both patients and healthcare providers.[184,189,394,395] A study assessing preferences towards a cancer PRS showed that a test that did not impact life insurance eligibility or premiums was preferred over one that did.[310] Multiple studies have identified the impact of genomic testing on life insurance to be a significant concern to consumers.[137,193,251,350,352,356] This may be an important barrier to the uptake of PRS testing, and may become more evident with increasing age. Furthermore, it is unclear whether testing and subsequent follow-up care or treatment will be covered by public or private insurance providers.

In response to the increasing availability of genetic testing used for diagnosis, prognosis and health risk assessment, such as polygenic risk testing, NHMRC has identified three integral areas for the appropriate delivery of polygenic risk testing.[396] This includes recognising the importance of professional involvement and education, the need for robust evidence, and necessity for consumer information and support.[396] Government response to testing will continue to evolve, and will be unique to each country and their health system.

Policy factors

Policy-level factors have the potential to create the greatest flow-on effect to healthcare systems, providers and individuals. Developing a whole-of-government and system-focused framework, with a person-centred approach to outcomes, is necessary to ensure consistency of action across Australia.The current model of genetic risk assessment for most complex conditions, including glaucoma, relies on the ability of healthcare providers to appropriately identify high risk patients, and is limited by the cost and specificity of testing. Polygenic risk testing will allow risk assessment to be personalised, although whether this is applied as a population-based screening model or as targeted screening is yet to be determined. A screening test must meet a number of criteria to be implemented by government health policy. In Australia, a screening test must be highly sensitive and specific, validated, safe, have relatively high positive and predictive values, and be acceptable to the target population. A glaucoma PRS test potentially fulfils these criteria, meaning consideration as a screening tool is appropriate.

Polygenic risk scores summarise an individual's risk based on the number of known disease-specific genetic associations identified from large population studies. It is therefore possible to generate a PRS for any individual. With this in mind, options for clinical application are broad. Targeted screening could be a more feasible option, especially in the early stages of implementation by limiting the number of tests performed and resources needed to facilitate them. Potential target groups could stem from risk assessment using traditional risk factors including family history, ethnicity, age and clinical features. First-degree relatives of individuals with glaucoma are recognised to be much more likely to develop glaucoma, however we have shown that not all demonstrate this genetic risk (Chapter 7). This could also be true for those of African ancestry, who are traditionally recognised to be more likely to develop glaucoma but are the population in which current PRS instruments are less effective. Either confirming increased genetic risk, or demonstrating average or low genetic risk could streamline treatment and follow-up, including limiting the amount of treatment and follow-up delivered to low-risk individuals.

Baseline clinical traits associated with developing glaucoma include higher IOP, greater cup-to-disc ratio, greater visual field mean or pattern standard deviation, and reduced central corneal thickness.[53] The Ocular Hypertension was a landmark study which found topical IOP-lowering therapy was effective in delaying or preventing progression from ocular hypertension to POAG.[54] Further classifying glaucoma suspects, including those with ocular hypertension, could clinically delineate those who are likely to progress and require treatment. Finally, with age being a progressive risk factor, offering screening to individuals above a particular age group could be useful. This is applied for other clinical screening programs such as for breast and bowel cancer.[397] Alternatively, PRS can be relevant to all individuals to understand their baseline risk of a condition.

PRS is well suited to population-based screening given it can be performed at any age and potentially requires only a once-in-a-lifetime test. In this way, it may be both easy and useful to screen everyone, similar to current newborn screening for monogenic conditions. Shifting to a population-based screening model would allow a larger number of high-risk individuals to be captured, with the potential to improve health outcomes. While the positive outcomes from PRS testing are exciting, it is important to also acknowledge the potential for negative health and cost outcomes, particularly in the early stages of clinical application. For example, based on PRS results, 'low-risk' individuals may still develop glaucoma resulting in blindness, or 'high-risk' individuals may experience complications for treatments. The cost of screening and the associated follow-up of patients has not yet been identified. A large population study including patient risk- and cost-benefit assessments is needed. Ongoing advances and updates of PRSs for glaucoma and other conditions will need to be planned for. Expanding data sets, refinement of genotyping and sequencing methods, and improved accuracy of polygenic risk models could require patients to be re-tested, or result in slight shifts in patient risk classifications, and therefore their advised screening and/or intervention when interpreted with traditional clinical risk factors. Healthcare practitioners who use PRS test results will need to consider whether to reorder a PRS test when an improved version becomes available. It is also unclear who will become responsible for overseeing the implementation and delivery of PRS testing, but regardless, will require close, evidence-based guidance. Like other population screening programs, this may become the responsibility of relevant governing bodies in each country.

Clear evidence of the clinical validity of PRS testing needs to be consistently demonstrated and replicable. Accuracy of PRS testing is an important factor for both patients and clinicians. A study

of the perspectives of patients towards breast cancer PRS testing found that concern about test inaccuracy was the reason for over a third of women declining the test.[137] Preferences for a polygenic test to estimate cancer risk was assessed in a general Australian population, with respondents found to be more likely to choose a PRS test that was more accurate.[310] Furthermore, patients have expressed an expectation that the PRS test should have an accuracy (of estimating disease risk) of at least 90%.[310,398] This is important given the performance of PRSs across ancestry groups has not yet been demonstrated. Demonstrated accuracy of PRS testing is also an essential element affecting clinicians attitudes towards a glaucoma PRS. Almost 80% of healthcare professionals indicated this was an important factor in our study (Chapter 3). Clear evidence of the efficacy needs to be established and readily communicated to clinicians to assist in gaining their support. While this indicates that accuracy of testing is important to consumers and healthcare professionals, interpreting this in the context of PRS is more complex. PRS is a tool for risk stratification rather than being a diagnostic test, and outcomes are measured on a continuous-scale. A PRS can be quantified and differentiated into binary outcomes using the area under the receiver operating characteristic curve (AUC), however, this does not inform individual risk, particularly for age-related conditions. The utility of the PRS lies in informing risk by identifying those at high-risk compared to low- or average-risk individuals. The outcomes of his thesis include the design and implementation of the GRADE Study, the first study to prospectively assess the efficacy of a glaucoma and AMD PRS to identify high-risk individuals (Chapter 8). While in its early stages, we hypothesise that a higher number of individuals with undiagnosed glaucoma and AMD will be detected in high risk PRS groups, providing evidence for clinical validity of the test.

Polygenic risk scores generated through large GWAS, differ depending on the size and heterogeneity of the sample, and the statistical methods used. There may be subtle or significant differences between PRSs used by different local, national or international stakeholders. Integration will need to accommodate for variability in PRS results due to disparities in the different datasets and methods used by different providers. This will occur regardless of whether PRS testing is Medicare subsidised or not, particularly as consumers can already access PRS results through commercial direct to consumer providers. Furthermore, a PRS developed using largely European genetic data will be less effective in individuals of non-European ancestry. One of the most challenging aspects of moving PRS to the clinical arena is ensuring that they are equally effective across ancestral groups, to avoid exacerbating health disparities that already exist. Current PRS methods rely on an individual's genetic ancestry being similar to the GWAS studies

from which the PRS was developed, and may require access to an ancestry-matched genotype-level reference panel. Such studies are currently only widely available in European ancestries.[399] Although a well-known weakness, the evidence base in non-European populations remains limited and must be addressed as part of ensuring equitable access to PRS testing. Previous studies have primarily focused on assessing the clinical utility and perspectives towards PRS testing for a single disease.[137,193,352,400] PRS performance in non-European ancestries has been shown to be lower, however still significant, and suggested that differences in associated genetic variants between ancestries may be suggestive of differences in disease aetiology.[401] The lack of diversity in the studies remains to be a key shortfall.[402] Further studies are needed to assess and improve PRS performance for all ancestral populations.

As a novel concept, care must be taken in the way the PRS testing is handled. It may be difficult to ensure PRS testing is understood to be a screening test rather than a diagnostic test, and therefore used as an additional risk assessment tool in combination with other factors. Accreditation of tests, performed by the National Association of Testing Authorities (NATA) in Australia, does not currently recognise this difference.[403] Recognition of the clinical utility of PRS testing needs to be interpreted within the scope of this technology to ensure appropriate usage and clinical application.

Once approved for clinical use, streamlined testing pathways are another critically important consideration. This includes referral recommendations and pathways; payment or reimbursement systems; test administration communication channels between test providers, patients, and clinicians; test reporting; and post-test risk-based recommendations and pathways. Integration challenges may vary between healthcare systems, and will need to be identified and overcome relevant to that system. Future testing may become more complex as technology advances and becomes more cost effective, whereby individuals could be screened for a multitude of conditions, either in parallel or sequentially, using a single sample.

Perhaps most importantly, clear guidelines need to be developed, encompassing recommendations for appropriate patient selection and direction for monitoring and treatment, tailored to each risk group. This will allow for the delivery of this personalised approach to be streamlined, and help address some of the influence of variance in confidence and knowledge of PRS or the condition being tested. An emphasis on education and availability of resources to help clinicians improve their knowledge and become more familiar with polygenic risk testing will be a

key factor to the success of clinical implementation. This will require change to university medical school curriculums, and investment in providing education to fully qualified clinicians.

Conclusion

Glaucoma encompasses a spectrum of optic neuropathies with recognised high heritability. Despite advances in our understanding of the genetic underpinnings of glaucoma, the path towards clinically applying this knowledge is not yet clear. Significant knowledge gaps surrounding acceptance and barriers to clinical implementation exist. The qualitative, cross-sectional and prospective studies included in this thesis have addressed several of these gaps, providing the first evidence to assist in progressing glaucoma PRS testing towards clinical implementation. My original contribution to knowledge included providing the first indication of the attitudes and perspectives of several groups towards genetic testing for glaucoma, factors affecting uptake of testing, and investigating methods to report PRS results for glaucoma. In addition, I have investigated the clinical validity of glaucoma PRS testing in various clinical subsets, further strengthening the evidence for its utility.

REFERENCES

1. Casson RJ, Chidlow G, Wood JPM, Crowston JG, Goldberg I. Definition of glaucoma: clinical and experimental concepts. *Clin Experiment Ophthalmol*. 2012;40(4):341-349.

2. Porciatti V, Ventura LM. Retinal ganglion cell functional plasticity and optic neuropathy: a comprehensive model. *J Neuroophthalmol*. 2012;32(4):354-358.

3. Shiga Y, Aizawa N, Tsuda S, et al. Preperimetric Glaucoma Prospective Study (PPGPS): Predicting Visual Field Progression With Basal Optic Nerve Head Blood Flow in Normotensive PPG Eyes. *Transl Vis Sci Technol*. 2018;7(1):11.

4. Hollows FC, Graham PA. Intra-ocular pressure, glaucoma, and glaucoma suspects in a defined population. *Br J Ophthalmol*. 1966;50(10):570-586.

5. Tham YC, Li X, Wong TY, Quigley HA, Aung T, Cheng CY. Global prevalence of glaucoma and projections of glaucoma burden through 2040: a systematic review and meta-analysis. *Ophthalmology*. 2014;121(11):2081-2090.

6. Zeppieri M, Tripathy K. Pigment Dispersion Glaucoma. In: *StatPearls*. Treasure Island (FL): StatPearls Publishing; 2022.

7. Desai MA, Lee RK. The medical and surgical management of pseudoexfoliation glaucoma. *Int Ophthalmol Clin*. 2008;48(4):95-113.

8. Källmark FP, Sakhi M. Evaluation of nasal and temporal anterior chamber angle with four different techniques. *Int J Clin Med*. 2013;04(12):548-555.

9. Quigley HA, Broman AT. The number of people with glaucoma worldwide in 2010 and 2020. *Br J Ophthalmol*. 2006;90(3):262-267.

10. Keel S, Xie J, Foreman J, et al. Prevalence of glaucoma in the Australian National Eye Health Survey. *Br J Ophthalmol*. 2019;103(2):191-195.

11. Zhang N, Wang J, Li Y, Jiang B. Prevalence of primary open angle glaucoma in the last 20 years: a meta-analysis and systematic review. *Sci Rep*. 2021;11(1):1-12.

12. World Health Organisation. *Ageing and Health*. World Health Organisation; 2021.

13. Enoch J, McDonald L, Jones L, Jones PR, Crabb DP. Evaluating Whether Sight Is the Most Valued Sense. *JAMA Ophthalmol*. 2019;137(11):1317-1320.

14. Taylor HR, Pezzullo ML, Keeffe JE. The economic impact and cost of visual impairment in Australia. *Br J Ophthalmol*. 2006;90(3):272-275.

15. Heijl A, Bengtsson B, Hyman L, Leske MC, Early Manifest Glaucoma Trial Group. Natural history of open-angle glaucoma. *Ophthalmology*. 2009;116(12):2271-2276.

16. Kwon YH, Kim CS, Zimmerman MB, Alward WL, Hayreh SS. Rate of visual field loss and long-term visual outcome in primary open-angle glaucoma. *Am J Ophthalmol*. 2001;132(1):47-56.

17. Eid TM, Spaeth GL, Bitterman A, Steinmann WC. Rate and amount of visual loss in 102 patients with open-angle glaucoma followed up for at least 15 years. *Ophthalmology*. 2003;110(5):900-907.

18. Fautsch MP, Johnson DH. Aqueous humor outflow: what do we know? Where will it lead us? *Invest Ophthalmol Vis Sci*. 2006;47(10):4181-4187.

19. Goel M, Picciani RG, Lee RK, Bhattacharya SK. Aqueous humor dynamics: a review. *Open Ophthalmol J*. 2010;4:52-59.

20. Weinreb RN, Aung T, Medeiros FA. The pathophysiology and treatment of glaucoma: a review. *JAMA*. 2014;311(18):1901-1911.

21. Keller KE, Peters DM. Pathogenesis of glaucoma: Extracellular matrix dysfunction in the trabecular meshwork-A review. *Clin Experiment Ophthalmol*. 2022;50(2):163-182.

22. Shoeb Ahmad S, Abdul Ghani S, Hemalata Rajagopal T. Current Concepts in the Biochemical Mechanisms of Glaucomatous Neurodegeneration. *J Curr Glaucoma Pract*. 2013;7(2):49-53.

23. Ahmad SS. Controversies in the vascular theory of glaucomatous optic nerve degeneration. *Taiwan J Ophthalmol*. 2016;6(4):182-186.

24. Kwon YH, Fingert JH, Kuehn MH, Alward WLM. Primary open-angle glaucoma. *N Engl J Med*. 2009;360(11):1113-1124.

25. Wong W, Landis B, Noyce N, Pappas AC. The Vascular Theory of Glaucoma - Quantifying Blood Vessels in Cadaveric Optic Nerve Head Specimens. *Invest Ophthalmol Vis Sci*. 2022;63(7):4026-A0411.

26. Khawaja AP, Crabb DP, Jansonius NM. The role of ocular perfusion pressure in glaucoma cannot be studied with multivariable regression analysis applied to surrogates. *Invest Ophthalmol Vis Sci*. 2013;54(7):4619-4620.

27. Ramdas WD, Wolfs RCW, Hofman A, de Jong PTVM, Vingerling JR, Jansonius NM. Ocular perfusion pressure and the incidence of glaucoma: real effect or artifact? The Rotterdam Study. *Invest Ophthalmol Vis Sci*. 2011;52(9):6875-6881.

28. Bonomi L, Marchini G, Marraffa M, Bernardi P, Morbio R, Varotto A. Vascular risk factors for primary open angle glaucoma: the Egna-Neumarkt Study. *Ophthalmology*. 2000;107(7):1287-1293.

29. Heijl A, Leske MC, Bengtsson B, Bengtsson B, Hussein M, Early Manifest Glaucoma Trial Group. Measuring visual field progression in the Early Manifest Glaucoma Trial. *Acta Ophthalmol Scand*. 2003;81(3):286-293.

30. Leske MC, Heijl A, Hyman L, et al. Predictors of long-term progression in the early manifest glaucoma trial. *Ophthalmology*. 2007;114(11):1965-1972.

31. Burr JM, Mowatt G, Hernández R, et al. The clinical effectiveness and cost-effectiveness of screening for open angle glaucoma: a systematic review and economic evaluation. *Health Technol Assess*. 2007;11(41):iii - iv, ix - x, 1-190.

32. Mitchell P, Smith W, Attebo K, Healey PR. Prevalence of open-angle glaucoma in Australia. The Blue Mountains Eye Study. *Ophthalmology*. 1996;103(10):1661-1669.

33. Weih LM, Nanjan M, McCarty CA, Taylor HR. Prevalence and predictors of open-angle glaucoma: results from the visual impairment project. *Ophthalmology*. 2001;108(11):1966-1972.

34. Coleman AL, Miglior S. Risk factors for glaucoma onset and progression. *Surv Ophthalmol*. 2008;53 Suppl1:S3-S10.

35. Leske MC, Connell AM, Wu SY, Hyman LG, Schachat AP. Risk factors for open-angle glaucoma. The Barbados Eye Study. *Arch Ophthalmol*. 1995;113(7):918-924.

36. Brandt JD, Beiser JA, Kass MA, Gordon MO. Central corneal thickness in the Ocular Hypertension Treatment Study (OHTS). *Ophthalmology*. 2001;108(10):1779-1788.

37. Nemesure B, Wu SY, Hennis A, Leske MC, Barbados Eye Studies Group. Factors related to the 4-year risk of high intraocular pressure: the Barbados Eye Studies. *Arch Ophthalmol*. 2003;121(6):856-862.

38. Rochtchina E, Mitchell P, Wang JJ. Relationship between age and intraocular pressure: the Blue Mountains Eye Study. *Clin Experiment Ophthalmol*. 2002;30(3):173-175.

39. Leske MC, Connell AM, Wu SY, Hyman L, Schachat AP. Distribution of intraocular pressure. The Barbados Eye Study. *Arch Ophthalmol*. 1997;115(8):1051-1057.

40. Comparison of glaucomatous progression between untreated patients with normal-tension glaucoma and patients with therapeutically reduced intraocular pressures. Collaborative Normal-Tension Glaucoma Study Group. *Am J Ophthalmol*. 1998;126(4):487-497.

41. Wolfs RC, Klaver CC, Ramrattan RS, van Duijn CM, Hofman A, de Jong PT. Genetic risk of primary open-angle glaucoma. Population-based familial aggregation study. *Arch Ophthalmol*. 1998;116(12):1640-1645.

42. Tielsch JM, Katz J, Sommer A, Quigley HA, Javitt JC. Family history and risk of primary open angle glaucoma. The Baltimore Eye Survey. *Arch Ophthalmol*. 1994;112(1):69-73.

43. Wang K, Gaitsch H, Poon H, Cox NJ, Rzhetsky A. Classification of common human diseases derived from shared genetic and environmental determinants. *Nat Genet*. 2017;49(9):1319-1325.

44. Kong X, Zhu W, Chen X, Chen Y, Sun X. Familial aggregation of primary open angle glaucoma in Shanghai, China. *Mol Vis*. 2013;19:1859-1865.

45. Kong X, Chen Y, Chen X, Sun X. Influence of family history as a risk factor on primary angle closure and primary open angle glaucoma in a Chinese population. *Ophthalmic Epidemiol*. 2011;18(5):226-232.

46. Nguyen RL, Raja SC, Traboulsi EI. Screening relatives of patients with familial chronic open angle glaucoma. *Ophthalmology*. 2000;107(7):1294-1297.

47. Green CM, Kearns LS, Wu J, et al. How significant is a family history of glaucoma? Experience from the Glaucoma Inheritance Study in Tasmania. *Clin Experiment*

Ophthalmol. 2007;35(9):793-799.

48. Gramer G, Weber BHF, Gramer E. Results of a patient-directed survey on frequency of family history of glaucoma in 2170 patients. *Invest Ophthalmol Vis Sci*. 2014;55(1):259-264.

49. Friedman DS, Wolfs RCW, O'Colmain BJ, et al. Prevalence of open-angle glaucoma among adults in the United States. *Arch Ophthalmol*. 2004;122(4):532-538.

50. Kapetanakis VV, Chan MPY, Foster PJ, Cook DG, Owen CG, Rudnicka AR. Global variations and time trends in the prevalence of primary open angle glaucoma (POAG): a systematic review and meta-analysis. *Br J Ophthalmol*. 2016;100(1):86-93.

51. Jonas JB, Aung T, Bourne RR, Bron AM, Ritch R, Panda-Jonas S. Glaucoma. *Lancet*. 2017;390(10108):2183-2193.

52. La Rosa FA, Gross RL, Orengo-Nania S. Central corneal thickness of Caucasians and African Americans in glaucomatous and nonglaucomatous populations. *Arch Ophthalmol*. 2001;119(1):23-27.

53. Gordon MO, Beiser JA, Brandt JD, et al. The Ocular Hypertension Treatment Study: baseline factors that predict the onset of primary open-angle glaucoma. *Arch Ophthalmol*. 2002;120(6):714-720; discussion 829-830.

54. Kass MA, Heuer DK, Higginbotham EJ, et al. The Ocular Hypertension Treatment Study: a randomized trial determines that topical ocular hypotensive medication delays or prevents the onset of primary open-angle glaucoma. *Arch Ophthalmol*. 2002;120(6):701-713; discussion 829-830.

55. Leske MC, Heijl A, Hyman L, Bengtsson B. Early Manifest Glaucoma Trial: design and baseline data. *Ophthalmology*. 1999;106(11):2144-2153.

56. Marcus MW, de Vries MM, Junoy Montolio FG, Jansonius NM. Myopia as a risk factor for open-angle glaucoma: a systematic review and meta-analysis. *Ophthalmology*. 2011;118(10):1989-1994.e2.

57. Wong TY, Klein BEK, Klein R, Knudtson M, Lee KE. Refractive errors, intraocular pressure, and glaucoma in a white population. *Ophthalmology*. 2003;110(1):211-217.

58. Grødum K, Heijl A, Bengtsson B. Refractive error and glaucoma. *Acta Ophthalmol Scand*. 2001;79(6):560-566.

59. Xu L, Wang Y, Wang S, Wang Y, Jonas JB. High myopia and glaucoma susceptibility the Beijing Eye Study. *Ophthalmology*. 2007;114(2):216-220.

60. Mitchell P, Hourihan F, Sandbach J, Wang JJ. The relationship between glaucoma and myopia: the Blue Mountains Eye Study. *Ophthalmology*. 1999;106(10):2010-2015.

61. Sohn SW, Song JS, Kee C. Influence of the extent of myopia on the progression of normal-tension glaucoma. *Am J Ophthalmol*. 2010;149(5):831-838.

62. Lee JY, Sung KR, Han S, Na JH. Effect of Myopia on the Progression of Primary Open-Angle Glaucoma. *Invest Ophthalmol Vis Sci*. 2015;56(3):1775-1781.

63. Doshi A, Kreidl KO, Lombardi L, Sakamoto DK, Singh K. Nonprogressive glaucomatous cupping and visual field abnormalities in young Chinese males. *Ophthalmology*. 2007;114(3):472-479.

64. Detry-Morel M. [Is myopia a risk factor for glaucoma?]. *J Fr Ophtalmol*. 2011;34(6):392-395.

65. Verhoeven VJM, Wong KT, Buitendijk GHS, Hofman A, Vingerling JR, Klaver CCW. Visual consequences of refractive errors in the general population. *Ophthalmology*. 2015;122(1):101-109.

66. Shukla AG, Sirinek PE, De Moraes CG, et al. Disc Hemorrhages Are Associated With the Presence and Progression of Glaucomatous Central Visual Field Defects. *J Glaucoma*. 2020;29(6):429-434.

67. Siegner SW, Netland PA. Optic disc hemorrhages and progression of glaucoma. *Ophthalmology*. 1996;103(7):1014-1024.

68. Begg IS, Drance SM, Sweeney VP. Ischaemic optic neuropathy in chronic simple glaucoma. *Br J Ophthalmol*. 1971;55(2):73-90.

69. Drance SM. Disc hemorrhages in the glaucomas. *Surv Ophthalmol*. 1989;33(5):331-337.

70. Founti P, Bunce C, Khawaja AP, et al. Risk Factors for Visual Field Deterioration in the United Kingdom Glaucoma Treatment Study. *Ophthalmology*. 2020;127(12):1642-1651.

71. Akagi T, Saunders LJ, Shoji T, et al. Association between Rates of Retinal Nerve Fiber Layer Thinning and Previous Disc Hemorrhage in Glaucoma. *Ophthalmol Glaucoma*. 2018;1(1):23-31.

72. Ocular Hypertension Treatment Study Group, European Glaucoma Prevention Study Group, Gordon MO, et al. Validated prediction model for the development of primary open-angle glaucoma in individuals with ocular hypertension. *Ophthalmology*. 2007;114(1):10-19.

73. Tonnu PA, Ho T, Newson T, El Sheikh A. The influence of central corneal thickness and age on intraocular pressure measured by pneumotonometry, non-contact tonometry, the Tono-Pen XL, and Goldmann …. *British Journal of*. 2005. https://bjo.bmj.com/content/89/7/851.short.

74. Qassim A, Mullany S, Abedi F, et al. Corneal Stiffness Parameters Are Predictive of Structural and Functional Progression in Glaucoma Suspect Eyes. *Ophthalmology*. 2021;128(7):993-1004.

75. Bae HW, Lee N, Lee HS, Hong S, Seong GJ, Kim CY. Systemic hypertension as a risk factor for open-angle glaucoma: a meta-analysis of population-based studies. *PLoS One*. 2014;9(9):e108226.

76. Mitchell P, Lee AJ, Rochtchina E, Wang JJ. Open-angle glaucoma and systemic hypertension: the blue mountains eye study. *J Glaucoma*. 2004;13(4):319-326.

77. Langman MJS, Lancashire RJ, Cheng KK, Stewart PM. Systemic hypertension and glaucoma: mechanisms in common and co-occurrence. *Br J Ophthalmol*. 2005;89(8):960-963.

78. Chan TCW, Bala C, Siu A, Wan F, White A. Risk Factors for Rapid Glaucoma Disease Progression. *Am J Ophthalmol*. 2017;180:151-157.

79. Marshall H, Mullany S, Qassim A, et al. Cardiovascular Disease Predicts Structural and Functional Progression in Early Glaucoma. *Ophthalmology*. 2021;128(1):58-69.

80. National Health and Medical Research Council. NHMRC Guidelines for the Screening, Prognosis, Diagnosis, Management and Prevention of Glaucoma. 2010:1-181. https://www.nhmrc.gov.au/about-us/publications/guidelines-screening-prognosis-diagnosis-management-and-prevention-glaucoma.

81. Tamura H, Akune Y, Hiratsuka Y, et al. Real-world effectiveness of screening programs for age-related macular degeneration: amended Japanese specific health checkups and augmented screening programs with OCT or AI. *Jpn J Ophthalmol*. 2022;66(1):19-32.

82. The first Toolkit for Glaucoma Management in Sub-Saharan Africa. The International Agency for the Prevention of Blindness. https://www.iapb.org/learn/resources/the-first-toolkit-for-glaucoma-management-in-sub-saharan-africa/. Published February 11, 2021. Accessed March 27, 2024.

83. Lee PP. Glaucoma Screening Standards. American Academy of Ophthalmology. www.aao.org/education/interview/glaucoma-screeningstandards. Published March 5, 2015. Accessed October 2023.

84. Chou R, Selph S, Blazina I, et al. Screening for Glaucoma in Adults: Updated Evidence Report and Systematic Review for the US Preventive Services Task Force. *JAMA*. 2022;327(20):1998-2012.

85. Hamid S, Desai P, Hysi P, Burr JM, Khawaja AP. Population screening for glaucoma in UK: current recommendations and future directions. *Eye* . 2022;36(3):504-509.

86. European glaucoma society terminology and guidelines for glaucoma, 5th edition. *Br J Ophthalmol*. 2021;105(Suppl 1):1-169.

87. Topouzis, F., Healey R.P., Weinreb R.N. WGA 5th Consensus Meething: Glaucoma Screening. Amsterdam: Kugler Publications; 2008. In: Kugler Publications; 2008.

88. Leske MC, Heijl A, Hussein M, et al. Factors for glaucoma progression and the effect of treatment: the early manifest glaucoma trial. *Arch Ophthalmol*. 2003;121(1):48-56.

89. Khawaja AP, Viswanathan AC. Are we ready for genetic testing for primary open-angle glaucoma? *Eye* . 2018;32(5):877-883.

90. Stone EM, Fingert JH, Alward WL, et al. Identification of a gene that causes primary open angle glaucoma. *Science*. 1997;275(5300):668-670.

91. Fingert JH. Primary open-angle glaucoma genes. *Eye* . 2011;25(5):587-595.

92. Jacobson N, Andrews M, Shepard AR, et al. Non-secretion of mutant proteins of the glaucoma gene myocilin in cultured trabecular meshwork cells and in aqueous humor. *Hum Mol Genet*. 2001;10(2):117-125.

93. Joe MK, Sohn S, Hur W, Moon Y, Choi YR, Kee C. Accumulation of mutant myocilins in ER

leads to ER stress and potential cytotoxicity in human trabecular meshwork cells. *Biochem Biophys Res Commun*. 2003;312(3):592-600.

94. Gobeil S, Rodrigue MA, Moisan S, et al. Intracellular sequestration of hetero-oligomers formed by wild-type and glaucoma-causing myocilin mutants. *Invest Ophthalmol Vis Sci*. 2004;45(10):3560-3567.

95. Liu Y, Vollrath D. Reversal of mutant myocilin non-secretion and cell killing: implications for glaucoma. *Hum Mol Genet*. 2004;13(11):1193-1204.

96. Souzeau E, Tram KH, Witney M, et al. Myocilin Predictive Genetic Testing for Primary Open-Angle Glaucoma Leads to Early Identification of At-Risk Individuals. *Ophthalmology*. 2017;124(3):303-309.

97. Shimizu S, Lichter PR, Johnson AT, et al. Age-dependent prevalence of mutations at the GLC1A locus in primary open-angle glaucoma. *Am J Ophthalmol*. 2000;130(2):165-177.

98. Vincent AL, Billingsley G, Buys Y, et al. Digenic inheritance of early-onset glaucoma: CYP1B1, a potential modifier gene. *Am J Hum Genet*. 2002;70(2):448-460.

99. Wiggs JL, Allingham RR, Vollrath D, et al. Prevalence of mutations in TIGR/Myocilin in patients with adult and juvenile primary open-angle glaucoma. *Am J Hum Genet*. 1998;63(5):1549-1552.

100. Souzeau E, Burdon KP, Dubowsky A, et al. Higher prevalence of myocilin mutations in advanced glaucoma in comparison with less advanced disease in an Australasian disease registry. *Ophthalmology*. 2013;120(6):1135-1143.

101. Chalasani MLS, Kumari A, Radha V, Swarup G. E50K-OPTN-induced retinal cell death involves the Rab GTPase-activating protein, TBC1D17 mediated block in autophagy. *PLoS One*. 2014;9(4):e95758.

102. Sarfarazi M, Rezaie T. Optineurin in primary open angle glaucoma. *Ophthalmol Clin North Am*. 2003;16(4):529-541.

103. VanderWall KB, Huang KC, Pan Y, et al. Retinal Ganglion Cells With a Glaucoma OPTN(E50K) Mutation Exhibit Neurodegenerative Phenotypes when Derived from Three-Dimensional Retinal Organoids. *Stem Cell Reports*. 2020;15(1):52-66.

104. Aung T, Ebenezer ND, Brice G, et al. Prevalence of optineurin sequence variants in adult primary open angle glaucoma: implications for diagnostic testing. *J Med Genet*. 2003;40(8):e101.

105. Fingert JH, Robin AL, Stone JL, et al. Copy number variations on chromosome 12q14 in patients with normal tension glaucoma. *Hum Mol Genet*. 2011;20(12):2482-2494.

106. Morton S, Hesson L, Peggie M, Cohen P. Enhanced binding of TBK1 by an optineurin mutant that causes a familial form of primary open angle glaucoma. *FEBS Lett*. 2008;582(6):997-1002.

107. Minegishi Y, Iejima D, Kobayashi H, et al. Enhanced optineurin E50K-TBK1 interaction evokes protein insolubility and initiates familial primary open-angle glaucoma. *Hum Mol Genet*. 2013;22(17):3559-3567.

108. Craig JE, Han X, Qassim A, et al. Multitrait analysis of glaucoma identifies new risk loci and enables polygenic prediction of disease susceptibility and progression. *Nat Genet*. 2020;52(2):160-166.

109. MacGregor S, Ong JS, An J, et al. Genome-wide association study of intraocular pressure uncovers new pathways to glaucoma. *Nat Genet*. 2018;50(8):1067-1071.

110. Qassim A, Souzeau E, Siggs OM, et al. An Intraocular Pressure Polygenic Risk Score Stratifies Multiple Primary Open-Angle Glaucoma Parameters Including Treatment Intensity. *Ophthalmology*. 2020;127(7):901-907.

111. Springelkamp H, Iglesias AI, Mishra A, et al. New insights into the genetics of primary open-angle glaucoma based on meta-analyses of intraocular pressure and optic disc characteristics. *Hum Mol Genet*. 2017;26(2):438-453.

112. Gao XR, Huang H, Kim H. Polygenic Risk Score Is Associated With Intraocular Pressure and Improves Glaucoma Prediction in the UK Biobank Cohort. *Transl Vis Sci Technol*. 2019;8(2):10.

113. Thorleifsson G, Walters GB, Hewitt AW, et al. Common variants near CAV1 and CAV2 are associated with primary open-angle glaucoma. *Nat Genet*. 2010;42(10):906-909.

114. Burdon KP, Macgregor S, Hewitt AW, et al. Genome-wide association study identifies susceptibility loci for open angle glaucoma at TMCO1 and CDKN2B-AS1. *Nat Genet*. 2011;43(6):574-578.

115. Gharahkhani P, Burdon KP, Fogarty R, et al. Common variants near ABCA1, AFAP1 and GMDS confer risk of primary open-angle glaucoma. *Nat Genet*. 2014;46(10):1120-1125.

116. Chen Y, Hughes G, Chen X, et al. Genetic Variants Associated With Different Risks for High Tension Glaucoma and Normal Tension Glaucoma in a Chinese Population. *Invest Ophthalmol Vis Sci*. 2015;56(4):2595-2600.

117. Springelkamp H, Iglesias AI, Cuellar-Partida G, et al. ARHGEF12 influences the risk of glaucoma by increasing intraocular pressure. *Hum Mol Genet*. 2015;24(9):2689-2699.

118. Bailey JNC, Loomis SJ, Kang JH, et al. Genome-wide association analysis identifies TXNRD2, ATXN2 and FOXC1 as susceptibility loci for primary open-angle glaucoma. *Nat Genet*. 2016;48(2):189-194.

119. Gharahkhani P, Jorgenson E, Hysi P, et al. Genome-wide meta-analysis identifies 127 open-angle glaucoma loci with consistent effect across ancestries. *Nat Commun*. 2021;12(1):1-16.

120. Han X, Gharahkhani P, Hamel AR, et al. Large-scale multitrait genome-wide association analyses identify hundreds of glaucoma risk loci. *Nat Genet*. 2023;55(7):1116-1125.

121. Khawaja AP, Cooke Bailey JN, Wareham NJ, et al. Genome-wide analyses identify 68 new loci associated with intraocular pressure and improve risk prediction for primary open-angle glaucoma. *Nat Genet*. 2018;50(6):778-782.

122. Lewis CM, Vassos E. Prospects for using risk scores in polygenic medicine. *Genome*

Med. 2017;9(1):96.

123. Ramdas WD, Amin N, van Koolwijk LME, et al. Genetic architecture of open angle glaucoma and related determinants. *J Med Genet*. 2011;48(3):190-196.

124. Tham YC, Liao J, Vithana EN, et al. Aggregate Effects of Intraocular Pressure and Cup-to-Disc Ratio Genetic Variants on Glaucoma in a Multiethnic Asian Population. *Ophthalmology*. 2015;122(6):1149-1157.

125. Mabuchi F, Mabuchi N, Sakurada Y, et al. Additive effects of genetic variants associated with intraocular pressure in primary open-angle glaucoma. *PLoS One*. 2017;12(8):e0183709.

126. Nannini DR, Kim H, Fan F, Gao X. Genetic Risk Score Is Associated with Vertical Cup-to-Disc Ratio and Improves Prediction of Primary Open-Angle Glaucoma in Latinos. *Ophthalmology*. 2018;125(6):815-821.

127. Aschard H, Kang JH, Iglesias AI, et al. Genetic correlations between intraocular pressure, blood pressure and primary open-angle glaucoma: a multi-cohort analysis. *Eur J Hum Genet*. 2017;25(11):1261-1267.

128. Siggs OM, Han X, Qassim A, et al. Association of Monogenic and Polygenic Risk With the Prevalence of Open-Angle Glaucoma. *JAMA Ophthalmol*. July 2021. doi:10.1001/jamaophthalmol.2021.2440

129. Siggs OM, Qassim A, Han X, et al. Association of High Polygenic Risk With Visual Field Worsening Despite Treatment in Early Primary Open-Angle Glaucoma. *JAMA Ophthalmol*. 2022;141(1):73-77.

130. Khera AV, Chaffin M, Aragam KG, et al. Genome-wide polygenic scores for common diseases identify individuals with risk equivalent to monogenic mutations. *Nat Genet*. 2018;50(9):1219-1224.

131. Deborah J. Thompson, Daniel Wells, Saskia Selzam, Iliana Peneva, Rachel Moore, Kevin Sharp, William A. Tarran, Edward J. Beard, Fernando Riveros-Mckay, Duncan Palmer, Priyanka Seth, James Harrison, Marta Futema, Genomics England Research Consortium, Gil McVean, Vincent Plagnol, Peter Donnelly, Michael E. Weale. UK Biobank release and systematic evaluation of optimised polygenic risk scores for 53 diseases and quantitative traits. *medRxiv 2022061622276246*. doi:10.1101/2022.06.16.22276246

132. Mavaddat N, Michailidou K, Dennis J, et al. Polygenic Risk Scores for Prediction of Breast Cancer and Breast Cancer Subtypes. *Am J Hum Genet*. 2019;104(1):21-34.

133. Mistry S, Harrison JR, Smith DJ, Escott-Price V, Zammit S. The use of polygenic risk scores to identify phenotypes associated with genetic risk of schizophrenia: Systematic review. *Schizophr Res*. 2018;197:2-8.

134. Khera AV, Emdin CA, Drake I, et al. Genetic Risk, Adherence to a Healthy Lifestyle, and Coronary Disease. *N Engl J Med*. 2016;375(24):2349-2358.

135. Choquet H, Wiggs JL, Khawaja AP. Clinical implications of recent advances in primary open-angle glaucoma genetics. *Eye* . 2020;34(1):29-39.

136. Qassim A, Souzeau E, Hollitt G, Hassall MM, Siggs OM, Craig JE. Risk Stratification and Clinical Utility of Polygenic Risk Scores in Ophthalmology. *Transl Vis Sci Technol*. 2021;10(6):14.

137. Yanes T, Meiser B, Kaur R, et al. Uptake of polygenic risk information among women at increased risk of breast cancer. *Clin Genet*. 2020;97(3):492-501.

138. Keogh LA, van Vliet CM, Studdert DM, et al. Is uptake of genetic testing for colorectal cancer influenced by knowledge of insurance implications? *Med J Aust*. 2009;191(5):255-258.

139. Dusic EJ, Theoryn T, Wang C, Swisher EM, Bowen DJ, EDGE Study Team. Barriers, interventions, and recommendations: Improving the genetic testing landscape. *Front Digit Health*. 2022;4:961128.

140. Gollust SE, Gordon ES, Zayac C, et al. Motivations and perceptions of early adopters of personalized genomics: perspectives from research participants. *Public Health Genomics*. 2012;15(1):22-30.

141. Pereira S, Muñoz KA, Small BJ, et al. Psychiatric polygenic risk scores: Child and adolescent psychiatrists' knowledge, attitudes, and experiences. *Am J Med Genet B Neuropsychiatr Genet*. 2022;189(7-8):293-302.

142. Yu MWC, Fung JLF, Ng APP, et al. Preparing genomic revolution: Attitudes, clinical practice, and training needs in delivering genetic counseling in primary care in Hong Kong and Shenzhen, China. *Mol Genet Genomic Med*. 2021;9(7):e1702.

143. Ayoub A, Lapointe J, Nabi H, Pashayan N. Risk-Stratified Breast Cancer Screening Incorporating a Polygenic Risk Score: A Survey of UK General Practitioners' Knowledge and Attitudes. *Genes* . 2023;14(3). doi:10.3390/genes14030732

144. Souzeau E, Goldberg I, Healey PR, et al. Australian and New Zealand Registry of Advanced Glaucoma: methodology and recruitment. *Clin Experiment Ophthalmol*. 2012;40(6):569-575.

145. Kass, Heuer, Higginbotham. The Ocular Hypertension Treatment Study: a randomized trial determines that topical ocular hypotensive medication delays or prevents the onset of primary open …. *Arch nat conserv landsc res*. https://jamanetwork.com/journals/jamaophthalmology/article-abstract/270953.

146. McNaught AI, Allen JG, Healey DL, et al. Accuracy and implications of a reported family history of glaucoma: experience from the Glaucoma Inheritance Study in Tasmania. *Arch Ophthalmol*. 2000;118(7):900-904.

147. Souzeau E, Glading J, Keane M, et al. Predictive genetic testing experience for myocilin primary open-angle glaucoma using the Australian and New Zealand Registry of Advanced Glaucoma. *Genet Med*. 2014;16(7):558-563.

148. Bottorff JL, Ratner PA, Balneaves LG, et al. Women's interest in genetic testing for breast cancer risk: the influence of sociodemographics and knowledge. *Cancer Epidemiol Biomarkers Prev*. 2002;11(1):89-95.

149. Anderson AE, Flores KG, Boonyasiriwat W, et al. Interest and informational preferences regarding genomic testing for modest increases in colorectal cancer risk. *Public Health Genomics*. 2014;17(1):48-60.

150. Bruno M, Digennaro M, Tommasi S, et al. Attitude towards genetic testing for breast cancer susceptibility: a comparison of affected and unaffected women. *Eur J Cancer Care* . 2010;19(3):360-368.

151. Lerman C, Seay J, Balshem A, Audrain J. Interest in genetic testing among first-degree relatives of breast cancer patients. *Am J Med Genet*. 1995;57(3):385-392.

152. Torkamani A, Wineinger NE, Topol EJ. The personal and clinical utility of polygenic risk scores. *Nat Rev Genet*. 2018;19(9):581-590.

153. Wray NR, Lin T, Austin J, et al. From Basic Science to Clinical Application of Polygenic Risk Scores: A Primer. *JAMA Psychiatry*. 2021;78(1):101-109.

154. Li J, Lee PP, Buckley S, Stinnett SS, Allingham RR, Challa P. Attitudes Toward Genetic Testing For Glaucoma: A Survey of Patients. *Invest Ophthalmol Vis Sci*. 2002;43(13):1077-1077.

155. Healey DL, Craig JE, Wilkinson CH, Stone EM, Mackey DA. Attitudes to predictive DNA testing for myocilin glaucoma: experience with a large Australian family. *J Glaucoma*. 2004;13(4):304-311.

156. Madlensky L, Vierkant RA, Vachon CM, et al. Preventive health behaviors and familial breast cancer. *Cancer Epidemiol Biomarkers Prev*. 2005;14(10):2340-2345.

157. Madlensky L, Flatt SW, Bardwell WA, Rock CL, Pierce JP, WHEL Study group. Is family history related to preventive health behaviors and medical management in breast cancer patients? *Breast Cancer Res Treat*. 2005;90(1):47-54.

158. Ochoa EM, Gómez-Acebo I, Rodríguez-Cundín P, Navarro-Córdoba M, Llorca J, Dierssen-Sotos T. Relationship between family history of breast cancer and health-related behavior. *Behav Med*. 2010;36(4):123-129.

159. Zebardast N, Sekimitsu S, Wang J, et al. Characteristics of Gln368Ter Myocilin Variant and Influence of Polygenic Risk on Glaucoma Penetrance in the UK Biobank. *Ophthalmology*. March 2021. doi:10.1016/j.ophtha.2021.03.007

160. Martínez-Ochoa E, Gómez-Acebo I, Beunza JJ, et al. Influence of family history of colorectal cancer on health behavior and performance of early detection procedures: the SUN Project. *Ann Epidemiol*. 2012;22(7):511-519.

161. Ceballos RM, Newcomb PA, Beasley JM, Peterson S, Templeton A, Hunt JR. Colorectal cancer cases and relatives of cases indicate similar willingness to receive and disclose genetic information. *Genet Test*. 2008;12(3):415-420.

162. Petersen GM, Larkin E, Codori AM, et al. Attitudes toward colon cancer gene testing: survey of relatives of colon cancer patients. *Cancer Epidemiol Biomarkers Prev*. 1999;8(4 Pt 2):337-344.

163. Courtney RJ, Paul CL, Carey ML, et al. A population-based cross-sectional study of

colorectal cancer screening practices of first-degree relatives of colorectal cancer patients. *BMC Cancer*. 2013;13:13.

164. Nusbaum R, Leventhal KG, Hooker GW, et al. Translational Genomic Research: Protocol Development and Initial Outcomes following SNP Testing for Colon Cancer Risk. *Transl Behav Med*. 2013;3(1):17-29.

165. Bronner K, Mesters I, Weiss-Meilnik A, et al. Do individuals with a family history of colorectal cancer adhere to medical recommendations for the prevention of colorectal cancer? *Familial Cancer*. 2013;12(4):629-637. doi:10.1007/s10689-013-9627-x

166. Hollands GJ, French DP, Griffin SJ, et al. The impact of communicating genetic risks of disease on risk-reducing health behaviour: systematic review with meta-analysis. *BMJ*. 2016;352:i1102.

167. World Health Organisation. Geneva. WHO Recommendations on the Diagnosis of HIV Infection in Infants and Children. 2010. https://www.ncbi.nlm.nih.gov/books/NBK138555/.

168. Hollitt GL, Siggs OM, Ridge B, et al. Attitudes towards polygenic risk testing in individuals with glaucoma. *Ophthalmol Glaucoma*. November 2021. doi:10.1016/j.ogla.2021.11.002

169. Moyer VA, U.S. Preventive Services Task Force. Screening for glaucoma: U.S. Preventive Services Task Force Recommendation Statement. *Ann Intern Med*. 2013;159(7):484-489.

170. The Royal College of Ophthalmologists Clinical Council for Eye Health Commissioning. *Commissioning Guide: Glaucoma (Recommendations)*. The Royal College of Ophthalmologists; 2016.

171. Dunlop K, Rankin NM, Smit AK, et al. Acceptability of risk-stratified population screening across cancer types: Qualitative interviews with the Australian public. *Health Expect*. 2021;24(4):1326-1336.

172. Taylor LC, Hutchinson A, Law K, Shah V, Usher-Smith JA, Dennison RA. Acceptability of risk stratification within population-based cancer screening from the perspective of the general public: A mixed-methods systematic review. *Health Expect*. 2023;26(3):989-1008.

173. Culler DD, Silberg J, Vanner-Nicely L, Ware JL, Jackson-Cook C, Bodurtha J. Factors Influencing Men's Interest in Gene Testing for Prostate Cancer Susceptibility. *J Genet Couns*. 2002;11(5):383-398.

174. Takeshima T, Okayama M, Ae R, Harada M, Kajii E. Influence of family history on the willingness of outpatients to undergo genetic testing for salt-sensitive hypertension: a cross-sectional study. *BMJ Open*. 2017;7(7):e016322.

175. Willis AM, Smith SK, Meiser B, et al. Influence of lived experience on risk perception among women who received a breast cancer polygenic risk score: "Another piece of the pie." *J Genet Couns*. 2021;30(3):849-860.

176. Austin JC. Re-conceptualizing risk in genetic counseling: implications for clinical practice. *J Genet Couns*. 2010;19(3):228-234.

177. Sivell S, Elwyn G, Gaff CL, et al. How risk is perceived, constructed and interpreted by clients in clinical genetics, and the effects on decision making: systematic review. *J Genet Couns*. 2008;17(1):30-63.

178. Tracy KA, Quillin JM, Wilson DB, et al. The impact of family history of breast cancer and cancer death on women's mammography practices and beliefs. *Genet Med*. 2008;10(8):621-625.

179. Walter FM, Emery J, Braithwaite D, Marteau TM. Lay understanding of familial risk of common chronic diseases: a systematic review and synthesis of qualitative research. *Ann Fam Med*. 2004;2(6):583-594.

180. Fraser L, Bramald S, Chapman C, et al. What motivates interest in attending a familial cancer genetics clinic? *Fam Cancer*. 2003;2(3-4):159-168.

181. Andrykowski MA, Munn RK, Studts JL. Interest in learning of personal genetic risk for cancer: a general population survey. *Prev Med*. 1996;25(5):527-536.

182. Murphy J, Scott J, Kaufman D, Geller G, LeRoy L, Hudson K. Public perspectives on informed consent for biobanking. *Am J Public Health*. 2009;99(12):2128-2134.

183. Hollitt GL, Siggs OM, Ridge B, et al. Attitudes towards glaucoma genetic risk assessment in unaffected individuals. *Transl Vis Sci Technol*. 2022;11(10):38.

184. Hann KEJ, Fraser L, Side L, et al. Health care professionals' attitudes towards population-based genetic testing and risk-stratification for ovarian cancer: a cross-sectional survey. *BMC Womens Health*. 2017;17(1):132.

185. Nippert I, Harris HJ, Julian-Reynier C, et al. Confidence of primary care physicians in their ability to carry out basic medical genetic tasks—a European survey in five countries—Part 1. *J Community Genet*. 2011;2(1):1-11.

186. Smit AK, Sharman AR, Espinoza D, et al. Knowledge, views and expectations for cancer polygenic risk testing in clinical practice: A cross-sectional survey of health professionals. *Clin Genet*. 2021;100(4):430-439.

187. Lapointe J, Buron AC, Mbuya-Bienge C, et al. Polygenic risk scores and risk-stratified breast cancer screening: Familiarity and perspectives of health care professionals. *Genet Med*. 2022;24(11):2380-2388.

188. Lapointe J, Côté JM, Mbuya-Bienge C, et al. Canadian Healthcare Professionals' Views and Attitudes toward Risk-Stratified Breast Cancer Screening. *J Pers Med*. 2023;13(7). doi:10.3390/jpm13071027

189. Falahee M, Simons G, Raza K, Stack RJ. Healthcare professionals' perceptions of risk in the context of genetic testing for the prediction of chronic disease: a qualitative metasynthesis. *J Risk Res*. 2018;21(2):129-166.

190. Genomics Advisory Working Group & Women's Health Committee and approved by the RANZCOG Board and Council. Genetic carrier screening. https://ranzcog.edu.au/wp-content/uploads/2022/05/Genetic-carrier-screeningC-Obs-63New-March-2019_1.pdf.

191. Royal Australian and New Zealand College of Ophthalmologists. Guidelines for the

assessment and management of patients with inherited retinal degenerations (IRD). https://ranzco.edu/wp-content/uploads/2020/05/RANZCO-Guidelines-for-the-assessment-and-management-of-patients-with-inherited-retinal-degenertions-060520.pdf.

192. Smit AK, Newson AJ, Keogh L, et al. GP attitudes to and expectations for providing personal genomic risk information to the public: a qualitative study. *BJGP Open*. 2019;3(1):bjgpopen18X101633.

193. Saya S, McIntosh JG, Winship IM, et al. A Genomic Test for Colorectal Cancer Risk: Is This Acceptable and Feasible in Primary Care? *Public Health Genomics*. 2020;23(3-4):110-121.

194. Benafif S, Ni Raghallaigh H, McGrowder E, et al. The BARCODE1 Pilot: a feasibility study of using germline single nucleotide polymorphisms to target prostate cancer screening. *BJU Int*. July 2021. doi:10.1111/bju.15535

195. Fisher A, Bonner C, Biankin AV, Juraskova I. Factors influencing intention to undergo whole genome screening in future healthcare: a single-blind parallel-group randomised trial. *Prev Med*. 2012;55(5):514-520.

196. Graves KD, Leventhal KG, Nusbaum R, et al. Behavioral and psychosocial responses to genomic testing for colorectal cancer risk. *Genomics*. 2013;102(2):123-130.

197. Stone EM, Aldave AJ, Drack AV, et al. Recommendations for genetic testing of inherited eye diseases: report of the American Academy of Ophthalmology task force on genetic testing. *Ophthalmology*. 2012;119(11):2408-2410.

198. Hollitt GL, Qassim A, Thomson D, et al. Genetic Risk Assessment of Degenerative Eye Disease (GRADE): study protocol of a prospective assessment of polygenic risk scores to predict diagnosis of glaucoma and age-related macular degeneration. *BMC Ophthalmol*. 2023;23(1):431.

199. Fan BJ, Bailey JC, Igo RP Jr, et al. Association of a Primary Open-Angle Glaucoma Genetic Risk Score With Earlier Age at Diagnosis. *JAMA Ophthalmol*. 2019;137(10):1190-1194.

200. Cole BS, Gudiseva HV, Pistilli M, et al. The Role of Genetic Ancestry as a Risk Factor for Primary Open-angle Glaucoma in African Americans. *Invest Ophthalmol Vis Sci*. 2021;62(2):28.

201. Kreft D, Doblhammer G, Guthoff RF, Frech S. Prevalence, incidence, and risk factors of primary open-angle glaucoma - a cohort study based on longitudinal data from a German public health insurance. *BMC Public Health*. 2019;19(1):851.

202. Souzeau E, Glading J, Ridge B, et al. Predictive genetic testing in minors for Myocilin juvenile onset open angle glaucoma. *Clin Genet*. 2015;88(6):584-588.

203. Katapodi MC, Lee KA, Facione NC, Dodd MJ. Predictors of perceived breast cancer risk and the relation between perceived risk and breast cancer screening: a meta-analytic review. *Prev Med*. 2004;38(4):388-402.

204. Ropka ME, Wenzel J, Phillips EK, Siadaty M, Philbrick JT. Uptake rates for breast

cancer genetic testing: a systematic review. *Cancer Epidemiol Biomarkers Prev*. 2006;15(5):840-855.

205. Sweeny K, Ghane A, Legg AM, Huynh HP, Andrews SE. Predictors of genetic testing decisions: a systematic review and critique of the literature. *J Genet Couns*. 2014;23(3):263-288.

206. Persky S, Kaphingst KA, Condit CM, McBride CM. Assessing hypothetical scenario methodology in genetic susceptibility testing analog studies: a quantitative review. *Genet Med*. 2007;9(11):727-738.

207. Wade CH, Shiloh S, Woolford SW, et al. Modelling decisions to undergo genetic testing for susceptibility to common health conditions: an ancillary study of the Multiplex Initiative. *Psychol Health*. 2012;27(4):430-444.

208. Sanderson SC, O'Neill SC, Bastian LA, Bepler G, McBride CM. What can interest tell us about uptake of genetic testing? Intention and behavior amongst smokers related to patients with lung cancer. *Public Health Genomics*. 2010;13(2):116-124.

209. Keogh LA, Niven H, Rutstein A, Flander L, Gaff C, Jenkins M. Choosing not to undergo predictive genetic testing for hereditary colorectal cancer syndromes: expanding our understanding of decliners and declining. *J Behav Med*. 2017;40(4):583-594.

210. Roberts JS. Assessing the Psychological Impact of Genetic Susceptibility Testing. *Hastings Cent Rep*. 2019;49 Suppl 1(Suppl 1):S38-S43.

211. Serr DM, Sachs L, Danon M. The diagnosis of sex before birth using cells from the amniotic fluid (a preliminary report). *Bull Res Counc Isr*. 1955;5B(2):137-138.

212. Jacobs C, Turbitt E, McEwen A, Atkins L. Australasian Genetic Counselors' Perceptions of Their Role in Supporting Clients' Behavior Change. *J Pers Med*. 2022;13(1). doi:10.3390/jpm13010030

213. Forrest LE, Sawyer SD, Hallowell N, James PA, Young MA. High-risk women's risk perception after receiving personalized polygenic breast cancer risk information. *J Community Genet*. 2019;10(2):197-206.

214. Davis KW, Hamby Erby L, Fiallos K, Martin M, Wassman ER. A comparison of genomic laboratory reports and observations that may enhance their clinical utility for providers and patients. *Mol Genet Genomic Med*. 2019;7(7):e00551.

215. Hudson B, Toop L, Mangin D, Pearson J. Risk communication methods in hip fracture prevention: a randomised trial in primary care. *Br J Gen Pract*. 2011;61(589):e469-e476.

216. Noordzij M, van Diepen M, Caskey FC, Jager KJ. Relative risk versus absolute risk: one cannot be interpreted without the other. *Nephrol Dial Transplant*. 2017;32(suppl_2):ii13-ii18.

217. Zipkin DA, Umscheid CA, Keating NL, et al. Evidence-based risk communication: a systematic review. *Ann Intern Med*. 2014;161(4):270-280.

218. Akl EA, Oxman AD, Herrin J, et al. Using alternative statistical formats for presenting risks and risk reductions. *Cochrane Database Syst Rev*. 2011;2011(3):CD006776.

219. Freeman ALJ. How to communicate evidence to patients. *Drug Ther Bull*. 2019;57(8):119-124.

220. Stacey D, Légaré F, Lewis K, et al. Decision aids for people facing health treatment or screening decisions. *Cochrane Database Syst Rev*. 2017;4(4):CD001431.

221. Fagerlin A, Zikmund-Fisher BJ, Ubel PA. Helping patients decide: ten steps to better risk communication. *J Natl Cancer Inst*. 2011;103(19):1436-1443.

222. Trevena LJ, Zikmund-Fisher BJ, Edwards A, et al. Presenting quantitative information about decision outcomes: a risk communication primer for patient decision aid developers. *BMC Med Inform Decis Mak*. 2013;13 Suppl 2:S7.

223. Zikmund-Fisher BJ, Ubel PA, Smith DM, et al. Communicating side effect risks in a tamoxifen prophylaxis decision aid: the debiasing influence of pictographs. *Patient Educ Couns*. 2008;73(2):209-214.

224. Hawley ST, Zikmund-Fisher B, Ubel P, Jancovic A, Lucas T, Fagerlin A. The impact of the format of graphical presentation on health-related knowledge and treatment choices. *Patient Educ Couns*. 2008;73(3):448-455.

225. Stuckey H, Williams JL, Fan AL, et al. Enhancing genomic laboratory reports from the patients' view: A qualitative analysis. *Am J Med Genet A*. 2015;167A(10):2238-2243.

226. Fenton GL, Smit AK, Freeman L, et al. Development and Evaluation of a Telephone Communication Protocol for the Delivery of Personalized Melanoma Genomic Risk to the General Population. *J Genet Couns*. 2018;27(2):370-380.

227. Smit AK, Keogh LA, Hersch J, et al. Public preferences for communicating personal genomic risk information: a focus group study. *Health Expect*. 2016;19(6):1203-1214.

228. Brockman DG, Petronio L, Dron JS, et al. Design and user experience testing of a polygenic score report: a qualitative study of prospective users. *BMC Med Genomics*. 2021;14(1):238.

229. 23andMe. Coronary artery disease (Report). https://medical.23andme.com/wp-content/uploads/2020/06/cad_increased.pdf. Accessed September 2023.

230. Multhaup ML, Kita R, Krock B, et al. The science behind 23andMe's Type 2 Diabetes report. *Sunnyvale (CA): 23andMe*. 2019:23-19.

231. Marjonen H, Marttila M, Paajanen T, et al. A Web Portal for Communicating Polygenic Risk Score Results for Health Care Use—The P5 Study. *Front Genet*. 2021;12:2170.

232. MyGeneRank. https://mygenerank.scripps.edu/. Accessed November 25, 2021.

233. Muse ED, Chen SF, Liu S, et al. Impact of polygenic risk communication: an observational mobile application-based coronary artery disease study. *npj Digital Medicine*. 2022;5(1):1-9.

234. DNA & Epigenetic testing kit with in-app results. https://www.mytoolboxgenomics.com/. Accessed November 25, 2021.

235. myRisk M. Myriad riskScore. https://myriadmyrisk.com/riskscore/. Accessed September 2023.

236. Genetics A. AmbryScore-Breast. https://web.archive.org/web/20210304005349/https://www.ambrygen.com/providers/ambryscore/breast. Accessed September 2023.

237. Carver T, Hartley S, Lee A, et al. CanRisk Tool-A Web Interface for the Prediction of Breast and Ovarian Cancer Risk and the Likelihood of Carrying Genetic Pathogenic Variants. *Cancer Epidemiol Biomarkers Prev*. 2021;30(3):469-473.

238. Genetics A. AmbryScore - Personalized Prostate Cancer Risk Estimate (Unaffected). https://www.ambrygen.com/file/material/view/976/AmbryScore%20Sample%20Report%202-%20Unaffected%20Male%20at%20Increased%20Risk.pdf.

239. Genetics A. AmbryScore - Personalized Prostate Cancer Risk Estimate (Affected). https://web.archive.org/web/20200929191018/https://www.ambrygen.com/file/material/view/978/AmbryScore%20Sample%20Report%204%20-%20Affected%20Male%20at%20Increased%20Risk.pdf. Accessed September 2023.

240. Linder JE, Allworth A, Bland HT, et al. Returning integrated genomic risk and clinical recommendations: The eMERGE study. *Genet Med*. 2023;25(4):100006.

241. Types of color vision deficiency. https://www.nei.nih.gov/learn-about-eye-health/eye-conditions-and-diseases/color-blindness/types-color-vision-deficiency. Accessed November 25, 2023.

242. Lipkus IM, Samsa G, Rimer BK. General performance on a numeracy scale among highly educated samples. *Med Decis Making*. 2001;21(1):37-44.

243. Milo Rasouly H, Cuneo N, Marasa M, et al. GeneLiFT: A novel test to facilitate rapid screening of genetic literacy in a diverse population undergoing genetic testing. *J Genet Couns*. 2021;30(3):742-754.

244. Young MA, Yanes T, Cust AE, et al. Human Genetics Society of Australasia Position Statement: Use of Polygenic Scores in Clinical Practice and Population Health. *Twin Res Hum Genet*. 2023;26(1):40-48.

245. Bunnik EM, Janssens ACJ, Schermer MHN. Personal utility in genomic testing: is there such a thing? *J Med Ethics*. 2015;41(4):322-326.

246. Lewis ACF, Perez EF, Prince AER, et al. Patient and provider perspectives on polygenic risk scores: implications for clinical reporting and utilization. *Genome Med*. 2022;14(1):114.

247. Wand H, Lambert SA, Tamburro C, et al. Improving reporting standards for polygenic scores in risk prediction studies. *Nature*. 2021;591(7849):211-219.

248. Fogg BJ, Soohoo C, Danielson DR, Marable L, Stanford J, Tauber ER. How do users evaluate the credibility of Web sites? a study with over 2,500 participants. In: *Proceedings of the 2003 Conference on Designing for User Experiences*. DUX '03. New York, NY, USA: Association for Computing Machinery; 2003:1-15.

249. Sillence E, Briggs P, Fishwick L, Harris P. Trust and mistrust of online health sites. In:

Proceedings of the SIGCHI Conference on Human Factors in Computing Systems. CHI '04. New York, NY, USA: Association for Computing Machinery; 2004:663-670.

250. Molster C, Charles T, Samanek A, O'Leary P. Australian study on public knowledge of human genetics and health. *Public Health Genomics*. 2009;12(2):84-91.

251. Metcalfe SA, Hickerton C, Savard J, et al. Australians' views on personal genomic testing: focus group findings from the Genioz study. *Eur J Hum Genet*. 2018;26(8):1101-1112.

252. Christensen KD, Jayaratne TE, Roberts JS, Kardia SLR, Petty EM. Understandings of basic genetics in the United States: results from a national survey of black and white men and women. *Public Health Genomics*. 2010;13(7-8):467-476.

253. Fortin JM, Hirota LK, Bond BE, O'Connor AM, Col NF. Identifying patient preferences for communicating risk estimates: a descriptive pilot study. *BMC Med Inform Decis Mak*. 2001;1:2.

254. Dorval M, Bouchard K, Chiquette J, et al. A focus group study on breast cancer risk presentation: one format does not fit all. *Eur J Hum Genet*. 2013;21(7):719-724.

255. Lautenbach DM, Christensen KD, Sparks JA, Green RC. Communicating genetic risk information for common disorders in the era of genomic medicine. *Annu Rev Genomics Hum Genet*. 2013;14:491-513.

256. Bennett RL, Waggoner D, Blitzer MG. Medical genetics and genomics education: how do we define success? Where do we focus our resources? *Genet Med*. 2017;19(7):751-753.

257. Johnson LM, Valdez JM, Quinn EA, et al. Integrating next-generation sequencing into pediatric oncology practice: An assessment of physician confidence and understanding of clinical genomics. *Cancer*. 2017;123(12):2352-2359.

258. Suther S, Goodson P. Barriers to the provision of genetic services by primary care physicians: a systematic review of the literature. *Genet Med*. 2003;5(2):70-76.

259. Selkirk CG, Weissman SM, Anderson A, Hulick PJ. Physicians' preparedness for integration of genomic and pharmacogenetic testing into practice within a major healthcare system. *Genet Test Mol Biomarkers*. 2013;17(3):219-225.

260. Marzuillo C, De Vito C, Boccia S, et al. Knowledge, attitudes and behavior of physicians regarding predictive genetic tests for breast and colorectal cancer. *Prev Med*. 2013;57(5):477-482.

261. Acton RT, Burst NM, Casebeer L, et al. Knowledge, attitudes, and behaviors of Alabama's primary care physicians regarding cancer genetics. *Acad Med*. 2000;75(8):850-852.

262. Klitzman R, Chung W, Marder K, et al. Attitudes and practices among internists concerning genetic testing. *J Genet Couns*. 2013;22(1):90-100.

263. Archer S, Babb de Villiers C, Scheibl F, et al. Evaluating clinician acceptability of the prototype CanRisk tool for predicting risk of breast and ovarian cancer: A multi-methods

study. *PLoS One*. 2020;15(3):e0229999.

264. Gedde SJ, Vinod K, Wright MM, et al. Primary Open-Angle Glaucoma Preferred Practice Pattern®. *Ophthalmology*. 2021;128(1):P71-P150.

265. Olsen CM, Green AC, Neale RE, et al. Cohort profile: the QSkin Sun and Health Study. *Int J Epidemiol*. 2012;41(4):929-929i.

266. Mitchell P, Rochtchina E, Lee AJ, Wang JJ. Bias in self-reported family history and relationship to glaucoma: the Blue Mountains Eye Study. *Ophthalmic Epidemiol*. 2002;9(5). doi:10.1076/opep.9.5.333.10335

267. Selzam S, Ritchie SJ, Pingault JB, Reynolds CA, O'Reilly PF, Plomin R. Comparing Within- and Between-Family Polygenic Score Prediction. *Am J Hum Genet*. 2019;105(2):351-363.

268. Tada H, Melander O, Louie JZ, et al. Risk prediction by genetic risk scores for coronary heart disease is independent of self-reported family history. *Eur Heart J*. 2016;37(6). doi:10.1093/eurheartj/ehv462

269. Hujoel MLA, Loh PR, Neale BM, Price AL. Incorporating family history of disease improves polygenic risk scores in diverse populations. *bioRxiv*. April 2021. doi:10.1101/2021.04.15.439975

270. Mars N, Lindbohm JV, Della Briotta Parolo P, et al. Systematic comparison of family history and polygenic risk across 24 common diseases. *Am J Hum Genet*. 2022;109(12):2152-2162.

271. Awadalla MS, Fingert JH, Roos BE, et al. Copy number variations of TBK1 in Australian patients with primary open-angle glaucoma. *Am J Ophthalmol*. 2015;159(1):124-130.e1.

272. Fan Q, Teo YY, Saw SM. Application of advanced statistics in ophthalmology. *Invest Ophthalmol Vis Sci*. 2011;52(9). doi:10.1167/iovs.10-7108

273. Qassim A, Mullany S, Awadalla MS, et al. A Polygenic Risk Score Predicts Intraocular Pressure Readings Outside Office Hours and Early Morning Spikes as Measured by Home Tonometry. *Ophthalmol Glaucoma*. 2021;4(4):411-420.

274. Hassanin E, May P, Aldisi R, et al. Breast and prostate cancer risk: The interplay of polygenic risk, rare pathogenic germline variants, and family history. *Genet Med*. 2022;24(3):576-585.

275. Kachuri L, Graff RE, Smith-Byrne K, et al. Pan-cancer analysis demonstrates that integrating polygenic risk scores with modifiable risk factors improves risk prediction. *Nat Commun*. 2020;11(1):6084.

276. Smith W, Assink J, Klein R, et al. Risk factors for age-related macular degeneration: Pooled findings from three continents. *Ophthalmology*. 2001;108(4):697-704.

277. Wong WL, Su X, Li X, et al. Global prevalence of age-related macular degeneration and disease burden projection for 2020 and 2040: a systematic review and meta-analysis. *Lancet Glob Health*. 2014;2(2):e106-e116.

278. Klaver CC, Assink JJ, van Leeuwen R, et al. Incidence and progression rates of age-related maculopathy: the Rotterdam Study. *Invest Ophthalmol Vis Sci*. 2001;42(10):2237-2241.

279. Age-Related Eye Disease Study Research Group. A randomized, placebo-controlled, clinical trial of high-dose supplementation with vitamins C and E, beta carotene, and zinc for age-related macular degeneration and vision loss: AREDS report no. 8. *Arch Ophthalmol*. 2001;119(10):1417-1436.

280. Rosenfeld PJ, Brown DM, Heier JS, et al. Ranibizumab for neovascular age-related macular degeneration. *N Engl J Med*. 2006;355(14):1419-1431.

281. Seddon JM, Cote J, Page WF, Aggen SH, Neale MC. The US twin study of age-related macular degeneration: relative roles of genetic and environmental influences. *Arch Ophthalmol*. 2005;123(3):321-327.

282. Lambert SA, Abraham G, Inouye M. Towards clinical utility of polygenic risk scores. *Hum Mol Genet*. 2019;28(R2):R133-R142.

283. Fritsche LG, Igl W, Bailey JNC, et al. A large genome-wide association study of age-related macular degeneration highlights contributions of rare and common variants. *Nat Genet*. 2016;48(2):134-143.

284. Ding Y, Liu Y, Yan Q, et al. Bivariate Analysis of Age-Related Macular Degeneration Progression Using Genetic Risk Scores. *Genetics*. 2017;206(1):119-133.

285. Heesterbeek TJ, de Jong EK, Acar IE, et al. Genetic risk score has added value over initial clinical grading stage in predicting disease progression in age-related macular degeneration. *Sci Rep*. 2019;9(1):1-8.

286. Warwick A, Lotery A. Genetics and genetic testing for age-related macular degeneration. *Eye* . 2018;32(5):849-857.

287. 1000 Genomes Project Consortium, Auton A, Brooks LD, et al. A global reference for human genetic variation. *Nature*. 2015;526(7571):68-74.

288. Han X, Gharahkhani P, Mitchell P, Liew G, Hewitt AW, MacGregor S. Genome-wide meta-analysis identifies novel loci associated with age-related macular degeneration. *J Hum Genet*. 2020;65(8):657-665.

289. Mitchell P, Smith W, Attebo K, Wang JJ. Prevalence of age-related maculopathy in Australia. The Blue Mountains Eye Study. *Ophthalmology*. 1995;102(10):1450-1460.

290. Data. https://www.internationalgenome.org/data/. Accessed December 15, 2023.

291. 1000 genomes. https://www.internationalgenome.org/. Accessed December 15, 2023.

292. Charlesworth J, Kramer PL, Dyer T, et al. The Path to Open-Angle Glaucoma Gene Discovery: Endophenotypic Status of Intraocular Pressure, Cup-to-Disc Ratio, and Central Corneal Thickness. *Invest Ophthalmol Vis Sci*. 2010;51(7):3509-3514.

293. Wang R, Wiggs JL. Common and rare genetic risk factors for glaucoma. *Cold Spring Harb Perspect Med*. 2014;4(12):a017244.

294. Mansberger SL, Cioffi GA. The probability of glaucoma from ocular hypertension determined by ophthalmologists in comparison to a risk calculator. *J Glaucoma*. 2006;15(5):426-431.

295. Seddon JM, Reynolds R, Maller J, Fagerness JA, Daly MJ, Rosner B. Prediction model for prevalence and incidence of advanced age-related macular degeneration based on genetic, demographic, and environmental variables. *Invest Ophthalmol Vis Sci*. 2009;50(5):2044-2053.

296. Csaky KG, Schachat AP, Kaiser PK, Small KW, Heier JS. The Use of Genetic Testing in the Management of Patients With Age-Related Macular Degeneration: American Society of Retina Specialists Genetics Task Force Special Report. *Journal of VitreoRetinal Diseases*. 2017;1(1):75-78.

297. Tandy-Connor S, Guiltinan J, Krempely K, et al. False-positive results released by direct-to-consumer genetic tests highlight the importance of clinical confirmation testing for appropriate patient care. *Genet Med*. 2018;20(12):1515-1521.

298. Sanfilippo PG, Kearns LS, Wright P, Mackey DA, Hewitt AW. Current landscape of direct-to-consumer genetic testing and its role in ophthalmology: a review. *Clin Experiment Ophthalmol*. 2015;43(6):578-590.

299. Hernández-Zimbrón LF, Zamora-Alvarado R, Ochoa-De la Paz L, et al. Age-Related Macular Degeneration: New Paradigms for Treatment and Management of AMD. *Oxid Med Cell Longev*. 2018;2018:8374647.

300. Age-Related Eye Disease Study Research Group, SanGiovanni JP, Chew EY, et al. The relationship of dietary carotenoid and vitamin A, E, and C intake with age-related macular degeneration in a case-control study: AREDS Report No. 22. *Arch Ophthalmol*. 2007;125(9):1225-1232.

301. Age-Related Eye Disease Study Research Group. Risk factors associated with age-related macular degeneration. A case-control study in the age-related eye disease study: Age-Related Eye Disease Study Report Number 3. *Ophthalmology*. 2000;107(12):2224-2232.

302. Blindness and vision impairment. https://www.who.int/news-room/fact-sheets/detail/blindness-and-visual-impairment. Accessed April 9, 2023.

303. Eye health. Australian Institute of Health and Welfare. https://www.aihw.gov.au/reports/eye-health/eye-health/contents/how-common-is-visual-impairment. Accessed April 9, 2023.

304. Kyari F, Wolvaardt E. Medicines for eye health. *Community Eye Health*. 2023;36(118):1.

305. Marques AP, Ramke J, Cairns J, et al. The economics of vision impairment and its leading causes: A systematic review. *EClinicalMedicine*. 2022;46:101354.

306. Khorrami-Nejad M, Sarabandi A, Akbari MR, Askarizadeh F. The Impact of Visual Impairment on Quality of Life. *Med Hypothesis Discov Innov Ophthalmol*. 2016;5(3):96-103.

307. Quaranta L, Riva I, Gerardi C, Oddone F, Floriani I, Konstas AGP. Quality of Life in

Glaucoma: A Review of the Literature. *Adv Ther*. 2016;33(6):959-981.

308. Marshall HN, Hollitt GL, Wilckens K, et al. High Polygenic Risk Is Associated with Earlier Trabeculectomy in Patients with Primary Open-Angle Glaucoma. *Ophthalmol Glaucoma*. 2023;6(1):54-57.

309. Marshall HN, Mullany S, Han X, et al. High Polygenic Risk Is Associated with Earlier Initiation and Escalation of Treatment in Early Primary Open-Angle Glaucoma. *Ophthalmology*. 2023;130(8):830-836.

310. Venning B, Saya S, De Abreu Lourenco R, Street DJ, Emery JD. Preferences for a polygenic test to estimate cancer risk in a general Australian population. *Genet Med*. 2022;24(10):2144-2154.

311. Wallingford CK, Kovilpillai H, Jacobs C, et al. Models of communication for polygenic scores and associated psychosocial and behavioral effects on recipients: A systematic review. *Genet Med*. 2023;25(1):1-11.

312. Widén E, Junna N, Ruotsalainen S, et al. How Communicating Polygenic and Clinical Risk for Atherosclerotic Cardiovascular Disease Impacts Health Behavior: an Observational Follow-up Study. *Circ Genom Precis Med*. 2022;15(2):e003459.

313. Cancer Australia. Genetic testing for breast/ovarian cancer risk. https://www.canceraustralia.gov.au/clinical-best-practice/gynaecological-cancers/familial-risk-assessment-fra-boc/genetic-testing. Accessed June 25, 2023.

314. Slunecka JL, van der Zee MD, Beck JJ, et al. Implementation and implications for polygenic risk scores in healthcare. *Hum Genomics*. 2021;15(1):46.

315. Lewis ACF, Green RC, Vassy JL. Polygenic risk scores in the clinic: Translating risk into action. *HGG Adv*. 2021;2(4):100047.

316. Pain O, Gillett AC, Austin JC, Folkersen L, Lewis CM. A tool for translating polygenic scores onto the absolute scale using summary statistics. *Eur J Hum Genet*. 2022;30(3):339-348.

317. Gigerenzer G, Gaissmaier W, Kurz-Milcke E, Schwartz LM, Woloshin S. Helping Doctors and Patients Make Sense of Health Statistics. *Psychol Sci Public Interest*. 2007;8(2):53-96.

318. Tinley ST, Houfek J, Watson P, et al. Screening adherence in BRCA1/2 families is associated with primary physicians' behavior. *Am J Med Genet A*. 2004;125A(1):5-11.

319. Krol D, Norman M, McHenry A, Stark E, Johnson K, Kaltman RD. Physician survey on utilization of clinical cancer genetics services at an academic institution. *Journal of Clinical Oncology*. 2018;36(15_suppl):e13507-e13507. doi:10.1200/jco.2018.36.15_suppl.e13507

320. Haga SB, Kim E, Myers RA, Ginsburg GS. Primary Care Physicians' Knowledge, Attitudes, and Experience with Personal Genetic Testing. *Journal of Personalized Medicine*. 2019;9(2):29.

321. Carroll JC, Makuwaza T, Manca DP, et al. Primary care providers' experiences with and perceptions of personalized genomic medicine. *Can Fam Physician*. 2016;62(10):e626-e635.

322. Chambers, Axell-House, Mills. Primary care physicians' experience and confidence with genetic testing and perceived barriers to genomic medicine. *J Fam Med*.

323. Isgut M, Sun J, Quyyumi AA, Gibson G. Highly elevated polygenic risk scores are better predictors of myocardial infarction risk early in life than later. *Genome Med*. 2021;13(1):13.

324. Lee A, Mavaddat N, Wilcox AN, et al. BOADICEA: a comprehensive breast cancer risk prediction model incorporating genetic and nongenetic risk factors. *Genet Med*. 2019;21(8):1708-1718.

325. Dite GS, MacInnis RJ, Bickerstaffe A, et al. Breast Cancer Risk Prediction Using Clinical Models and 77 Independent Risk-Associated SNPs for Women Aged Under 50 Years: Australian Breast Cancer Family Registry. *Cancer Epidemiol Biomarkers Prev*. 2016;25(2):359-365.

326. Lakeman IMM, Hilbers FS, Rodríguez-Girondo M, et al. Addition of a 161-SNP polygenic risk score to family history-based risk prediction: impact on clinical management in non-BRCA1/2 breast cancer families. *J Med Genet*. 2019;56(9):581-589.

327. Läll K, Lepamets M, Palover M, et al. Polygenic prediction of breast cancer: comparison of genetic predictors and implications for risk stratification. *BMC Cancer*. 2019;19(1):557.

328. Allman R, Dite GS, Hopper JL, et al. SNPs and breast cancer risk prediction for African American and Hispanic women. *Breast Cancer Res Treat*. 2015;154(3):583-589.

329. Terry MB, Liao Y, Whittemore AS, et al. 10-year performance of four models of breast cancer risk: a validation study. *Lancet Oncol*. 2019;20(4):504-517.

330. Jeon J, Du M, Schoen RE, et al. Determining Risk of Colorectal Cancer and Starting Age of Screening Based on Lifestyle, Environmental, and Genetic Factors. *Gastroenterology*. 2018;154(8):2152-2164.e19.

331. Hsu L, Jeon J, Brenner H, et al. A model to determine colorectal cancer risk using common genetic susceptibility loci. *Gastroenterology*. 2015;148(7):1330-1339.e14.

332. Jenkins MA, Makalic E, Dowty JG, et al. Quantifying the utility of single nucleotide polymorphisms to guide colorectal cancer screening. *Future Oncol*. 2016;12(4):503-513.

333. Jenkins MA, Win AK, Dowty JG, et al. Ability of known susceptibility SNPs to predict colorectal cancer risk for persons with and without a family history. *Fam Cancer*. 2019;18(4):389-397.

334. Ibáñez-Sanz G, Díez-Villanueva A, Alonso MH, et al. Risk Model for Colorectal Cancer in Spanish Population Using Environmental and Genetic Factors: Results from the MCC-Spain study. *Sci Rep*. 2017;7:43263.

335. Smith T, Gunter MJ, Tzoulaki I, Muller DC. The added value of genetic information in colorectal cancer risk prediction models: development and evaluation in the UK Biobank prospective cohort study. *Br J Cancer*. 2018;119(8):1036-1039.

336. Na R, Labbate C, Yu H, et al. Single-Nucleotide Polymorphism-Based Genetic Risk Score and Patient Age at Prostate Cancer Diagnosis. *JAMA Netw Open*. 2019;2(12):e1918145.

337. Butoescu V, Ambroise J, Stainier A, Dekairelle AF, Gala JL, Tombal B. Does genotyping of risk-associated single nucleotide polymorphisms improve patient selection for prostate biopsy when combined with a prostate cancer risk calculator? *Prostate*. 2014;74(4):365-371.

338. Chen H, Na R, Packiam VT, et al. Reclassification of prostate cancer risk using sequentially identified SNPs: Results from the REDUCE trial. *Prostate*. 2017;77(11):1179-1186.

339. Conran CA, Na R, Chen H, et al. Population-standardized genetic risk score: the SNP-based method of choice for inherited risk assessment of prostate cancer. *Asian J Androl*. 2016;18(4):520-524.

340. Kaiser I, Pfahlberg AB, Uter W, Heppt MV, Veierød MB, Gefeller O. Risk Prediction Models for Melanoma: A Systematic Review on the Heterogeneity in Model Development and Validation. *Int J Environ Res Public Health*. 2020;17(21). doi:10.3390/ijerph17217919

341. Abraham G, Havulinna AS, Bhalala OG, et al. Genomic prediction of coronary heart disease. *Eur Heart J*. 2016;37(43):3267-3278.

342. Iribarren C, Lu M, Jorgenson E, et al. Clinical Utility of Multimarker Genetic Risk Scores for Prediction of Incident Coronary Heart Disease: A Cohort Study Among Over 51 000 Individuals of European Ancestry. *Circ Cardiovasc Genet*. 2016;9(6):531-540.

343. Marston NA, Kamanu FK, Nordio F, et al. Predicting Benefit From Evolocumab Therapy in Patients With Atherosclerotic Disease Using a Genetic Risk Score: Results From the FOURIER Trial. *Circulation*. 2020;141(8):616-623.

344. Ripatti S, Tikkanen E, Orho-Melander M, et al. A multilocus genetic risk score for coronary heart disease: case-control and prospective cohort analyses. *Lancet*. 2010;376(9750):1393-1400.

345. Tikkanen E, Havulinna AS, Palotie A, Salomaa V, Ripatti S. Genetic risk prediction and a 2-stage risk screening strategy for coronary heart disease. *Arterioscler Thromb Vasc Biol*. 2013;33(9):2261-2266.

346. Inouye M, Abraham G, Nelson CP, et al. Genomic Risk Prediction of Coronary Artery Disease in 480,000 Adults: Implications for Primary Prevention. *J Am Coll Cardiol*. 2018;72(16):1883-1893.

347. Elliott J, Bodinier B, Bond TA, et al. Predictive Accuracy of a Polygenic Risk Score-Enhanced Prediction Model vs a Clinical Risk Score for Coronary Artery Disease. *JAMA*. 2020;323(7):636-645.

348. Mosley JD, Gupta DK, Tan J, et al. Predictive Accuracy of a Polygenic Risk Score Compared With a Clinical Risk Score for Incident Coronary Heart Disease. *JAMA*. 2020;323(7):627-635.

349. Patient experiences. Australian Bureau of Statistics. https://www.abs.gov.au/statistics/health/health-services/patient-experiences/latest-release. Published November 18, 2022. Accessed June 25, 2023.

350. Allen NL, Karlson EW, Malspeis S, Lu B, Seidman CE, Lehmann LS. Biobank participants' preferences for disclosure of genetic research results: perspectives from the OurGenes, OurHealth, OurCommunity project. *Mayo Clin Proc*. 2014;89(6):738-746.

351. Savard J, Hickerton C, Tytherleigh R, et al. Australians' views and experience of personal genomic testing: survey findings from the Genioz study. *Eur J Hum Genet*. 2019;27(5):711-720.

352. Smit AK, Espinoza D, Newson AJ, Morton RL. A Pilot Randomized Controlled Trial of the Feasibility, Acceptability, and Impact of Giving Information on Personalized Genomic Risk of Melanoma to the …. *Biomarkers & Prevention*. 2017. https://aacrjournals.org/cebp/article-abstract/26/2/212/71280.

353. Committee AMWA, Others. *The General Practice Workforce in Australia: Supply and Requirements to 2013: Summary of Findings and Recommendations*. Australian Medical Workforce Advisory Committee; 2005.

354. Norris S, Belcher A, Howard K, Ward RL. Evaluating genetic and genomic tests for heritable conditions in Australia: lessons learnt from health technology assessments. *J Community Genet*. 2022;13(5):503-522.

355. Kiflen M, Le A, Mao S, et al. Cost-Effectiveness of Polygenic Risk Scores to Guide Statin Therapy for Cardiovascular Disease Prevention. *Circ Genom Precis Med*. 2022;15(5):e003423.

356. Goranitis I, Best S, Christodoulou J, Stark Z, Boughtwood T. The personal utility and uptake of genomic sequencing in pediatric and adult conditions: eliciting societal preferences with three discrete choice experiments. *Genet Med*. 2020;22(8):1311-1319.

357. Labs E. Eugene. Eugene Labs. https://eugenelabs.com/. Accessed October 10, 2023.

358. Rural and remote health. Australian Institute of Health and Welfare. https://www.aihw.gov.au/reports/rural-remote-australians/rural-and-remote-health. Accessed December 6, 2022.

359. Telehealth. https://www.digitalhealth.gov.au/healthcare-providers/initiatives-and-programs/telehealth. Accessed August 27, 2023.

360. [No title]. https://www.researchgate.net/profile/Khurshid-Ahmad-5/publication/285655783_845_The_Role_of_Specialist_Terminology_Management_in_Artificial_Intelligence_and_Knowledge_Acquisition/links/5695a49d08ae3ad8e33d8d5b/845-The-Role-of-Specialist-Terminology-Management-in-Artificial-Intelligence-and-Knowledge-Acquisition.pdf. Accessed August 27, 2023.

361. Akkara JD, Kuriakose A. Role of artificial intelligence and machine learning in ophthalmology. *Kerala Journal of Ophthalmology*. 2019;31(2):150.

362. Zheng C, Johnson TV, Garg A, Boland MV. Artificial intelligence in glaucoma. *Curr Opin Ophthalmol*. 2019;30(2):97.

363. Martin KR, Mansouri K, Weinreb RN. Use of machine learning on contact lens sensor–derived parameters for the diagnosis of primary open-angle glaucoma. *American journal of*.

2018. https://www.sciencedirect.com/science/article/pii/S0002939418303866?casa_token=OwpgyOAYzHoAAAAA:1kxMwuTLnInhEiV68SdM8wdLcM-gZjOvYQLnKA5A2DbcKmyLfQfgXzHgZGz7GZUswy3CTXkkbobV.

364. Li Z, He Y, Keel S, Meng W, Chang RT, He M. Efficacy of a Deep Learning System for Detecting Glaucomatous Optic Neuropathy Based on Color Fundus Photographs. *Ophthalmology*. 2018;125(8):1199-1206.

365. Al-Aswad LA, Kapoor R, Chu CK, et al. Evaluation of a Deep Learning System For Identifying Glaucomatous Optic Neuropathy Based on Color Fundus Photographs. *J Glaucoma*. 2019;28(12):1029-1034.

366. Cerentini A, Welfer D, Cordeiro d'Ornellas M, Pereira Haygert CJ, Dotto GN. Automatic Identification of Glaucoma Using Deep Learning Methods. *Stud Health Technol Inform*. 2017;245:318-321.

367. Haleem MS, Han L, van Hemert J, et al. A Novel Adaptive Deformable Model for Automated Optic Disc and Cup Segmentation to Aid Glaucoma Diagnosis. *J Med Syst*. 2017;42(1):20.

368. Thompson AC, Jammal AA, Medeiros FA. A Deep Learning Algorithm to Quantify Neuroretinal Rim Loss From Optic Disc Photographs. *Am J Ophthalmol*. 2019;201:9-18.

369. Cifuentes-Canorea P, Ruiz-Medrano J, Gutierrez-Bonet R, et al. Analysis of inner and outer retinal layers using spectral domain optical coherence tomography automated segmentation software in ocular hypertensive and glaucoma patients. *PLoS One*. 2018;13(4):e0196112.

370. Omodaka K, An G, Tsuda S, et al. Classification of optic disc shape in glaucoma using machine learning based on quantified ocular parameters. *PLoS One*. 2017;12(12):e0190012.

371. Muhammad H, Fuchs TJ, De Cuir N, et al. Hybrid Deep Learning on Single Wide-field Optical Coherence tomography Scans Accurately Classifies Glaucoma Suspects. *J Glaucoma*. 2017;26(12):1086-1094.

372. Asaoka R, Murata H, Hirasawa K, et al. Using Deep Learning and Transfer Learning to Accurately Diagnose Early-Onset Glaucoma From Macular Optical Coherence Tomography Images. *Am J Ophthalmol*. 2019;198:136-145.

373. Christopher M, Belghith A, Weinreb RN, et al. Retinal Nerve Fiber Layer Features Identified by Unsupervised Machine Learning on Optical Coherence Tomography Scans Predict Glaucoma Progression. *Invest Ophthalmol Vis Sci*. 2018;59(7):2748-2756.

374. Barella KA, Costa VP, Gonçalves Vidotti V, Silva FR, Dias M, Gomi ES. Glaucoma Diagnostic Accuracy of Machine Learning Classifiers Using Retinal Nerve Fiber Layer and Optic Nerve Data from SD-OCT. *J Ophthalmol*. 2013;2013:789129.

375. Bizios D, Heijl A, Hougaard JL, Bengtsson B. Machine learning classifiers for glaucoma diagnosis based on classification of retinal nerve fibre layer thickness parameters measured by Stratus OCT. *Acta Ophthalmol*. 2010;88(1):44-52.

376. Larrosa JM, Polo V, Ferreras A, García-Martín E, Calvo P, Pablo LE. Neural Network Analysis of Different Segmentation Strategies of Nerve Fiber Layer Assessment for Glaucoma Diagnosis. *J Glaucoma*. 2015;24(9):672-678.

377. Fu H, Xu Y, Lin S, et al. Segmentation and Quantification for Angle-Closure Glaucoma Assessment in Anterior Segment OCT. *IEEE Trans Med Imaging*. 2017;36(9):1930-1938.

378. Niwas SI, Lin W, Bai X, et al. Automated anterior segment OCT image analysis for Angle Closure Glaucoma mechanisms classification. *Comput Methods Programs Biomed*. 2016;130:65-75.

379. Asaoka R, Murata H, Iwase A, Araie M. Detecting Preperimetric Glaucoma with Standard Automated Perimetry Using a Deep Learning Classifier. *Ophthalmology*. 2016;123(9):1974-1980.

380. Li F, Wang Z, Qu G, et al. Automatic differentiation of Glaucoma visual field from non-glaucoma visual filed using deep convolutional neural network. *BMC Med Imaging*. 2018;18(1):35.

381. Goldbaum MH, Sample PA, Zhang Z, et al. Using unsupervised learning with independent component analysis to identify patterns of glaucomatous visual field defects. *Invest Ophthalmol Vis Sci*. 2005;46(10):3676-3683.

382. Andersson S, Heijl A, Bizios D, Bengtsson B. Comparison of clinicians and an artificial neural network regarding accuracy and certainty in performance of visual field assessment for the diagnosis of glaucoma. *Acta Ophthalmol*. 2013;91(5):413-417.

383. Bowd C, Weinreb RN, Balasubramanian M, et al. Glaucomatous patterns in Frequency Doubling Technology (FDT) perimetry data identified by unsupervised machine learning classifiers. *PLoS One*. 2014;9(1):e85941.

384. Oh E, Yoo TK, Hong S. Artificial Neural Network Approach for Differentiating Open-Angle Glaucoma From Glaucoma Suspect Without a Visual Field Test. *Invest Ophthalmol Vis Sci*. 2015;56(6):3957-3966.

385. Department of Health and Ageing Staff. *Population Based Screening Framework*. Australian Government - Department of Health - Publications; 2007.

386. Genetic discrimination. https://www.nhmrc.gov.au/about-us/publications/genetic-discrimination. Accessed June 24, 2023.

387. Life insurance products and genetic testing in Australia. https://www.genetics.edu.au/SitePages/Life-insurance-products-and-genetic-testing-in-Australia.aspx. Accessed August 27, 2023.

388. Otlowski M, Taylor S, Bombard Y. Genetic discrimination: international perspectives. *Annu Rev Genomics Hum Genet*. 2012;13:433-454.

389. Klein R. *Genetics and Life Insurance: A View Into the Microscope of Regulation*. Geneva Association - International Association for the Study of Insurance Economics; 2017.

390. Kim H, Ho CWL, Ho CH, et al. Genetic discrimination: introducing the Asian perspective to the debate. *npj Genomic Medicine*. 2021;6(1):1-8.

391. Tiller J, Winship I, Otlowski MF, Lacaze PA. Monitoring the genetic testing and life insurance moratorium in Australia: a national research project. *Med J Aust*. 2021;214(4):157-159.e1.

392. Code on genetic testing and insurance. GOV.UK. https://www.gov.uk/government/publications/code-on-genetic-testing-and-insurance. Published October 23, 2018. Accessed August 29, 2023.

393. Wallace H. Genetic discrimination. *Lancet*. 2004;363(9416):1238.

394. Freedman AN, Wideroff L, Olson L, et al. US physicians' attitudes toward genetic testing for cancer susceptibility. *Am J Med Genet A*. 2003;120A(1):63-71.

395. Chowdhury S, Dent T, Pashayan N, et al. Incorporating genomics into breast and prostate cancer screening: assessing the implications. *Genet Med*. 2013;15(6):423-432.

396. National Health and Medical Research Council (NHMRC). DNA Genetic Testing in the Australian Context: A Statement from the National Health and Medical Research Council (NHMRC).1-6.

397. Australian Government Department of Health, Care A. Population-based health screening. Australian Government Department of Health and Aged Care. https://www.health.gov.au/our-work/population-based-health-screening. Published August 29, 2023. Accessed August 29, 2023.

398. Wong XY, Groothuis-Oudshoorn CG, Tan CS, et al. Women's preferences, willingness-to-pay, and predicted uptake for single-nucleotide polymorphism gene testing to guide personalized breast cancer screening strategies: a discrete choice experiment. *Patient Prefer Adherence*. 2018;12:1837-1852.

399. Lewis CM, Vassos E. Polygenic risk scores: from research tools to clinical instruments. *Genome Med*. 2020;12(1):44.

400. Wong XY, Chong KJ, van Til JA, Wee HL. A qualitative study on Singaporean women's views towards breast cancer screening and Single Nucleotide Polymorphisms (SNPs) gene testing to guide personalised screening strategies. *BMC Cancer*. 2017;17(1):776.

401. Faro VL, Bhattacharya A, Zhou W, et al. Genome-wide association meta-analysis identifies novel ancestry-specific primary open-angle glaucoma loci and shared biology with vascular mechanisms and cell proliferation. *bioRxiv*. December 2021. doi:10.1101/2021.12.16.21267891

402. Cooke Bailey JN, Funk KL, Cruz LA, et al. Diversity in Polygenic Risk of Primary Open-Angle Glaucoma. *Genes* . 2022;14(1). doi:10.3390/genes14010111

403. About Us. NATA. https://nata.com.au/about-us/. Published February 9, 2021. Accessed December 1, 2023.

APPENDIX

APPENDIX A: INVITATION LETTERS AND QUESTIONNAIRES

Appendix A.1: Affected individual invitation letter and questionnaire

AUSTRALIAN & NEW ZEALAND
REGISTRY OF ADVANCED GLAUCOMA

Australian & New Zealand
Registry of Advanced Glaucoma
Department of Ophthalmology
Flinders Medical Centre
Flinders Drive
Bedford Park 5042
South Australia
T (08) 8404 2035
F (08) 8204 6722
E info@anzrag.com
www.anzrag.com

Date

Name
Address

Dear participant,

We are writing to you because you are a participant in **the Australian and New Zealand Registry of Advanced Glaucoma.** Thank you for your ongoing contribution to the project. Your participation allows us the opportunity to continue research within the field of glaucoma.

Recent research is looking at the feasibility of developing a test to predict a person's risk of developing glaucoma based on genetic information extracted from blood or saliva samples. The results from such a test would be able to predict a person's likelihood of developing glaucoma (e.g. a genetic risk score that would predict if a person has a higher or a lower risk). We wish to assess whether people who have glaucoma would have been interested in such testing if it had been available before they developed glaucoma.

Enclosed is a brief questionnaire which takes approximately 10 minutes to complete. We would appreciate your help in completing the questionnaire and returning it to us using the reply-paid envelope enclosed. Alternatively if you would prefer to complete the questionnaire with someone over the phone, please call or email us using our details above. Your answers will remain confidential and will not be shared with members outside of the research team. You have the option to receive a summary of the findings at the end of the study.

Your participation through the completion of the questionnaire is completely voluntary and your decision to participate will not affect your glaucoma care or registry participation in any way.

If you have any questions regarding the questionnaire or would like to subsequently withdraw your answers, please contact Georgina Hollitt at georgina.hollitt@sa.gov.au or members of the research team using the details above.

Sincerely,

Georgina Hollitt
Department of Ophthalmology
Flinders University
Bedford Park SA 5042
08 8404 6986
georgina.hollitt@sa.gov.au

Bronwyn Ridge
Department of Ophthalmology
Flinders University
Bedford Park SA 5042
08 8404 2035
Bronwyn.Usher@flinders.edu.au

SURVEY ON GLAUCOMA GENETIC RISK TESTING AFFECTED INDIVIDUAL

Glaucoma is a progressive eye disease which can lead to permanent vision loss and blindness. It is often referred to as the 'sneak thief of sight' because there are no symptoms in its early stages. With early detection and treatment, vision loss can often be prevented.

DEMOGRAPHIC DETAILS

DATE: _____/_____/_____

NAME: _____

DOB: _____/_____/_____ SEX: MALE / FEMALE

ADDRESS: _____

_____

PHONE: ( ) MOBILE:

EMAIL: _____

ETHNIC BACKGROUND: MOTHER: _____ FATHER: _____

HIGHEST LEVEL OF EDUCATION:

☐ Primary School ☐ Secondary School ☐ Vocational training/TAFE ☐ University

Would you like to receive a summary of the findings at the conclusion of this study? ☐ YES ☐ NO

If yes, what is your preferred method of receiving information? (please ensure details are provided above)

☐ Email ☐ Mail ☐ Phone

MEDICAL HISTORY

Does anyone in your family have glaucoma? ☐ YES ☐ NO ☐ Unsure ☐ Don't know

If yes, how many? _____

How are they related to you?

_____

_____

Do you wear glasses or contact lenses for distance vision (driving/watching television)?

☐ YES ☐ NO ☐ Sometimes

When was the last time you had your eyes checked?

☐ Within 6 months ☐ Within 6-12 months ☐ Within 1-2 years ☐ > 2 years ☐ Never

How often do you have your eyes checked by an optometrist or ophthalmologist?

☐ 3 monthly ☐ 6 monthly ☐ Annually ☐ Every 2 years ☐ More than every 2 years ☐ Never

Do you have diabetes?	☐ YES	☐ NO	☐ Unsure
Do you have high blood pressure or take medication for it?	☐ YES	☐ NO	☐ Unsure
Do you get migraines?	☐ YES	☐ NO	☐ Unsure
Do you have a history of Raynaud's disease or poor circulation?	☐ YES	☐ NO	☐ Unsure
Do you have a history of heart attack, angina, or stenting?	☐ YES	☐ NO	☐ Unsure
Do you have a history of stroke or mini stroke?	☐ YES	☐ NO	☐ Unsure

QUESTIONS REGARDING GLAUCOMA

Before being diagnosed, how much did you know about glaucoma?

☐ A lot ☐ A fair amount ☐ A little ☐ Nothing

How satisfied are you with your understanding of glaucoma?

☐ Very dissatisfied ☐ Dissatisfied ☐ Moderately satisfied ☐ Very satisfied ☐ Unsure

Do you consider glaucoma as being a severe medical condition?

☐ Not severe ☐ Slightly severe ☐ Moderately severe ☐ Very severe ☐ Unsure

Before being diagnosed, what was your perception of your risk of developing glaucoma in your lifetime?

☐ Highly unlikely ☐ Unlikely ☐ Likely ☐ Highly likely ☐ N/A (didn't know about glaucoma)

Before being diagnosed, were you worried about developing glaucoma?

☐ Not worried ☐ Slightly worried ☐ Moderately worried ☐ Very worried ☐ Unsure

How likely would you have taken a genetic test to predict your risk of developing glaucoma if it had been offered to you before you were diagnosed?

☐ Highly unlikely ☐ Unlikely ☐ Likely ☐ Highly likely ☐ Unsure

If a cost were involved, how much would you be willing to pay for the test?

☐ I would not be willing to pay ☐ $50-100 ☐ $100-200 ☐ $200-300 ☐ $300-500

What information about the test would you want to know? (Choose as many as appropriate)

☐ Cost

☐ Process involved in taking the test

☐ Follow-up

☐ Implications of results

☐ Other:

_____

_____

_____

What would your preferred method of receiving results be? (Choose as many as appropriate)

☐ Face to face

☐ Letter / Mailout

☐ Telephone call

☐ Email

☐ Other: _____

Do you think you would have changed your behaviour regarding your eye health if you had known your risk of developing glaucoma? (For example, more frequent eye checks, better treatment compliance)

☐ Definitely not ☐ Probably not ☐ Possibly ☐ Probably ☐ Definitely

Reason: _____

_____

Would you recommend your family members to have a genetic test to predict their risk of developing glaucoma?

☐ Definitely not ☐ Probably not ☐ Possibly ☐ Probably ☐ Definitely

Would you recommend non-family members to have a genetic test to predict their risk of developing glaucoma?

☐ Definitely not ☐ Probably not ☐ Possibly ☐ Probably ☐ Definitely

Would you take a test to predict your risk of rapid progression or developing severe disease if stronger treatments could prevent blindness?

☐ Definitely not ☐ Probably not ☐ Possibly ☐ Probably ☐ Definitely

COMMENTS: _____

_____

_____

_____

Appendix A.2: Unaffected individual invitation letters and questionnaire

AUSTRALIAN & NEW ZEALAND
REGISTRY OF ADVANCED GLAUCOMA

Australian & New Zealand
Registry of Advanced Glaucoma

Department of Ophthalmology
Flinders Medical Centre
Flinders Drive
Bedford Park 5042
South Australia

T (08) 8404 2035
F (08) 8204 6722
E info@anzrag.com

www.anzrag.com

Date

Name
Address

Dear participant,

We are writing to you because you are a participant in **the Australian and New Zealand Registry of Advanced Glaucoma.** Thank you for your ongoing contribution to the project. Your participation allows us the opportunity to continue research within the field of glaucoma.

Recent research is looking at the feasibility of developing a test to predict a person's risk of developing glaucoma based on genetic information extracted from blood or saliva samples. The results from such a test would be able to predict a person's likelihood of developing glaucoma (e.g. a genetic risk score that would predict if a person has a higher or a lower risk). We wish to assess whether people with relatives who have glaucoma would be interested in such testing if it were available.

Enclosed is a brief questionnaire which takes approximately 10 minutes to complete. We would appreciate your help in completing the questionnaire and returning it to us using the reply-paid envelope enclosed. Alternatively if you would prefer to complete the questionnaire with someone over the phone, please call or email us using our details above. Your answers will remain confidential and will not be shared with members outside of the research team. You have the option to receive a summary of the findings at the end of the study.

Your participation through the completion of the questionnaire is completely voluntary and your decision to participate will not affect your current health care or registry participation in any way.

If you have any questions regarding the questionnaire or would like to subsequently withdraw your answers, please contact Georgina Hollitt at georgina.hollitt@sa.gov.au or members of the research team using the details above.

Sincerely,

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08 8404 6986
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Bronwyn Ridge
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Flinders University
Bedford Park SA 5042
08 8404 2035
Bronwyn.Usher@flinders.edu.au

Tarrget

Targeting
at-risk relatives
of glaucoma
patients for
early diagnosis
& treatment

Department of
Ophthalmology
Flinders Medical Centre
1 Flinders Drive
Bedford Park SA 5042
08 8404 2035
www.tarrget.com.au

Date

Name
Address

Dear participant,

We are writing to you because you are a participant in **the TARRGET study.** Thank you for your ongoing contribution to the project. Your participation allows us the opportunity to continue research within the field of glaucoma.

Recent research is looking at the feasibility of developing a test to predict a person's risk of developing glaucoma based on genetic information extracted from blood or saliva samples. The results from such a test would be able to predict a person's likelihood of developing glaucoma (e.g. a genetic risk score that would predict if a person has a higher or a lower risk). We wish to assess whether people with relatives who have glaucoma would be interested in such testing if it were available.

Enclosed is a brief questionnaire which takes approximately 10 minutes to complete. We would appreciate your help in completing the questionnaire and returning it to us using the reply-paid envelope enclosed. Alternatively if you would prefer to complete the questionnaire with someone over the phone, please call or email us using our details above. Your answers will remain confidential and will not be shared with members outside of the research team. You have the option to receive a summary of the findings at the end of the study.

Your participation through the completion of the questionnaire is completely voluntary and your decision to participate will not affect your participation in research in any way.

If you have any questions regarding the questionnaire or would like to subsequently withdraw your answers, please contact Georgina Hollitt at georgina.hollitt@sa.gov.au or members of the research team using the details above.

Sincerely,

Georgina Hollitt
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08 8404 6986
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Bronwyn Ridge
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Flinders University
Bedford Park SA 5042
08 8404 2035
Bronwyn.Usher@flinders.edu.au

Date

Name
Address

Dear participant,

Thank you for your participation in our project on individuals' perspective on glaucoma genetic risk assessment. Glaucoma is the leading cause of irreversible vision loss worldwide. Moreover, approximately half of those with glaucoma remain undiagnosed. This is largely due to the condition developing without symptoms or signs in the early stages of disease. This is a statistic we hope will change in the near future, through effective implementation of genetic testing.

Recent research is looking at the feasibility of developing a test to predict a person's risk of developing glaucoma based on genetic information extracted from blood or saliva samples. The results from such a test would be able to predict a person's likelihood of developing glaucoma (e.g. a genetic risk score that would predict if a person has a higher or a lower risk). We wish to assess whether people who go to the optometrist but do not have glaucoma would be interested in such testing if it were available.

Enclosed is a brief questionnaire which takes approximately 10 minutes to complete. We would appreciate your help in completing the questionnaire in person or by returning it to us using the reply-paid envelope enclosed. Your answers will remain confidential and will not be shared with members outside of the research team. You have the option to receive a summary of the findings at the end of the study.

Your participation through the completion of the questionnaire is completely voluntary and your decision to participate will not affect your current health care in any way.

If you have any questions regarding the questionnaire or would like to subsequently withdraw your answers, please contact Georgina Hollitt at georgina.hollitt@sa.gov.au or members of the research team using the details above.

Sincerely,

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Bronwyn.Usher@flinders.edu.au

SURVEY ON GLAUCOMA GENETIC RISK TESTING UNAFFECTED INDIVIDUAL

Glaucoma is a progressive eye disease which can lead to permanent vision loss and blindness. It is often referred to as the 'sneak thief of sight' because there are no symptoms in its early stages. With early detection and treatment, vision loss can often be prevented.

DEMOGRAPHIC DETAILS

DATE: _____/_____/_____

NAME: _____

DOB: _____/_____/_____ SEX: MALE / FEMALE

ADDRESS: _____

_____

PHONE: ( ) MOBILE:

EMAIL: _____

ETHNIC BACKGROUND: MOTHER: _____ FATHER: _____

HIGHEST LEVEL OF EDUCATION:

☐ Primary School ☐ Secondary School ☐ Vocational training/TAFE ☐ University

Would you like to receive a summary of the findings at the conclusion of this study? ☐ YES ☐ NO

If yes, what is your preferred method of receiving information? (please ensure details are provided above)

☐ Email ☐ Mail ☐ Phone

MEDICAL HISTORY

Does anyone in your family have glaucoma? ☐ YES ☐ NO ☐ Unsure ☐ Don't know

If yes, how many? _____

How are they related to you?

_____

_____

Do you wear glasses or contact lenses for distance vision (driving/watching television)?

☐ YES ☐ NO ☐ Sometimes

When was the last time you had your eyes checked?

☐ Within 6 months ☐ Within 6-12 months ☐ Within 1-2 years ☐ > 2 years ☐ Never

How often do you have your eyes checked by an optometrist or ophthalmologist?

☐ 3 monthly ☐ 6 monthly ☐ Annually ☐ Every 2 years ☐ More than every 2 years ☐ Never

Do you have diabetes? ☐ YES ☐ NO ☐ Unsure

Do you have high blood pressure or take medication for it? ☐ YES ☐ NO ☐ Unsure

Do you get migraines? ☐ YES ☐ NO ☐ Unsure

Do you have a history of Raynaud's disease or poor circulation? ☐ YES ☐ NO ☐ Unsure

Do you have a history of heart attack, angina, or stenting? ☐ YES ☐ NO ☐ Unsure

Do you have a history of stroke or mini stroke? ☐ YES ☐ NO ☐ Unsure

QUESTIONS REGARDING GLAUCOMA

Do you think glaucoma is a hereditary condition? (ie can be inherited)

☐ Not at all ☐ Somewhat ☐ Definitely ☐ Unsure

Do you consider glaucoma as being a severe medical condition?

☐ Not severe ☐ Slightly severe ☐ Moderately severe ☐ Very severe ☐ Unsure

How likely do you think you are to develop glaucoma in your lifetime?

☐ Highly unlikely ☐ Unlikely ☐ Likely ☐ Highly likely ☐ Unsure

Are you worried about developing glaucoma?

☐ Not worried ☐ Slightly worried ☐ Moderately worried ☐ Very worried ☐ Unsure

How likely would you be to take a genetic test which could predict your risk of developing glaucoma?

☐ Highly unlikely ☐ Unlikely ☐ Likely ☐ Highly likely ☐ Unsure

Would you want to know more about glaucoma before having a test predicting your risk of developing it?

☐ Definitely not ☐ Probably not ☐ Possibly ☐ Probably ☐ Definitely

If a cost were involved, how much would you be willing to pay for the test?

☐ I would not be willing to pay ☐ $50-100 ☐ $100-200 ☐ $200-300 ☐ $300-500

What information about the test would you want to know? (Choose as many as appropriate)

☐ Cost

☐ Process involved in taking the test

☐ Follow-up

☐ Implications of results

☐ Other:

_____

_____

_____

Which of the following factors would affect your decision to be tested? (Choose as many as appropriate)

☐ To be able to take appropriate measures regarding my glaucoma risk and future eyesight

☐ To be able to provide advice to my children about their potential risk

☐ To be able to provide advice to my family members about their potential risk

☐ Personal advice – if someone from my family recommended the test

☐ Would rather know/to prepare for the future

☐ Medical advice – if your doctor or optometrist recommended the test

☐ Other:

_____

_____

Which of the following factors would concern you about having the test? (Choose as many as appropriate)

☐ Personal anxiety/fear if results showed an increased glaucoma risk

☐ Would rather not know if at risk

☐ Concerns about cost

☐ Concerns about attending ongoing follow-up appointments

☐ Concern regarding whether it could affect insurance

☐ Concern regarding whether it could affect employment

☐ Concern regarding confidentiality

☐ Other:

_____

_____

How would you change the frequency of eye checks if the test results showed that you were at **LOWER** risk of developing glaucoma?

☐ I would not change the frequency ☐ I would have less frequent eye checks

How frequently would you be willing to have an eye check?

Every: ☐ 6 ☐ 12 ☐ 18 ☐ 24 months

What would your preferred method of receiving results be? (Choose as many as appropriate)

☐ Face to face

☐ Letter / Mailout

☐ Telephone call

☐ Email

☐ Other: _____

COMMENTS: _____

_____

_____

_____

_____

Appendix A.3: Healthcare Professionals' questionnaire

Introduction

.

Dear Clinician/Healthcare Professional,

You are invited to take part in a research project assessing healthcare professionals' perspective about polygenic risk testing for glaucoma (e.g. ophthalmologists, optometrists, orthoptists, clinical geneticists, genetic counsellors, general practitioners, lab scientists). Glaucoma is the leading cause of irreversible vision loss in the world. Moreover, approximately half of those with glaucoma remain undiagnosed. This is largely due to the condition developing without symptoms or signs in the early stages of the disease. This is a situation we hope will change in the near future, through effective implementation of genetic testing.

Polygenic risk scores stratify an individual's risk based on the cumulative effect of many common genetic variants. Polygenic risk testing is emerging as an effective approach to identify individuals at higher risk of developing disease for complex genetic conditions.

Healthcare workers will be at the forefront of the delivery of personalised medicine. As a result, we wish to understand their perspectives of polygenic risk testing for glaucoma. The results from this survey will help develop training and resources for health care workers who may be involved in offering, referring or counselling patients as well as interpreting results from the test.

The survey will take approximately 15 minutes to complete. Your answers will remain confidential. Your participation through the completion of the questionnaire is completely voluntary.

This study is conducted by Prof Jamie Craig, Dr Emmanuelle Souzeau and PhD student Dr Georgie Hollitt at Flinders University and has received Ethics approval from the Southern Adelaide Clinical Human Research Ethics Committee (HREC 279.19).

If you have any questions regarding the questionnaire or would like to subsequently withdraw your answers, please contact Georgie Hollitt at georgie.hollitt@flinders.edu.au or members of the research team.

Department of Ophthalmology

Flinders University
1 Flinders Drive, Bedford Park
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Tel: +61 8 8204 5737; Fax: +61 8 8277 0899

Demographic detials

Q1. What is your primary occupation:

- ○ Ophthalmologist
- ○ General Practitioner
- ○ Clinical Geneticist
- ○ Genetic Counsellor
- ○ Optometrist
- ○ Orthoptist
- ○ Laboratory Scientist
- ○ Other (please specify) _____

Qa. What percentage of your patients have glaucoma?

_____

Q2. What is your age?

- ○ < 30 years
- ○ 30-39 years
- ○ 40-49 years
- ○ 50-59 years
- ○ 60-69 years
- ○ ≥ 70 years

Q3. What is your gender?

- ○ Male
- ○ Female
- ○ Non-binary
- ○ Prefer not to say
- ○ Other (please specify) _____

Q4. What is your ancestry? (choose as many as apply)

- ☐ European
- ☐ African
- ☐ Asian
- ☐ Australian Aboriginal
- ☐ Hispanic
- ☐ Middle Eastern
- ☐ Pacific Islander
- ☐ Unknown
- ☐ Other (please specify) _____

Q5. How many years has it been since you completed your training for your current profession or specialty?

_____

Q6. How much of your professional training did you complete in Australia?

- ○ All
- ○ Most
- ○ Some
- ○ None

Q7. How many years have you been practicing full time in your profession?

Q8. What is your primary workplace?

- ○ Private hospital
- ○ Public hospital
- ○ Private clinic/practice
- ○ Public clinic/practice
- ○ Corporate practice
- ○ Academic institution/University
- ○ Laboratory
- ○ Other (please specify)

Q9. What is the structure of your primary practice? (choose as many as apply)

- ☐ Solo practice
- ☐ Single specialty group
- ☐ Multi-specialty group
- ☐ Not applicable
- ☐ Prefer not to answer
- ☐ Other (please specify)

Q10. How much exposure to genetics did you have during your training?

None	A little	A moderate amount	A lot	A great deal	Not applicable
○	○	○	○	○	○

Q11. Have you undertaken any post-graduate training courses in genetics?

- ○ Yes
- ○ No

Q12. Do you hold an academic position?

- ○ No
- ○ Yes, part-time
- ○ Yes, full-time
- ○ Yes, honorary (please specify)

Q13. Have you ever been diagnosed with glaucoma?

- ○ Yes
- ○ No

Q14. Are you aware of anyone in your family ever having been diagnosed with glaucoma?

- ○ Yes
- ○ No
- ○ Unknown

Q14a. If yes, how many family members are affected? (including those who are deceased)

Q14b. How are they related to you? (list all, including those who are deceased, eg. brother, paternal grandmother, maternal aunt)

General Questions

Q15. When seeing a new patient, how important do you think it is to assess their

family history of glaucoma in consideration of:

	Not at all important	Slightly important	Moderately important	Very important	Extremely important	Not applicable
Affected first-degree relatives? (eg mother, father, sibling)	○	○	○	○	○	○
Affected second-degree relatives? (grandparents, aunt, uncle, cousins)	○	○	○	○	○	○

Q16. If a patient reported a family history of glaucoma, how important do you think it is to ask about the age of diagnosis of their affected family member(s)?

Not at all important	Slightly important	Moderately important	Very important	Extremely important	Not applicable
○	○	○	○	○	○

Q17. During the past 12 months, have you counselled a patient on a genetic issue for any of the following?

	No	Yes	Not applicable
For any genetic condition?	○	○	○
For an eye condition?	○	○	○
For glaucoma?	○	○	○

Q18. If yes, who initiated the conversation about the genetic issue?

- ○ Patient
- ○ Yourself
- ○ Both
- ○ Other (please specify) _____

Q19. During the past 12 months, have any of your patients asked you if they can get a genetic test?

	No	Yes	Not applicable
For any genetic condition?	○	○	○
For an eye condition?	○	○	○
For glaucoma?	○	○	○

Q20. During the past 12 months, have you referred a patient for a genetic test?

	No	Yes	Not applicable
For any genetic condition?	○	○	○
For an eye condition?	○	○	○
For glaucoma?	○	○	○

Q21. During the past 12 months, have you ordered a genetic test for a patient?

	Yes	No	Not applicable
For any genetic condition?	○	○	○
For an eye condition?	○	○	○
For glaucoma?	○	○	○

Q22. If you have received genetic results for a patient, how did you receive the results? (Choose as many as appropriate)

- ☐ Verbal - in person
- ☐ Verbal - via telephone call
- ☐ Written report
- ☐ Written - email
- ☐ Electronic medical records
- ☐ Electronic results

☐ Other (please specify): ____

Q22b. If you received written results, how were the results presented?

☐ Words only
☐ Words and graphics - black and white
☐ Words and graphics - colour
☐ Graphics only
☐ Other (please specify) ____

Q22c. Did you communicate the results to the patient yourself?

○ Yes
○ No

Genetics

Q23. How would you rate your level of knowledge on genetics and disease susceptibility? (scale from lowest to highest)

0	1	2	3	4	5	6	7	8	9	10
○	○	○	○	○	○	○	○	○	○	○

Q24. How qualified do you feel to order genetic tests?

Not at all qualified	Slightly qualified	Moderately qualified	Very qualified	Extremely qualified
○	○	○	○	○

Q25. How confident do you feel to perform the following?

	Not at all confident	Slightly confident	Moderately confident	Very confident	Extremely confident	Not applicable
Take a family history?	○	○	○	○	○	○
Identify family history of a	○	○	○	○	○	○

	Not at all confident	Slightly confident	Moderately confident	Very confident	Extremely confident	Not applicable
potentially inherited condition?						
Determine the mode of inheritance from a pedigree?	○	○	○	○	○	○
Estimate the risk of your patient having or developing a genetic condition based on their family and medical history?	○	○	○	○	○	○
Identify specialist genetic services in your area?	○	○	○	○	○	○
Counsel patients on genetic testing?	○	○	○	○	○	○
Interpret the results of a genetic test?	○	○	○	○	○	○

Q26. Do you feel you would benefit from more training in any of the following?

	Definitely no	No, not really	Yes, probably	Definitely yes	Not applicable
Genetic testing	○	○	○	○	○
Interpretation of genetic test results	○	○	○	○	○
Polygenic risk scores	○	○	○	○	○

Q27. What would your preferred method of training be? (Please rank in order of preference - with 1 being most preferred)

Day-long conference/workshop

Genetic education grand rounds

Mailed information - email or post

Online course

Online information e.g. websites, journals

Department meeting/in-service

Other

Q27a. Specify other preferred method of training:

Glaucoma

Q28. How would you rate your knowledge of the following? (with 0 being no knowledge)

	0	1	2	3	4	5	6	7	8	9	10
Glaucoma?	○	○	○	○	○	○	○	○	○	○	○
Risk factors for open-angle glaucoma?	○	○	○	○	○	○	○	○	○	○	○
Diagnosing glaucoma?	○	○	○	○	○	○	○	○	○	○	○
The genetics of glaucoma?	○	○	○	○	○	○	○	○	○	○	○
Current screening recommendations for glaucoma?	○	○	○	○	○	○	○	○	○	○	○

Q29. Are you aware of any commercially available tests for the following?

	Yes	No	Unsure
Inherited genes for glaucoma?	○	○	○

	Yes	No	Unsure
Polygenic risk testing for glaucoma?	○	○	○

Attitudes toward genetic testing

Q30. How familiar are you with the concept of polygenic risk?

	Not at all familiar	Slightly familiar	Moderately familiar	Very familiar	Extremely familiar
For any condition	○	○	○	○	○
For an eye condition	○	○	○	○	○
For glaucoma	○	○	○	○	○

Q30a. How qualified do you consider yourself to recommend polygenic risk testing for glaucoma to your patients?

Not at all qualified	Slightly qualified	Moderately qualified	Very qualified	Extremely qualified
○	○	○	○	○

Q30b. If polygenic risk testing were to become available, how likely would you be to recommend a polygenic risk test for glaucoma in:

	Highly unlikely	Unlikely	Likely	Highly likely	Not applicable
First degree relatives of patients with glaucoma?	○	○	○	○	○
People aged >50 years?	○	○	○	○	○
Individuals of Asian ancestry?	○	○	○	○	○
Individuals of African ancestry?	○	○	○	○	○
Second degree relatives of patients with glaucoma?	○	○	○	○	○

	Highly unlikely	Unlikely	Likely	Highly likely	Not applicable
Individuals aged >70 years?	○	○	○	○	○

Q30c.

How important are each of the following in your decision whether or not to recommend polygenic risk testing for glaucoma?

	Not at all important	Slightly important	Moderately important	Very important	Extremely important	Not applicable
The individual's attitude toward genetic testing	○	○	○	○	○	○
Recommendations and guidelines from your institution or practice	○	○	○	○	○	○
Recommendations and guidelines from medical societies (including your professional college or peak body)	○	○	○	○	○	○
Recommendations and guidelines from government agencies	○	○	○	○	○	○
Discussion with your colleagues	○	○	○	○	○	○
Information you obtained through continuing medical education	○	○	○	○	○	○
Commercial advertisements and promotions	○	○	○	○	○	○
Availability of genetic testing services	○	○	○	○	○	○
Clinical data published in the	○	○	○	○	○	○

	Not at all important	Slightly important	Moderately important	Very important	Extremely important	Not applicable
medical literature						
Confidence to interpret test results	○	○	○	○	○	○
Lack of time to review or discuss test results	○	○	○	○	○	○
Coverage of genetic tests by Medicare	○	○	○	○	○	○

Q31. Which of the following health care provider(s) would you consider are appropriate to do the following? (Tick as many as appropriate)

	Ophthalmologist	General Practitioner	Medical geneticist	Optometrist	Genetic counsellor	Orthoptis
Order polygenic risk testing for glaucoma?	☐	☐	☐	☐	☐	☐
Communicate polygenic risk test results showing LOW individual risk	☐	☐	☐	☐	☐	☐
Communicate polygenic risk test results showing HIGH individual risk	☐	☐	☐	☐	☐	☐

Q31a. Specify other health care provider qualified to order polygenic testing for glaucoma?

Q31b. Specify other health care provider qualified to communicate test results showing low individual risk for glaucoma?

Q31c. Specify other health care provider qualified to communicate test results showing HIGH individual risk for glaucoma?

Q32. What is your preferred method of communicating results to patients' for the following? (Number as many as appropriate in order from 1 to 4, with 1 being most preferred)

	In person	Telephone call	Email	Mail
If results were shown to be high risk				
If results were shown to be low risk				

Q33. Based on your preferred method of receiving results, would a graph or figure be helpful with written information?

- ○ Yes
- ○ No

Q34. When ordering a test, how important are the following factors?

	Not at all important	Slightly important	Moderately important	Very important	Extremely important	Not applicable
Performance characteristics of the test (positive/negative predictive value, sensitivity/specificity)	○	○	○	○	○	○
Ability of the test to provide prognostic information	○	○	○	○	○	○
Ability of the test to predict clinical benefit of specific	○	○	○	○	○	○

	Not at all important	Slightly important	Moderately important	Very important	Extremely important	Not applicable
treatments need for surgery						
Ability to provide information about family members risk	○	○	○	○	○	○
Other	○	○	○	○	○	○

Q34a. Specify other factor:

Block 6

Q60. This is the end of the questionnaire. Please use the arrows to navigate back if you would like to review your responses before completion, or forward if you are happy to finalise your answers.

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APPENDIX B: REPORTING RESULTS QUESTIONNAIRE

Trial Reports

Start of Block: Default Question Block

Introduction Thank you for your participation in our study evaluating the use of genetic testing for glaucoma and age-related macular degeneration (AMD) - the Genetic Risk Assessment of Degenerative Eye disease (GRADE) study.

In this part of the study, you consented to provide feedback on different fictional reports. Participation requires you to complete this online questionnaire that should take approximately 20 minutes to help us understand how you interpret numbers, graphs and genetic information. We will then contact you to participate in a group discussion or individual interview, depending on personal preference and COVID-19 restrictions, to discuss each aspect of the mock reports.

If you have any questions regarding the study, please contact Georgie Hollitt on 0493 057 068 or georgie.hollitt@flinders.edu.au.

End of Block: Default Question Block

Start of Block: Demographic Details

Q1 First name:

_____

Q2 Surname:

_____

Q3 What is your age?

- ○ 50-59 years (1)
- ○ 60-69 years (2)
- ○ ≥70 years (3)

Q4 What is your gender?

- ○ Male (1)
- ○ Female (2)
- ○ Non-binary / third gender (3)
- ○ Prefer not to say (4)
- ○ Other (please specify) (5) _____

Q5 What is the highest level of education you have completed?

- ○ No formal education (1)
- ○ Primary School (2)
- ○ Secondary School (3)
- ○ Vocational Training/TAFE (4)
- ○ University (5)

Q6 What is your ethnicity/family background? (choose as many as apply)

- [ ] European/Caucasian (1)
- [ ] African (2)
- [ ] Asian (3)
- [ ] Australian Aboriginal (4)
- [ ] Middle Eastern (9)
- [ ] Hispanic (5)
- [ ] Pacific Islander (6)
- [ ] Unknown (7)
- [ ] Other (please specify) (8) _____

Q7 Are you colour blind?

- ○ Yes (1)
- ○ No (2)
- ○ Unsure (3)

End of Block: Demographic Details

Start of Block: Genetic Literacy

Q1 The following list contains some real medical words related to genetic testing. The list also contains items that may look or sound like medical words but are not real words. As you read through the list, mark the items that you know are real words. You should not guess. Only mark an item if you are sure it is a real word.

- [ ] Genetolisis (1)
- [ ] Preventative (2)
- [ ] Recessive (3)
- [ ] Citozygous (4)
- [ ] Allele (5)
- [ ] Pathogynia (6)
- [ ] Sequation (7)
- [ ] Microtation (8)
- [ ] Lenotype (9)
- [ ] Chromosome (10)
- [ ] Chemosomal (11)
- [ ] Heteroygous (12)
- [ ] Pathochemia (13)
- [ ] Biocromity (14)
- [ ] Penetrance (15)
- [ ] Microbiotion (16)

- [ ] Genotype (17)
- [ ] Hereditary (18)
- [ ] Treatious (19)
- [ ] Autosome (20)
- [ ] Prevotype (21)
- [ ] Homozygous (22)
- [ ] Predisposition (23)
- [ ] Offspring (24)
- [ ] Genome (25)
- [ ] Potient (26)
- [ ] Nucleotisity (27)
- [ ] Carrier (28)
- [ ] Depretion (29)
- [ ] DNA (30)
- [ ] Likelihood (31)
- [ ] Prebiocular (32)
- [ ] Consent (33)
- [ ] Pathogenicity (34)

- [ ] Sequencing (35)
- [ ] Exome (36)
- [ ] Variant (37)
- [ ] Mutation (38)
- [ ] Heloux (39)
- [ ] Prevelative (40)
- [ ] Exon (41)
- [ ] Moleculative (42)
- [ ] Gene (43)
- [ ] Phenotype (44)
- [ ] Inheritance (45)
- [ ] Diagnosis (46)
- [ ] Karyotype (47)
- [ ] Behaviose (48)
- [ ] Molecular (49)
- [ ] Dominant (50)
- [ ] Actionability (51)

Q2 Indicate whether you think the following statements are either 'true' or 'false'.

	True (1)	False (2)
Most genetic disorders are caused by a single gene (1)	○	○
A "complex disease" is a health condition brought on by many genes and lifestyle and environment (2)	○	○
If your close relatives have glaucoma, you are more likely to develop it (3)	○	○
Glaucoma screening is only recommended for people with a family history of glaucoma (4)	○	○
Each of us has variations in our genes that make it more likely that we will get certain diseases (5)	○	○
If a person has a genetic predisposition for a disease, this person will always get the disorder (6)	○	○
The exact chance of developing a genetic condition can be determined through genetic testing (7)	○	○
Once a genetic marker for a disorder is identified in a person, the disorder can usually be prevented or cured (8)	○	○

End of Block: Genetic Literacy

Start of Block: Numeracy

Q1 Imagine that we rolled a fair, six-sided die 1000 times. Out of 1000 rolls, how many times do you think the die would come up even (2, 4, or 6)

_____

Q2 If the chance of getting a disease is 10%, how many people out of 1000 would be expected to get the disease?

_____

Q3 If the chance of getting a disease is 1 in 1000, what percent of people would be expected to get the disease?

_____

End of Block: Numeracy

Start of Block: Graph Literacy

Q1
Enter answer below:

_____

Q2
Select one answer below:

- ○ Crosicol (4)
- ○ Hertinol (5)
- ○ They are equal (6)
- ○ Can't say (7)

Q3
Enter answer below:

_____

Q4
Enter answer below:

_____

End of Block: Graph Literacy

Start of Block: Report questions

Q1 In preparing for discussing the reports, could you tell us which of the following questions and explanations would you want to see included in a report for glaucoma genetic risk? (choose as many as appropriate)

- [ ] What is glaucoma? (1)
- [ ] What are the risk factors for developing glaucoma? (i.e. why me?) (2)
- [ ] What can I do to reduce my risk? (3)
- [ ] How is glaucoma detected? (4)
- [ ] What are the treatment options? (5)
- [ ] What do my results mean for my family? (6)
- [ ] Who else can I talk to/where can I get support? (7)
- [ ] How will my result affect my insurance? (8)
- [ ] Other (please specify) (9)

_____

End of Block: Report questions

APPENDIX C: MOCK REPORTS

Polygenic Risk Score (PRS) Report - Glaucoma

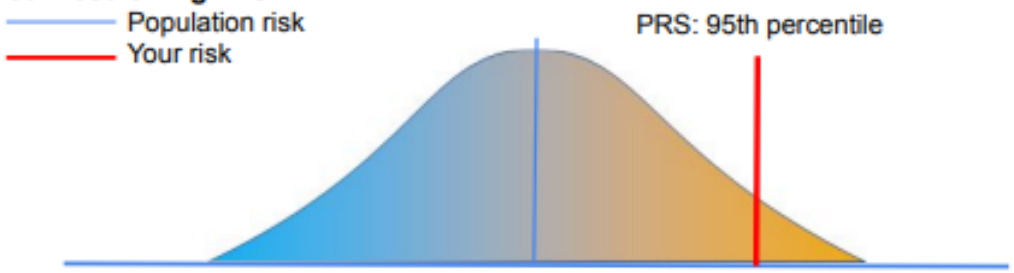
ID: xxxxxxxxxxx

First Name: .....
Surname: .....
DOB: .....
Gender: .....

Ordered by: .....
Sample collection date: .....
Received at lab: .....
Date of reporting: .....

1. PRS Result - Glaucoma

Your result - high risk



Your Glaucoma PRS indicates you are in the 95th percentile of risk within the population. This means that your risk of developing glaucoma is higher than 95% of the population. Out of 100 people, you have a higher genetic risk of glaucoma than 95 people.

2. Polygenic Risk Scores (PRS) explained

- Polygenic risk scores give an indication of a person's overall genetic risk for a particular condition.
- Your test results were based on 2673 genetic changes (or variants) that we know influence a person's risk of developing glaucoma.
- Some variants are more strongly associated with glaucoma than others.
- Polygenic risk scores add up all of the genetic variants associated with a particular condition that a person has, accounting for how strongly they are associated with the condition.
- The score is represents your risk compared to other people within the population.

What are the limitations of the test?

- This test estimates your risk of primary-open angle glaucoma (the most common subtype of glaucoma).
- It does not estimate your risk of other types of glaucoma or other conditions.
- Although the polygenic score predicts risk in all ancestries, it has been best validated in individuals of European ancestry.
- This test does not account for some rare variants known to cause glaucoma. Therefore, your risk may be higher, especially if you have a strong family history of glaucoma.
- PRS results represent a probability of individual disease risk and are therefore not diagnostic, and results should be interpreted by a suitably qualified clinician in conjunction with other established clinical risk factors, in particular age. It does not take into account non-genetic risk factors.

3. What does my test result mean for me?

- Your result suggests you are at 2.3 times higher risk of developing glaucoma than most other people.
- Your result does not mean that you have glaucoma now.
- It does not mean you will definitely develop glaucoma.
- Because you are at increased risk, we recommend that you see an *ophthalmologist* or *optometrist* every *6 months* so they can check your eyes to see if you have glaucoma.
- There are several treatment options available for glaucoma. Please refer to the back of the page for more information on glaucoma and treatment options.
- Your results also means that other people in your family may be at increased risk as well. They can talk to their GP or eye specialist about it.

Polygenic Risk Score (PRS) Report - Glaucoma

ID: xxxxxxxxxxx

First Name: .....
Surname: .....
DOB: .....
Gender: .....

Ordered by: .....
Sample collection date: .....
Received at lab: .....
Date of reporting: .....

1. PRS Result - Glaucoma

Your result - high risk

Your result indicates your risk of developing glaucoma is approximately 7%. This means that 7 out of 100 people of the same gender and age as you will develop glaucoma over their lifetime.

Average population

Average population - the average population risk is approximately 3%. This means that on average, 3 in 100 people of the same age and gender as you will develop glaucoma.

2. Polygenic Risk Scores (PRS) explained

- Polygenic risk scores give an indication of a person's overall genetic risk for a particular condition.
- Your test results were based on 2673 genetic changes (or variants) that we know influence a person's risk of developing glaucoma.
- Some variants are more strongly associated with glaucoma than others.
- Polygenic risk scores add up all of the genetic variants associated with a particular condition that a person has, accounting for how strongly they are associated with the condition.
- Your score (and genetic risk) can be compared to other people within the population.

What are the limitations of the test?

- This test estimates your risk of primary-open angle glaucoma (the most common subtype of glaucoma).
- It does not estimate your risk of other types of glaucoma or other conditions.
- Although the polygenic score predicts risk in many ancestries, it has been best validated in individuals of European ancestry.
- This test does not account for some rare variants known to cause glaucoma. Therefore, your risk may be higher, especially if you have a strong family history of glaucoma.
- PRS results represent a probability of individual disease risk and are therefore not diagnostic, and results should be interpreted by a suitably qualified clinician in conjunction with other established clinical risk factors, in particular age. It does not take into account non-genetic risk factors.

3. What does my test result mean for me?

- Your result suggests you are at 2.3 times higher risk of developing glaucoma than most other people.
- Your result does not mean that you have glaucoma now.
- It does not mean you will definitely develop glaucoma.
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- There are several treatment options available for glaucoma. Please refer to the back of the page for more information on glaucoma and treatment options.
- Your result also means that other people in your family may be at increased risk as well. They can talk to their GP or eye care professional about it.

Polygenic Risk Score (PRS) Report - Glaucoma

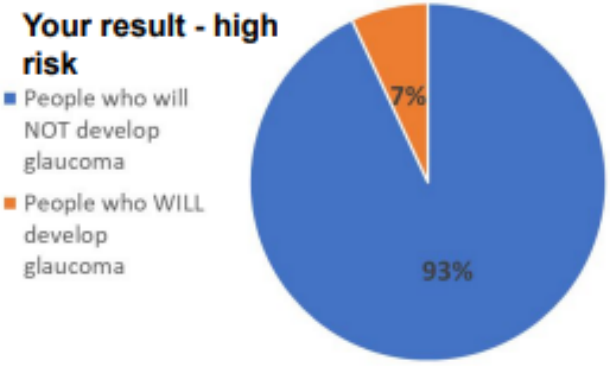
ID: xxxxxxxxxxx

First Name: .....
Surname: .....
DOB: .....
Gender: .....

Ordered by: .....
Sample collection date: .....
Received at lab: .....
Date of reporting: .....

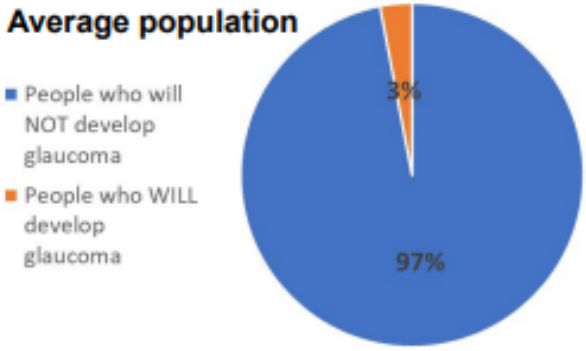
1. PRS Result - Glaucoma

Your result - high risk



Your result indicates your risk of developing glaucoma is approximately 7%. This means that 7 out of 100 people of the same gender and age as you will develop glaucoma over their lifetime.

Average population



Average population - the average population risk is approximately 3%. This means that on average, 3 in 100 people of the same age and gender as you will develop glaucoma.

2. Polygenic Risk Scores (PRS) explained

- Polygenic risk scores give an indication of a person's overall genetic risk for a particular condition.
- Your test results were based on 2673 genetic changes (or variants) that we know influence a person's risk of developing glaucoma.
- Some variants are more strongly associated with glaucoma than others.
- Polygenic risk scores add up all of the genetic variants associated with a particular condition that a person has, accounting for how strongly they are associated with the condition.
- The score is represents your risk compared to other people within the population.

What are the limitations of the test?

- This test estimates your risk of primary-open angle glaucoma (the most common subtype of glaucoma).
- It does not estimate your risk of other types of glaucoma or other conditions.
- Although the polygenic score predicts risk in all ancestries, it has been best validated in individuals of European ancestry.
- This test does not account for some rare variants known to cause glaucoma. Therefore, your risk may be higher, especially if you have a strong family history of glaucoma.
- PRS results represent a probability of individual disease risk and are therefore not diagnostic, and results should be interpreted by a suitably qualified clinician in conjunction with other established clinical risk factors, in particular age. It does not take into account non-genetic risk factors.

3. What does my test result mean for me?

- Your result suggests you are at 2.3 times higher risk of developing glaucoma than most other people.
- Your result does not mean that you have glaucoma now.
- It does not mean you will definitely develop glaucoma.
- Because you are at increased risk, we recommend that you see an *ophthalmologist* or *optometrist* every *6 months* so they can check your eyes to see if you have glaucoma.
- There are several treatment options available for glaucoma. Please refer to the back of the page for more information on glaucoma and treatment options.
- Your results also means that other people in your family may be at increased risk as well. They can talk to their GP or eye specialist about it.

Polygenic Risk Score Report - Glaucoma

ID: xxxxxxxxxxx

Your Polygenic Risk in Detail

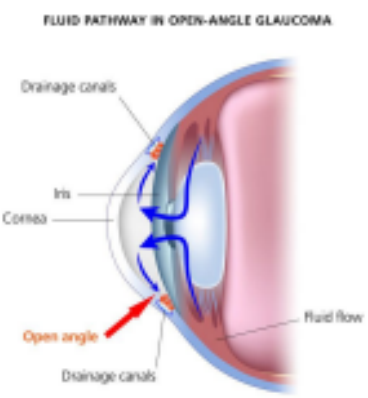
- Researchers have identified genetic variants which are associated with glaucoma by comparing those with the disease to those without
- A PRS collates the combined risk of multiple genetic risk variants into a single score, typically by weighting the relative effect size of each variant.
- Scores may be combined with conventional risk factors to estimate overall disease risk.

For a full explanation on genetic risk and calculation of PRS:
https://www.genome.gov/Health/Genomics-and-Medicine/Polygenic-risk-scores

Frequently Asked Questions

What is glaucoma?

- Glaucoma is a group of neurodegenerative conditions that affect the optic nerve
- Glaucoma is usually a complex disease, influenced by both genetic and environmental factors.
- Primary open-angle glaucoma is the most common form of glaucoma
  - Open-angle means the area where the fluid drains out of the eye is not obstructed
  - Primary means there is no other known cause (such as trauma or surgery)



What are the symptoms?

- Usually there are no symptoms in early disease
- Vision loss may only be noticeable in later stages of disease
- Vision loss from glaucoma is irreversible and cannot be restored
- Only an eye exam performed by an eye specialist can tell if someone has glaucoma

Are there any risk factors?

- Yes, there are a number of risk factors (examples listed below)
- Family history of glaucoma
  - Those who have a first-degree relative (parent, sibling, child) with glaucoma are at almost 10 times increased risk of also developing glaucoma compared to those who do not
- African ancestry
- Age over 50 years
- Elevated eye pressure

What are the treatment options?

- Treatment options are highly effective at slowing or preventing disease progression in most people
- Treatments include topical eye drops, laser therapy, and in very advanced cases, surgery.

What does this mean for my family?

- We know that people who have a family member with glaucoma are at higher risk of also developing glaucoma
- If you have been identified to be at high risk, it is possible that your closest relatives are also at increased risk

Resources - for more information and where to get help

Glaucoma Australia:
https://glaucoma.org.au/home

Glaucoma Australia
Saving sight

Vision Australia:
https://www.vision2020australia.org.au/

VISION 2020 AUSTRALIA

Speak to your:
- Ophthalmologist
- Optometrist
- GP

APPENDIX D: SEMI-STRUCTURED INTERVIEW GUIDE

Focus Group/Interview Guide

Outline of topics to cover

Purpose/Aim:
To explore the preferences of individuals towards various formats of reporting polygenic risk results.

Discussion points:

- Overall impression of the different reports
- Impact of colour on report preference
- Impact of font on report preference
- Whether the report content is appropriate
- Whether the report content is easy to understand
- Whether there was anything missing from the reports
- Does the report raise any questions
- What do they understand about the representation of the risk
- Whether the report adequately communicates the results to ensure the individual would feel confident that they have correctly understood it.

Focus Group/Interview semi-structured interview guide

Introduction and Purpose

Thank you for volunteering and taking the time to participate in this project. My name is Georgie, and I am conducting this study as part of my PhD within the Department of Ophthalmology at Flinders University. You have been asked to participate as your view is important. We appreciate your time and willingness to participate. This focus group/interview is designed to explore your thoughts and preferences towards 3 reports which we have recently created to communicate polygenic risk score results to members of the community.

As you may remember, the GRADE study is working towards integrating polygenic risk testing for glaucoma into clinical practice, so it will be important to ensure that the results are reported effectively. That is why we are asking for your honest opinions.

There are absolutely no right or wrong answers. We want to hear any feedback you may have. You might find that you don't agree with someone and that's okay; it does not mean either of you are right or wrong, it just means that you see things differently and we want to hear why that is (for focus groups only).

We are recording your answers so that we don't miss anything, but your answers will be kept confidential. The recordings are stored safely. When they are transcribed, nobody's name will be attached to their comments.

Can I ask you again for your permission to record the discussion? *[Upon yes from all, start recording]*

Ground Rules

1. The most important rule is that only one person speaks at a time. Please wait until the person speaking has finished before starting your comment (focus group only).
2. There are no right or wrong answers.
3. You do not have to speak in a particular order (focus group only)
4. You do not have to agree with the views of other people in the group (focus group only)
5. Do you/Does anyone have any questions? (answers).

General

- Have you ever received a genetic report before? If yes, did you find it useful?
- Have you ever received any other medical results in a written report before?
    - If yes, did you find it useful?
    - What did you like about the report?
    - What didn't you like about the report

First Impressions

- What is your first impression of the report?
- What is the first thing you looked at on the report?

- Is there anything you would suggest changing to improve your first impression of the report?
- After reading the report, can you tell me what the main thing this report is telling you?

Risk figure (each):

- Do you think this figure is easy to understand? If not, what is difficult to understand?
- What do you like about this figure?
- What don't you like about this figure?
- What is the key message of the figure?
- What is your interpretation of the risk that is shown in the figure? Do you think this person is at higher risk, lower risk, or average risk of developing glaucoma compared to other people?
- On a scale of 1-10, How worried would you be if you saw this result, with 1 being not at all worried and 10 being extremely worried?
- Do you think most people would be able to understand this figure? Why/why not?

Preference:

- Out of the three figures you have been shown, which did you prefer? Why?
- Can you rank the three figures in order of most preferred to least preferred?
- Can you rank the three figures in order of easiest to hardest to understand?
- Do you have any suggestions about how they could be improved?
- Do you have any further comments about these figures?

Report layout and appearance

- Do you think the report was easy to read and interpret?
- Do you think the content of the report was appropriate?
- Was there any information missing from the reports that you would like to see?
- What did you like about the report?
- What did you not like about the report?
- Can you comment on the balance of text and visual elements? Is there too much of one and not the other?
- Did you like the font that was used?
- Did you like the colours that were used?

Confidence

- Would you feel confident that you correctly understood the report?
- Would you want to review the report with your doctor?
- Do you have any other comments/feedback on the report/s?

Summary

Thank you very much for sharing your thoughts and preferences on the reports. We are reaching the end of our time now, so I would like to finish by summarising the key ideas that I have heard.

Is there anything I have missed or anything anyone/you would like to add?

If you think of anything later that you would like to feed back, you are welcome to contact me via email or phone. My contact details can be found on the Information Sheet.

Thank you again for your contribution to this project. Your honest discussion has been very helpful in furthering our understanding of how we can effectively communicate genetic results for glaucoma to members of the community.

Useful prompts to use throughout focus group/interview:

- Does anyone think differently about what was said? (focus group only)
- Can you tell us a little bit more about that?
- Can you give an example of what you mean?
- For negative responses
    - Can you give a suggestion on how it could be changed/improved?

APPENDIX E: LITERATURE REVIEW - EXAMPLES OF PRS REPORTS

Peter MacCallum Cancer Centre
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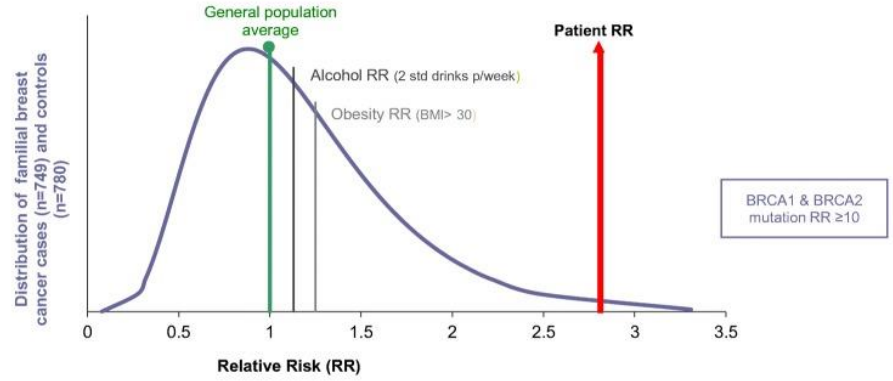
Lab episode: XXXXX
Report date: DD/MM/YYYY

Patient name: TEST, test
Date of birth: DD/MM/YYYY
Sex: Female

Clinical details: Personal history of breast cancer (unilateral)
Previous screening of BRCA1 and BRCA2 genes did not identify a mutation.

Test requested: **Common Genetic Variants**

Test result: **Polygenic risk catergory is HIGH**



Interpretation: **This patient's breast cancer relative risk is 2.8**
This is based on the polygenic risk score of 1.5

This result suggests that this patient is at high risk of breast cancer.

Research has found that women with a high polygenic risk score have a two-fold increase in the incidence of a second primary cancer when compared to women with a low polygenic risk score.

Methods:

Patient sample has been genotyped for 22 common genomic variants that have a clinical and statistical significance with breast cancer risk.

Polygenic risk score calculated through the sum of the log odds ratios for each common genomic variant tested within specimen. Score has been standardized to the same metric using population controls.

Polygenic risk score category ranges:

High:	*Greater than*	0.59
Intermediate:	-0.07	0.58
Low:	*Less than*	-0.08

Common genomic variants tested:

rs2981582 (FGFR2), rs3803662 (TOX3), rs17468277 (CASP8), rs889312 (MAP3K1), rs3817198 (LSP1), rs2046210 (ESR1), rs4973798 (SLC4A7), rs6504950 (STXBP4), rs999737 (RAD51L), rs13387042, rs13281615, rs10941679, rs11249433, rs1011970, rs2380205, rs704010, rs614367, rs10509168, rs12662670, rs1975930, rs8170, rs865686.

Reported By: **A/Prof Paul James**
Sarah Sawyer

Reference: Sawyer, S, James, PA et al 30(35)4330 2012 Journal of Clinical Oncology

Family Report: Whole Genome Sequencing Results

		Patient Information		Clinician	
Patient Name:	Walter Jones	15 State Street	Preferred Contact:	**Ordering Clinician:**	Geoff Genes, MD
DOB:	1/11/2003	Bloomsburg, PA	Janine Jones		100 N Academy Ave.
Sex:	Male	17815	(mother)		Danville, PA 17822
Date of sample collection:	3/20/2013	123-456-7890	1-2jones@gmail.com		570-555-5555
Age at sample collection:	10 years, 2 months	**Patient Representative** *(if applicable)*:	Janine Jones, Mother	Contact:	Nancy Nurse
Reason for testing:	Myopathy (muscle weakness)	**Family Samples Submitted**		Referring Clinician:	Peter Peds, MD Primary Pediatrics
		Name	Relationship		
		Janine Jones	Mother		
		Jimmy Jones	Father		

Test Results and Explanation

Part I: Results Related to the Reason for Testing

Summary of Results	A likely genetic cause for symptoms was found with a probable diagnosis of Salih myopathy
Associated Gene	*TTN*

Was at least one relevant genetic mutation found? **Yes**

Two changes were found in **one** gene called *TTN* that may be the cause of your child's symptoms. These results are shown on the next page. It is important to talk with your doctor about the meaning of these results for your child.

Whole genome sequencing testing was ordered to identify a possible genetic cause for your child's symptoms. Your child's symptoms were reported to include **muscle weakness (myopathy)**, **delay in physical development**, and **drooping of the eyelids (ptosis)**. A muscle biopsy showed findings that a possible muscle disease could explain these symptoms.

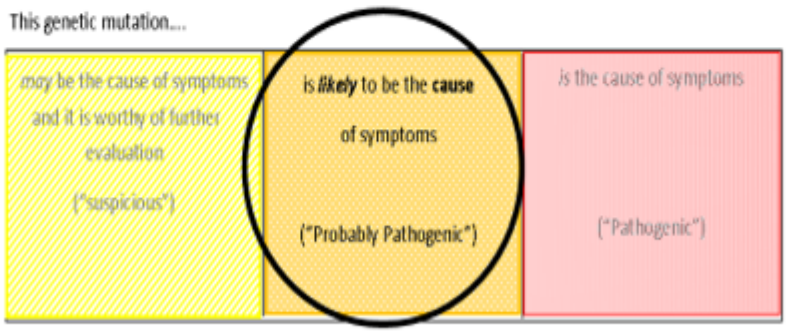
Glossary:

Myopathy is muscle weakness. There are many different types of myopathy. Different causes lead to the different kinds of muscle weakness.

Salih myopathy is a specific type of myopathy that is caused by change in a gene that works to produce energy for cells in the body.

Mutation is also known as a variant. This is a change in the code of a gene. Most variants do not change the function or activity of a gene. This type of variant is called benign. Some variants will be harmful and lead to health problems. These kinds of variants cause genetic conditions.

RESULTS	
Name of the gene	***TTN***
What does this gene do?	The ***TTN*** gene contains instructions for your body to make a large protein that is important for the muscles of your body and heart to work. The protein is called "titin" (also known as "connectin").
What variant(s) does your child have?	Two different genetic variants of the *TTN* gene were identified: ***TTN*** c.A9857G:p.KI3286R (from mother) and ***TTN*** c.G13738C:p.V4580L (from father)
How do variants in this gene cause health problems?	Each person has two copies of the *TTN* gene (one from their mother and one from their father). Some variants stop the *TTN* gene from working. When both copies of the *TTN* gene are not working, titin cannot be made correctly and patients develop symptoms. • **When a person has two non-working copies of the *TTN* gene, they have a condition called "Salih myopathy"** • **When a person has one normal copy of the *TTN* gene and one non-working copy of the *TTN* gene, they are said to be a "carrier". They do not have symptoms of Salih myopathy**
Are the variants the cause of your child's symptoms?	These two variants are **probably the cause** for your child's symptoms because each variant is believed to stop the *TTN* gene from making titin. We are still learning more about this gene and this protein.

EXPLANATION	
How certain are we that the <u>TTN variants</u> are the cause for your child's symptoms?	

Part II. Next Steps

1) Specific issues to discuss with your doctor

Some things to ask about:

- **Treatment Options** — *Are there are any new options to consider? Should there be a change in our child's current care? If there are several options, how will we move forward?*
- **Lifestyle Changes** — *Are there certain activities our child should avoid? Would it help to adopt certain behaviors? Would any dietary changes be helpful? Should we make changes to our house?*
- **Support Services:** *Would our child benefit from occupational therapy, speech therapy or any other types of intervention services? Would the therapist or provider benefit from learning specific things about these results?*
- **Monitoring:** *Are there certain medical symptoms to look out for? Are there new health problems we should prepare for?*
- **Additional Medical Specialists:** *What are other medical specialists should be consulted? How can a referral to these specialists be arranged?*

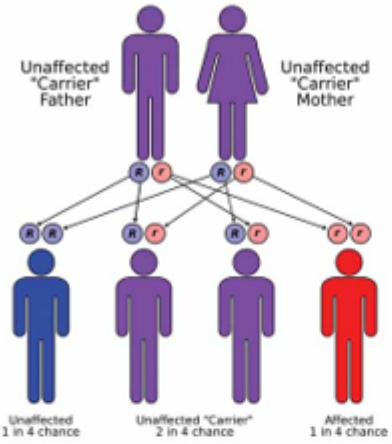
2) Diagnosis specific guidance

[*This information will be included as SimulConsult and provider-curated information. An example of this information is attached separately and labeled as "Concept Sheets". We recognize that many gene findings will not have significant amounts of information available.]*

3) How these findings might affect your family members

- **How is the myopathy passed on in families?** This condition is caused because your child two copies of the *TTN* genes that do not work correctly. One *TTN* gene came from Mom and one came from Dad. We know that each of you must have one copy of the *TTN* gene that works correctly and one copy that is not working. People who have at least one working *TTN* gene, do not have myopathy. Copies of the *TTN* gene that do not work have a change in the structure that is called a "mutation". Inheritance that is caused by two copies of non-working genes is called autosomal recessive inheritance.
- **Could our other children also have this condition?** This condition is identified early in infancy. We would know already whether or not your other children have myopathy. Other children in the family may carry a single non-working copy of the *TTN* gene. They are not at risk for health problems for themselves. Any of your children could have testing to find out if they carry one or the other of the *TTN* gene changes.
- **How do these results affect future pregnancies?** You have a 1 in 4 chance or a 25% chance with each pregnancy that each child could inherit two non-working copies of the same gene. There are 3 out of 4 chances or 75% chance that each child in the future would not have this myopathy. If you are thinking about children in the future we can talk about possible testing options before or during pregnancy.
- **What about other family members?** It is possible that your siblings or parents also carry one non-working copy of the *TTN* gene. These types of gene changes can be carried silently for many generations and no one knows that the change is in the family. It becomes known once someone marries someone who also carries a change in the very same gene. Then there is a 25% chance or 1 in 4 chances to have a child with this myopathy. Family members can be tested for the gene changes found in the *TTN* gene in your child.

4) How is this variant passed on in families?



In this diagram,
R = usual *TTN* gene
r = *TTN* gene variant
Autosomal Recessive Inheritance

4) Other Resources

General Resources:

- To learn more about a genetic condition, it can be helpful to start at the Genetic and Rare Disease Information Center: *http://rarediseases.info.nih.gov/*
- Another helpful resource is "Genetics Home Reference", which provides links to condition-specific patient support groups: *http://ghr.nlm.nih.gov*
- To learn more information about genetics concepts and terms, visit *Genes in Life* from The Genetic Alliance: *http://www.genesinlife.org*

Resources Specific to Condition:

- **https://www.childrenshospital.org/research-and-innovation/research-labs/beggs-laboratory/recent-developments/nemaline-animation**
- **http://www.joshuafrase.org/life-with-cnm-mtm/the-disorder.php**

5) How to stay up-to-date on new information about your Whole Genome Sequencing results

- **Important:** Please stay in touch and call us once a year so that we can talk about any new information about your Whole Genome Sequencing test results.
- Please feel free to call Dr. Marc Williams or Janet Williams at 570-214-7942.

- A support group that focuses on myopathy may also be helpful.
  - http://www.childrenscardiomyopathy.org/
- If you are interested in taking part in research about this condition, go to *www.clinicaltrials.gov* once or twice a year to see if you qualify for any research studies.

What do Whole Genome Sequencing test results mean?

We have a reasonable cause for your child's muscle condition. There may be other important genetic findings in the test results. At this time we looked for the genes that might explain your child's diagnosis. As time goes on, we may be able to look at other genes and other conditions. We also looked at the genes that can tell us about certain health conditions recommended to be included in results from whole genome sequencing.

It is estimated that the human body contains about 25,000 genes. We currently understand the function of only about 3,000 genes and how certain types of variants affect this function. Some genetic variants can have no effect at all, some may even be helpful, and others may be harmful.

We suggest that you stay in contact with your healthcare provider and/or genetics professional at least once a year to learn if there is any new information related to your Whole Genome Sequencing test results.

Part II of this report will tell you about the other genes looked at as a part of this study. There are genes that are not linked to your child's condition, but are important for your health.

Part III: Additional Important Findings

These genetic test results are <u>not</u> related to your child's myopathy. However, these results are important for you and your family's health.

Summary of Additional Findings	*BRCA1* mutation
Why is this result being shared?	This variant in the *BRCA1* gene leads to greater risk for getting breast or ovarian cancer as an adult. These types of cancers **can either be prevented or cured if found early.** Knowledge of these higher cancer risks will help you and your family doctor to discuss your cancer risks. <u>Your child is NOT believed to be at any increased risk for childhood cancers.</u> **It is likely that other members of the family carry this mutation**. This information could also affect their own medical care.

RESULTS & EXPLANATION	
Name of the gene	***BRCA1***
What does this gene do?	The *BRCA1* gene contains instructions for your body to make a protein that helps repair damaged DNA.
What variant was found?	**Cys64Gly mutation of the *BRCA1* gene**
How do variants in this gene cause health problems?	This variant (mutation) in the *BRCA1* gene makes it harder for the body to repair damaged DNA. This can increase a person's risk of certain types of cancer. People who carry a *BRCA1* mutation have a condition called Hereditary Breast and Ovarian Cancer syndrome (also known as "HBOC syndrome").

Information about Hereditary Breast and Ovarian Cancer Syndrome (HBOC)

(General Information about **HBOC,** continued)

Adults with HBOC syndrome are at a higher risk to develop breast, ovarian and other cancer than people in the general population.

Childhood cancers are NOT known to be associated with HBOC.

Your child is NOT considered to be at a higher risk to develop a childhood cancer

Estimated cancer risks for WOMEN:	**Woman with *BRCA1* gene mutation**	Woman in General Population
Lifetime risk of breast cancer	50-85%	12% (or 1 in 9)
Risk of breast cancer by age 50	30-50%	2%
Lifetime risk of ovarian cancer	11-44%	1.4%
Estimated cancer risks for MEN:	**Man with *BRCA1* gene mutation**	Man in General Population
Lifetime risk of male breast cancer	Up to 7-8%	.05% (or 1 in 2000)
Lifetime risk of prostate cancer	20%	15%

Some families with HBOC syndrome also have a higher risk for certain other cancers, such as pancreatic cancer.

While it is possible to have HBOC and **never** develop cancer, it is important for anyone who has HBOC follow specific early cancer screening and prevention guidelines.

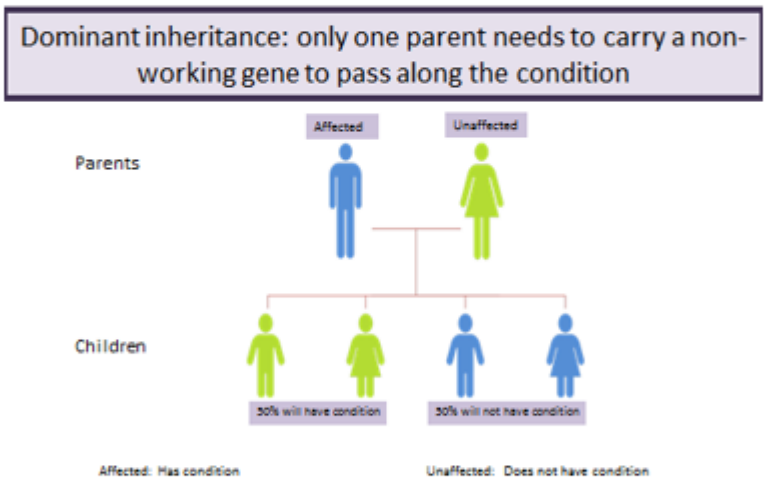
What these results mean for you and other members of the family:	This is important medical information for you and your relatives because it is likely that one or more of you also carry this *BRCA1* mutation and have HBOC syndrome. **Your child inherited this *BRCA* mutation from one of you, his parents.** Since nearly all *BRCA* mutations are inherited, it is highly unlikely that your child's *BRCA1* mutation is "new". This means that one of you has HBOC syndrome and are at a higher risk for certain cancers. A genetic test is available that can determine which of you could benefit from discussing early cancer screening and prevention options with your healthcare provider. **Each one of your child's full siblings has a 50% chance to also carry this *BRCA* mutation.** A "full sibling" is a brother or sister who has the same mother and same father as your child. This information. **Other members of your family are at risk to carry this *BRCA* mutation as well.** Your child's aunts, uncles, cousins and other relatives also have a chance of having this *BRCA* mutation. *Please see "Next Steps" to learn about how to arrange testing for you and other members of your family.*

Part IV. Next Steps

1) **How these Additional Findings affect the medical care for your child**
Your child's current medical care should **not** be affected by these results. Currently, there are no medical recommendations for children who carry a *BRCA* mutation. When your child is an adult, it is important for her/him to share these results with their doctor so that an appropriate care plan can be made.

   It is possible that new information and recommendations for individuals with HBOC syndrome will become available over the coming years, so it is important to include these Additional Findings in your yearly discussion with a genetics professional about these Whole Genome Sequencing results.

2) **How these Additional Findings might affect your family members**
BRCA mutations are inherited in an **autosomal dominant** manner. In this type of condition only one copy of the non-working gene is needed to cause disease. The non-working copy of the gene is inherited from one parent or the other, but not both. People who have a non-working copy of the gene have a 50% chance (1 in 2) to pass along the non-working copy of the gene and a 50% chance to pass on the working copy. Genetic testing is available for family members and will find who has a non-working copy of the gene.



3) **Genetic testing for family members**

At-risk family members will not need to have Whole Genome Sequencing (as your child did). To learn whether they also carry this *BRCA* mutation, they can have a much simpler genetic testing done that is called a "single-site mutation test" – this test looks specifically for this one *BRCA* mutation. A genetic counselor or physician can help to arrange for testing.

Name of the test for you and your family members.	Single-site Mutation Analysis for the *BRCA1* Cys64Gly mutation.

At the end of this report, there is a Family Letter to help you explain this information to your relatives and their doctors and to help them get appropriate genetic testing.

4) **Treatment recommendations for adults with HBOC syndrome (carriers of *BRCA1* mutation)**

Your family may benefit from speaking to a genetics professional that can provide a detailed review of the most up-to-date recommendations for people with this condition.

Summary of current recommendations for adult individuals with *BRCA1* mutations to discuss with their healthcare providers (as of the date of this report):

	To discuss with your physician:
Adult women with HBOC syndrome	▪ **Yearly mammograms and breast MRI beginning at age 25** ▪ **Monthly self-breast examination and clinical breast exams two times a year beginning at age 18** ▪ **Serious consideration of removal of ovaries and fallopian tubes ("bilateral salpingo-oophorectomy," or "BSO") at ages 30-40 or after child-bearing is completed. This procedure significantly lowers the risk of ovarian cancer, as well as the risk of breast cancer.** ▪ **If BSO is not performed: Transvaginal ultrasound, screening of CA-125 levels in the blood, and pelvic exam performed twice a year** ▪ **Consideration of medications and/or breast removal ("mastectomies") to lower the risk of breast cancer.**
Adult men with HBOC syndrome	▪ **Monthly breast self-exams and clinical breast exams two times a year beginning at age 35** ▪ **Consideration of a baseline mammogram at age 40** ▪ **Follow population screening guidelines for prostate cancer (for example, PSA blood test and annual digital rectal exam)**

5) Other Resources

- **Fact Sheet:** This is a basic fact sheet from the National Cancer Institute about *BRCA 1* and *2*-related cancer risks and genetic testing *http://www.cancer.gov/cancertopics/factsheet/Risk/BRCA*
- **FORCE:** This non-profit organization, "Facing Our Risk of Cancer Empowered (FORCE)" serves women and their families at risk for hereditary breast and ovarian cancer. Their website provides a variety of resources such as information about support services or how to talk to family members about HBOC. *http://www.facingourrisk.org*
- **Bright Pink:** Bright Pink is an organization that focuses on the prevention and early detection of breast and ovarian cancer in young women. Their "PinkPal" program provides one-on-one personalized mentoring to young women with HBOC. www.brightpink.org
- **Find a Genetic Counselor tool:** You can locate a genetic counselor in a specific geographic area by using this tool from the National Society of Genetic Counselors. http://nsgc.org/p/cm/ld/fid=164

A Letter To Share With Relatives

Dear _____,

We recently learned that some members of our family may have inherited a genetic condition that increases one's risk to develop certain cancers. A mutation in the *BRCA1* gene was identified in _____. This genetic mutation causes Hereditary Breast and Ovarian Cancer (HBOC) syndrome and it is possible that other members our family may also have HBOC.

Since HBOC is associated with an increased risk for certain cancers that can be either prevented or detected at an early, curable stage, I am writing to all of my relatives who may have inherited the same genetic change as me. In comparison to the general population, female carriers of a *BRCA1* mutation have a much higher risk for breast and ovarian cancer, while male carriers have an increased risk for prostate and male breast cancer.

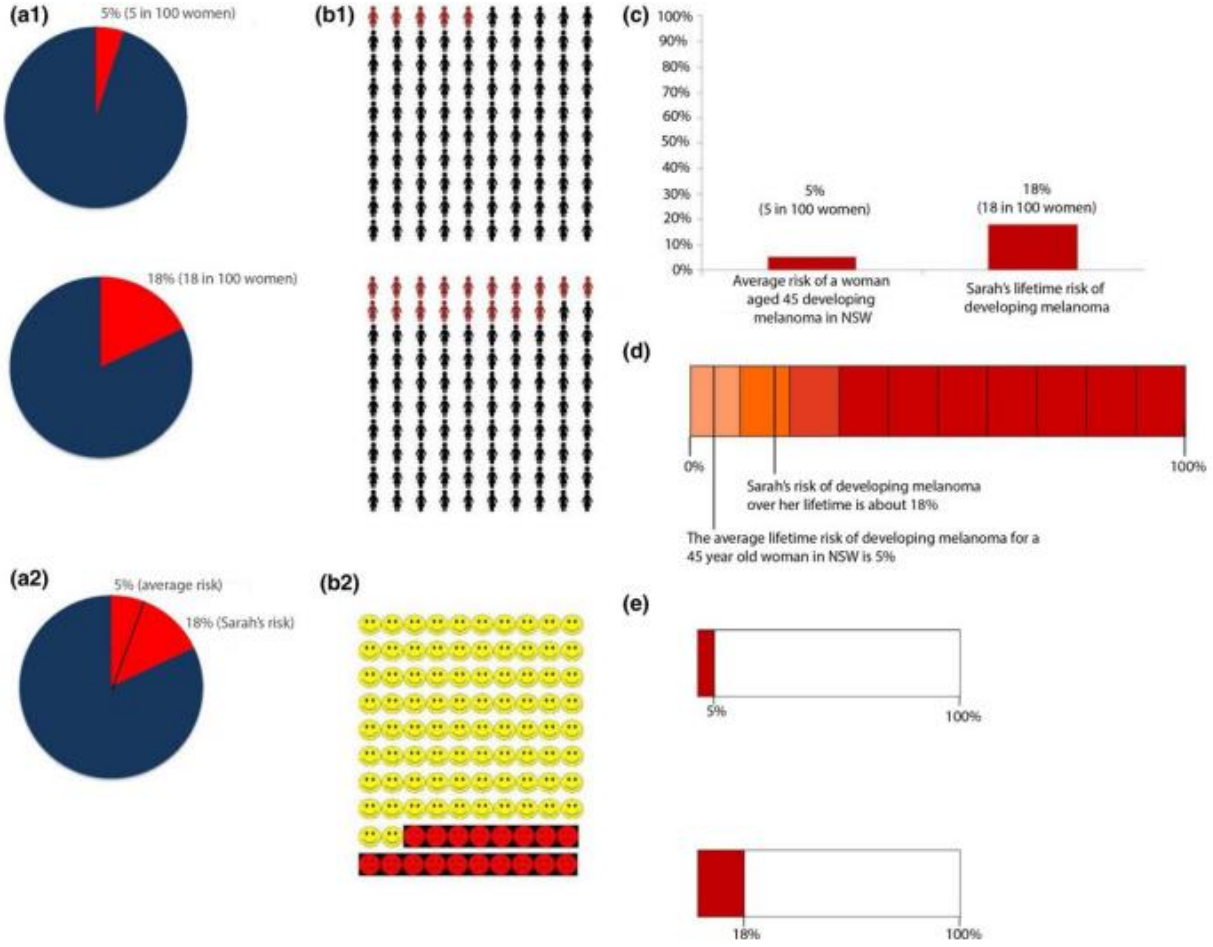
When someone has a *BRCA* mutation, each one of his or her children, brothers, and sister has a 50% (1 in 2) chance of also carrying this mutation. Men and women are equally likely to inherit this genetic mutation.

You may wish to speak to a genetic counselor or your healthcare provider about whether genetic testing for this mutation makes sense for you. To locate a genetic counselor, you can use a feature on the website for the National Society of Genetic Counselors at ***www.nsgc.org*** that is called "Find a Genetic Counselor".

Name of this condition	Hereditary Breast and Ovarian Cancer Syndrome
Genetic test results	A Cys64Gly mutation of the *BRCA1* gene was found. This mutation is associated with a higher risk for breast, ovarian and other cancers.
The genetic test that can be ordered for my relatives	Single-site Mutation Analysis for the ***BRCA1*** Cys64Gly mutation.

I have also included a copy of the test result with this letter, which you can share. Please bring this letter and those results to your healthcare provider or genetic counselor so that genetic testing can be arranged for you. I hope you find this information helpful. Please let me know if you have any questions.

Sincerely,


(a1)
5% (5 in 100 women)
18% (18 in 100 women)
(b1)
(c)
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
5%
(5 in 100 women)
18%
(18 in 100 women)
Average risk of a woman aged 45 developing melanoma in NSW
Sarah's lifetime risk of developing melanoma
(d)
0%
100%
Sarah's risk of developing melanoma over her lifetime is about 18%
The average lifetime risk of developing melanoma for a 45 year old woman in NSW is 5%
(a2)
5% (average risk)
18% (Sarah's risk)
(b2)
(e)
5%
100%
18%
100%

Coronary Artery Disease

POWERED BY 23ANDME RESEARCH

Coronary artery disease, sometimes called CAD, is a type of heart disease that is typically caused by the buildup of a waxy, cholesterol-containing substance called plaque inside the coronary arteries, which are the major blood vessels that supply the heart with oxygen-rich blood. When plaque builds up in the coronary arteries, the vessels narrow and blood flow to the heart is decreased.

Jamie, your genetic result is associated with an **increased likelihood** of developing coronary artery disease.

An estimated **20%** of males with genetic results like yours develop coronary artery disease **by their 70s**. This is based on data from 23andMe research participants of European descent.

This estimate is based on currently available data and may be updated over time.

Ways to take action

Your overall likelihood of developing coronary artery disease also depends on other factors, including lifestyle. Experts agree that healthy lifestyle habits can help lower the chances of developing this condition.

- Maintain a healthy weight
- Eat a heart-healthy diet
- Exercise regularly
- Avoid smoking
- Limit alcohol consumption

Start taking action

About coronary artery disease

How can coronary artery disease impact your health?

In people with coronary artery disease, plaque buildup in the coronary arteries causes the vessels to narrow and decreases blood flow to the heart. At first, this may not cause any symptoms. However, as more plaque builds up over time, people can experience chest pain (called angina), shortness of breath, and fatigue. The heart can also become weak and unable to pump blood effectively to the rest of the body (called heart failure).

If a piece of plaque inside an artery breaks off and a blood clot forms, blood flow to the heart may be blocked, causing a heart attack. If blood flow to the brain is blocked, this can cause a stroke.

Estimate your risk* for complications of heart disease, including things like heart attack and stroke. This tool from the American Heart Association uses non-genetic factors, and is for individuals who are at least 40 years old.

Other factors that can impact your chances of developing coronary artery disease

According to the Centers for Disease Control and Prevention, up to 16% of people in the U.S. are expected to develop coronary artery disease by their 70s. Besides genetics, weight, and lifestyle, some factors that can increase a person's chances of developing coronary artery disease include:

- Age (this condition becomes more common as people get older)
- Sex (more males than females are diagnosed with coronary artery disease, but females are likely under-diagnosed)
- Family history (especially if a parent had a heart attack at a young age)
- Certain health conditions (including high blood pressure, high cholesterol, and type 2 diabetes)

Keep in mind

This report **does not diagnose** coronary artery disease. It also does not provide information about or diagnose other forms of heart disease. **Consult with a healthcare professional** if you are concerned about your likelihood of developing coronary artery disease, have a personal or family history of coronary artery disease, or before making any major lifestyle changes.

If you have already been diagnosed with coronary artery disease by a healthcare professional, it is important to **continue any treatment plans**, including medications and lifestyle modifications, that they prescribe.

The likelihood of developing coronary artery disease also depends on **other factors**, including age, sex, family history, and lifestyle.

This report **does not account for every possible genetic variant** that could affect your likelihood of developing coronary artery disease, and it **does not include rare variants that individually have a large impact** on the likelihood of developing this condition.

This report is based on a genetic model **created using data from 23andMe research participants** and has not been clinically validated.

How we got your result ^

Methods

This report is based on a statistical model that takes into account your genetic results at more than 2,400 genetic markers, along with the ethnicity and sex you reported in your account settings, to estimate the likelihood of developing coronary artery disease. We used data from 23andMe research participants as well as data reported in the scientific literature to calculate this estimate. Results and estimates may be updated over time as the model or scientific understanding about this condition improves. Note that this report does not include genetic variants that have a large impact on the likelihood of developing coronary artery disease, such as variants linked to familial hypercholesterolemia (FH).

About the result

People whose result is associated with odds of developing coronary artery disease that are at least 1.5 times higher than average are considered to have an increased likelihood. Between 2% and 19% of individuals receive an "increased likelihood" result, depending on ethnicity. These results are based on thousands of genetic markers, and random test error at one or more of these markers can lead to a small margin of error in your estimated likelihood of developing coronary artery disease. For people whose estimates are near the boundary between typical and increased likelihood, this margin of error may introduce some uncertainty about whether their estimated likelihood is considered "typical" or "increased". Your genetic result is associated with an increased likelihood. Based on the available genetic markers used to calculate your result, there is a less than 1% chance your genetic likelihood estimate could fall on the other side of the boundary and be in the range that is considered typical.

Scientific validity across ethnicities

We verified that the model meets our scientific standards for individuals of European, Hispanic/Latino, East/Southeast Asian, South Asian, Sub-Saharan African/African American, and Northern African/Central & Western Asian descent.

How we may use ethnicity and sex to customize this result

- If you indicated in your account settings that you are of European, Hispanic/Latino, East/Southeast Asian, South Asian, Sub-Saharan African/African American, or Northern African/Central & Western Asian (Middle Eastern) descent, your result is tailored based on data from individuals of that ancestry.
- Otherwise, your result may be based on data from individuals of European descent because there is not enough data from individuals of your ancestry at this time. Data from individuals of European descent is used because the most data is available for this population.
- Your Coronary Artery Disease result also takes into account the sex you indicated in your account settings.

See our **white paper** to learn more about the science behind this report.

Read More:

Arnett DK et al. (2019). "2019 ACC/AHA Guideline on the Primary Prevention of Cardiovascular Disease: Executive Summary: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines." J Am Coll Cardiol. 74(10):1376-1414. ˅

Benjamin EJ et al. (2019). "Heart Disease and Stroke Statistics-2019 Update: A Report From the American Heart Association." Circulation. 139(10):e56-e528. ˅

Centers for Disease Control and Prevention. (2017). "Behavioral Risk Factor Surveillance System (BRFSS) Prevalence & Trends Data." Retrieved May 4, 2020, from https://www.cdc.gov/brfss/brfssprevalence/. ˅

Khera AV et al. (2017). "Genetics of coronary artery disease: discovery, biology and clinical translation." Nat Rev Genet. 18(6):331-344. ˅

Lloyd-Jones DM et al. (2004). "Parental cardiovascular disease as a risk factor for cardiovascular disease in middle-aged adults: a prospective study of parents and offspring." JAMA. 291(18):2204-11. ˅

Mayo Clinic. "Coronary artery disease." Retrieved August 1, 2019, from https://www.mayoclinic.org/diseases-conditions/coronary-artery-disease/symptoms-causes/syc-20350613. ˅

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Explore how genetics and other factors add up

How common is type 2 diabetes in 23andMe research participants with genetics like yours who have different ages, weights, and other characteristics?

Your genetics: **Increased likelihood**

Ethnicity: **European**

Age

Height

ft

in

Weight

lb

How many times do you eat fast food **each week?**

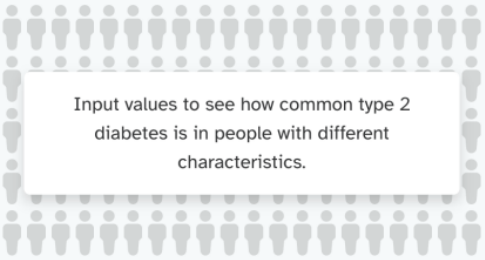
Select fast food frequency

How many times do you exercise **each week?**

Select exercise frequency

Calculate

Percent who have type 2 diabetes



Input values to see how common type 2 diabetes is in people with different characteristics.

You can **update your ethnicity** in your account settings. Estimates are not available for all ethnicities. European is used as the default for people of mixed ancestry and for those of ancestries for which we do not yet have enough research participants. Keep in mind that this tool does not include all possible factors that affect the likelihood of developing type 2 diabetes and does not predict your personal overall likelihood of developing type 2 diabetes.

Ways to take action

Experts agree that healthy lifestyle choices can reduce the likelihood of developing type 2 diabetes.

If you have already been diagnosed with prediabetes by a healthcare professional

If you have already been diagnosed with type 2 diabetes by a healthcare professional

- **Maintain a healthy weight**
- **Get active**
- **Eat healthy foods**
- **Don't smoke**
- **Talk to a healthcare professional**
- **Consider a diabetes prevention program**

Keep in mind

Consult with a healthcare professional if you are concerned about your likelihood of developing type 2 diabetes, have a personal or family history of diabetes, or before making any major lifestyle changes.

This report **does not diagnose** type 2 diabetes. It also does not provide information about or diagnose other forms of diabetes.

The likelihood of developing type 2 diabetes also depends on **other factors**, including age, weight, ethnicity, and family history.

This report **does not account for every possible genetic variant** that could affect your likelihood of developing type 2 diabetes.

This report is based on a genetic model created **using data from 23andMe research participants** and has not been clinically validated.

About the type 2 diabetes genetic model

Summary

This report is based on a statistical model that estimates the likelihood of developing type 2 diabetes by looking at genetic variants at 1,244 places in your DNA. We identified these variants and created this model using data from more than 1,110,000 23andMe research participants of European descent.

About the likelihood estimate

The estimated likelihood of developing type 2 diabetes is based on your type 2 diabetes genetic score, self-reported ethnicity, and current age. This estimate assumes you do not already have type 2 diabetes. For every year you do not develop type 2 diabetes, your estimated likelihood of developing type 2 diabetes in the future and the "typical range" for your age will both decrease slightly. This estimate is based on data from 23andMe research participants of European descent with your genetic score, combined with Centers for Disease Control data on the average likelihood of developing type 2 diabetes in people of European descent. Overall, about 78% of people have estimated likelihoods that fall into the typical range, while about 22% have increased likelihoods. See our white paper to learn more about how we determined which genetic scores correspond to "typical" vs. "increased" likelihoods.

About "Explore how genetics and other factors add up"

This calculation shows estimates of type 2 diabetes prevalence, or how common type 2 diabetes is, in people with different characteristics, including type 2 diabetes genetic score as well as ethnicity, age, BMI, fast food consumption, and exercise frequency. It was developed using data from more than 530,000 23andMe research participants of European, African, Hispanic/Latino, East Asian, and South Asian descent.

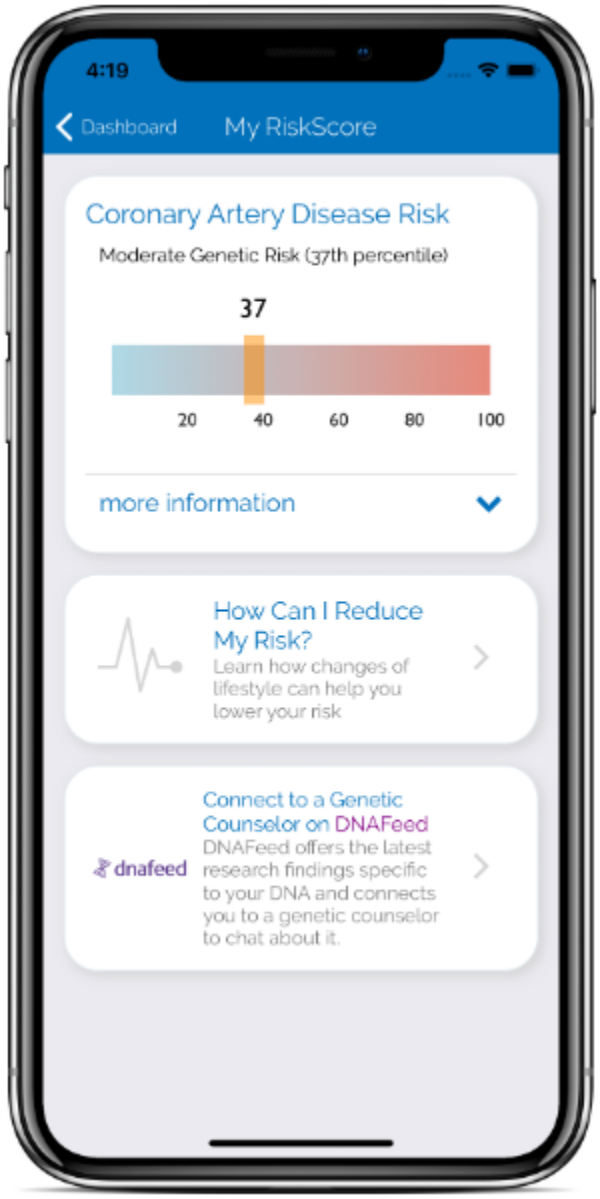
See our **white paper** to learn more about the science behind this report.

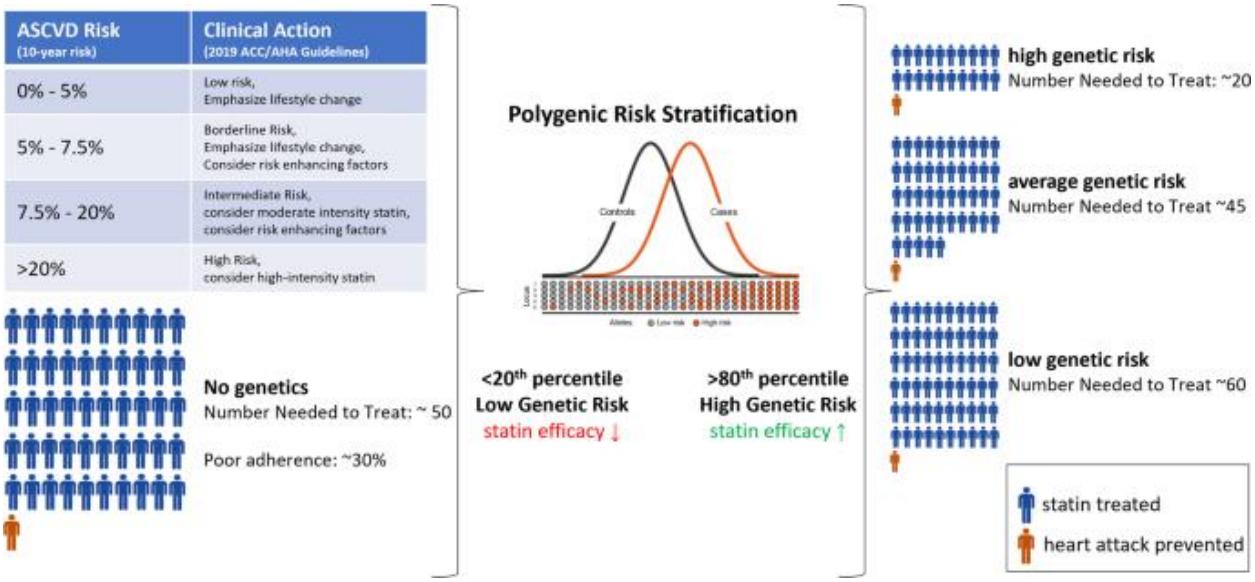
Performance across ethnicities

We evaluated model performance for people of European, African, Hispanic/Latino, East Asian, and South Asian descent. This analysis included data from 17,000 or more research participants of each of these ethnicities. The predictive power of the model (AUC) varies across ethnicities, possibly due to factors like limitations in the amount of data available from each ethnicity.

ETHNICITY	AUC VALUE
European	0.652
South Asian	0.603
Hispanic/Latino	0.638
East Asian	0.609
African	0.588

The "Area Under the receiver operating characteristic Curve" (AUC or AUROC) measures how well a statistical model predicts whether or not people have a trait. AUC values usually range from 0.5 to 1, where higher numbers mean the model has more predictive power. The predictive power of the type 2 diabetes model is limited by the fact that type 2 diabetes depends not only on the genetic factors included in this model, but also on non-genetic factors and likely on additional genetic factors that are not yet known.


4:19
Dashboard
My RiskScore
Coronary Artery Disease Risk
Moderate Genetic Risk (37th percentile)
37
20
40
60
80
100
more information
How Can I Reduce My Risk?
Learn how changes of lifestyle can help you lower your risk
dnafeed
Connect to a Genetic Counselor on DNAFeed
DNAFeed offers the latest research findings specific to your DNA and connects you to a genetic counselor to chat about it.


ASCVD Risk
(10-year risk)
Clinical Action
(2019 ACC/AHA Guidelines)
0% - 5%
Low risk,
Emphasize lifestyle change
5% - 7.5%
Borderline Risk,
Emphasize lifestyle change,
Consider risk enhancing factors
7.5% - 20%
Intermediate Risk,
consider moderate intensity statin,
consider risk enhancing factors
>20%
High Risk,
consider high-intensity statin
No genetics
Number Needed to Treat: ~ 50
Poor adherence: ~30%
Polygenic Risk Stratification
Controls
Cases
<20th percentile
Low Genetic Risk
statin efficacy ↓
>80th percentile
High Genetic Risk
statin efficacy ↑
high genetic risk
Number Needed to Treat: ~20
average genetic risk
Number Needed to Treat ~45
low genetic risk
Number Needed to Treat ~60
statin treated
heart attack prevented


10:05
MyTOOLBOX
DNA RESULTS
Metabolic Rate
Slow Metabolism
Summary
Diet
Health
Physical
Vitamins
SUMMARY
Sort
Filter
Metabolic Rate
Status: SLOW METABOLISM
Choline Benefit
Status: NOT ESSENTIAL
Glutamine Benefit
Home
Results
Insights
Epigenetics
More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MyRisk Management Tool
MyRisk™
Hereditary Cancer Test
RECEIVING HEALTHCARE PROVIDER
Test HCP, MD
Test Medical Center
6689 BLANCO RD STE 200
SAN ANTONIO, TX 78216
SPECIMEN
Specimen Type: Blood
Draw Date: Dec 03, 2021
Accession Date: Dec 03, 2021
Report Date: Dec 03, 2021
PATIENT
Name: Pt Last Name, Pt First Name
Date of Birth: Dec 03, 1976
Patient ID: Patient id
Gender: Female
Accession #: 07007253-BLD
Requisition #: 90026822
GENETIC RESULT: POSITIVE - CLINICALLY SIGNIFICANT MUTATION IDENTIFIED
Note: "CLINICALLY SIGNIFICANT," as defined in this report, is a genetic change that is associated with the potential to alter medical intervention.
CLINICAL HISTORY ANALYSIS: NO ADDITIONAL MANAGEMENT GUIDELINES IDENTIFIED BASED ON THE CLINICAL HISTORY PROVIDED
Other clinical factors may influence individualized management. This analysis may be incomplete if details about cancer diagnoses, ages, family relationships or other factors were omitted or ambiguous. If this patient also has a clinically significant mutation, the recommendations based on the clinical history analysis should be considered in light of the possibility that this mutation explains all or some of the cancer history in the family.
GENE
MUTATION
THIS GENETIC TEST RESULT IS ASSOCIATED WITH THE FOLLOWING CANCER RISKS:
BRCA2
c.1163_1164dupTA (p.Pro389Tyrfs*11)
Heterozygous
HIGH RISK: Breast, Ovarian, Pancreatic
ELEVATED RISK: Skin
Please see the Genetic Test Result for more details on any variant(s) detected in this patient, including variant classification information.
ADDITIONAL FINDINGS: NO VARIANT(S) OF UNCERTAIN SIGNIFICANCE (VUS) IDENTIFIED
TYRER-CUZICK BREAST CANCER RISK CALCULATION
REMAINING LIFETIME BREAST CANCER RISK: Not Calculated
5-YEAR BREAST CANCER RISK: Not Calculated
The Tyrer-Cuzick breast cancer risk estimate is only calculated for women who meet the following criteria: 1) age is younger than 85 years, 2) no known mutation or inconclusive result has been found in the woman or any of her relatives, and 3) the sample was submitted with a current Test Request Form that includes all of the fields required to collect the information used in the calculation, and the provider has not indicated on the Test Request Form that the Tyrer-Cuzick calculation is not appropriate for the patient. Version 7.02 of the Tyrer-Cuzick model was used for this risk estimate. Tyrer-Cuzick model Versions 7.02 and 8.0 are available for download at the EMS-Trials website, http://www.ems-trials.org/riskevaluator.
CLINICAL OVERVIEW OF GENETIC FINDINGS
Hereditary Breast and Ovarian Cancer syndrome (HBOC)
This patient has been found to have a mutation in the BRCA2 gene. Individuals with mutations in BRCA2 have Hereditary Breast and Ovarian Cancer syndrome (HBOC).
Women with HBOC have a risk for breast cancer that is greatly increased over the 12.5% lifetime risk for women in the general population of the United States.
Women with HBOC also have high risks for ovarian, fallopian tube, and primary peritoneal cancer.
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MyRisk Genetic Result

MyRisk™ Hereditary Cancer Test

RECEIVING HEALTHCARE PROVIDER	SPECIMEN		PATIENT	
Test HCP, MD	Specimen Type:	Blood	Name:	Pt Last Name, Pt First Name
Test Medical Center	Draw Date:	Dec 03, 2021		
6609 BLANCO RD STE 200	Accession Date:	Dec 03, 2021	Date of Birth:	Dec 03, 1982
SAN ANTONIO, TX 78216	Report Date:	Dec 03, 2021	Patient ID:	Patient id
			Gender:	Female
			Accession #:	07007255-BLD
			Requisition #:	90026826

GENETIC RESULT: NEGATIVE - NO CLINICALLY SIGNIFICANT MUTATION IDENTIFIED

Note: "CLINICALLY SIGNIFICANT," as defined in this report, is a genetic change that is associated with the potential to alter medical intervention.

BREAST CANCER RISKSCORE®: REMAINING LIFETIME RISK 35.3%

This level of risk is at or above 20% threshold for consideration of modified medical management.
See RiskScore Interpretation Section for more information.

CLINICAL HISTORY ANALYSIS: NO ADDITIONAL MANAGEMENT GUIDELINES IDENTIFIED BASED ON THE CLINICAL HISTORY PROVIDED

Other clinical factors may influence individualized management. This analysis may be incomplete if details about cancer diagnoses, ages, family relationships or other factors were omitted or ambiguous. If this patient also has a clinically significant mutation, the recommendations based on the clinical history analysis should be considered in light of the possibility that this mutation explains all or some of the cancer history in the family.

ADDITIONAL FINDINGS: NO VARIANT(S) OF UNCERTAIN SIGNIFICANCE (VUS) IDENTIFIED

Details About Non-Clinically Significant Variants: All individuals carry DNA changes (i.e., variants), and most variants do not increase an individual's risk of cancer or other diseases. When identified, variants of uncertain significance (VUS) are reported. Likely benign variants (Favor Polymorphisms) and benign variants (Polymorphisms) are not reported and available data indicate that these variants most likely do not cause increased cancer risk. Present evidence does not suggest that non-clinically significant variant findings be used to modify patient medical management beyond what is indicated by the personal and family history and any other clinically significant findings.

Variant Classification: Myriad's myVision™ Variant Classification Program performs ongoing evaluations of variant classifications. In certain cases, healthcare providers may be contacted for more clinical information or to arrange family testing to aid in variant classification. When new evidence about a variant is identified and determined to result in clinical significance and management change, that information will automatically be made available to the healthcare provider through an amended report.

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MyRisk Genetic Result

Name: **Pt Last Name, Pt First Name** DOB: **Dec 03, 1982** Accession #: **07007255-BLD** Report Date: **Dec 03, 2021**

ADDITIONAL INFORMATION

Genes Analyzed: Sequencing (seq) and large rearrangement analyses were performed for all coding exons in the following genes, unless otherwise indicated:
APC, ATM, AXIN2, BAP1, BARD1, BMPR1A, BRCA1, BRCA2, BRIP1, CDH1, CDK4, CDKN2A, CHEK2, CTNNA1, FH, FLCN, HOXB13 (seq only), *MEN1, MET, MLH1, MSH2, MSH3* (excluding repetitive portions of exon 1), *MSH6, MUTYH, NTHL1, PALB2, PMS2, PTEN, RAD51C, RAD51D, SDHA, SDHB, SDHC, SDHD, SMAD4, STK11, TP53, TSC1, TSC2, VHL.*
Limited promoter regions may also be analyzed for large rearrangements.

Sequencing (seq) and/or large rearrangement (LR) analyses were performed only for the gene portions indicated in parenthesis for the following genes:
EGFR (exons 18-21, seq and LR), *EPCAM* (exons 8-9, LR only), *GREM1* (exon 1 and upstream regulatory regions, LR only), *MITF* (c.952, seq only), *POLE* (exonuclease domain, seq only), *POLD1* (exonuclease domain, seq only), *RET* (exons 5, 8, 10, 11, 13-16 seq and LR), *TERT* (promoter c.DNA -1 to -70, seq only).

** Other genes not analyzed with this test may also be associated with cancer.

Indication for Testing: It is our understanding that this individual was identified for testing due to a personal or family history suggestive of a hereditary predisposition for cancer.

Associated Cancer Risks and Clinical Management: The "MyRisk Management Tool" associated with this report provides a summary of cancer risk and professional society medical management guidelines that may be useful in developing a plan for this patient based on any clinically significant test results and/or reported personal/family history. In some cases, a MyRisk Management Tool cannot be provided, such as when the result has a special interpretation or includes a mutation with unusual characteristics.

Analysis Description: The Technical Specifications summary (myriad.com/technical-specifications) describes the analysis, method, performance, nomenclature, and interpretive criteria of this test. Current testing technologies are unable to definitively determine whether a variant is germline or somatic in origin, which may significantly impact risk estimates and medical management; therefore, these results should be correlated with this patient's personal and family history. The interpretation of this test may also be impacted if the patient has a hematologic malignancy or an allogeneic bone marrow transplant.

CLASSIFICATION DISCLAIMER

THE CLASSIFICATION AND INTERPRETATION OF ALL VARIANTS IDENTIFIED IN THIS ASSAY REFLECTS THE CURRENT STATE OF MYRIAD'S SCIENTIFIC UNDERSTANDING AT THE TIME THIS REPORT WAS ISSUED. VARIANT CLASSIFICATION AND INTERPRETATION MAY CHANGE FOR A VARIETY OF REASONS, INCLUDING BUT NOT LIMITED TO, IMPROVEMENTS TO CLASSIFICATION TECHNIQUES, AVAILABILITY OF ADDITIONAL SCIENTIFIC INFORMATION, AND OBSERVATION OF A VARIANT IN MORE PATIENTS.

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MyRisk Genetic Result

Name: **Pt Last Name, Pt First Name** DOB: **Dec 03, 1982** Accession #: **07007255-BLD** Report Date: **Dec 03, 2021**

Breast Cancer RiskScore®

Breast Cancer RiskScore®:

35.3%

RESULT: 35.3% Remaining Lifetime Breast Cancer Risk
1.7% 5-Year Breast Cancer Risk

Remaining Lifetime Breast Cancer Risk (Age-Adjusted)

BREAST CANCER RISKSCORE® INTERPRETATION

The breast cancer RiskScore provides an estimate of the remaining lifetime risk for breast cancer. A risk estimate at or above 20% is associated with specific modified medical recommendations, including consideration of more aggressive breast cancer screening and additional risk reduction measures. If applicable, details of these recommendations are provided in the accompanying MyRisk Medical Management Tool or other supplemental material. Women with a risk estimate below 20% may still be appropriate for consideration of modified medical management based on other clinical factors or estimates from other breast cancer risk models, such as Tyrer-Cuzick, Claus, and Gail.

TYRER-CUZICK BREAST CANCER RISK CALCULATION

REMAINING LIFETIME BREAST CANCER RISK: 18.5% **5-YEAR BREAST CANCER RISK: 0.8%**

The National Comprehensive Cancer Network (NCCN) provides medical management recommendations for women with an estimated remaining lifetime breast cancer risk greater than 20% based on Tyrer-Cuzick. These recommendations are summarized on the MyRisk Management Tool (MMT). If an MMT is not included with this report, current management recommendations from the NCCN Breast Cancer Screening and Diagnosis panel can be accessed at www.nccn.org. Version 7.02 of the Tyrer-Cuzick model was used for this risk estimate. Tyrer-Cuzick model Versions 7.02 and 8.0 are available for download at the EMS-Trials website, http://www.ems-trials.org/riskevaluator.

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MyRisk Genetic Result

Name: **Pt Last Name, Pt First Name** DOB: **Dec 03, 1982** Accession #: **07007255-BLD** Report Date: **Dec 03, 2021**

BREAST CANCER RISKSCORE® ANALYSIS DESCRIPTION

The breast cancer RiskScore provides 5-year and remaining lifetime breast cancer risks, based on an analysis of genetic markers combined with patient clinical and family history data. The Technical Specifications summary (http://myriad.com/technical-specifications) describes the RiskScore eligibility criteria, analysis, method, performance and interpretive criteria of this test. Data from 149 biomarkers are analyzed during next generation sequencing (NGS). The allele status of these markers has been weighted to generate a polygenic odds ratio of 2.1 for this patient, which is combined with clinical and family history information to generate the final RiskScore. This odds ratio is adjusted for overlap between the risk captured by the biomarkers and the clinical factors and has not been validated for use with other risk models. The Clinical and Cancer Family History Information section of this report displays the data used for this analysis and explains important limitations on the accuracy of RiskScore (including significant over- or under-estimates of breast cancer risk) that can be caused by errors and/or omissions in the reported clinical and family history data.

Please contact Myriad Medical Services at 1-800-469-7423 X 3850 to discuss any questions regarding this result.

This **Authorized Signature** pertains to this laboratory report:

Benjamin B. Roa, PhD
Diplomate ABMG
Laboratory Director

These test results should only be used in conjunction with the patient's clinical history and any previous analysis of appropriate family members. The patient's clinical history and test results should not be disclosed to a third party, unless related to treatment or payment for treatment, without the patient's express written authorization. It is strongly recommended that these results be communicated to the patient in a setting that includes appropriate genetic consultation. This test was developed and its performance characteristics determined by Myriad Genetic Laboratories. It has not been cleared or approved by the U.S. Food and Drug Administration (FDA). The FDA has determined that clearance or approval for laboratory-developed tests is not required.

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Clinical & Cancer Family History Information

MyRisk™ Hereditary Cancer Test

RECEIVING HEALTHCARE PROVIDER

Test HCP, MD
Test Medical Center
6609 BLANCO RD STE 200
SAN ANTONIO, TX 78216

SPECIMEN

Specimen Type:	Blood
Draw Date:	Dec 03, 2021
Accession Date:	Dec 03, 2021
Report Date:	Dec 03, 2021

PATIENT

Name:	Pt Last Name, Pt First Name
Date of Birth:	Dec 03, 1982
Patient ID:	Patient id
Gender:	Female
Accession #:	07007255-BLD
Requisition #:	90026826

PERSONAL / FAMILY CANCER HISTORY SUMMARY

FAMILY MEMBER	CANCER / CLINICAL DIAGNOSIS	AGE AT DIAGNOSIS
Patient	None	
Aunt Paternal	Breast, Invasive	62

PATIENT CLINICAL HISTORY SUMMARY

Woman's age	39	Hormone Replacement Therapy (HRT)	No
Ancestry	White/Non-Hispanic	- HRT: Treatment Type	N/A
Height	5 ft 7 in	- HRT: Current user	N/A
Weight	175 lbs	- Number of years ago started	N/A
Age of menarche	13	- Additional years of intended use	N/A
Patient's menopausal status	Pre-menopausal	- HRT: Past user	N/A
- Age of onset	N/A	- Number of years ago ended	N/A
Age of first live birth	27	Breast biopsy	Not Specified

NUMBER OF PATIENT'S FEMALE RELATIVES

Daughters	1	Sisters	2	Maternal Aunts	2	Paternal Aunts	2

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Clinical & Cancer Family History Information

Name: **Pt Last Name, Pt First Name** DOB: **Dec 03, 1982** Accession #: **07007255-BLD** Report Date: **Dec 03, 2021**

The clinical information displayed here was provided by a qualified healthcare provider on the Test Request Form and other documents, and was not verified by Myriad. Family members listed as "other" are not included in a Tyrer-Cuzick breast cancer risk estimate or other personal/family history assessments. For more information see the Specifications for Personal/Family History Analysis at http://myriad.com/technical-specifications.

The accuracy and completeness of the information provided in the Clinical and Cancer Family History Information section of the report (e.g. height and weight, age of menarche) may significantly affect the accuracy of breast cancer risk estimates provided based on either Tyrer-Cuzick or RiskScore.

RiskScore is not valid, and may significantly over- or under-estimate breast cancer risk for women who do not meet the eligibility criteria in effect when the testing was performed. The current criteria are: 1) age is 18 to 84 years, 2) no personal history of breast cancer, LCIS, hyperplasia (with or without atypia), or a breast biopsy with unknown results, 3) there is no mutation detected in a breast cancer risk gene (other than a monoallelic *CHEK2* mutation in a White/Non-Hispanic or Ashkenazi Jewish individual), 4) the woman's relatives have not been found to have a mutation in a high-penetrance breast cancer risk gene and 5) the sample was submitted with a current Test Request Form and the ordering healthcare provider has not determined that RiskScore is inappropriate for the patient. If this is an amended report for a patient tested in the past, please refer to the MyRisk Technical Specifications at http://myriad.com/technical-specifications for the eligibility criteria in effect at the time of the original testing.

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MyRisk Management Tool

MyRisk™ Hereditary Cancer Test

RECEIVING HEALTHCARE PROVIDER	SPECIMEN		PATIENT	
Test HCP, MD	Specimen Type:	Blood	Name:	Pt Last Name, Pt First Name
Test Medical Center	Draw Date:	Dec 03, 2021	Date of Birth:	Dec 03, 1982
6609 BLANCO RD STE 200	Accession Date:	Dec 03, 2021	Patient ID:	Patient id
SAN ANTONIO, TX 78216	Report Date:	Dec 03, 2021	Gender:	Female
			Accession #:	07007255-BLD
			Requisition #:	90026826

GENETIC RESULT: NEGATIVE - NO CLINICALLY SIGNIFICANT MUTATION IDENTIFIED

Note: "CLINICALLY SIGNIFICANT," as defined in this report, is a genetic change that is associated with the potential to alter medical intervention.

BREAST CANCER RISKSCORE®: REMAINING LIFETIME RISK 35.3%

This level of risk is at or above 20% threshold for consideration of modified medical management.
See RiskScore Interpretation Section for more information.

CLINICAL HISTORY ANALYSIS: NO ADDITIONAL MANAGEMENT GUIDELINES IDENTIFIED BASED ON THE CLINICAL HISTORY PROVIDED

Other clinical factors may influence individualized management. This analysis may be incomplete if details about cancer diagnoses, ages, family relationships or other factors were omitted or ambiguous. If this patient also has a clinically significant mutation, the recommendations based on the clinical history analysis should be considered in light of the possibility that this mutation explains all or some of the cancer history in the family.

BREAST CANCER RISKSCORE®	THIS BREAST CANCER RISKSCORE® IS ASSOCIATED WITH THE FOLLOWING CANCER RISKS:
At or above 20%	ELEVATED RISK: Female Breast

No clinically significant mutations were identified in this patient. However, based on personal/family history, the patient's cancer risks may still be increased over the general population. See information below.

Please see the Genetic Test Result for more details on any variant(s) detected in this patient, including variant classification information.

ADDITIONAL FINDINGS: NO VARIANT(S) OF UNCERTAIN SIGNIFICANCE (VUS) IDENTIFIED

TYRER-CUZICK BREAST CANCER RISK CALCULATION

REMAINING LIFETIME BREAST CANCER RISK: 18.5%	5-YEAR BREAST CANCER RISK: 0.8%

The Tyrer-Cuzick breast cancer risk estimate is only calculated for women who meet the following criteria: 1) age is younger than 85 years, 2) no known mutation or inconclusive result has been found in the woman or any of her relatives, and 3) the sample was submitted with a current Test Request Form that includes all of the fields required to collect the information used in the calculation, and the provider has not indicated on the Test Request Form that the Tyrer-Cuzick calculation is not appropriate for the patient. Version 7.02 of the Tyrer-Cuzick model was used for this risk estimate. Tyrer-Cuzick model Versions 7.02 and 8.0 are available for download at the EMS-Trials website, http://www.ems-trials.org/riskevaluator.

CLINICAL OVERVIEW OF GENETIC FINDINGS

Remaining Lifetime Breast Cancer Risk Estimated to be 20% or Higher

- This woman has an estimated remaining lifetime risk for breast cancer at or above the 20% threshold based on RiskScore. This is the estimated risk of developing breast cancer from this woman's current age to age 85.

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MyRisk Management Tool

Name: **Pt Last Name, Pt First Name** DOB: **Dec 03, 1982** Accession #: **07007255-BLD** Report Date: **Dec 03, 2021**

- RiskScore is partially based on the analysis of selected genetic markers known to have an impact on breast cancer risk. Although the level of risk associated with each individual marker is small, results from the combined analysis of multiple markers can have a significant impact on breast cancer risk estimates.
- The RiskScore estimate is also based on information about the woman's personal medical history and any history of breast and ovarian cancer in her relatives, as reported by the healthcare provider. RiskScore will be less accurate if any of the information that was provided is incomplete or incorrect. The RiskScore estimate is not valid, and may significantly over- or under-estimate risk, if the woman is ineligible for RiskScore based on the criteria described on the RiskScore Clinical & Family History page of the report.
- Currently there are no guidelines for the medical management of breast cancer risk in women based on RiskScore. However, it may be appropriate to consider options based on guidelines for other situations where the estimated remaining lifetime breast cancer risk is at or above the 20% threshold.

WHAT ARE THE PATIENT'S CANCER RISKS?

These risk tables show the clinically significant cancer risks identified as part of this patient's testing. Testing for some patients does not include some of the analyses listed:

- GENETIC RESULT: Mutations detected in any of the hereditary cancer genes included on the MyRisk panel.
- BREAST CANCER RISKSCORE: RiskScore estimate of remaining lifetime breast cancer risk if greater than 20%
- CLINICAL HISTORY ANALYSIS for breast cancer risk: Tyrer-Cuzick model estimate of remaining lifetime breast cancer risk greater than 20%
- CLINICAL HISTORY ANALYSIS for breast, colorectal, pancreatic, prostate and melanoma cancer: Analysis of the patient's personal and family history.

The risks for each of these results are provided separately. If the risk for any individual cancer is affected by more than one of these results, the risk associated with each finding is listed in a separate table. At this time, there is not enough information to estimate risks for cancers affected by more than one gene mutation, or risks based on both gene mutations and personal/family history.

Breast cancer risk estimate at or above the 20% threshold based on RiskScore® and/or the Tyrer-Cuzick model

CANCER TYPE	CANCER RISK	RISK FOR GENERAL POPULATION	RELATED TO
FEMALE BREAST			
Remaining lifetime risk (age at time of testing to age 85)	35.3%	13.0%	RiskScore at or above the 20% threshold

WHAT MANAGEMENT FOR CANCER RISKS SHOULD BE CONSIDERED?

This overview of clinical management guidelines is based on the patient's genetic test results and the Clinical History Analysis. Medical management guidelines are summarized from established medical societies, primarily the National Comprehensive Cancer Network (NCCN). If there are overlapping management guidelines for any individual cancer due to more than one of these results, the guidelines associated with each finding are listed in separate tables, even if they are the same. At this time, there are no medical society guidelines for how to adjust management when there are multiple sources of risk, such as from more than one gene mutation, or a mutation and a personal/family history of cancer. In these cases, it may be appropriate to use the most aggressive of the management options provided.

The overview provided below should not be used as the sole source of information to determine medical management. The references cited should always be consulted for more details and updates to the recommendations.

No information is provided related to treatment of a previous or existing cancer or polyps. The recommendation summaries below may require modification due to the patient's personal medical history, past surgeries and other treatments. Patients with a past history of cancer, benign tumors, or pre-cancerous findings may be candidates for long term surveillance and risk-reduction strategies beyond what is necessary for the treatment of their initial diagnosis. Any discussion of medical management options is for general information purposes only and does not constitute a recommendation. While genetic testing and medical society recommendations provide important and useful information, medical management decisions should be made in consultation between each patient and his or her healthcare provider.

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MyRisk Management Tool

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Management Options for a breast cancer risk estimate at or above the 20% threshold based on RiskScore® and/or the Tyrer-Cuzick model

PROCEDURE	AGE TO BEGIN	FREQUENCY Unless otherwise indicated by findings	RELATED TO
FEMALE BREAST			
Currently there are no specific medical management guidelines for breast cancer risk based on RiskScore. However, the estimated remaining lifetime risk at or above the 20% threshold warrants consideration of risk-reduction strategies similar to those listed below, which are recommended for women with an estimated lifetime risk greater than 20% based on other risk prediction methods. [1,2]	At age identified as being at increased risk	NA	RiskScore at or above the 20% threshold
Breast awareness - Women should be familiar with their breasts and promptly report changes to their healthcare provider. Periodic, consistent breast self-examination (BSE) may facilitate breast awareness. [1]	Individualized	NA	RiskScore at or above the 20% threshold
Clinical encounter, including clinical breast exam, ongoing risk assessment and risk-reduction counseling [1]	At age identified as being at increased risk, but not before age 21	Every 6 to 12 months	RiskScore at or above the 20% threshold
Mammography, with consideration of tomosynthesis [1]	10 years younger than the earliest diagnosis in the family, but to begin no younger than age 30 but no later than age 40	Annually	RiskScore at or above the 20% threshold
Breast MRI with contrast [1]	10 years younger than the earliest diagnosis in the family, but no later than age 40	Annually	RiskScore at or above the 20% threshold
Consider additional risk-reduction strategies. [1]	Individualized	NA	RiskScore at or above the 20% threshold

1. Bevers TB, et al. NCCN Clinical Practice Guidelines in Oncology®: Breast Cancer Screening and Diagnosis. V 1.2021. May 6. Available at https://www.nccn.org.
2. Daly M et al. NCCN Clinical Practice Guidelines in Oncology®: Genetic/Familial High-Risk Assessment: Breast, Ovarian and Pancreatic. V 1.2022. Aug 11. Available at https://www.nccn.org.

Notes for Personalized Management:

INFORMATION ON HOW CANCER RISKS AND MANAGEMENT ARE DETERMINED

The MyRisk Management Tool provides cancer risk levels based on analysis of genetic test results (see MyRisk Genetic Result) and a summary of medical society management recommendations based on both the genetic test results and a limited analysis of the patient's clinical history related to the risk for breast, colorectal, prostate, melanoma and pancreatic cancers. Here are some important points to understand as you interpret this test report and decide on the best plan for management:

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- Comprehensive patient management. The management recommendations presented in this report are a summary of management options recommended by the National Comprehensive Cancer Network (NCCN) and other established medical societies and are general in nature. The patient's actual management should be modified based on personal medical history, surgeries and other treatments. A comprehensive risk assessment and management plan may take into account this report and other aspects of the patient's personal/family medical history (e.g., all known clinical diagnoses), as well as lifestyle, environmental and other factors.
- Risk estimates based on provider-supplied information. Some of the risk estimates and management recommendation summaries provided in this report are based on our interpretation of information supplied by the ordering health care provider on the test request form (see Specifications for Personal/Family History analysis at myriad.com/technical-specifications). The patient's actual risks and appropriate management may be significantly different if details provided for cancer diagnoses, ages, family relationships or other factors were incorrect, omitted, ambiguous or have since changed. Please review the clinical history listed on the Clinical & Family History Information page of this report to make sure that the information used was provided and interpreted correctly.
- Variability in Tyrer-Cuzick risk estimates. Tyrer-Cuzick estimates of breast cancer risk can vary significantly based on the way in which the model is used, and the estimate provided here may be higher or lower than what would be calculated by other users. For complete details of how Myriad calculates Tyrer-Cuzick risk estimates, including how Myriad handles information provided in a format not compatible with the model, please see the Specifications for Personal/Family History analysis at myriad.com/technical-specifications. These Specifications also include information for recalculating the Tyrer-Cuzick breast cancer risk estimate if desired.
- What is meant by "High Risk" and "Elevated Risk"? In the Genetic Test Result Summary, a gene-associated cancer risk is described as "High Risk" for a cancer type if all of the following conditions are met: the absolute risk of cancer is approximately 5% or higher, the increase in risk over the general population is approximately 2 to 3-fold or higher, and there is significant data from multiple studies supporting the cancer risk estimate. A gene is described as "Elevated Risk" for a cancer type if there is sufficient data to support an increase in cancer risk over the general population risk, but not all criteria for "High Risk" are met.

INFORMATION FOR FAMILY MEMBERS

Family members should talk to their healthcare providers about hereditary cancer testing to help define their own risk and assist in the interpretation of this patient's genetic test result.

Additional Information for Remaining Lifetime Breast Cancer Risk Estimated to be 20% or Higher

- This patient has an estimated remaining lifetime risk of breast cancer at or above the 20% threshold based on the breast cancer RiskScore estimate, which includes both genetic and non-genetic factors that may be shared within the family. Female relatives of this patient may also be at a significantly increased risk for breast cancer and should consult with a healthcare provider to discuss their own risk.

Please contact Myriad Medical Services at 1-800-469-7423 X 3850 to discuss any questions regarding this result.

END OF MANAGEMENT TOOL

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SAMPLE REPORT

Ordered By Contact ID:405956 Org ID:249	Patient Name: **Last, First**
Physician: Sample Doctor, A	Accession #: **00-332049** Specimen #: 44-55-66
Client: Sample Organization (00403)	AP2 Order #: 205725 Specimen: Blood EDTA (Purple top)
Additional Authorized Recipient:	Birthdate: 01/01/1980 Gender: F
Sample Genetic Counselor MS, OGC	MRN #: #### Collected: 05/18/2018
	Indication: Diagnostic/Family History Received: 05/19/2018

BRCA1/2 Analyses with CancerNext®

RESULTS

Pathogenic Mutation(s): None Detected
Variant(s) of Unknown Significance: None Detected
Gross Deletion(s)/Duplication(s): None Detected

SUMMARY

NEGATIVE: No Clinically Significant Variants Detected

INTERPRETATION

- No pathogenic mutations, variants of unknown significance, or gross deletions or duplications were detected.
- **Risk Estimate:** low likelihood of variants in the genes analyzed contributing to this individual's clinical history.
- Genetic counseling is a recommended option for all individuals undergoing genetic testing.

Genes Analyzed (36 total): ***APC, ATM, AXIN2, BARD1, BMPR1A, BRCA1, BRCA2, BRIP1, CDH1, CDK4, CDKN2A, CHEK2, DICER1, MLH1, MSH2, MSH3, MSH6, MUTYH, NBN, NF1, NTHL1, PALB2, PMS2, PTEN, RAD51C, RAD51D, RECQL, SMAD4, SMARCA4, STK11*** **and** ***TP53*** **(sequencing and deletion/duplication);** ***HOXB13, POLD1*** **and** ***POLE*** **(sequencing only);** ***EPCAM*** **and** ***GREM1*** **(deletion/duplication only).**

Order Summary: The following products were included in the test order for this individual. Please note: tests on hold and those that have been cancelled (including reflex testing steps cancelled due to a positive result in a preceding test) are excluded. For additional information, please contact Ambry Genetics.

- BRCA1/2 seq and del/dup (Product Code 8838)
- CancerNext® (Product Code 8824)

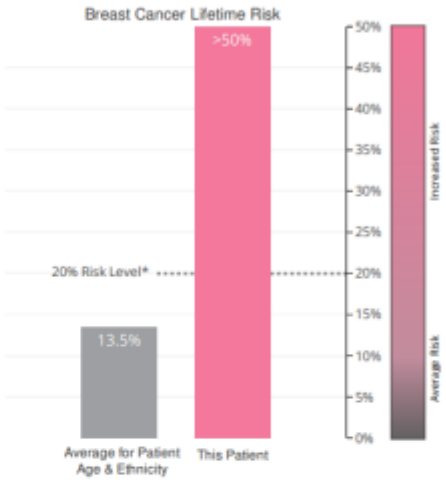
AmbryScore: Personalized Breast Cancer Risk Estimate

Supplement to Test Results

Remaining Lifetime Risk:

>50%

- This individual's remaining lifetime risk for breast cancer is estimated to be >50%, which is increased above the general population risk of 13.5%.
- Modified breast cancer screening should be considered.



*Recommendations for modified breast cancer screening exist for women estimated above 20% lifetime risk by models such as Tyrer-Cuzick.

Interpretation

- This individual's remaining lifetime breast cancer risk of >50% is increased above the average risk based on age and ethnicity and exceeds the 20% threshold for consideration of modified medical management.
- The below scores were used to estimate the remaining lifetime risk for this individual:
  - **Tyrer-Cuzick remaining lifetime risk estimate:** 33.4%
  - **Polygenic risk score (PRS): RR=** 1.7
- Use of the AmbryScore risk estimate in medical management and planning should be left to the discretion of the healthcare provider and interpreted in the context of patient age, clinical history, and family history.

Technical Details

The AmbryScore tool provides a personalized estimate of remaining lifetime breast cancer risk (up to age 85) based on the following patient-specific factors: age at testing, ethnicity, clinical and family history data, and results of single nucleotide polymorphism (SNP) profiling. A population-standardized PRS is computed as the sum of the patient's risk alleles across 180 SNPs, weighted by the SNP-specific effects reported in large breast cancer studies, and ethnicity-specific allele frequencies [PMID: 20956782, 26589314, 28477205, 29059683]. A patient's absolute risk of breast cancer is computed according to the Tyrer-Cuzick model (v.8) [PMID: 29029312], which is based on her age, family history and clinical information, and combined with her PRS to produce an estimate of her remaining lifetime risk. The AmbryScore calculation is highly-dependent on the accuracy of clinician-provided clinical data. Other factors not accounted for in the AmbryScore calculation may impact lifetime breast cancer risk including, but not limited to, germline mutations not analyzed by the ordered genetic test. The AmbryScore provided is patient-specific and cannot be used to infer risk to relatives. Additional technical details and supporting references can be found here: www.ambrygen.com/ambryscore.

ASSAY INFORMATION

General Information: Cancer is a complex, multifactorial disease diagnosed in approximately 1 out of every 2 men and 1 out of every 3 women over the course of a lifetime. Mutations in cancer predisposition genes appear to be responsible for between 5-10% of cancer diagnoses.

Methodology: The **CancerNext®** test is a comprehensive screen of 36 genes associated with hereditary cancer predisposition. Genomic deoxyribonucleic acid (gDNA) is isolated from the patient's specimen using standardized methodology and quantified. Sequence enrichment of the targeted coding exons and adjacent intronic nucleotides is carried out by a bait-capture methodology using long biotinylated oligonucleotide probes followed by polymerase chain reaction (PCR) and Next-Generation sequencing. Additional Sanger sequencing is performed for any regions missing or with insufficient read depth coverage for reliable heterozygous variant detection. Variants in regions complicated by pseudogene interference, variant calls not satisfying depth of coverage and variant allele frequency quality thresholds, and potentially homozygous variants are verified by Sanger sequencing. The *BRCA2* Portuguese founder mutation, c.156_157InsAlu (also known as 384insAlu), and the *MSH2* coding exons 1-7 inversion are detected by next generation sequencing and confirmed by multiplex ligation-dependent probe amplification (MLPA) or PCR and agarose gel electrophoresis. Gross deletion/duplication analysis for the genes sequenced (excluding *HOXB13, POLD1, POLE, PMS2*) is performed using a custom pipeline based on read-depth from NGS data and/or targeted chromosomal microarray with confirmatory MLPA when applicable. Gross deletion/duplication analysis of *PMS2* is performed using MLPA kit P008-B1. If a deletion is detected in exons 13, 14, or 15 of *PMS2*, double stranded sequencing of the appropriate exon(s) of the pseudogene *PMS2CL* will be performed to determine if the deletion is located in the *PMS2* gene or pseudogene. Sequence analysis is based on the following NCBI reference sequences: *APC*- NM_000038.5 & NM_001127511.2, *ATM*- NM_000051.3, *AXIN2*- NM_004655.3, *BARD1*- NM_000465.2, *BMPR1A*- NM_004329.2, *BRCA1*- NM_007294.3, *BRCA2*- NM_000059.3, *BRIP1*- NM_032043.2, *CDH1*- NM_004360.3, *CDK4*- NM_000075.3, *CDKN2A*- NM_000077.4 and NM_058195.3 (p14ARF), *CHEK2*- NM_007194.3, *DICER1*-NM_177438.2, *HOXB13*- NM_006361.5, *MUTYH*- NM_001128425.1, *MLH1*- NM_000249.3, *MSH2*- NM_000251.1, *MSH3*- NM_002439.3, *MSH6*- NM_000179.2, *NBN*- NM_002485.4, *NF1*- NM_000267.3, *NTHL1*- NM_002528.5, *PALB2*- NM_024675.3, *PMS2*- NM_000535.5, *POLD1*-NM_002691.2, *POLE*-NM_006231.2, *PTEN*- NM_000314.4, *RAD51C*- NM_058216.1, *RAD51D*- NM_002878.3, *RECQL*- NM_002907.3, *SMAD4*- NM_005359.5, *SMARCA4*- NM_001128849.1, *STK11*- NM_000455.4, *TP53*- NM_000546.4.

Analytical Range: The **CancerNext®** test targets detection of DNA sequence mutations in the sequenced genes (*APC, ATM, AXIN2, BARD1, BMPR1A, BRCA1, BRCA2, BRIP1, CDH1, CDK4, CDKN2A, CHEK2, DICER1, HOXB13, MLH1, MSH2, MSH3, MSH6, MUTYH, NBN, NF1, NTHL1, PALB2, POLD1, POLE, PMS2, PTEN, RAD51C, RAD51D, RECQL, SMAD4, SMARCA4, STK11,* and *TP53)* by either Next-Generation or Sanger sequencing of all coding domains and well into the flanking 5' and 3' ends of all the introns and untranslated regions. For *HOXB13*, only variants impacting codon 84 are routinely reported. For *POLD1* and *POLE*, only missense variants and in-frame insertions/deletions in the exonuclease domains (codons 311-541 and 269-485, respectively) are routinely reported. For *RECQL*, only missense variants in the helicase and RCQ domains (codons 63-592) and exonic truncating variants are routinely reported. The *MSH3* polyalanine repeat region is excluded from analysis. Gross deletion/duplication analysis determines gene copy number for the covered exons and untranslated regions of sequenced genes (excluding *HOXB13, POLD1*, and *POLE*) as well as *GREM1* and *EPCAM*. For *GREM1*, only the status of the 40kb 5'UTR gross duplication is analyzed and reported. For *EPCAM*, only gross deletions encompassing the 3' end of the gene are reported. For *NTHL1*, only full-gene gross deletions and duplications are detected. For *APC*, all promoter 1B gross deletions as well as single nucleotide substitutions within the promoter 1B YY1 binding motif (NM_001127511 c.-196_-186) are analyzed and reported.

Result Reports: Results reported herein may be of constitutional or somatic origin. This methodology cannot differentiate between these possibilities. In result reports, alterations in the following classifications are always reported, and are based on the following definitions and clinical recommendations:

- **Pathogenic Mutation**: alterations with sufficient evidence to classify as pathogenic (capable of causing disease). Targeted testing of at-risk relatives and appropriate changes in medical management for pathogenic mutation carriers recommended. Previously described pathogenic mutations, including intronic mutations at any position, are always reported when detected.
- **Variant, Likely Pathogenic (VLP)**: alterations with strong evidence in favor of pathogenicity. Targeted testing of at-risk relatives and appropriate changes in medical management for VLP carriers typically recommended. Previously described likely pathogenic variants, including intronic VLPs at any position, are always reported when detected.
- **Variant, Unknown Significance (VUS)**: alterations with limited and/or conflicting evidence regarding pathogenicity. Familial testing via the Family Studies Program recommended. Medical management to be based personal/family clinical histories, not VUS carrier status. Note, intronic VUSs are always reported out to 5 basepairs from the splice junction when detected.

Alterations of unlikely clinical significance (those with strong/very strong evidence to argue against pathogenicity) are not routinely included on results reports. These include findings classified as "likely benign" and "benign" alterations.

Assay Information Continued on Next Page

ASSAY INFORMATION *(Supplement to Test Results - Continued)*

Resources: The following references are used in variant analysis and classification when applicable for observed genetic alterations.

1. The 1000 Genomes Project Consortium. An integrated map of genetic variation from 1092 human genomes. *Nature*. 2012;491:56-65.
2. ACMG Standards and guidelines for the interpretation of sequence variants. *Genet Med*. 2015 May;17(5):405-23.
3. Ambry Genetics Variant Classification Scheme. http://www.ambrygen.com/variant-classification.
4. Berkeley Drosophila Genome Project [Internet]. Reese MG et al. *J Comp Biol*. 1997;4:311-23. http://www.fruitfly.org/seq_tools/splice.html.
5. Database of Single Nucleotide Polymorphisms (dbSNP) [Internet]. Bethesda (MD): National Center for Biotechnology Information, National Library of Medicine (dbSNP Build ID:135) Available from: www.ncbi.nlm.nih.gov/SNP. Accessed Jan 2012).
6. ESEfinder [Internet]. Smith PJ, et al. (2006) *Hum Mol Genet*. 15(16):2490-2508 and Cartegni L, et al. *Nucleic Acid Research*. 2003;31(13):3568-3571. http://rulai.cshl.edu/cgi-bin/tools/ESE3/esefinder.cgi?process=home.
7. Exome Variant Server, NHLBI Exome Sequencing Project (ESP) [Internet], Seattle WA. Available from: evs.gs.washington.edu/EVS.
8. Grantham R. Amino acid difference formula to help explain protein evolution. *Science*. 1974;185(4151):862-864.
9. HGMD® [Internet]: Stenson PD et al. *Genome Med*. 2009;1(1):13. www.hgmd.cf.ac.uk.
10. Landrum MJ et al. ClinVar: public archive of relationships among sequence variation and human phenotype. *Nucleic Acids Res*. 2014 Jan 1;42(1):D980-5. doi: 10.1093/nar/gkt1113. PubMed PMID: 24234437.
11. Online Mendelian Inheritance in Man, OMIM®. McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University (Baltimore, MD), Copyright® 1966-2012. World Wide Web URL: http://omim.org.
12. Feng BJ. PERCH: A Unified Framework for Disease Gene Prioritization. *Hum Mutat*. 2017 Mar;38(3):243-251.
13. Exome Aggregation Consortium (ExAC) [Internet], Cambridge, MA. Available from: http://exac.broadinstitute.org.
14. Genome Aggregation Database (gnomAD) [Internet], Cambridge, MA. Available from: http://gnomad.broadinstitute.org.
15. Lek M et al. Analysis of protein-coding genetic variation in 60,706 humans. *Nature*. 2016 Aug 17;536(7616):285-91. PMID: 27535533
16. Mu W et al. *J Mol Diagn*. 2016 Oct 4. PubMed PMID: 27720647

Disclaimer: This test was developed and its performance characteristics were determined by Ambry Genetics Corporation. It has not been cleared or approved by the US Food and Drug Administration. The FDA does not require this test to go through premarket FDA review. It should not be regarded as investigational or for research. This test should be interpreted in context with other clinical findings. This laboratory is certified under the Clinical Laboratory Improvement Amendments (CLIA) as qualified to perform high complexity clinical laboratory testing. This test analyzes the following types of mutations: nucleotide substitutions, small deletions (up to 25 bp), small insertions (up to 10 bp), small indels and gross deletions/duplications. Unless otherwise noted in the methodology section above, it is not intended to analyze the following types of alterations: gross rearrangements, deep intronic variations, Alu element insertions, and other unknown abnormalities. The pattern of mutation types varies with the gene tested and this test detects a high but variable percentage of known and unknown mutants of the classes stated. A negative result from the analysis cannot rule out the possibility that the tested individual carries a rare unexamined mutation or mutation in the undetectable group. This test is designed and validated to be capable of detecting ~99% of described mutations in the 36 genes represented on the panel (analytical sensitivity). The clinical sensitivity of this test may vary widely according to the specific clinical and family history. Breast, ovarian and colon cancers are complex clinical disorders. Mutations in other genes or the regions not analyzed by this test can also give rise to clinical conditions similar to breast cancer, ovarian or colon cancer. Although molecular tests are highly accurate, rare diagnostic errors may occur. Possible diagnostic errors include sample mix-up, erroneous paternity identification, technical errors, clerical errors, and genotyping errors. Genotyping errors can result from trace contamination of PCR reactions, from maternal cell contamination in fetal samples, from rare genetic variants that interfere with analysis, low-level mosaicism, presence of pseudogenes, technical difficulties in regions with high GC content or homopolymer tracts, presence of pre-malignant or malignant cells in the sample, or from other sources. Rare variants present in the human genome reference sequence (GRCh37.p5/hg19) or rare misalignment due to presence of pseudogenes can lead to misinterpretation of patient sequence data can lead to misinterpretation of patient sequence data. This report does not represent medical advice. Any questions, suggestions, or concerns regarding interpretation of results should be forwarded to a genetic counselor, medical geneticist, or physician skilled in interpretation of the relevant medical literature.

Ambry Genetics

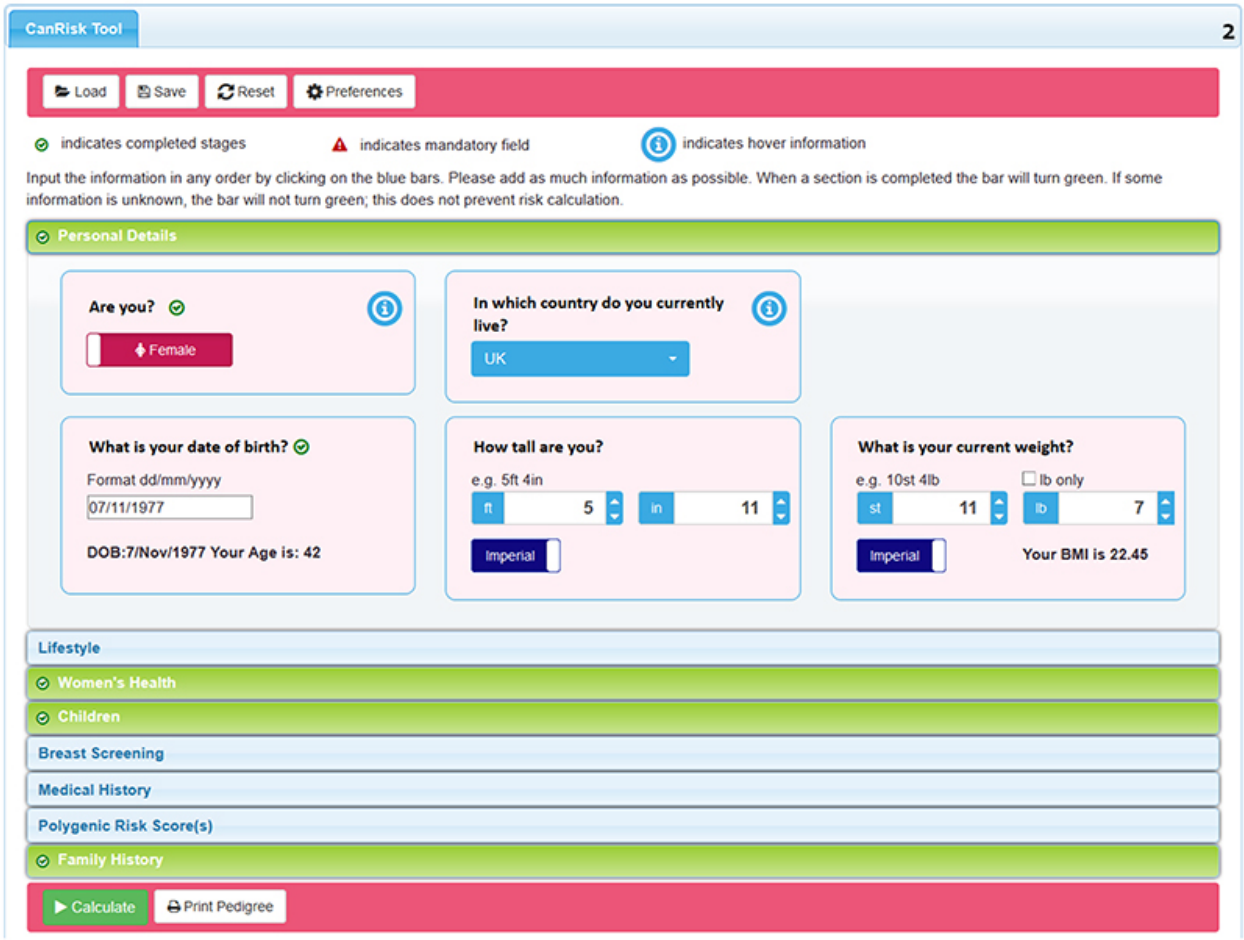
Understanding Your Negative Hereditary Cancer Genetic Test Result

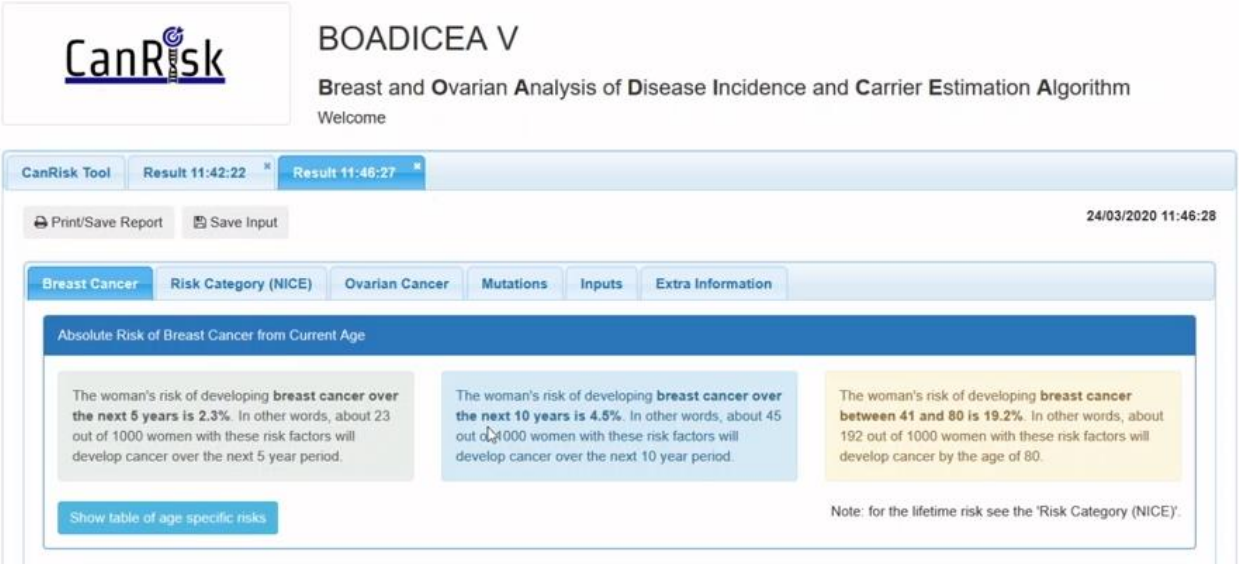
INFORMATION FOR PATIENTS

Result	NEGATIVE	Your testing did not find any disease-causing mutations (changes, like spelling mistakes) in the genes tested.
Cancer Risks	VARIES	Even though no mutation was found, you may still have an increased risk of developing cancer based on other possible factors, including the following: • Your medical and/or family history • You could have a mutation in the genes tested that cannot be found with current testing methods • You could have a mutation in a gene that has not yet been linked to cancer or was not tested Your healthcare provider can help you learn more about this.
Risk Management	VARIES	Risk management decisions are very personal, and depend on many factors. Talk to your healthcare provider about which, if any, options may be right for you.
Family Members	VARIABLE RISKS	Depending on your medical and/or family history, your relatives may still have an increased risk of developing cancer and may be eligible for genetic testing and/or increased cancer screening. They should discuss this with a healthcare provider.
Next Steps	DISCUSS	Please share this with family members so they can talk with their healthcare providers and learn more. Stay in contact with your healthcare provider for any relevant updates in genetic testing and/or cancer screening. Also, remember to update him/her with any new information about your family history, especially new cancer diagnoses, as this may change how they determine your cancer risks.
Reach Out	RESOURCES	• Ambry's Hereditary Cancer Site for Families patients.ambrygen.com/cancer • American Cancer Society cancer.org • Genetic Information Nondiscrimination Act (GINA) ginahelp.org • National Society of Genetic Counselors nsgc.org • Canadian Association of Genetic Counsellors cagc-accg.ca

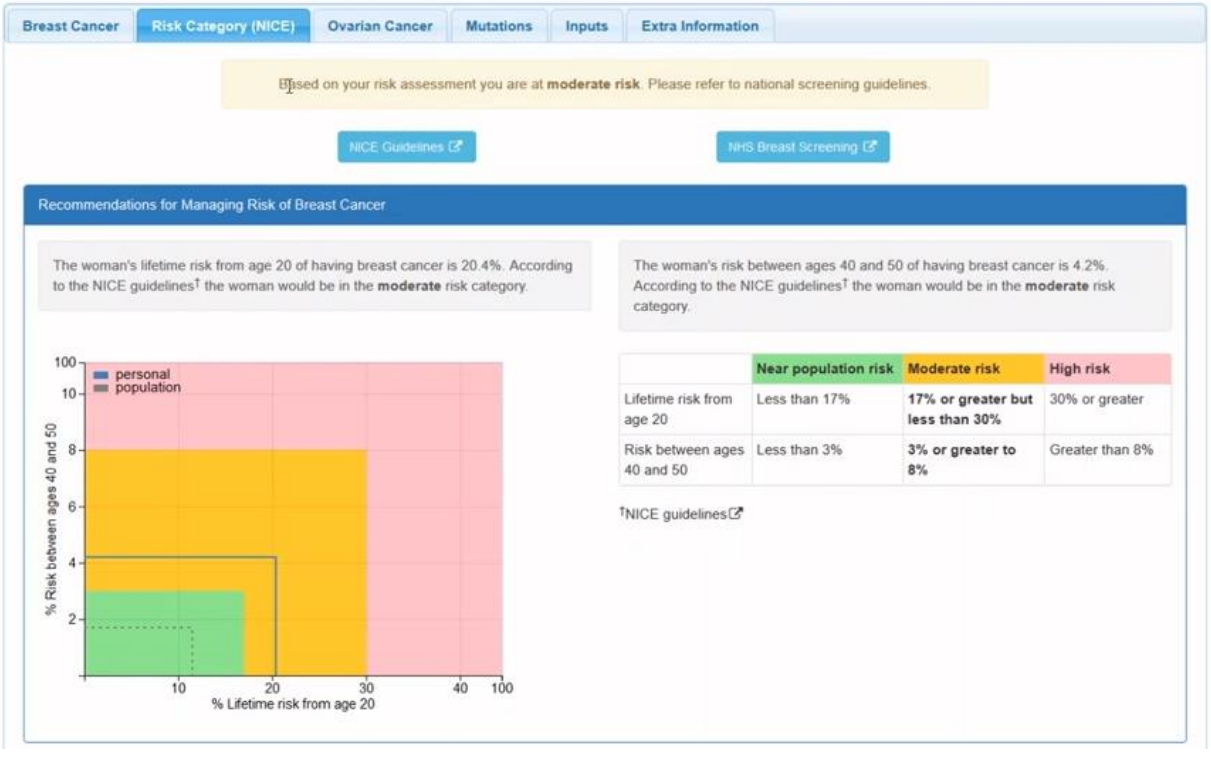
Please discuss this information with your healthcare provider. The cancer genetics field is continuously evolving, so updates related to your genetic test result, medical recommendations, genetic testing options, and/or potential treatments may be available over time. This information is not meant to replace a discussion with a healthcare provider, and should not be considered or interpreted as medical advice.

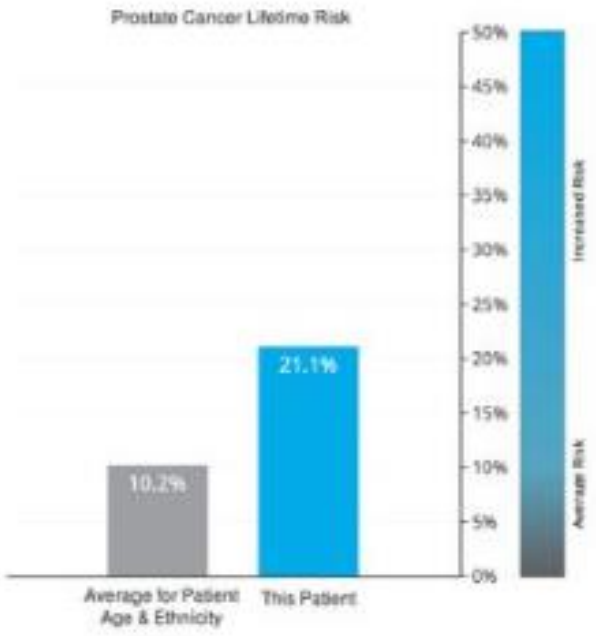
7 Argonaut, Aliso Viejo, CA 92656 USA Toll Free +1 866 262 7943 Fax +1 949 900 5501 ambrygen.com

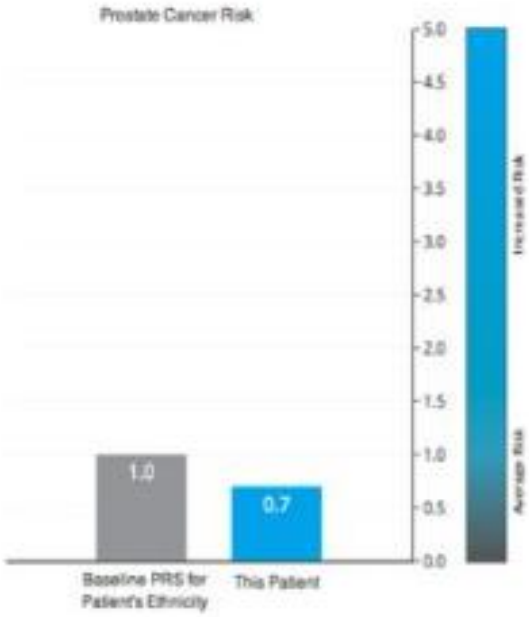

CanRisk Tool
2
Load
Save
Reset
Preferences
indicates completed stages
indicates mandatory field
indicates hover information
Input the information in any order by clicking on the blue bars. Please add as much information as possible. When a section is completed the bar will turn green. If some information is unknown, the bar will not turn green; this does not prevent risk calculation.
Personal Details
Are you?
Female
In which country do you currently live?
UK
What is your date of birth?
Format dd/mm/yyyy
07/11/1977
DOB:7/Nov/1977 Your Age is: 42
How tall are you?
e.g. 5ft 4in
ft 5
in 11
Imperial
What is your current weight?
e.g. 10st 4lb
lb only
st 11
lb 7
Imperial
Your BMI is 22.45
Lifestyle
Women's Health
Children
Breast Screening
Medical History
Polygenic Risk Score(s)
Family History
Calculate
Print Pedigree


CanRisk
BOADICEA V
Breast and Ovarian Analysis of Disease Incidence and Carrier Estimation Algorithm
Welcome
CanRisk Tool
Result 11:42:22
Result 11:46:27
Print/Save Report
Save Input
24/03/2020 11:46:28
Breast Cancer
Risk Category (NICE)
Ovarian Cancer
Mutations
Inputs
Extra Information
Absolute Risk of Breast Cancer from Current Age
The woman's risk of developing breast cancer over the next 5 years is 2.3%. In other words, about 23 out of 1000 women with these risk factors will develop cancer over the next 5 year period.
The woman's risk of developing breast cancer over the next 10 years is 4.5%. In other words, about 45 out of 1000 women with these risk factors will develop cancer over the next 10 year period.
The woman's risk of developing breast cancer between 41 and 80 is 19.2%. In other words, about 192 out of 1000 women with these risk factors will develop cancer by the age of 80.
Show table of age specific risks
Note: for the lifetime risk see the 'Risk Category (NICE)'.


Next 5 Year Risk
Next 10 Year Risk
Risk by Age 80
Breast Cancer 5 Year Risk
The woman's risk of developing breast cancer over the next 5 years is 2.3%. In other words, about 23 out of 1000 women with these risk factors will develop cancer over the next 5 year period.
23 women are likely to develop breast cancer
977 women are unlikely to develop breast cancer
Breast Cancer Remaining Lifetime Risk
The woman's risk of developing breast cancer by the age of 80 is 19.2%. In other words, about 192 out of 1000 women with these risk factors will develop breast cancer by the age of 80.
192 women are likely to develop breast cancer
808 women are unlikely to develop breast cancer


Breast Cancer
Risk Category (NICE)
Ovarian Cancer
Mutations
Inputs
Extra Information
Based on your risk assessment you are at moderate risk. Please refer to national screening guidelines.
NICE Guidelines
NHS Breast Screening
Recommendations for Managing Risk of Breast Cancer
The woman's lifetime risk from age 20 of having breast cancer is 20.4%. According to the NICE guidelines† the woman would be in the moderate risk category.
The woman's risk between ages 40 and 50 of having breast cancer is 4.2%. According to the NICE guidelines† the woman would be in the moderate risk category.
personal
population
% Risk between ages 40 and 50
% Lifetime risk from age 20
Near population risk
Moderate risk
High risk
Lifetime risk from age 20
Less than 17%
17% or greater but less than 30%
30% or greater
Risk between ages 40 and 50
Less than 3%
3% or greater to 8%
Greater than 8%
†NICE guidelines


Prostate Cancer Lifetime Risk
50%
45%
40%
35%
30%
25%
20%
15%
10%
5%
0%
21.1%
10.2%
Increased Risk
Average Risk
Average for Patient Age & Ethnicity
This Patient


Prostate Cancer Risk
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
1.0
0.7
Increased Risk
Average Risk
Baseline PRS for Patient's Ethnicity
This Patient

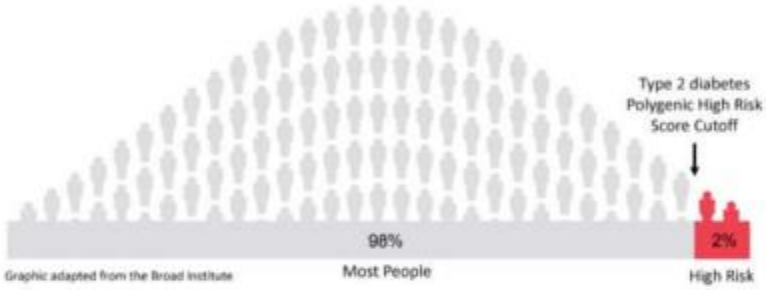
Type 2 Diabetes: Understanding Your Results

What is Type 2 Diabetes?

- Type 2 diabetes is a condition where the level of sugar (glucose) in your blood is too high.
- Your blood always has some sugar in it, but too much sugar isn't good for your health.
- If your blood sugar level remains high over time, it can cause serious damage to your heart, eyes, kidneys, and feet.
- Genetic and lifestyle factors like too little exercise, poor diet and obesity can lead to type 2 diabetes. Risk for developing type 2 diabetes increases with age.

What does high risk for type 2 diabetes mean?

- Your polygenic risk score (PRS) is in the top 2%. This means that you may have a higher risk for type 2 diabetes than 98 out of 100 people.



- On average, 1 in 10 people, or 10%, will get type 2 diabetes in their lifetime. High risk for type 2 diabetes means that your genetic risk is 3-7 times higher for developing type 2 diabetes compared to a person not in the high risk category.
- This result does not mean that you have type 2 diabetes or that you will definitely develop in your lifetime.
- This PRS was created using genetic information from large research studies of people with European, Asian, African, and Hispanic/Latino descent. We outline how this score was created below:
  - DNA differences in each population were picked up that are linked to type 2 diabetes risk
  - This score was tested using genetic information from other research studies with different populations and was accurate
- Larger research studies are needed in people of other descents to provide risk ranges for other populations - see the Broad PRS report attached.

GIRA Report

Risk Result:	**High Risk for Type 2 Diabetes**

Monogenic Results: Not Evaluated

No gene sequencing was completed for genes related to this phenotype.

Polygenic Risk: High Risk

A high polygenic risk score for type 2 diabetes was found in this individual. A high polygenic risk score is associated with 3-7 times increased risk for developing type 2 diabetes relative to a person not in the high risk category. The data is based on populations of African, European, East Asian and Hispanic/Latino descent. Information is insufficient or not available for populations of other descent.

Family History:

Family history provided by this participant did not meet the criteria for elevated risk. Family history may be incomplete or unknown.

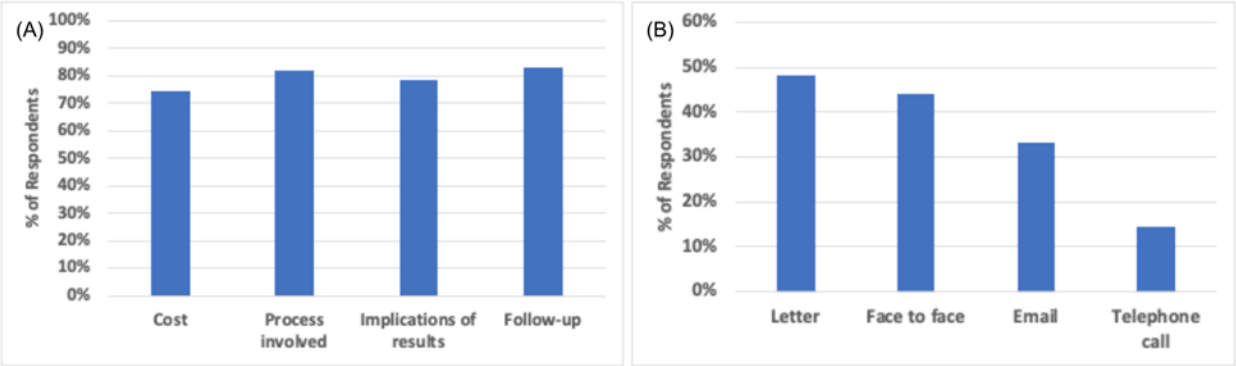
Clinical Factors:

This patient has a history of one or more of the clinical risk factors listed below. Screening should be considered in overweight or obese adults who have one or more of the additional risk factors listed. Screening should also be considered for all adults over 45 years of age.

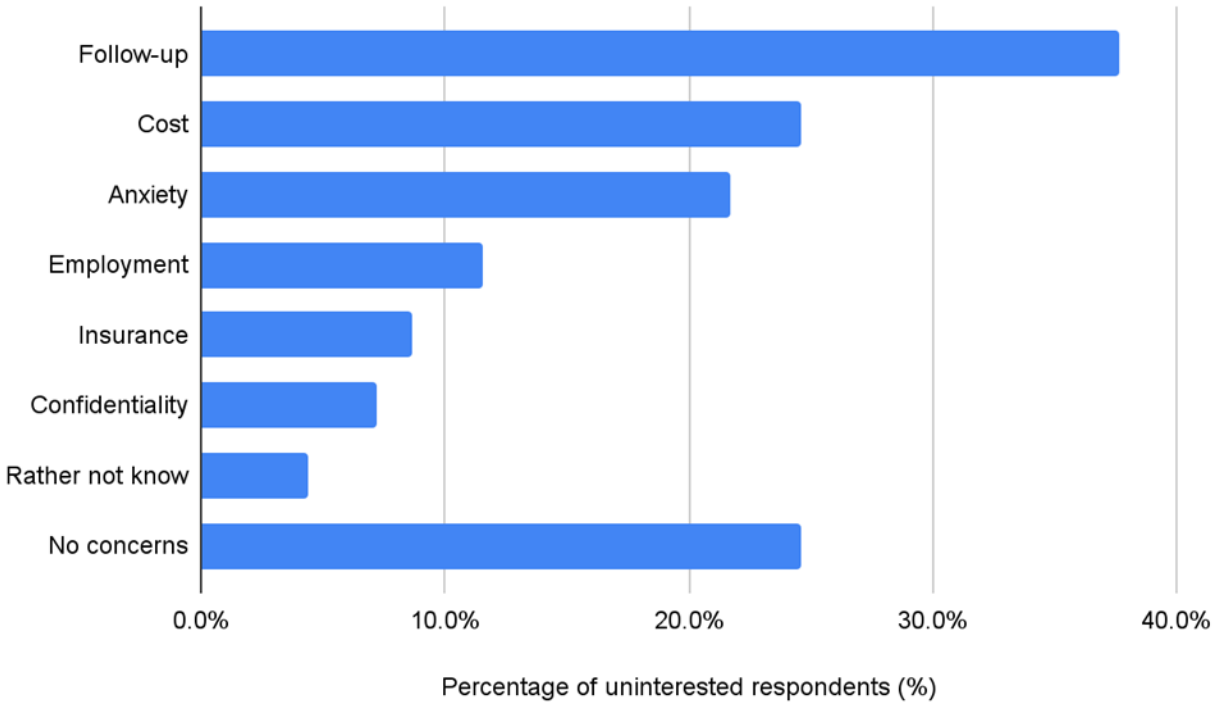
Risk Factor	Sub-Category	Present
Overweight or obese	BMI ≥ 25 kg/m2	Present
	Pediatrics: > 85%	Unknown
Demographics	Self-reported non-white race, non-Hispanic	Present
	Age ≥ 45	Yes
Diagnoses	Hypertension	Present
	Gestational Diabetes (female only)	Not Present
	Polycystic ovarian syndrome (female only)	Not Present
Lab tests	HDL < 35 mg/dL	Present
	Triglycerides >250 mg/dl	Not Present
	A1C ≥ 5.7%	Not Present

Limitations of polygenic risk: This polygenic risk does not take into account the individual's non-genetic factors such as lifestyle, habits and historyof other diseases, which could affect risk. These results should be viewed in the context of the individual's medicalcare, family history, and racial/ethnic background. See the full methods and limitations for additional information.

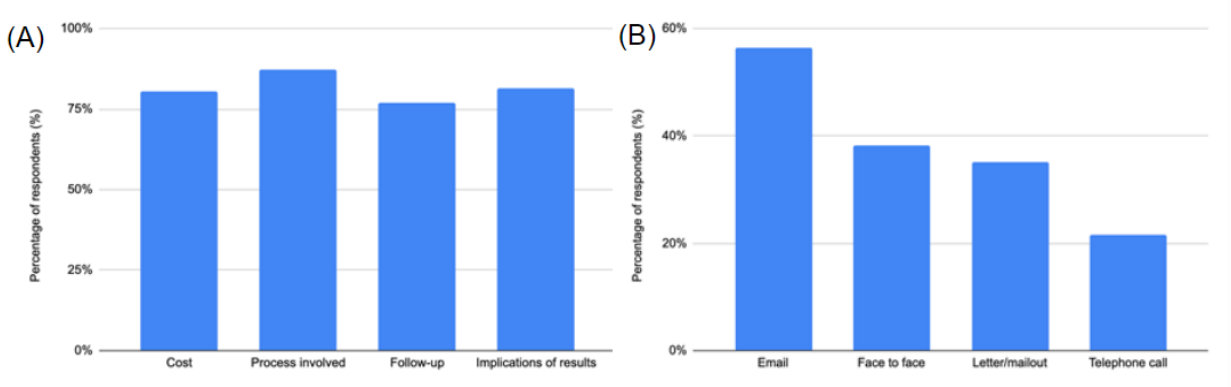
APPENDIX F: SUPPLEMENTARY FIGURES



Supplementary Figure 1: Participants' preferences for information content surrounding genetic risk testing. Preferences were expressed for information delivered prior to having the test (A), and their preferred method of receiving the results (B). Responses to the questions 'What information about the test would you want to know? (Choose as many as appropriate)' and 'What would your preferred method of receiving results be? (Choose as many as appropriate).



Supplementary Figure 2: Factors concerning participants about having the test among those who indicated being uninterested in undergoing testing. Responses to the question 'Which of the following factors would concern you about having the test? (Choose as many as appropriate)'.



Supplementary Figure 3: Participant's preferences for information content surrounding genetic risk testing. Preferences were expressed for information delivered prior to having the test (A), and their preferred method of receiving the results (B). Responses to the questions 'What information about the test would you want to know? (Choose as many as appropriate)' and 'What would your preferred method of receiving results be? (Choose as many as appropriate)'.

APPENDIX G: SUPPLEMENTARY TABLES

Question	**Number (%)**
Before being diagnosed, how much did you know about glaucoma? n = 1154	Nothing: 459 (39.3) A little: 497 (42.5) A fair amount: 171 (14.6) A lot: 27 (2.3) Missing: 15 (1.3)
How satisfied are you with your understanding of glaucoma? n = 1150	Very dissatisfied: 71 (6.2) Dissatisfied: 19 (1.6) Moderately satisfied: 516 (44.9) Very satisfied: 507 (44.1) Unsure: 37 (3.2) Missing: 19 (1.6)
Do you consider glaucoma as being a severe medical condition? n = 1147	Not severe: 36 (3.1) Slightly severe: 83 (7.2) Moderately severe: 392 (34.2) Very severe: 597 (52.1) Unsure: 39 (3.4) Missing: 22 (1.9)
Before being diagnosed, what was your perception of your risk of developing glaucoma in your lifetime? n = 1148	Highly unlikely: 114 (9.9) Unlikely: 319 (27.8) Likely: 264 (23.0) Highly likely: 82 (7.2) N/A (didn't know about glaucoma): 369 (32.1) Missing: 21 (1.8)
Before being diagnosed, were you worried about developing glaucoma? n = 1148	Not worried: 760 (66.2) Slightly worried: 194 (16.9) Moderately worried: 118 (10.3) Very worried: 22 (1.9) Unsure: 54 (4.7) Missing: 21 (1.8)

How likely would you have been to take a genetic test to predict your risk of developing glaucoma if it had been offered to you before you were diagnosed? n = 1150	Highly unlikely: 108 (9.4) Unlikely: 184 (16.0) **Likely: 430 (37.4)** **Highly likely: 368 (32.0)** Unsure: 60 (5.2) Missing: 19 (1.6)
Do you think you would have changed your behaviour regarding your eye health if you had known your risk of developing glaucoma? (For example, more frequent eye checks, better treatment compliance) n = 1122	Definitely not: 61 (5.5) Probably not: 180 (16.0) Possibly: 183 (16.3) Probably: 233 (20.8) Definitely: 465 (41.4) Missing: 47 (4.0)
Would you recommend your family members to have a genetic test to predict their risk of developing glaucoma? n = 1144	Definitely not: 6 (0.5) Probably not: 48 (4.2) Possibly: 138 (12.1) Probably: 254 (22.2) Definitely: 698 (61.0) Missing: 25 (2.1)
Would you recommend non-family members to have a genetic test to predict their risk of developing glaucoma? n = 1149	Definitely not: 24 (2.1) Probably not: 184 (16.0) Possibly: 267 (23.3) Probably: 308 (26.8) Definitely: 366 (31.8) Missing: 20 (1.7)
Would you take a test to predict your risk of rapid progression or developing severe disease if stronger treatments could prevent blindness? n = 1136	Definitely not: 12 (1.1) Probably not: 31 (2.7) Possibly: 117 (10.3) Probably: 237 (20.9) Definitely: 739 (65.0) Missing: 33 (2.8)

Supplementary Table 1: Summary of responses to survey questions relating to glaucoma and interest in testing.

The table shows the number of participants who answered each survey question.

Question	**Number (%)**

Do you think glaucoma is a hereditary condition? (ie can be inherited) n =412	Not at all: 6 (1.4) Somewhat: 123 (29.4) Definitely: 118 (28.2) Unsure: 165 (39.5) Missing: 6 (1.4)
Do you consider glaucoma as being a severe medical condition? n =414	Not severe: 6 (1.4) Slightly severe: 15 (3.6) Moderately severe: 144 (34.4) Very severe: 225 (53.8) Unsure: 24 (5.7) Missing: 4 (1.0)
How likely do you think you are to develop glaucoma in your lifetime? n =414	Highly unlikely: 18 (4.3) Unlikely: 109 (26.1) Likely: 113 (27.0) Highly likely: 20 (4.8) Unsure: 154 (36.8) Missing: 4 (1.0)
Are you worried about developing glaucoma? n =412	Not worried: 115 (27.5) Slightly worried: 149 (35.6) Moderately worried: 107 (25.6) Very worried: 23 (5.5) Unsure: 18 (4.3) Missing: 6 (1.4)
How likely would you be to take a genetic test which could predict your risk of developing glaucoma? n =412	Highly unlikely: 25 (6.0) Unlikely: 47 (11.2) Likely: 151 (36.1) Highly likely: 147 (35.2) Unsure: 42 (10.0) Missing: 6 (1.4)
Would you want to know more about glaucoma before having a test predicting your risk of developing it? n =413	Definitely not: 21 (5.0) Probably not: 75 (17.9) Possibly: 97 (23.2) Probably: 120 (28.7) Definitely: 100 (23.9) Missing: 5 (1.2)

Which of the following factors would affect your decision to be tested? (Choose as many as appropriate) Average number of responses selected: 3.7	
- To be able to take appropriate measures regarding my glaucoma risk and future eyesight	326 (78.0)
- To be able to provide advice to my children about their potential risk	267 (63.9)
- To be able to provide advice to my family members about their potential risk	231 (55.3)
- Personal advice - if someone from my family recommended the test	145 (34.7)
- Would rather know/to prepare for the future	270 (64.6)
- Medical advice - if your doctor or optometrist recommended the test	308 (73.7)
Which of the following factors would concern you about having the test? (Choose as many as appropriate) Average number of responses selected: 1.4	
- Personal anxiety/fear if results showed an increased glaucoma risk	124 (29.7)
- Would rather not know if at risk	12 (2.9)
- Concerns about cost	177 (42.3)
- Concerns about attending ongoing follow-up appointments	85 (20.3)
- Concerns regarding whether it could affect insurance	87 (20.8)
- Concern regarding whether it could affect employment	55 (13.2)
- Concern regarding confidentiality	47 (11.2)
- No concerns	101 (24.2)
How would you change the frequency of eye checks if the test results showed that you were at lower risk of developing glaucoma? n = 413	I would not change the frequency: 383 (91.6) I would have less frequent eye checks: 30 (7.2)
How would you change the frequency of eye checks if the test results showed that you were at higher risk of developing glaucoma? n = 412	I would not change the frequency: 92 (22.0) I would have more frequent eye checks: 320 (76.6)

Supplementary Table 2 - Summary of responses to survey questions relating to glaucoma and interest in testing

APPENDIX H: FIRST AUTHORED PUBLICATIONS

Publication 1: Attitudes towards polygenic risk testing in individuals with glaucoma

AMERICAN ACADEMY OF OPHTHALMOLOGY®
In partnership with the AMERICAN GLAUCOMA SOCIETY

Attitudes Towards Polygenic Risk Testing in Individuals with Glaucoma

Georgina L. Hollitt, MBBS,[1] Owen M. Siggs, MD, DPhil,[1] Bronwyn Ridge, BA,[1] Miriam C. Keane, PhD,[1] David A. Mackey, MBBS, MD,[2,3] Stuart MacGregor, MSc, PhD,[4] Alex W. Hewitt, MBBS, PhD,[2] Jamie E. Craig, MBBS, PhD,[1] Emmanuelle Souzeau, MSci, PhD[1]

Purpose: Glaucoma is the leading cause of irreversible blindness worldwide; however, vision loss resulting from glaucoma generally can be prevented through early identification and timely implementation of treatment. Recently, polygenic risk scores (PRSs) have shown promise in stratifying individual risk and prognostication for primary open-angle glaucoma (POAG) to reduce disease burden. Integrating PRS testing into clinical practice is becoming increasingly realistic; however, little is known about the attitudes of patients toward such testing.

Design: Cross-sectional, questionnaire-based study.

Participants: Among the participants in the Australian and New Zealand Registry of Advanced Glaucoma, 2369 were invited to participate who fit the inclusion criteria of adults with a diagnosis of POAG who had not received genetic results that explain their condition, were not known to be deceased, resided in Australia, and had agreed to receive correspondence.

Methods: One thousand one hundred sixty-nine individuals (response rate, 49%) with POAG completed the survey evaluating their attitudes towards polygenic risk testing for glaucoma.

Main Outcome Measures: Sociodemographic, health, perception, and emotional factors were examined to assess associations with interest in PRS testing. Interest in PRS testing was evaluated through assessing likelihood to take the test to predict personal risk of disease and disease severity, and whether the individual would recommend the test to family members or others.

Results: Our results show strong interest in the test, with 69.4% of individuals (798 of 1150) indicating a keenness in testing before diagnosis, had it been available. In particular, interest was seen in those from an urban area (odds ratio [OR], 1.70; 95% confidence interval [CI], 1.15–2.49; $P = 0.007$), those who perceived their risk of developing glaucoma as higher (OR, 2.05; 95% CI, 1.28–3.29; $P = 0.003$), and those who were worried about developing glaucoma (OR, 2.07; 95% CI, 1.27–3.37; $P = 0.004$). People who were interested in testing were more likely to change their eye health-seeking intentions and to recommend testing to family members and others, as well as to undergo testing for prognostication.

Conclusions: These findings will help to facilitate the clinical implementation of PRS testing for glaucoma to reduce irreversible vision loss. *Ophthalmology Glaucoma 2022;5:436-446 © 2021 by the American Academy of Ophthalmology. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).*

Supplemental material available at www.ophthalmologyglaucoma.org.

Glaucoma is currently the leading cause of irreversible blindness worldwide and the second most common cause of vision loss after cataract.[1] It is a genetically complex, heterogeneous disease that manifests as progressive optic neuropathy with corresponding visual field defects.[2] Primary open-angle glaucoma (POAG) is the most common subtype, affecting 76 million individuals worldwide and predicted to affect 112 million people by 2040.[3] Raised intraocular pressure (IOP) is the only known modifiable risk factor for glaucoma. Current treatment principles focus on reducing IOP, and this approach is highly effective at slowing disease progression as well as being the only evidence-based treatment option currently available.[4] Despite the risk of irreversible blindness, even in developed countries with excellent health care provisions, approximately half of all individuals with glaucoma remain undiagnosed.[1,5] This is largely because the condition is asymptomatic in the early stages of disease, and also indicates that current screening methods are inadequate.

Historically, glaucoma risk assessment has been based on the cumulative sum of clinical features and traditional risk factors. These include IOP, age, ancestry, and family history.[6,7] Glaucoma prevalence differs between different ethnicities: those with African ancestry are at greater risk of POAG than those with European ancestry.[3] Genetic risk largely has been estimated through family history, with a 9.2-fold

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https://doi.org/10.1016/j.ogla.2021.11.002
ISSN 2589-4196/21

increased risk for first-degree relatives of patients with glaucoma compared with controls.[8] However, family history is often an unreliable measure because many patients are unaware of family members with diagnosed or undiagnosed glaucoma or have erroneous views as to the cause of relatives' vision loss.[9]

Early diagnosis can be difficult, even in individuals identified as being at increased risk, because of the overlap of the phenotypic spectrum of healthy optic nerves and nerves affected by early glaucoma.[10] However, early identification and timely intervention are critical because available treatments cannot restore lost vision. Furthermore, rates of progression vary widely in those with an established diagnosis of glaucoma, which may result in delayed treatment or overtreatment in those with rapid or slow progression, respectively.

Glaucoma is one of the most heritable human diseases, with the heritability of POAG underpinned by both rare and common variants.[11–13] High-penetrance variants in genes such as *MYOC*, *OPTN*, and *TBK1* account for up to 5% of adult-onset POAG.[14] Currently, the use of clinical genetic testing for glaucoma has been restricted to rare genetic variants that cause Mendelian glaucoma.[14,15] However, these are relevant to only a very small portion of the population and often can be anticipated in individuals with a strong family history of glaucoma and young age at disease onset. Increasingly, large genome-wide association studies and the derivation of polygenic risk scores (PRSs) from these studies offers the prospect of performing genetic risk assessment on a much broader population and suggests that genetic risk profiling could be used to identify individuals at high risk of glaucoma developing, more rapid disease progression, and treatment intensity.[16] Other studies have used PRS of glaucoma endophenotypes, such as IOP, to provide further insight into the relationship between genetic variants implicated in IOP and POAG outcomes.[17–19]

Recommendations from the American Academy of Ophthalmology in 2014 did not support routine genetic testing for complex eye diseases such as adult-onset glaucoma because of the limited number of genetic loci associated with the disease and the lack of clinical usefulness at the time.[15] Recent findings regarding the genetic stratification of risk and progression of POAG raise the possibility that PRS testing may become part of glaucoma screening and management if validated in prospective clinical trials. Given that POAG affects approximately 3% of individuals 50 years of age and older and that timely treatment can prevent progression to blindness, enhanced risk prediction has the potential to impact glaucoma management significantly and to reduce vision loss worldwide.[20] Despite the significant progress made in genetic risk prediction, no studies have assessed the interest toward polygenic risk testing for glaucoma. In this study, we assessed patients' perception of genetic testing for glaucoma and identified demographic as well as psychosocial factors influencing their attitudes toward polygenic risk testing.

Methods

Study Sample

This was a cross-sectional, questionnaire-based study approved by the Southern Adelaide Clinical Human Research Ethics Committee that adhered to the tenets of the revised Declaration of Helsinki. The study sample included participants with diagnosed glaucoma drawn from the Australian and New Zealand Registry of Advanced Glaucoma (ANZRAG), one of the largest databases of clinical and genetic data for primary glaucoma in the world.[21] A letter of invitation and a questionnaire were mailed to 2369 of the living ANZRAG participants who met the inclusion criteria of adults with a diagnosis of POAG who had not received genetic results that explain their condition, resided in Australia, and had agreed to receive correspondence. Consent to participate was implied by completion of the survey.

Independent Variables

Sociodemographic, health, perception, and emotional factors were examined to assess associations with interest in genetic testing. Perception and emotional variables were assessed in a retrospective sense, with participants asked to consider their possible perspective before receiving a diagnosis of glaucoma.

Sociodemographic Variables

Age, gender, ethnicity, education, and urban or rural residence were collected. Family history was acquired from the ANZRAG database and was self-reported by respondents at the time of recruitment. Family history of glaucoma, the number of family members affected, and their degree of relation was collected. Ethnicity was self identified by respondents and defined in parallel to the ANZRAG classification.[21] Urban or rural status was based on the Australian Bureau of Statistics census data using the participants' postcodes. Urban status was classified as those postcodes with more than 50 000 residents.

Health Factors

Eye health factors included history of myopia, time since last eye examination (by an optometrist or ophthalmologist), and frequency of eye examinations. In addition to the information obtained from the questionnaires, clinical data related to glaucoma were acquired from the ANZRAG database. This included classification as advanced or nonadvanced glaucoma, age at diagnosis, and specific indicators of glaucoma severity, including best-corrected visual acuity (BCVA) and vertical cup-to-disc ratio (VCDR). In the ANZRAG database, advanced glaucoma was defined as central visual field loss related to glaucoma with at least 2 of the 4 central fixation squares having a pattern standard deviation probability of less than 0.5% on a reliable Humphrey 24-2 field analysis (Carl Zeiss Meditec) or a mean deviation worse than −15 dB or, in the absence of visual field testing, BCVA worse than 20/200 because of glaucoma.[21] Additionally, evidence of glaucoma was required to be present in the less severely affected eye, demonstrated through glaucomatous visual field defects with corresponding optic disc rim thinning, including an enlarged cup-to-disc ratio ($\geq$ 0.7) or cup-to-disc asymmetry ($>$ 0.2) between both eyes.[21] Best-corrected visual acuity was converted to a decimal equivalent for ease of analysis and interpretation. Legal blindness was defined by a visual acuity of 20/200 or worse. The poorest recorded result between the right and left eye of the clinical indicators of severity were used for analysis.

Perception and Emotional Factors

Perceptive factors were assessed through single-item measures with Likert-like scale response options. Variables included perceived knowledge regarding glaucoma, perceived severity of disease, and perceived glaucoma susceptibility before diagnosis. To assess the influence of emotion on interest in testing, participants were asked about their anxiety related to the possibility of glaucoma developing before diagnosis.

Outcome Variable

Interest in genetic testing was evaluated through assessing likelihood to undergo the test to predict personal risk of disease and disease severity and whether the individual would recommend the test to family members or others. A Likert like scale was used to assess personal interest and attitude toward testing for others. Participants were given the opportunity to comment on their selected responses regarding their interest in genetic testing and how they might have changed their health-seeking intentions toward glaucoma screening and management.

Additional Factors

Other factors relating to the test itself and communication of results were assessed. Aspects of the test that were considered important to know about before undergoing genetic testing and preferred method of receiving genetic test results were assessed. Participants were given the opportunity to comment on any additional aspects of concern or interest regarding the test itself.

Statistical Analysis

Before the distribution, the survey was trialed with 10 volunteers at Flinders Medical Centre and members of the community to ensure ease of completion and that questions were comprehensible. In addition, the survey was trialed with clinicians; however, it was not validated for its effectiveness by an expert panel. Data were analyzed using the Statistics Package for the Social Sciences version 25.0 (SPSS, Inc). Descriptive statistics were used to characterize the study sample. Responses were combined into bivariate outcomes: "highly unlikely" and "unlikely" responses were merged into a single "uninterested" group, and "likely" and "highly likely" were merged into a single "interested" group. "Unsure" responses for all questions were excluded. Univariate logistic regression was performed between level of interest and covariables (sociodemographic, emotional, and perception variables). Variables that showed significance levels of $P < 0.1$ in the univariate analysis were included in the multivariate regression model. Multivariate logistic regression modeling was used to identify factors independently associated with interest in testing ($P < 0.05$) using a backward stepwise approach. Where multiple comparisons were made of the same data, Bonferroni correction was applied.

Nonrespondents

Demographic and clinical data were obtained for those who did not complete the survey (obtained at referral to the ANZRAG) and were analyzed for comparison. These demographic data included age, gender, and urban or rural status, and clinical data included family history of glaucoma, age at diagnosis, classification of severity (advanced or nonadvanced), VCDR, and BCVA.

Results

Demographic Characteristics

In total, 2369 ANZRAG participants were invited to participate in the study, with 1169 completing the questionnaire, yielding a response rate of 49.3%. The demographic and personal characteristics of respondents are shown in Table 1. In summary, 53.5% of respondents were women, 92.9% were White, and 51.7% had an education level of more than secondary school. The mean age of the cohort was 75.7 ± 10.3 years, with 93.8% being older than 60 years. A positive family history of glaucoma was reported by 65.9% of respondents, with 87.5% of those with a positive family history having at least 1 affected first-degree relative. Of the 1200 participants who did not respond, limited demographic and clinical data were obtained from the ANZRAG database. In summary, 55.2% of nonrespondents were women and 83.1% were White. The mean age of nonrespondents was 77.7 ± 14.5 years. Respondents and nonrespondents did not differ by gender, age at diagnosis, family history, or residency. However, respondents were more likely to be younger ($P < 0.001$), to have White ancestry ($P < 0.001$), and to have less severe glaucoma reflected by nonadvanced disease classification ($P < 0.001$) and rate of legal blindness ($P < 0.001$) compared with nonrespondents (Table 1).

Understanding of Glaucoma and Perception of Severity and Risk

Before being diagnosed with glaucoma, only 16.9% of respondents believed that they knew a fair amount or a lot about glaucoma (Table S1, available at www.ophthalmologyglaucoma.org). This was associated significantly with family history, with those having a family history of glaucoma being more likely to have a better understanding of the condition before diagnosis (68.3% vs. 50.2%; $P < 0.001$). Furthermore, having a higher number of affected family members was associated with increased awareness of glaucoma (odds ratio [OR], 1.43; 95% confidence interval [CI], 1.29–1.59; $P < 0.001$). Most participants (86.3%) considered glaucoma to be a moderately severe or very severe medical condition. Approximately one-third believed that glaucoma was either likely or highly likely to develop in their lifetime (29.2%) and were either slightly worried, moderately worried, or very worried about glaucoma developing (29.1%) before receiving a diagnosis. A belief of being at risk and being worried about glaucoma developing were both associated with the presence of a family history (self-reported; OR, 6.01 [95% CI, 4.18–8.62; $P < 0.001$] and OR, 3.0 [95% CI, 2.23–4.10; $P < 0.001$], respectively) and increasing number of affected family members (OR, 1.88 [95% CI, 1.65–2.15; $P < 0.001$] and OR, 1.42 [95% CI, 1.30–1.57; $P < 0.001$], respectively).

Interest in Genetic Risk Testing for Glaucoma

Responses to survey questions are summarized in Table S1. Overall, participants were in favor of glaucoma PRS testing. More than two-thirds of individuals (69.4%) were likely or highly likely to have undergone a genetic test to predict their risk of glaucoma developing if it had been offered to them before they received a diagnosis (Fig 1). Additionally, 96.2% of participants would possibly, probably, or definitely take a test to predict their

Table 1. Characteristics of the Study Respondents and Nonrespondents

Variable	Respondents (n = 1169; 49.3%)	Nonrespondents (n = 1200; 50.7%)	P Value
Age (yrs)			<0.001*
Range	22.7–101.8	20.4–108.5	
Mean ± SD	75.7 ± 10.3	77.7 ± 14.5	
Median	76.1	79.7	
Age at diagnosis (yrs)			0.58*
Range	20.0–89.0	17.0–94.0	
Mean ± SD	59.1 ± 12.8	59.2 ± 14.4	
Median	60.0	60.0	
Unknown (excluded from analysis)	n = 80	n = 93	
Gender, no. (%)			0.43†
Female	625 (53.5)	662 (55.2)	
Male	544 (46.5)	538 (44.8)	
Ethnicity, no. (%)			<0.001†
White	1086 (92.9)	971 (83.1)	
Non-White	76 (6.5)	123 (10.5)	
Asian	46 (3.9)	81 (6.9)	
Mixed Ethnicity	19 (1.6)	19 (1.6)	
Middle Eastern	5 (0.4)	8 (0.7)	
African	4 (0.3)	10 (0.9)	
Australian Aboriginal	1 (0.1)	0 (0.0)	
Hispanic	1 (0.1)	5 (0.4)	
Unknown (excluded from analysis)	7 (0.6)	106 (8.8)	
Residency, no. (%)			0.20†
Urban	881 (75.4)	932 (77.7)	
Rural	288 (24.6)	268 (22.3)	
Highest level of education, no. (%)		—	—
Primary school	73 (6.3)		
Secondary school	487 (42.0)		
Vocational training	285 (24.6)		
University	314 (27.1)		
Unknown (excluded from analysis)	10 (0.9)		
Family history of glaucoma			0.12†
Positive	770 (65.9)	740 (63.2)	
Negative	389 (33.3)	431 (36.7)	
Unknown (excluded from analysis)	12 (10.9)	29 (2.4)	0.26†
Positive			
First-degree relative	674 (87.5)	647 (87.4)	
Second-degree relative	86 (11.2)	74 (10.0)	
Third-degree relative	7 (0.9)	12 (1.6)	
Fourth-degree relative	3 (0.4)	3 (0.4)	
Unknown (excluded from analysis)	2 (0.3)	4 (0.5)	
Glaucoma severity, no. (%)			<0.001†
Advanced	534 (45.7)	735 (61.2)	
Nonadvanced	635 (54.3)	465 (38.8)	
BCVA			
> 20/200	1063 (90.9)	987 (82.3)	<0.001†
≤ 20/200	72 (6.2)	179 (14.9)	
Unknown (excluded from analysis)	34 (2.9)	34 (2.8)	
VCDR			
Range	0.10–1.0	0.2–1.0	
Mean ± SD	0.81 ± 0.13	0.84 ± 0.12	<0.001*
Median	0.80	0.90	
< 0.9	728 (62.3)	570 (47.5)	
≥ 0.9	409 (35.0)	592 (49.3)	
Unknown (excluded from analysis)	32 (2.7)	38 (3.2)	<0.001†
Last ophthalmic review, no. (%)		—	—
Within 6 mos	886 (77.5)		
6–12 mos	182 (15.9)		
1–2 yrs	60 (5.3)		
More than 2 yrs	15 (1.3)		
Unknown (excluded from analysis)	26 (2.2)		

(Continued)

Table 1. (Continued.)

Variable	Respondents (n = 1169; 49.3%)	Nonrespondents (n = 1200; 50.7%)	P Value
Frequency of clinical reviews, no. (%)		—	—
Every 3 mos	172 (15.1)		
Every 6 mos	678 (59.3)		
Annually	229 (20.0)		
Every 2 yrs	47 (4.1)		
More than every 2 yrs	11 (1.0)		
Unknown (excluded from analysis)	32 (2.7)		

BCVA = best-corrected visual acuity; SD = standard deviation; VCDR = vertical cup-to-disc ratio.
*Paired Mann–Whitney *U* test for differences in median rank.
†Chi-square test for association. Differences in ethnicity were assessed between White people and non-White people, positive and negative family history, and VCDR between < 0.9 and ≥ 0.9 groups. Boldface indicates statistical significance.

risk of rapid progression or severe disease developing if stronger treatments could prevent blindness.

Factors Affecting Interest in Genetic Risk Testing for Glaucoma

The association between demographic, perception, and emotional predictor variables and interest in genetic risk testing for glaucoma was analyzed (Table 2). Age, age at glaucoma diagnosis, gender, ethnicity, level of education, BCVA, VCDR, timing of last eye examination, and frequency of eye examinations were not associated with interest in glaucoma genetic risk testing in the univariate logistic regression. Variables that reached a significance level of $P \leq 0.1$ were included in a multivariate logistic regression to identify the impact of these variables on a positive interest in genetic risk testing for glaucoma (either likely or highly likely to have undergone testing if it were available). After adjusting for other predictor variables, urban residency was associated with increased interest in testing (OR, 1.70; 95% CI, 1.15–2.49; $P = 0.007$).

Level of knowledge of glaucoma before diagnosis and perceived severity of glaucoma were not associated with increased interest in testing. Level of glaucoma awareness before diagnosis, perceived risk of glaucoma, and concern regarding glaucoma developing before diagnosis were associated significantly with interest in genetic risk testing for glaucoma in univariate analysis. Increased interest in testing was associated with an increased perceived risk of glaucoma (OR, 2.05; 95% CI, 1.28–3.29; $P = 0.003$) and worry before diagnosis about glaucoma developing (OR, 2.07; 95% CI, 1.27–3.37; $P = 0.004$) in the multivariate logistic regression model (Table 2).

Health-Seeking Intentions

We assessed whether interest in glaucoma genetic risk testing was associated with an individual's eye health-seeking intentions or their likelihood to recommend genetic testing to others (Table 2). Interest in testing was associated significantly with a likeliness to change health-seeking intentions relating to eye health (OR, 1.53; 95% CI, 1.11–2.11; $P = 0.009$). In addition, interest was associated positively with increased likelihood of recommending testing to family (OR, 12.83; 95% CI, 6.33–25.99; $P < 0.001$) and others (OR, 3.67; 95% CI, 2.66–5.06; $P < 0.001$) and the likelihood of undergoing testing for the purpose of predicting prognosis and disease severity (OR, 4.97; 95% CI, 2.47–10.00; $P < 0.001$) using univariate logistic regression.

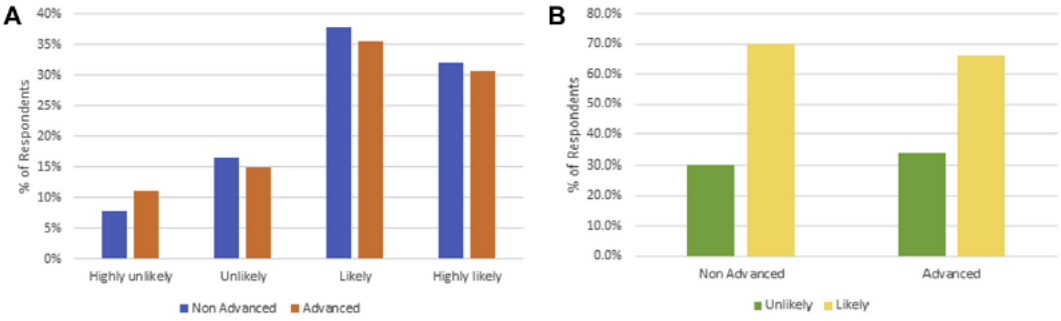

Figure 1. Bar graphs showing level of interest in polygenic risk testing for glaucoma according to (A) disease severity and (B) positive versus negative attitude. Responses to the question, "How likely would you have been to take a genetic test to predict your risk of developing glaucoma if it had been offered to you before you were diagnosed?" Responses (A) were grouped by disease severity (advanced or nonadvanced) and by individual response (highly unlikely, unlikely, likely, or highly likely) or (B) were grouped into a positive (likely or highly likely) or negative (highly unlikely or unlikely) expressed interest. Sixty respondents indicated being unsure (5.2%).

Table 2. Univariate and Multivariate Logistic Regression Analysis Assessing Predictors of a Positive Interest in Genetic Risk Testing and the Impact of Interest in Testing on Health-Seeking Intentions

Variable	Univariate Logistic Regression		Multivariate Logistic Regression	
	OR (95% CI)	*P Value*	*OR (95% CI)*	*P Value*
Age (per increasing yr)	1.00 (0.99–1.01)	0.871		
Age at diagnosis (per increasing yr)	1.00 (0.99–1.02)	0.487		
Sex				
Male	1.00			
Female	1.15 (0.88–1.50)	0.312		
European ancestry				
Yes	1.00			
No	1.05 (0.61–1.83)	0.857		
Residency				
Rural	1.00			
Urban	**1.44 (1.06–1.83)**	**0.018**	**1.70 (1.15–2.49)**	**0.007**
Level of education		0.797		
Primary school	1.00			
Secondary school	0.88 (0.48–1.63)	0.682		
TAFE/vocational education	0.80 (0.43–0.78)	0.502		
University	0.78 (0.41–1.46)	0.428		
Family history		**0.002**		0.638
Unaffected	1.00			
First-degree relative	**1.66 (1.25–2.22)**	**<0.001**	1.06 (0.67–1.68)	0.802
Other relative	**1.28 (0.77–2.12)**	**0.338**	1.39 (0.70–2.73)	0.348
No. of family members affected (per extra)	**1.16 (1.04–1.29)**	**0.009**	1.06 (0.92–1.13)	0.429
Glaucoma severity				
Advanced				
Nonadvanced	1.00	0.341		
BCVA (per improvement of 0.1 on decimal scale)	1.14 (0.87–1.49)			
BCVA	1.09 (0.70–1.71)	0.698		
≤ 20/200	1.00			
> 20/200	1.13 (0.65–1.95)	0.676		
VCDR (per increase of 0.1)	0.67 (0.23–1.92)			
VCDR	1.00	0.455		
≥ 0.9	1.19 (0.90–1.57)	0.236		
<0.9				
Last eye examination		0.229		
> 2 yrs	1.00			
1–2 yrs	0.60 (0.14–2.49)	0.479		
6–12 mos	1.12 (0.28–4.40)	0.875		
Within 6 mos	1.08 (0.28–4.10)	0.913		
Frequency of eye examinations		0.754		
> 2 yrs	1.00			
1–2 yrs	1.38 (0.30–6.38)	0.684		
Annually	1.46 (0.35–6.01)	0.605		
Every 6 mos	1.47 (0.36–5.93)	0.592		
Every 3 mos	1.14 (0.28–4.76)	0.854		
Awareness before glaucoma diagnosis				
Not aware	1.00			
Aware	**1.65 (1.26–2.16)**	**<0.001**	1.03 (0.67–1.06)	0.883
Knowledge of glaucoma				
No knowledge	1.00			
Good knowledge	1.19 (0.66–2.12)	0.565		
Perceived severity				
Severe	1.00			
Not severe	1.33 (0.62–2.87)	0.462		
Perceived risk				
Not at risk	1.00			
At risk	**3.14 (2.15–4.59)**	**<0.001**	**2.05 (1.28–3.29)**	**0.003**
Worry before diagnosis				
Not worried	1.00			
Worried	**3.24 (2.28–4.62)**	**<0.001**	**2.07 (1.27–3.37)**	**0.004**

(*Continued*)

Table 2. (Continued.)

Variable	Univariate Logistic Regression		Multivariate Logistic Regression	
	OR (95% CI)	P Value	OR (95% CI)	P Value
Likeliness to change health-seeking intentions				
Not interested in PRS	1.00			
Interested in PRS	1.53 (1.11–2.11)	**0.009**		
Interest in test for prognosis				
Not interested in PRS	1.00			
Interested in PRS	4.97 (2.47–10.00)	**<0.001**		
Interest in test recommendation outside family				
Not interested in PRS	1.00			
Interested in PRS	3.67 (2.66–5.06)	**<0.001**		
Interest in family test recommendation				
Not interested in PRS	1.00			
Interested in PRS	12.83 (6.33–25.99)	**<0.001**		

BCVA = best-corrected visual acuity; CI = confidence interval; PRS = polygenic risk score; TAFE = Technical and Further Education; VCDR = vertical cup-to-disc ratio.
Boldface indicates statistical significance.
An odds ratio (OR) of more than 1 indicates that participants were more likely to be interested in testing.

Factors about Testing and Follow-up

We assessed the aspects of glaucoma genetic risk testing and follow-up that respondents would like to know before undergoing analysis, regardless of their indicated interest in testing. All 4 options provided were deemed important by more than 70% of respondents (cost, process involved, meaning of results, and follow-up). We assessed the preferred method of receiving results, identifying that most participants would prefer to receive results in person, in a letter, or via e-mail, rather than via a telephone call. The factors about testing and follow-up are summarized in Figure S1A (available at www.ophthalmologyglaucoma.org), and the preferred methods of receiving results are summarized in Figure S1B. Several participants commented that their preferred method would depend on the result; if at high risk, face-to-face delivery would be preferred, and if low risk, other methods would be sufficient. It also was noted that if results were received nonverbally or via telephone, an option to speak with someone in person would be appreciated to discuss implications and answer questions. Regarding cost, approximately 80% of participants would be willing to pay for testing, with more than half of those willing to pay indicating that a cost of AU$50 to AU$100 would be appropriate (Fig 2). Others commented that they would expect the test to be covered by Medicare (Australia's universal health insurance system), particularly if they themselves were a senior citizen or pensioner.

Additional Results

Participants were given the opportunity to make additional comments on aspects of the test they would like to know more about and how such testing would change their behavior regarding their eye health. Some noted the accuracy of the test would be important to know before undergoing testing with respect to false-positive and false-negative rates and the

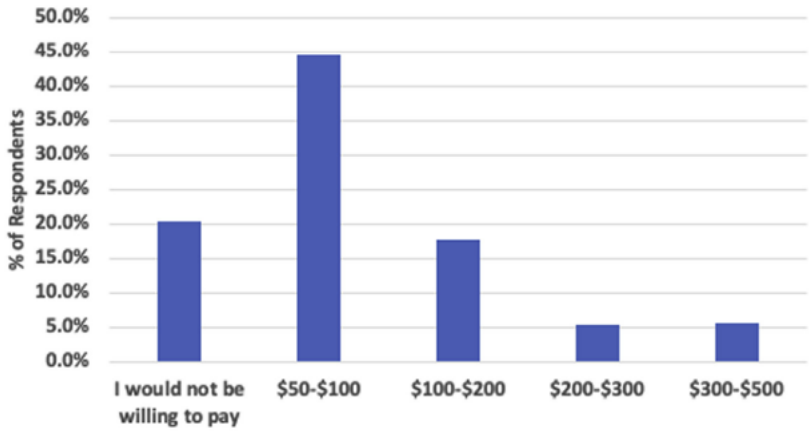

Figure 2. Bar graph showing the cost that participants would be willing to pay for a glaucoma genetic risk test. Responses to the question, "If a cost were involved, how much would you be willing to pay for the test?"

specificity and sensitivity of the test. Privacy also was highlighted as a concern, given the need to provide genetic material and the implications that results may have on employment or insurance. Some were interested in whether any additional risks (other than glaucoma risk) could be identified from the test and whether the test would be recommended to family members automatically based on their results. Recommended age to undergo the test, treatment options, adverse effects of the test and their available treatments, and short- and long-term prognosis were also identified as information of interest.

Discussion

Recent studies of PRS have shown that implementing PRS for clinical use in ophthalmology (and other fields of medicine) is becoming increasingly realistic.[14,16–18,22–29] For conditions such as glaucoma, PRS testing has strong clinical usefulness given the complex nature and heritability of the disease and its treatability, as well as the difficulties associated with diagnosis.[28] Polygenic risk testing has the potential to improve disease prediction, diagnosis, and management of vision loss, from reactive and responsive to predictive and preventative. For this adaptation to be successful, thorough understanding of stakeholders' attitudes toward such testing is required first to develop implementation frameworks and successful uptake.

To our knowledge, no studies have assessed the attitude of individuals with glaucoma toward PRS testing for the condition, and critical gaps in understanding barriers to implementing such testing persist. This study provides useful insights into the potential uptake of PRS testing for glaucoma. Our results supported the hypothesis that individuals with glaucoma have a positive attitude toward genetic risk testing for glaucoma (69.4% being likely or highly likely to have undergone testing), as well as testing to predict risk of severe disease or rapid progression. The reported interest was similar to that of a previous study investigating attitudes toward single gene testing for glaucoma, with 61.8% reporting interest among a large glaucoma pedigree.[30,31] In addition, our results are comparable with those of studies on predictive genetic testing in other conditions, including inherited breast and colorectal cancer.[22–26,32–34] In particular, studies assessing interest in predictive genetic testing for breast cancer among individuals affected by the disease reported similar levels of interest, ranging from 57.0% to 61.8%.[24,25] Further research is needed to validate the effectiveness of PRS testing in an unaffected population.

Increasing interest in glaucoma genetic risk testing was associated with a positive family history of glaucoma and a higher number of affected family members. However, it was shown that the affected relative must be at least a second-degree relative or closer. These results are in line with other studies that found interest in genetic testing was particularly supported if there was a family history of the condition.[22,23,26,32–34] However, although significant in the univariate logistic regression, these variables were not statistically significant when controlling for other associated variables. This may be attributable to some respondents recognizing their predisposition from having an affected family member. Several respondents commented that glaucoma development was somewhat expected given their family history of glaucoma, and therefore they believed that a genetic test was not necessary, given that they were already undergoing regular eye examinations. However, it has been shown that glaucoma risk can vary significantly even in individuals with high penetrant variants, ranging from very high to average population risk depending on the PRS.[16,35] This indicates a need for community education regarding genetic risk. Studies in inherited breast and colorectal cancer reported positive interest particularly in those with a positive family history of the condition.[23,26,32–34,36–41] However, it should be recognized that screening for these conditions via colonoscopy or mammography generally is more invasive than an eye examination, so genetic testing may be preferable by those at an increased risk to avoid this type of investigation.

Previous studies have shown that individuals who have a higher perceived risk of glaucoma are the most motivated to reduce their risk of vision loss.[42] This is consistent with our results showing that those who had a higher perceived risk of glaucoma developing were more interested in genetic risk testing for glaucoma. Similarly, those who had been worried about glaucoma developing before receiving a diagnosis were more interested in testing. Interest in testing was associated with having an intention to change behavior toward eye health. This is not in keeping with other genetic studies that have shown that knowledge of risk has little effect on risk-reducing behaviors.[43] However, this may be less relevant to glaucoma because, unlike most common conditions, no established environmental risk factors exist that could be modified through risk-reducing lifestyle changes.

A positive attitude toward genetic risk testing for glaucoma seems to extend beyond personal interest. Increasing interest was associated with increased likelihood to recommend testing to family members and others. Interestingly, a positive family history of glaucoma was not associated with an increased likelihood to recommend testing to family members. However, although not significant, having an affected first-degree relative was associated with an increased interest in testing, suggesting that close affected relatives still may influence interest in testing.

Nonrespondents were significantly older, more likely to be of non-European ancestry, and more likely to have advanced disease and legal blindness. Those who are of an older age may believe that a genetic test regarding risk and prognosis is not relevant at their stage of life. These individuals may also have added difficulty completing a questionnaire that requires reading and comprehension and the dexterity to record their responses. However, it is possible that the disparity in age seen between groups is the result of ANZRAG participants who have died remaining in the database. Although the database is updated regularly, if the database manager is not notified of a participant's death, it may not be recorded. Similarly, individuals of non-European ancestry may not speak English as their primary language and may have difficulty completing the questionnaire, which was delivered only in English. In addition, it is

not yet clear how a glaucoma PRS may perform across non-European populations, and participants of non-European ethnicity may not be aware of this. Finally, those with advanced disease may have had more difficulty completing the questionnaire because of their impaired vision. Moreover, they may not have believed that genetic risk testing would be personally relevant given their severe disease. These individuals may have expected glaucoma to develop, regardless of the potential calculated genetic risk, based on a strong family history of the disease. However, no significant association was found between advanced glaucoma and the presence of a family history of glaucoma ($P = 0.245$).

We asked participants which components of the test they would like to know about before undergoing the test. The cost of the test, the process involved in taking the test, the implications of the results, and likely follow-up were roughly of equal importance to respondents, with more than 70% indicating each would be important to know. Respondents indicated mail, in person, and e-mail to be the most preferred methods of receiving results. In addition, the largest proportion of participants (approximately 45%) indicated a cost of AU$50 to AU$100 for the test would be reasonable. Some who indicated an unwillingness to pay for the test commented on the challenges of affording additional health care costs while receiving a pension. This is important to consider given the older age of those most commonly affected by glaucoma.

The findings from this study should be interpreted in light of the following limitations. Questions assessing glaucoma knowledge, risk, and interest in genetic testing for the study population were framed as a retrospective concept given that individuals in this group had already received a diagnosis. This may be difficult for some to interpret and answer without the bias of hindsight influencing their response. The study participants were drawn from an existing glaucoma research registry that may have introduced a selection bias. By participating in the ANZRAG (a study in which participants must consent for genetic testing in a research context), participants may be more interested in genetic research, and therefore may be more likely to be interested in such testing in a clinical context. Participants were asked to indicate their level of interest in such testing from a retrospective point of view, which may reduce this bias. Almost 95% of the study sample was of European ancestry, highlighting the need for further validation across other ancestral backgrounds before implementation, which also is pertinent to the predominantly European-derived PRS instruments themselves.

Additional challenges to clinical implementation of PRS testing for glaucoma remain. One challenge of conveying PRS results is to ensure that these results are communicated as absolute and relative risk values in conjunction with other established and validated clinical risk factors, and not as predictive or prognostic risk.[27] Clinical implementation of PRS will require that clinicians and the public receive education about the significance and limitations of the results. Furthermore, additional issues will arise in public health infrastructure and policy including economically balancing the cost of screening with the cost of management, identifying the most appropriate target screening population, and ensuring adequate access to testing and follow-up treatment.[44]

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Footnotes and Disclosures

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The author(s) have made the following disclosure(s): O.M.S.: Equity owner – StratifEYE Pty Ltd.

S.M.: Equity owner – StratifEYE Pty Ltd; Patent (pending) – Use of genetic risk scores to determine risk and guide treatment for glaucoma

A.W.H.: Equity owner – StratifEYE Pty Ltd; Patent (pending) – Use of genetic risk scores to determine risk and guide treatment for glaucoma

J.E.C.: Equity owner – StratifEYE Pty Ltd; Patent (pending) – Use of genetic risk scores to determine risk and guide treatment for glaucoma

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The data that support the findings of this study are available from the corresponding author on reasonable request.

HUMAN SUBJECTS: Human subjects were included in this study. This study was approved by the Southern Adelaide Clinical Human Research Ethics Committee, and it adhered to the Declaration of Helsinki. The study questionnaire was mailed to participants from the Australian and New Zealand Registry of Advanced Glaucoma. Consent to participation was implied by completion of the survey.

No animal subjects were included in this study.

Author Contributions:

Conception and design: Hollitt, Siggs, Craig, Souzeau

Analysis and interpretation: Hollitt, Siggs, Ridge, Keane, Mackey, MacGregor, Hewitt, Craig, Souzeau

Data collection: Hollitt, Ridge, Souzeau

Obtained funding: N/A; Study was performed as part of regular employment duties at listed affiliation sites. No additional funding was provided.

Overall responsibility: Hollitt, Siggs, Ridge, Keane, Mackey, MacGregor, Hewitt, Craig, Souzeau

Abbreviations and Acronyms:
ANZRAG = Australian and New Zealand Registry of Advanced Glaucoma; **BCVA** = best-corrected visual acuity; **CI** = confidence interval; **IOP** = intraocular pressure; **OR** = odds ratio; **POAG** = primary open-angle glaucoma; **PRS** = polygenic risk score; **VCDR** = vertical cup-to-disc ratio.

Keywords:
Attitude, Genetic testing, Glaucoma, POAG, Polygenic risk score.

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References

1. Quigley HA, Broman AT. The number of people with glaucoma worldwide in 2010 and 2020. *Br J Ophthalmol*. 2006;90(3):262–267.
2. Casson RJ, Chidlow G, Wood JPM, et al. Definition of glaucoma: clinical and experimental concepts. *Clin Exp Ophthalmol*. 2012;40(4):341–349.
3. Tham Y-C, Li X, Wong TY, et al. Global prevalence of glaucoma and projections of glaucoma burden through 2040: a systematic review and meta-analysis. *Ophthalmology*. 2014;121(11):2081–2090.
4. National Health and Medical Research Council. NHMRC guidelines for the screening, prognosis, diagnosis, management and prevention of glaucoma. 2010:1–181. Available at: https://www.nhmrc.gov.au/about-us/publications/guidelines-screening-prognosis-diagnosis-management-and-prevention-glaucoma. Accessed November 30, 2020.
5. Mitchell P, Smith W, Attebo K, Healey PR. Prevalence of open-angle glaucoma in Australia. The Blue Mountains Eye Study. *Ophthalmology*. 1996;103(10):1661–1669.
6. McMonnies CW. Glaucoma history and risk factors. *J Optom*. 2017;10(2):71–78.
7. Weih LM, Nanjan M, McCarty CA, Taylor HR. Prevalence and predictors of open-angle glaucoma: results from the visual impairment project. *Ophthalmology*. 2001;108(11):1966–1972.
8. Wolfs RC, Klaver CC, Ramrattan RS, et al. Genetic risk of primary open angle glaucoma. Population-based familial aggregation study. *Arch Ophthalmol*. 1998;116(12):1640–1645.
9. McNaught AI, Allen JG, Healey DL, et al. Accuracy and implications of a reported family history of glaucoma: experience from the Glaucoma Inheritance Study in Tasmania. *Arch Ophthalmol*. 2000;118(7):900–904.
10. Weinreb RN, Khaw PT. Primary open-angle glaucoma. *Lancet*. 2004;363(9422):1711–1720.
11. Charlesworth J, Kramer PL, Dyer T, et al. The path to open-angle glaucoma gene discovery: endophenotypic status of intraocular pressure, cup-to-disc ratio, and central corneal thickness. *Invest Ophthalmol Vis Sci*. 2010;51(7):3509–3514.
12. Wang R, Wiggs JL. Common and rare genetic risk factors for glaucoma. *Cold Spring Harb Perspect Med*. 2014;4(12):a017244.
13. Wang K, Gaitsch H, Poon H, et al. Classification of common human diseases derived from shared genetic and environmental determinants. *Nat Genet*. 2017;49(9):1319–1325.
14. Khawaja AP, Viswanathan AC. Are we ready for genetic testing for primary open-angle glaucoma? *Eye*. 2018;32(5):877–883.
15. Stone EM, Aldave AJ, Drack AV, et al. Recommendations for genetic testing of inherited eye diseases: report of the American Academy of Ophthalmology Task Force on Genetic Testing. *Ophthalmology*. 2012;119(11):2408–2410.
16. Craig JE, Han X, Qassim A, et al. Multitrait analysis of glaucoma identifies new risk loci and enables polygenic prediction of disease susceptibility and progression. *Nat Genet*. 2020;52(2):160–166.
17. Qassim A, Souzeau E, Siggs OM, et al. An intraocular pressure polygenic risk score stratifies multiple primary open-angle glaucoma parameters including treatment intensity. *Ophthalmology*. 2020;127(7):901–907.
18. MacGregor S, Ong J-S, An J, et al. Genome-wide association study of intraocular pressure uncovers new pathways to glaucoma. *Nat Genet*. 2018;50(8):1067–1071.
19. Gao XR, Huang H, Kim H. Polygenic risk score is associated with intraocular pressure and improves glaucoma prediction in the UK Biobank Cohort. *Transl Vis Sci Technol*. 2019;8(2):10.
20. Kreft D, Doblhammer G, Guthoff RF, Frech S. Prevalence, incidence, and risk factors of primary open-angle glaucoma—a cohort study based on longitudinal data from a German public health insurance. *BMC Public Health*. 2019;19(1):851.
21. Souzeau E, Goldberg I, Healey PR, et al. Australian and New Zealand Registry of Advanced Glaucoma: methodology and recruitment. *Clin Exp Ophthalmol*. 2012;40(6):569–575.
22. Bottorff JL, Ratner PA, Balneaves LG, et al. Women's interest in genetic testing for breast cancer risk: the influence of sociodemographics and knowledge. *Cancer Epidemiol Biomarkers Prev*. 2002;11(1):89–95.
23. Anderson AE, Flores KG, Boonyasiriwat W, et al. Interest and informational preferences regarding genomic testing for modest increases in colorectal cancer risk. *Public Health Genomics*. 2014;17(1):48–60.
24. Bruno M, Digennaro M, Tommasi S, et al. Attitude towards genetic testing for breast cancer susceptibility: a comparison of affected and unaffected women. *Eur J Cancer Care*. 2010;19(3):360–368.
25. Yanes T, Meiser B, Kaur R, et al. Uptake of polygenic risk information among women at increased risk of breast cancer. *Clin Genet*. 2020;97(3):492–501.
26. Lerman C, Seay J, Balshem A, Audrain J. Interest in genetic testing among first-degree relatives of breast cancer patients. *Am J Med Genet*. 1995;57(3):385–392.
27. Torkamani A, Wineinger NE, Topol EJ. The personal and clinical utility of polygenic risk scores. *Nat Rev Genet*. 2018;19(9):581–590.
28. Lewis CM, Vassos E. Prospects for using risk scores in polygenic medicine. *Genome Med*. 2017;9(1):96.
29. Wray NR, Lin T, Austin J, et al. From basic science to clinical application of polygenic risk scores: a primer. *JAMA Psychiatry*. 2021;78(1):101–109.
30. Li J, Lee PP, Buckley S, et al. Attitudes toward genetic testing for glaucoma: a survey of patients. *Invest Ophthalmol Vis Sci*. 2002;43(13), 1077–1077.
31. Healey DL, Craig JE, Wilkinson CH, et al. Attitudes to predictive DNA testing for myocilin glaucoma: experience with a large Australian family. *J Glaucoma*. 2004;13(4):304–311.

32. Madlensky L, Vierkant RA, Vachon CM, et al. Preventive health behaviors and familial breast cancer. *Cancer Epidemiol Biomarkers Prev*. 2005;14(10):2340–2345.
33. Madlensky L, Flatt SW, Bardwell WA, et al. Is family history related to preventive health behaviors and medical management in breast cancer patients? *Breast Cancer Res Treat*. 2005;90(1):47–54.
34. Ochoa EM, Gómez-Acebo I, Rodríguez-Cundín P, et al. Relationship between family history of breast cancer and health-related behavior. *Behav Med*. 2010;36(4):123–129.
35. Zebardast N, Sekimitsu S, Wang J, et al. Characteristics of Gln368Ter myocilin variant and influence of polygenic risk on glaucoma penetrance in the UK Biobank. *Ophthalmology*. 2021;128(9):1300–1311.
36. Martínez-Ochoa E, Gómez-Acebo I, Beunza J-J, et al. Influence of family history of colorectal cancer on health behavior and performance of early detection procedures: the SUN Project. *Ann Epidemiol*. 2012;22(7):511–519.
37. Ceballos RM, Newcomb PA, Beasley JM, et al. Colorectal cancer cases and relatives of cases indicate similar willingness to receive and disclose genetic information. *Genet Test*. 2008;12(3):415–420.
38. Petersen GM, Larkin E, Codori AM, et al. Attitudes toward colon cancer gene testing: survey of relatives of colon cancer patients. *Cancer Epidemiol Biomarkers Prev*. 1999;8(4 Pt 2):337–344.
39. Courtney RJ, Paul CL, Carey ML, et al. A population-based cross-sectional study of colorectal cancer screening practices of first-degree relatives of colorectal cancer patients. *BMC Cancer*. 2013;13:13.
40. Nusbaum R, Leventhal K-G, Hooker GW, et al. Translational genomic research: protocol development and initial outcomes following SNP testing for colon cancer risk. *Transl Behav Med*. 2013;3(1):17–29.
41. Bronner K, Mesters I, Weiss-Meilnik A, et al. Do individuals with a family history of colorectal cancer adhere to medical recommendations for the prevention of colorectal cancer? *Familial Cancer*. 2013;12(4):629–637.
42. Souzeau E, Glading J, Keane M, et al. Predictive genetic testing experience for myocilin primary open-angle glaucoma using the Australian and New Zealand Registry of Advanced Glaucoma. *Genet Med*. 2014;16(7):558–563.
43. Hollands GJ, French DP, Griffin SJ, et al. The impact of communicating genetic risks of disease on risk-reducing health behaviour: systematic review with meta-analysis. *BMJ*. 2016;352:i1102.
44. World Health Organization. WHO recommendations on the diagnosis of HIV infection in infants and children. Available at: https://www.ncbi.nlm.nih.gov/books/NBK138555/; 2010. Accessed December 2, 2020.

Publication 2: Attitudes towards glaucoma genetic risk assessment in unaffected individuals

tvst

Glaucoma

Attitudes Toward Glaucoma Genetic Risk Assessment in Unaffected Individuals

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Purpose: Integrating polygenic risk scores (PRS) into healthcare has the potential to stratify an individual's risk of glaucoma across a broad population. Glaucoma is the most common cause of irreversible blindness worldwide, therefore effective screening for glaucoma endorsed by the population is highly important. This study assessed the attitude of unaffected individuals toward PRS testing for glaucoma, and sought to identify factors associated with interest in testing.

Methods: We surveyed 418 unaffected individuals including 193 with a first-degree relative with glaucoma, 117 who had a recent eye examination, and 108 general members of the community.

Results: Overall, 71.3% of the individuals indicated an interest in taking a polygenic risk test for glaucoma. Interest was more likely in those who believed glaucoma to be a severe medical condition (odds ratio [OR] = 14.58, 95% confidence interval [CI] = 1.15–185.50, $P = 0.039$), those concerned about developing glaucoma (OR = 4.37, 95% CI = 2.32–8.25, $P < 0.001$), those with an intention to take appropriate measures regarding eye health (OR = 2.39, 95% CI = 1.16–4.95, $P = 0.019$), and those preferring to know if considered to be at-risk or not (OR = 4.52, 95% CI = 2.32–8.83, $P < 0.001$).

Conclusions: Our results show strong interest in genetic risk assessment for glaucoma among unaffected individuals in Australia.

Translational Relevance: These findings represent a valuable assessment of interest in glaucoma polygenic risk testing among potential target populations, which will be integral to the implementation and uptake of novel PRS-based tests into clinical practice.

Introduction

Glaucoma is a degenerative condition affecting the optic nerve and can result in irreversible vision loss and blindness if left untreated. Primary open-angle glaucoma (POAG) is the most common subtype, affecting over 60 million people worldwide,[1] including 3% of the population over the age of 50 years in Australia.[2] It is associated with, but not dependent on, raised intraocular pressure (IOP).[3] IOP is the major modifiable risk factor for POAG and is therefore the target of treatment approaches, including topical eye drops, laser treatment, or incisional surgical intervention. Other risk factors relate to individual genetic risk, including ethnicity and family history, with a 9.2-fold increased risk for first-degree relatives of individuals with glaucoma compared with controls.[4] Due to the asymptomatic nature of early-stage disease, limitations of screening techniques, and challenges in diagnosis, over half of all individuals with glaucoma in developed countries and over 90% in developing countries

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are estimated to be undiagnosed.[2,5] Early diagnosis is paramount given that vision cannot be restored once it is lost,[6] and existing treatments are highly effective in preventing or slowing disease progression.[7,8] Because glaucoma is the most common cause of irreversible vision loss in the world, improving screening methods and identifying at-risk individuals has the potential to significantly reduce the social and economic burden of disease.

Glaucoma is one of the most heritable common complex diseases.[9,10] Both monogenic and polygenic factors contribute to glaucoma.[9,11] Disease-causing variants in genes, such as *MYOC* and *OPTN*, or copy number variants in *TBK1* account for less than 5% of POAG with Mendelian inheritance patterns.[12] Currently, the clinical use of genetic testing for glaucoma has been limited to these genes.[12,13] With recent advances in the scale of genomewide association studies (GWAS), there is increasing interest in the application of polygenic risk scores (PRS) across a variety of common diseases, including glaucoma. A PRS collates the combined risk of multiple common genetic risk variants into a single score, typically by weighting the relative effect size of each variant.[14] Such scores may be combined with conventional risk factors to estimate overall disease risk.[15]

Recent studies have demonstrated the utility of glaucoma PRS in risk stratification. A recent glaucoma PRS was associated with higher glaucoma risk (top 10% PRS compared to remaining 90% glaucoma OR = 4.2) as well as more rapid disease progression, and higher treatment intensity.[16] Individuals in the top PRS decile were at 15-fold increased risk of developing advanced glaucoma compared to the bottom decile.[16] Furthermore, high polygenic risk confers a comparable risk to monogenic variants, whereas being over 15 times more prevalent in the general population, and can also influence the penetrance and age at diagnosis.[16–18] By stratifying individuals across the risk spectrum for developing glaucoma and likelihood of progression, high-risk individuals would benefit from treatment before vision loss is diagnosed, whereas low-risk individuals could benefit from community-based monitoring.

With more data supporting the clinical validity of PRS in risk stratification, such tests may soon become part of routine clinical care. Before this can occur, it is necessary to understand how such testing may be received by the general population and what key social and behavioral elements may impact implementation. The attitudes of affected individuals have been previously assessed,[19,20] including for glaucoma,[21] however, they have not been assessed in unaffected individuals who will be the ones benefiting from the test. In this study, we addressed this gap and reported the attitudes of individuals without diagnosed glaucoma toward glaucoma PRS testing, and the demographic and psychosocial factors that influence this.

Methods

Study Sample

This was a cross-sectional, questionnaire-based study approved by the Southern Adelaide Clinical Human Research Ethics Committee (SAC HREC) that adhered to the Revised Declaration of Helsinki. The study sample included three different groups of individuals who may be target populations for polygenic risk testing for glaucoma and who were recruited between March 2020 and March 2021. We aimed to recruit 100 participants in each group. Using a one-sided test with multiple test correction (alpha = 0.01), 100 participants in each group will yield 100% power to detect a difference in levels of interest of 20% or more. The first group included unaffected first-degree relatives of individuals with a known glaucoma diagnosis, with participants drawn from the Australian and New Zealand Registry of Advanced Glaucoma (ANZRAG) and the Targeting At Risk Relatives of Glaucoma patients for Early diagnosis and Treatment (TARRGET) study. ANZRAG is one of the largest databases of clinical and genetic data for glaucoma in the world (regardless of glaucoma severity),[22] whereas TARRGET is designed to provide educational material to first-degree relatives of individuals with glaucoma, with their personalized risk of developing the disease according to their family member's clinical phenotype. The second group included people attending an optometrist for an eye assessment for conditions other than glaucoma, or those with no ocular health history who had undergone an eye assessment within the last 6 months. This group is referred to as the "optometry group." These participants were recruited from private (Specsavers) and public (Flinders University) optometry clinics. The third group comprised members of the general community without an ocular health history, who had not undergone a recent eye examination. Recruitment occurred at Flinders Medical Centre (including the Flinders Volunteer service) and Noarlunga Hospital in Adelaide, Australia, and included Flinders volunteer members, patients, and their relatives in outpatient hospital clinics. Individuals for the first two groups were also recruited from these clinics if they had a first-degree relative with glaucoma or had a recent eye examination. Recruitment from

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public hospital settings as well as public and private clinics was opportunistic. Participants were included if they had the capacity to complete the questionnaire without assistance (except if needing an interpreter). Participants were excluded if they were <18 years old or did not have the cognitive capacity to complete the questionnaire.

Data Collection

The questionnaire was adapted from previously published surveys[23] and used Likert-like scale items. The questionnaire was first tested with 10 individuals from Flinders Medical Centre. Sociodemographic, health, cognitive, emotional, and influencing factors were used to assess association with interest in genetic testing.

Sociodemographic

Age, gender, ethnicity, highest level of education, and urban/rural residency were collected. Ethnicity was self-reported and classified into 10 ethnic groupings, then into categories of "European" and "non-European" ancestry. Those recorded as "unknown" were excluded from analyses involving ethnicity. Residency was based on the Australian Bureau of Statistics census data using the participants' postcodes. Urban residency was classified as postcodes with populations greater than 50,000 persons. Rural residency included regional, rural, and remote areas of populations less than 50,000 persons.

Health Factors

Family history, including the number of family members affected by any form of glaucoma and their degree of relation, was self-reported by participants. Eye health factors assessed included a history of myopia, most recent eye check, and the frequency of eye checks.

Cognitive Factors

Cognitive factors were assessed through single-item measures with Likert-like scale response options. We assessed participants' understanding of the heritability of glaucoma, perception of the severity of glaucoma, and perceived likelihood of developing glaucoma.

Emotional Factors

To assess the influence of emotion on interest in genetic testing for glaucoma, we asked participants to indicate their level of worry related to the possibility of developing glaucoma in the future using Likert-like scale response options.

Factors Affecting Decision to be Tested and Concerns

We assessed several factors which could affect the participants' decision to be tested related to their own risk, their family's risk, and advice from others. We assessed factors which would concern participants about testing, including personal anxiety, cost, future requirements, and issues relating to confidentiality and implications of results. Participants could also include additional factors or comments.

Outcome Variable

Interest in genetic testing for glaucoma was evaluated by assessing the likelihood to undergo genetic testing to predict personal glaucoma risk with Likert-like scale response options.

Additional Factors

Participants were asked about aspects of the test that would be considered important to know prior to undergoing genetic testing, the cost participants would be willing to pay, and their preferred method of receiving their results. Participants were asked to indicate how their behavior toward their eye health might change based on theoretical results of higher and lower risk of developing glaucoma, and the frequency of eye checks which they would be willing to undergo.

Statistical Analysis

Data were analyzed using Statistics Package for the Social Sciences (version 27.0, SPSS Inc., Chicago, IL). Descriptive statistics were used to characterize the study sample. Responses from the three groups were combined for the statistical analysis. Responses were combined into bivariate outcomes; for example, "highly unlikely" and "unlikely" were merged into an "uninterested" group, and "likely" and "highly likely" were merged as an "interested" group. Unsure or missing responses for all questions were excluded. Associations of different variables among the three groups were analyzed using 1-way ANOVA and chi-square test for association for continuous and categorical variables, respectively. The association between level of interest and covariables (sociodemographic, emotional, and cognitive variables) was performed using a univariate logistic regression model. Variables that had significance levels of $P < 0.1$ in the univariate analysis were initially included in the multivariate regression model. Multivariate logistic regression models were performed to identify factors independently associated with interest in testing ($P < 0.05$) using a backward stepwise approach.

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Results

Demographic and Personal Characteristics

In total, 418 participants completed the questionnaire; 193 had at least one affected first-degree relative, 117 had had a recent eye review, and 108 were from the community. In total, 243 unaffected family members in ANZRAG and TARRGET were invited to participate in the study, and 143 completed the questionnaire, yielding a response rate of 58.8%. The other 50 participants with a first-degree relative were recruited from outpatient clinics and hospital settings. The demographic and personal characteristics of each group and the whole study sample are shown in Table 1. In summary, 66.5% were women, 95.0% were of European ancestry, 75.4% were from an urban area, and 63.8% had an education level above secondary school. The mean age of the total cohort was 62.1 years ± 13.3 years, with 28 individuals being under the age of 40 years. There was a significant difference in residency, family history, timing of last eye check, and frequency of eye checks among groups (Table 1–significant results in bold). Participants with affected first-degree relatives, those who had a recent eye check, and members of the general community did not differ by age, gender, and level of education (see Table 1). The majority (74.9%) of participants had undergone an eye check within at least the last year and over half (55.0%) reported undergoing eye checks at least annually.

Understanding of Glaucoma and Perception of Severity and Risk

In the overall cohort, 57.7% believed glaucoma was at least somewhat hereditary, with 57.7% of those having an affected first-degree relative. A large proportion (39.5%) of the total cohort were unsure about the hereditary nature of glaucoma. The majority (91.9%) of respondents considered glaucoma to be a severe medical condition, with an approximately equivalent proportion with (47.9%) and without (52.1%) an affected first-degree relative. Perception of glaucoma as a severe condition was associated with being likely to increase the frequency of eye checks if found to be at high risk (odds ratio [OR] = 7.36, 95% confidence interval [CI] = 1.32–40.89, $P = 0.023$). Almost a third (31.8%) of the participants believed they were likely or highly likely to develop glaucoma in their lifetime, and 89.1% of these expressed worry about this belief. Those with at least one first-degree relative with glaucoma were more likely to believe they were at risk of developing glaucoma (OR = 5.06, 95% CI = 2.99–8.58, $P < 0.001$), and were worried about this (OR = 3.75, 95% CI = 2.33–6.06, $P < 0.001$). Being worried about the possibility of developing glaucoma was associated with a preference to know the glaucoma risk (OR = 2.19, 95% CI = 1.40–3.43, $P < 0.001$).

Interest in Genetic Risk Prediction Testing for Glaucoma

Overall, the majority of individuals expressed an interest in genetic risk prediction testing for glaucoma, with 71.3% of respondents indicating they would be either likely or highly likely to take a test if it were available. The attitudes of each group are shown in Figure 1. Over half of those who were interested in testing (62.2%) also reported they would probably or definitely like to know more about glaucoma before being tested. Individuals with at least one affected first-degree relative were more likely to be interested in genetic testing for glaucoma than those without (OR = 2.90, 95% CI = 1.65–5.09, $P < 0.001$; Table 2). There was no significant difference between the level of interest between those aged below and above the age of 40 years (75.0% vs. 81.2%, respectively, $P = 0.459$).

Factors Affecting Interest in Genetic Risk Prediction Testing for Glaucoma

We assessed the factors that may affect the participants' decision to be tested (Fig. 2) and factors that may concern participants about genetic risk prediction testing (Fig. 3). After adjusting for all variables that were significant in univariate regression, interest in glaucoma genetic risk prediction testing was more common in those who believed glaucoma to be a severe medical condition (OR = 14.58, 95% CI = 1.15–185.50, $P = 0.039$), were concerned about developing glaucoma (OR = 4.37, 95% CI = 2.32–8.25, $P < 0.001$), had an intention to take appropriate measures regarding eye health (OR = 2.39, 95% CI = 1.16–4.95, $P = 0.019$), or who preferred to know if they were at risk of glaucoma or not (OR = 4.52, 95% CI = 2.32–8.83, $P < 0.001$; see Table 2). The average number of factors which may affect the participants' decision to be tested was 3.7.

The majority (75.8%) of individuals had at least one concern about genetic risk prediction testing for glaucoma, with cost being the most frequent (42.3%), followed by personal anxiety about the possibility of the test showing increased glaucoma risk (29.7%; see Fig. 3). The average number of concerns per

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Table 1. Characteristics of the Study Sample (Including Individuals With a First-Degree Relative With Glaucoma [First-Degree Relative], Those Who had Undergone a Recent Eye Check [Optometry] and General Members of the Community [Community])

Variable	First-Degree Relative $n = 193$	Optometry $n = 117$	Community $n = 108$	TOTAL $n = 418$	P Value
Age, y					
Range	33.0–89.8	21.0–89.3	19.4–94.6	19.4–94.6	$P = 0.573$*
Mean (standard deviation)	61.7 (11.2)	63.2 (15.4)	61.5 (14.3)	62.1 (13.3)	
Median	62.1	65.4	66.3	63.3	
Missing		$n = 1$	$n = 1$	$n = 2$	
Gender, *n* (%)					
Female	134 (69.4)	73 (62.4)	71 (65.7)	278 (66.5)	$P = 0.437$†
Male	59 (30.6)	44 (37.6)	37 (34.3)	140 (33.5)	
Ethnicity, *n* (%)					
European ancestry	185 (95.9)	114 (97.4)	98 (90.7)	397 (95.0)	**$P = 0.028$**†
Non-European ancestry	6 (3.1)	2 (1.7)	9 (8.3)	17 (4.1)	
- African	2 (1.0)	0	0	2 (0.5)	
- Asian	3 (1.6)	1 (0.8)	3 (2.8)	7 (1.7)	
- Hispanic	0	1 (0.8)	1 (0.9)	2 (0.5)	
- Middle Eastern	0	0	2 (1.9)	2 (0.5)	
- Mixed	1 (0.5)	0	3 (2.8)	4 (1.0)	
Unknown	2 (1.0)	1 (0.9)	1 (0.9)	4 (1.0)	
Residency, *n* (%)					
Urban	137 (71.0)	90 (76.9)	88 (81.5)	315 (75.4)	**$P = 0.019$**†
Rural	55 (28.5)	21 (17.9)	16 (14.8)	92 (22.0)	
Unknown	1 (0.5)	6 (5.1)	4 (3.7)	11 (2.6)	
Highest level of education, *n* (%)					
Primary school	2 (1.0)	4 (3.2)	1 (1.0)	7 (1.7)	$P - 0.056$†
Secondary school	60 (31.1)	43 (36.8)	39 (36.1)	142 (34.1)	
Vocational training	52 (26.9)	39 (33.3)	40 (37.0)	131 (31.3)	
University	77 (39.9)	31 (26.5)	28 (5.9)	136 (32.5)	
Unknown	2 (1.0)	0	0	2 (0.5)	
Family history, *n* (%)					
Positive	193 (100.0)	9 (7.7)	7 (7.4)	209 (50.0)	**$P < 0.001$**†
Negative	0	107 (95.1)	87 (80.6)	209 (50.0)	
Unknown	0	1 (0.9)	14 (13.0)	0	
Positive (closest affected relative):					
- First-degree	193 (100.0)	0	0	193 (92.3)	
- Second-degree	0	7 (6.0)	5 (4.6)	12 (5.7)	
- Third-degree	0	1 (0.9)	1 (0.9)	2 (1.0)	
- Unknown	0	1 (0.9)	1 (0.9)	2 (1.0)	
Last eye check, *n* (%)					
Within 6 mo	63 (32.6)	117 (100.0)	0	180 (43.1)	**$P < 0.001$**†
6–12 mo	82 (42.5)	0	51 (47.2)	133 (31.8)	
1–2 y	41 (21.2)	0	30 (27.8)	71 (17.0)	
More than 2 y	4 (2.1)	0	25 (23.1)	29 (6.9)	
Never	1 (0.5)	0	0	1 (0.2)	
Missing	2 (1.0)		2 (1.9)	4 (1.0)	
Frequency of eye checks, *n* (%)					
3 mo	2 (1.0)	1 (0.9)	0	3 (0.7)	**$P = 0.003$**†
6 mo	9 (4.7)	5 (4.3)	2 (1.9)	16 (3.8)	
Annually	107 (55.4)	61 (52.1)	43 (39.8)	211 (50.5)	
Every 2 y	61 (31.6)	32 (27.4)	34 (31.5)	127 (30.4)	
More than every 2 y	10 (5.2)	13 (11.1)	22 (20.4)	45 (10.8)	
Never	2 (1.0)	3 (2.6)	6 (5.6)	11 (2.6)	
Missing	2 (1.0)	2 (1.7)	1 (0.9)	5 (1.2)	

*Denotes P value calculated using one-way ANOVA.

†Denotes P value calculated using chi-square test for association. Differences in ethnicity were assessed between European and non-European ancestry.

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individual was 1.4. We assessed the factors concerning individuals about undergoing genetic risk assessment for glaucoma and why participants may be less likely to take the test. These are summarized in Supplementary Figure S2. Of those who indicated being uninterested in testing, 24.6% had no concerns about the test. Having to attend follow-up appointments was the most concerning factor (37.7%), followed by the cost of the test (23.6%), potential anxiety caused by the results (20.8%), concern about how the results would affect employment (11.1%) and insurance (8.3%), confidentiality concerns (6.9%), and rather not knowing their risk (4.2%).

Behavior

In addition to assessing which factors may influence the decision to undergo genetic risk prediction testing, we assessed whether the potential result would influence attitudes toward the frequency of future eye checks. If testing were to indicate a low risk of developing glaucoma, 91.6% of individuals indicated they would not change the frequency of their eye checks. However, if testing were to indicate a high risk of developing glaucoma, 76.6% of individuals indicated they would have more frequent eye examinations. Those with an affected first-degree relative were not likely to change the current frequency of their eye examinations, regardless of whether a test indicated they were at either low risk ($P = 0.344$) or high risk ($P = 0.092$). Individuals indicated that their decision to undergo testing would be influenced more by medical advice compared to advice from family or friends (74.6% vs. 35.1%, $P < 0.001$).

Factors About Testing and Follow-Up

Finally, we surveyed aspects of genetic risk prediction testing that participants wanted to know prior to undergoing testing. These are summarized in Supplementary Figure S1. Over 77% of participants deemed cost, the test process, possible implications of results, and follow-up to be important factors to understand prior to undergoing testing. Email was the most preferred method to receive results (56.5%), followed by face to face (38.3%), and receiving a letter (35.2%), with a telephone call being the least preferred (21.5%). Several individuals commented that their preference would depend on the result, with face to face being preferred if results showed high glaucoma risk, and other methods, particularly email, being preferred if results showed low risk. A majority of participants (64.6%) indicated they would be willing to pay at least $50 for a glaucoma genetic test if required, with AUD

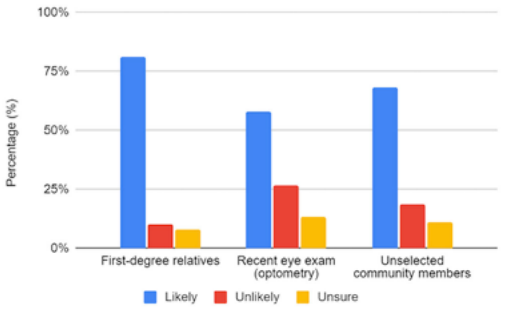

Figure 1. Level of interest in polygenic risk testing for glaucoma (positive versus negative) according to group classification. Responses to the question "How likely would you be to take a genetic test which could predict your risk of developing glaucoma?" Responses were grouped by group classification (first-degree relatives, recent eye examination [optometry], and general members of the community [community]), and grouped into interested (likely or highly likely) or uninterested (highly unlikely or unlikely) expressed interest. Forty-two respondents indicated being "unsure" (10.0%).

$50 to $100 (approximately USD $40–$70 at the time of writing) being the most acceptable range (Fig. 4). Those who were willing to pay were more likely to be interested in testing (OR = 1.81, 95% CI = 1.07–3.07, $P = 0.028$) and to have completed tertiary education (OR = 1.95, 95% CI = 1.28 2.98, $P = 0.002$). Regarding the possible frequency of eye checks, 88.8% of all participants indicated they would be willing to have either biannual or annual eye examinations if required (Fig. 5).

Discussion

Genetic risk stratification for diseases with complex inheritance will become increasingly accessible with the development of PRS. Studies have previously assessed interest and attitudes toward such testing in affected and high-risk individuals for breast and colorectal cancer.[19,20] To the best of our knowledge, the attitudes of those outside of an already identified at-risk population have not been investigated for any condition. Given one of the greatest potential advantages of PRS testing is population-scale risk stratification, it is crucial to understand the attitudes of the broader population toward this form of testing. Our findings provide useful insights into the attitude of unaffected individuals toward glaucoma genetic risk testing, and demonstrated a similar level of interest toward PRS testing for glaucoma among unaffected individuals (71.3%)

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Table 2. Univariate and Multivariate Logistic Regression Assessing Predictors for Interest in Polygenic Risk Testing

	Univariate Logistic Regression		Multivariate Logistic Regression	
Variable (Demographic)	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value
Age, y	0.99 (0.96–1.01)	0.165		
Gender				
- Male	**1.00**	**0.045**	1.67 (0.85–3.30)	0.138
- Female	**1.71 (1.01–2.89)**			
Ethnicity				
- Non-European	1.00	0.312		
- European	1.86 (0.56–6.23)			
Residency				
- Urban	1.00			
- Rural	1.05 (0.56–1.98)	0.877		
Education				
- School (primary or secondary)	**1.00**		1.28 (0.51–3.24)	0.593
- Tertiary (vocational training or university	**1.67 (0.99–2.84)**	**0.056**		
Family history				
- Negative	**1.00**	**<0.001**		0.111
- First-degree relative	**2.89 (1.64–5.11)**	**<0.001**	2.05 (0.76–2.17)	0.156
- Other relative	0.98 (0.25–3.85)	0.980	0.24 (0.03–2.17)	0.205
Last eye check				
- <1 y (0)	1.00			
- >1 y (1)	1.15 (0.62–2.12)	0.665		
Frequency of eye checks				
- At least annually (0)	1.00			
- Every 2 y or more (1)	1.02 (0.60–1.71)	0.954		
Perceived glaucoma heredity				
- Non hereditary	**1.00**	**0.018**	1.9 (0.02–193.52)	0.779
- Hereditary	**9.14 (1.47–56.86)**			
Perceived severity				
- Not severe	**1.00**	**0.009**	**14.58 (1.15–185.50)**	**0.039**
- Severe	**18.69 (2.05–170.14)**			
Perceived risk				
- Not at risk	**1.00**	**0.005**	1.88 (0.81–4.35)	0.139
- At risk	**2.47 (1.32–4.63)**			
Concern of developing glaucoma				
- Not worried	**1.00**		**4.37 (2.32–8.25)**	**<0.001**
- Worried	**5.00 (2.87–8.72)**	**<0.001**		
Interest in obtaining more information about the test				
- Not interested	**1.00**		1.71 (0.71–4.11)	0.233
- Interested	**2.04 (1.16–3.59)**	**0.013**		
Intention to take appropriate measures				
- Would not change behavior	**1.00**		**2.39 (1.16–4.95)**	**0.019**
- Would change behavior	**5.00 (2.83–8.83)**	**<0.001**		
Advice to children				
- No	**1.00**		1.15 (0.25–5.39)	0.860
- Yes	**3.00 (1.77–5.08)**	**<0.001**		
Advice to family members				
- No	**1.00**		0.49 (0.18–1.32)	0.160
- Yes	**2.92 (1.71–4.99)**	**<0.001**		
Personal advice				
- No	1.00			
- Yes	1.34 (0.77–2.33)	0.304		
Medical advice				
- No	1.00			
- Yes	1.17 (0.64–2.13)	0.614		
Would rather know				
- No	**1.00**			
- Yes	**6.78 (3.86–11.90)**	**<0.001**	**4.52 (2.32–8.83)**	**<0.001**

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Table 2. Continued

Variable (Demographic)	Univariate Logistic Regression OR (95% CI)	*P* Value	Multivariate Logistic Regression OR (95% CI)	*P* Value
Would rather not know				
- No	1.00			
- Yes	0.378 (0.09–1.62)	0.190		
Anxiety				
- No	1.00			
- Yes	1.55 (0.83–2.89)	0.170		
Cost				
- No	1.00			
- Yes	1.37 (0.80–2.34)	0.254		
Follow-up				
- Yes	1.00			
- No	1.29 (0.69–2.38)	0.424		
Insurance				
- No	**1.00**		3.11 (0.99–9.79)	0.052
- Yes	**3.44 (1.43–8.27)**	**0.006**		
Employment				
- No	1.00			
- Yes	1.26 (0.56–2.82)	0.573		
Confidentiality				
- No	1.00			
- Yes	1.89 (0.71–4.98)	0.201		
Concerns				
- No concerns	1.00			
- At least 1 concern	1.23 (0.69–2.20)	0.485		

Bold text in the multivariate logistic regression indicates variables which were retained in the final model. Where a variable was excluded, the listed values given related to the point at which the variable was removed from the model. Results reflect questionnaire answers provided by participants, although the authors acknowledge that some responses are not logical.

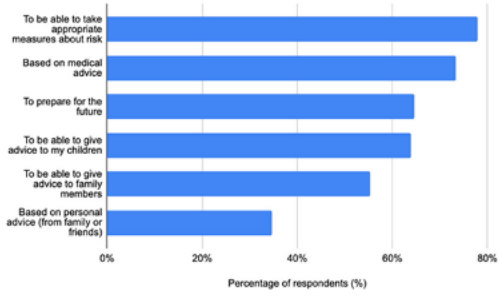

Figure 2. **Factors affecting participants' decision to be tested.** Responses to the question "Which of the following factors would affect your decision to be tested? (Choose as many as appropriate)."

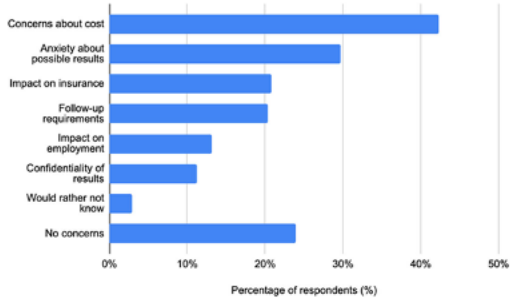

Figure 3. **Factors concerning participants about having the test.** Responses to the question "Which of the following factors would concern you about having the test? (Choose as many as appropriate)."

compared with individuals with diagnosed glaucoma (69.4%).[21]

Although glaucoma is the most common cause of irreversible vision loss, current screening methods are insufficient and not cost-effective at the population level.[24,25] Evidence of the benefit of PRS testing was demonstrated by a previous study showing that individuals in the top decile of a glaucoma PRS distribution reach the same absolute risk of developing the disease 10 years earlier than those in the bottom decile.[16] Glaucoma PRS testing could improve current screening strategies given the disease's high and complex heritability, lack of environmental risk factors, asymptomatic nature of early disease, and effectiveness of early treatment options to slow

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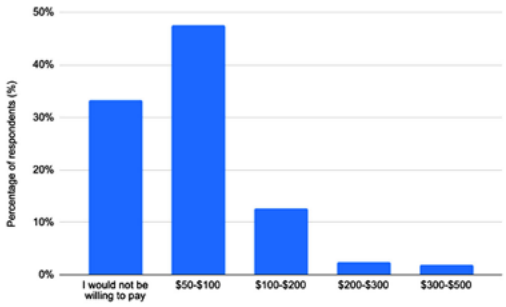

Figure 4. Cost participants would be willing to pay for a glaucoma genetic risk test. Responses to the question "If a cost were involved, how much would you be willing to pay for the test?"

Figure 5. Frequency of eye checks participants would be willing to undergo. Responses to the question "How frequently would you be willing to have an eye check?"

disease progression.[26] Risk stratification may help to guide monitoring and treatment of high-risk individuals, as well as potentially avoiding unnecessarily regular follow-up or over-treatment of low-risk individuals. PRS may assist in deciding on monitoring frequency or context, such as by an ophthalmologist or optometrist, particularly given the difficulty in diagnosing glaucoma in the early stage of disease and the large number of individuals who are diagnosed as glaucoma suspects.[12]

Interest in the test was not significantly associated with having a family history in the multivariate analysis, even though individuals with a family history were more likely to be interested in polygenic risk testing than those without. Previous studies have reported increased interest in PRS testing among first-degree relatives of individuals with breast cancer or colorectal cancer.[19,20,27–31] These discrepancies may be due to an assumed predisposition to glaucoma and frequent monitoring already in place in this cohort. The majority of those with an affected first-degree relative (74.1%) were drawn from existing glaucoma research databases. As part of their participation in these registries, individuals will have received information about the purpose of the research being to investigate the genetic nature of glaucoma as well as targeted glaucoma educational material, and may be more aware of the risk associated with having a family history. This is supported by our results which showed that those with an affected first-degree relative were more likely to believe they were at risk of developing glaucoma. Previous studies have shown that risk perception is often influenced by lived experience[32–36] and that PRS may not alter perceived risk in these cases.[32] Interestingly, in this study, individuals with an affected first-degree relative were not more likely to change the current frequency of eye examinations, regardless of whether a test indicated they were at either low or high risk. However, this cohort was also the one reporting the highest frequency of eye examination and may therefore feel that additional testing is not necessary.

These issues may represent a potential barrier to the uptake of PRS testing for glaucoma in this high-risk group and will need to be further investigated for successful implementation of the test and combination with existing screening methods. This is highly relevant in the context of a prediction model which showed that approximately one quarter of people will have a PRS counteracting their risk due to their family history.[16] These individuals may be unaware of any underlying risk and will not be identified early through current screening guidelines given earlier age at screening is only recommended for those with a family history.[6]

Individuals who believed glaucoma to be a severe condition were more likely to be interested in PRS testing for glaucoma, and were more likely to increase the frequency of their eye examinations if shown to be at high risk. Furthermore, being worried about the possibility of developing glaucoma in the future appears to be a strong motivating factor to undergo testing. However, despite 76.7% of participants indicating being likely to have more frequent eye checks if results showed increased glaucoma risk, this was not associated with interest in PRS testing for glaucoma. This is in keeping with other studies which have shown that knowledge of risk does not correspond to a change in risk-reducing behaviours.[37,38] Previous studies have shown that motivation for undergoing genetic testing commonly stems from a conviction to altruism and desire to understand more about personal health, rather than to make preventative lifestyle behavior changes or change screening behaviours.[39–43] The option to choose to know of a genetic susceptibility

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to disease may seem to be valued more than the results and their possible implications.[43] Future research should examine whether knowledge of risk from the actual uptake of the test leads to change in glaucoma screening behaviors.

We asked participants which components of the test they would like to know more about prior to undergoing the test. The cost of the test, process involved in taking the test, implications of the results, and likely follow-up were each equally important to respondents with over 75% indicating they would want to know. Respondents indicated email as the preferred method of receiving results, with face to face, letter, and telephone call being approximately equally preferred. The majority of those who expressed willingness to pay for the test indicated AUD $50 to $100 to be an appropriate cost for the test. Whereas early indications of the likely cost of PRS testing are above $100, public preference is relevant in order to consider future cost subsidization and possible impact on uptake of the test. Moreover, concerns about insurance were significantly associated with testing in the univariate regression analysis and close to significance in the multivariate analysis. This may be particularly important in an older population who are more likely to be at risk. Our results may reflect the study population, with many being recruited from public hospitals where the provision of health services, including investigations and treatments for glaucoma, are not associated with any out-of-pocket costs for patients in Australia. Furthermore, Medicare (Australia's universal health insurance system) subsidizes the cost of most pathology tests, thus the Australian population are generally not accustomed to paying for such tests. However, genetic tests are currently not widely subsidized. It will be important to address concerns associated with costs in the future, especially given some respondents commented that they would expect that the test would be subsidized by Medicare and cost was one of the main reasons for not being interested in testing.

Given the potential for broad population screening, ordering PRS testing, interpreting results, and communication of their significance to patients will extend beyond the clinicians directly involved in glaucoma diagnosis and management. Clinical implementation of PRS will rely on sound clinician understanding of the test and its results. It will be important to emphasize that PRS results represent a probability of individual disease risk and are therefore not diagnostic, and results will need to be interpreted in conjunction with other established clinical risk factors, in particular age.[15] Integrated risk models that incorporate established clinical and demographic risk factors will need to be developed. Genetic counsellors have the skill set to assist individuals in making informed decisions about their results and the implications for their family members. However, their role may be most necessary for those who receive high-risk results, as the current workforce will not be able to carry the entire burden of a population-based screening test. Further research will need to evaluate the views and the needs of clinicians and healthcare professionals who may be involved in ordering PRS testing, interpreting results, and communicating their significance to the patients. Adequate resources will need to be available to upskill all clinicians and healthcare professionals who may be involved in glaucoma PRS testing.

Results should be interpreted in light of the study's strengths and limitations. Of the total participants, 34.4% were drawn from existing glaucoma research registries (ANZRAG and TARRGET). These participants have previously demonstrated interest in glaucoma research, particularly regarding genetic studies and family history, and may therefore be more likely to report interest in glaucoma genetic testing. However, the interest toward PRS testing was still strong among individuals who were not part of existing research projects (65.6%). The majority of our study sample (95.0%) was of self-reported European ancestry, highlighting the need for further validation across other ancestral backgrounds prior to implementation. It will also be pertinent to ensure the utility of predominantly European-derived PRS instruments themselves in non-European ancestries. Furthermore, the attitudes of individuals of European ancestry may vary depending on cultural and geographic differences, such as among individuals in Australia, Northern America, and Europe. Although we have included unaffected individuals from three different groups, the study cohort may not be representative of a broader population of unaffected individuals. Additional studies would be needed to extrapolate these results to the general population. Finally, the methodology of this study relates to anticipated behaviors and future intentions and is not a representation of actual behavior. Further research should compare the uptake of PRS testing for glaucoma in those with reported interest.

PRS has the potential to stratify individual risk across a broad population for many common conditions with complex inheritance, including glaucoma. We found positive interest toward glaucoma PRS testing among three different groups of unaffected individuals and have identified possible target populations for initial clinical implementation. We have also identified factors affecting interest toward the test and potential barriers to address. Acceptability of genetic risk testing by the general population is crucial for clinical implementation to be successful.

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Acknowledgments

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References

1. Zhang N, Wang J, Li Y, Jiang B. Prevalence of primary open angle glaucoma in the last 20 years: a meta-analysis and systematic review. *Sci Rep*. 2021;11(1):1–12.
2. Mitchell P, Smith W, Attebo K, Healey PR. Prevalence of open-angle glaucoma in Australia. The Blue Mountains Eye Study. *Ophthalmology*. 1996;103(10):1661–1669.
3. Weinreb RN, Khaw PT. Primary open-angle glaucoma. *Lancet*. 2004;363(9422):1711–1720.
4. Wolfs RC, Klaver CC, Ramrattan RS, van Duijn CM, Hofman A, de Jong PT. Genetic risk of primary open-angle glaucoma. Population-based familial aggregation study. *Arch Ophthalmol*. 1998;116(12):1640–1645.
5. Quigley HA, Broman AT. The number of people with glaucoma worldwide in 2010 and 2020. *Br J Ophthalmol*. 2006;90(3):262–267.
6. National Health and Medical Research Council. NHMRC Guidelines for the Screening, Prognosis, Diagnosis, Management and Prevention of Glaucoma. 2010:1–181. Available at: https://www.nhmrc.gov.au/about-us/publications/guidelines-screening-prognosis-diagnosis-management-and-prevention-glaucoma.
7. Kass MA, Heuer DK, Higginbotham, et al. The Ocular Hypertension Treatment Study: A randomized trial determines that topical ocular hypotensive medication delays or prevents the onset of primary open-angle glaucoma. *Arch Ophthalmol*. 2002;120(6):701–713. Available at: https://jamanetwork.com/journals/jamaophthalmology/article-abstract/270953.
8. AGIS Investigators. The Advanced Glaucoma Intervention Study (AGIS): 9. Comparison of glaucoma outcomes in black and white patients within treatment groups. *Am J Ophthalmol*. 2001;132(3):311–320.
9. Charlesworth J, Kramer PL, Dyer T, et al. The Path to Open-Angle Glaucoma Gene Discovery: Endophenotypic Status of Intraocular Pressure, Cup-to-Disc Ratio, and Central Corneal Thickness. *Invest Ophthalmol Vis Sci*. 2010;51(7):3509–3514.
10. Springelkamp H, Iglesias AI, Mishra A, et al. New insights into the genetics of primary open-angle glaucoma based on meta-analyses of intraocular pressure and optic disc characteristics. *Hum Mol Genet*. 2017;26(2):438–453.
11. Wang R, Wiggs JL. Common and rare genetic risk factors for glaucoma. *Cold Spring Harb Perspect Med*. 2014;4(12):a017244.
12. Khawaja AP, Viswanathan AC. Are we ready for genetic testing for primary open-angle glaucoma? *Eye*. 2018;32(5):877–883.
13. Stone EM, Aldave AJ, Drack AV, et al. Recommendations for genetic testing of inherited eye diseases: report of the American Academy of Ophthalmology task force on genetic testing. *Ophthalmology*. 2012;119(11):2408–2410.
14. Dudbridge F. Power and predictive accuracy of polygenic risk scores. *PLoS Genet*. 2013;9(3):e1003348.
15. Torkamani A, Wineinger NE, Topol EJ. The personal and clinical utility of polygenic risk scores. *Nat Rev Genet*. 2018;19(9):581–590.
16. Craig JE, Han X, Qassim A, et al. Multitrait analysis of glaucoma identifies new risk loci and enables polygenic prediction of disease susceptibility and progression. *Nat Genet*. 2020;52(2):160–166.
17. Zebardast N, Sekimitsu S, Wang J, et al. Characteristics of p.Gln368Ter Myocilin Variant and Influence of Polygenic Risk on Glaucoma Penetrance in the UK Biobank. *Ophthalmology*. 2021;128(9):1300–1311.
18. Siggs OM, Han X, Qassim A, et al. Association of Monogenic and Polygenic Risk With the Prevalence of Open-Angle Glaucoma. *JAMA Ophthalmol*. 2021;139(9):1023–1028.
19. Yanes T, Meiser B, Kaur R, et al. Uptake of polygenic risk information among women at increased

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risk of breast cancer. *Clin Genet*. 2020;97(3):492–501.
20. Lerman C, Seay J, Balshem A, Audrain J. Interest in genetic testing among first-degree relatives of breast cancer patients. *Am J Med Genet*. 1995;57(3):385–392.
21. Hollitt GL, Siggs OM, Ridge B, et al. Attitudes towards polygenic risk testing in individuals with glaucoma. *Ophthalmol Glaucoma*. 2022;5(4):436–446.
22. Souzeau E, Goldberg I, Healey PR, et al. Australian and New Zealand Registry of Advanced Glaucoma: methodology and recruitment. *Clin Experiment Ophthalmol*. 2012;40(6):569–575.
23. Souzeau E, Glading J, Keane M, et al. Predictive genetic testing experience for myocilin primary open-angle glaucoma using the Australian and New Zealand Registry of Advanced Glaucoma. *Genet Med*. 2014;16(7):558–563.
24. Moyer VA, U.S. Preventive Services Task Force. Screening for glaucoma: U.S. Preventive Services Task Force Recommendation Statement. *Ann Intern Med*. 2013;159(7):484–489.
25. Royal College of Ophthalmologists. Commissioning guide: glaucoma. 2016. Available at: https://www.college-optometrists.org/coo/media/media/documents/clinical%20council%20-%20ccehc/commissioning-guide-glaucoma-recommendations-executive-summary.pdf missioning-guide-glaucoma-recommendations-executive-summary.pdf(college-optometrists.org).
26. Lewis CM, Vassos E. Prospects for using risk scores in polygenic medicine. *Genome Med*. 2017;9(1):96.
27. Ceballos RM, Newcomb PA, Beasley JM, Peterson S, Templeton A, Hunt JR. Colorectal cancer cases and relatives of cases indicate similar willingness to receive and disclose genetic information. *Genet Test*. 2008;12(3):415–420.
28. Anderson AE, Flores KG, Boonyasiriwat W, et al. Interest and informational preferences regarding genomic testing for modest increases in colorectal cancer risk. *Public Health Genomics*. 2014;17(1):48–60.
29. Culler DD, Silberg J, Vanner-Nicely L, Ware JL, Jackson-Cook C, Bodurtha J. Factors Influencing Men's Interest in Gene Testing for Prostate Cancer Susceptibility. *J Genet Couns*. 2002;11(5):383–398.
30. Takeshima T, Okayama M, Ae R, Harada M, Kajii E. Influence of family history on the willingness of outpatients to undergo genetic testing for salt-sensitive hypertension: a cross-sectional study. *BMJ Open*. 2017;7(7):e016322.
31. Bottorff JL, Ratner PA, Balneaves LG, et al. Women's interest in genetic testing for breast cancer risk: the influence of sociodemographics and knowledge. *Cancer Epidemiol Biomarkers Prev*. 2002;11(1):89–95.
32. Willis AM, Smith SK, Meiser B, et al. Influence of lived experience on risk perception among women who received a breast cancer polygenic risk score: "Another piece of the pie". *J Genet Couns*. 2021;30(3):849–860.
33. Austin JC. Re-conceptualizing risk in genetic counseling: implications for clinical practice. *J Genet Couns*. 2010;19(3):228–234.
34. Sivell S, Elwyn G, Gaff CL, et al. How risk is perceived, constructed and interpreted by clients in clinical genetics, and the effects on decision making: systematic review. *J Genet Couns*. 2008;17(1):30–63.
35. Tracy KA, Quillin JM, Wilson DB, et al. The impact of family history of breast cancer and cancer death on women's mammography practices and beliefs. *Genet Med*. 2008;10(8):621–625.
36. Walter FM, Emery J, Braithwaite D, Marteau TM. Lay understanding of familial risk of common chronic diseases: a systematic review and synthesis of qualitative research. *Ann Fam Med*. 2004;2(6):583–594.
37. Hollands GJ, French DP, Griffin SJ, et al. The impact of communicating genetic risks of disease on risk-reducing health behaviour: systematic review with meta-analysis. *BMJ*. 2016;352:i1102.
38. Bronner K, Mesters I, Weiss-Meilnik A, et al. Do individuals with a family history of colorectal cancer adhere to medical recommendations for the prevention of colorectal cancer? *Familial Cancer*. 2013;12(4):629–637.
39. Bruno M, Digennaro M, Tommasi S, et al. Attitude towards genetic testing for breast cancer susceptibility: a comparison of affected and unaffected women. *Eur J Cancer Care*. 2010;19(3):360–368.
40. Fraser L, Bramald S, Chapman C, et al. What motivates interest in attending a familial cancer genetics clinic? *Fam Cancer*. 2003;2(3-4):159–168.
41. Gollust SE, Gordon ES, Zayac C, et al. Motivations and perceptions of early adopters of personalized genomics: perspectives from research participants. *Public Health Genomics*. 2012;15(1):22–30.
42. Andrykowski MA, Munn RK, Studts JL. Interest in learning of personal genetic risk for cancer: a general population survey. *Prev Med*. 1996;25(5):527–536.
43. Murphy J, Scott J, Kaufman D, Geller G, LeRoy L, Hudson K. Public perspectives on informed consent for biobanking. *Am J Public Health*. 2009;99(12):2128–2134.

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Publication 3: High polygenic risk is associated with earlier trabeculectomy in primary open-angle glaucoma

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High Polygenic Risk Is Associated with Earlier Trabeculectomy in Patients with Primary Open-Angle Glaucoma

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Purpose: To evaluate the association between a polygenic risk score (PRS) for primary open-angle glaucoma (POAG) and the age at the first trabeculectomy and the need for bilateral trabeculectomy.

Design: Retrospective observational cohort study.

Participants: Nine hundred and three genotyped participants with POAG from the Australian and New Zealand Registry of Advanced Glaucoma.

Methods: The ocular surgical history of these participants was reviewed and the following parameters were recorded: age at diagnosis, age at trabeculectomy, and lateraly of trabeculectomy. Multivariate linear regression analyses correlated glaucoma PRSs with age at trabeculectomy, and laterality of trabeculectomy. For descriptive purposes, the participants were stratified into the top decile, intermediate group (10th-89th percentile), and bottom decile.

Main Outcome Measures: Age at trabeculectomy, and laterality of trabeculectomy.

Results: Higher PRS was associated with younger age at the first trabeculectomy (β, −1.94 years/standard deviation; 95% confidence interval [CI], − 0.41 to −3.47; $P = 0.014$). Participants in the top decile underwent their first trabeculectomy approximately 7 years earlier than participants in the lowest decile (mean difference, −7.04 years; 95% CI, 2.82−11.26). Participants in the top decile were 1.41-fold more likely to require bilateral trabeculectomy than participants in the bottom decile (odds ratio, 1.41; 95% CI, 1.06−1.91; $P = 0.021$).

Conclusions: This report identified clinically relevant correlations between glaucoma PRS and the need for surgical intervention in patients with glaucoma. Further work is required to investigate the association between PRS and other clinical end points such as treatment initiation. *Ophthalmology Glaucoma 2023;6:54-57 © 2022 by the American Academy of Ophthalmology*

Supplemental material available at www.ophthalmologyglaucoma.org.

Primary open-angle glaucoma (POAG) is a highly heritable progressive optic neuropathy.[1,2] Polygenic risk scores (PRSs) represent the summation of an individual's genetic susceptibility to a disease or trait based on the number of inherited risk alleles and can be used to predict the onset and severity of diseases, including glaucoma.[1] Higher glaucoma PRSs have been associated with a greater risk of glaucoma diagnosis, younger age at diagnosis, and a greater need for surgery.[1,3]

Trabeculectomy is a therapeutic incisional procedure that aims to lower intraocular pressure (IOP) and is usually considered only in advanced cases that are refractory to topical medical or laser treatments.[4] Predicting which patients may require this procedure remains a clinical challenge. It is unknown if genetic risk scoring aids the prediction of which patients will need earlier surgery.

Methods

The ocular surgical history was reviewed for all participants with POAG and self-reported European ancestry in the Australian and New Zealand Registry of Advanced Glaucoma,[5] recruited at clinics in the state of South Australia. Participants with secondary forms of glaucoma (e.g., pseudoexfoliation glaucoma) or a documented Mendelian form of POAG were excluded. The age at and laterality of trabeculectomy were recorded. The following covariates were also recorded: age at glaucoma diagnosis, self-reported sex, highest recorded IOP, and family history of glaucoma. This research was approved by local human research ethics committees, and all research adhered to the tenets of the Declaration of Helsinki. Informed written consent was obtained from all participants.

The glaucoma PRS was calculated for each individual using a previously described multitrait glaucoma PRS.[1] Genotyping was performed on DNA extracted from peripheral blood samples using the Illumina Omni1M, OmniExpress, or HumanCoreExome array

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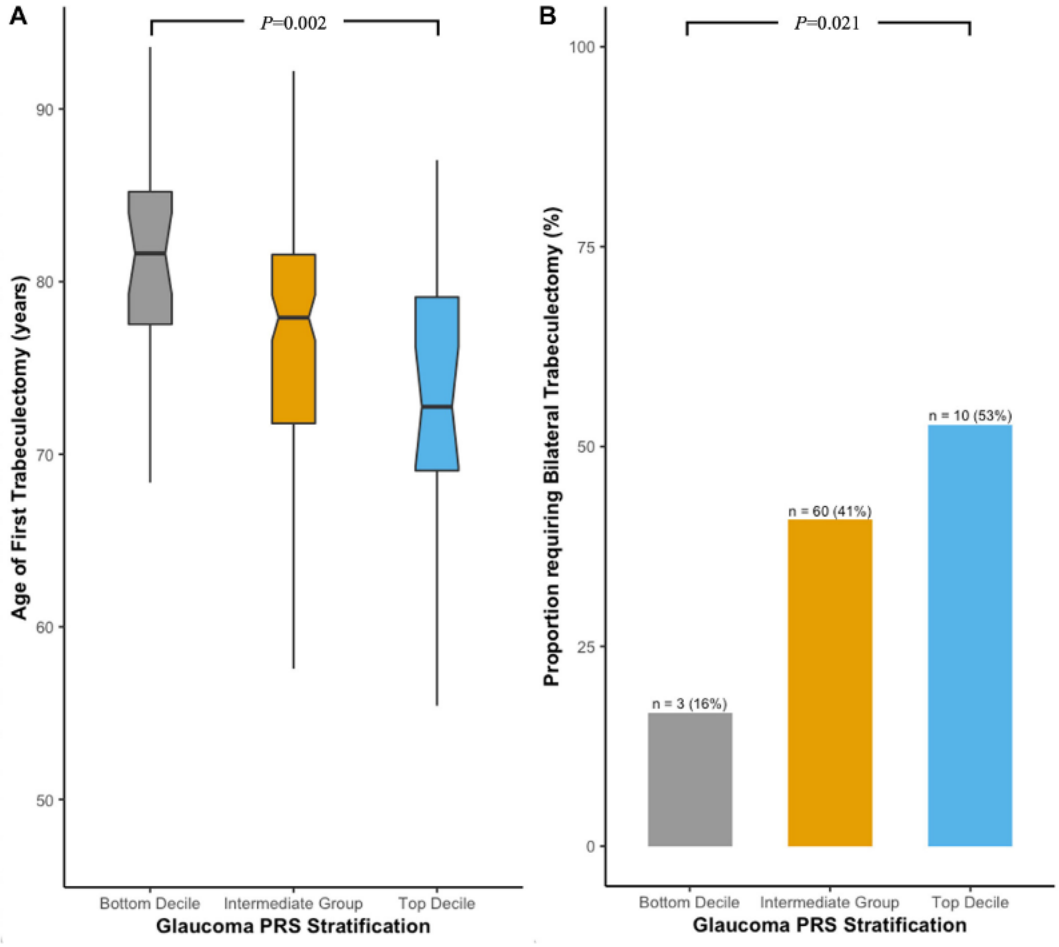

Figure 1. Comparison of glaucoma polygenic risk scores with age at trabeculectomy and the need for bilateral trabeculectomy. **A,** Comparison of age at the first trabeculectomy between polygenic risk score stratifications. **B,** Comparison of proportion requiring bilateral trabeculectomy between polygenic risk score stratifications. The gray box indicates the bottom decile, the orange box indicates the intermediate group (10th to 98th percentile), and the blue box indicates the top decile. Participants in the top decile underwent their first trabeculectomy approximately 7 years earlier than those in the bottom decile (mean age at the first trabeculectomy, 73.45 ± 7.82 years vs. 80.9 ± 6.44 years, respectively; multivariate $P = 0.002$). Participants in the top decile were also 1.41-fold more likely to require bilateral trabeculectomy, as determined using a multivariate analysis (odds ratio, 1.41; 95% confidence interval, 1.06–1.92; $P = 0.021$) (**B**). PRS = polygenic risk score.

(Illumina). The PRSs were calculated using PLINK (version: 1.90 beta) and normalized as z-scores using 17 642 normative individuals from the QSkin Sun and Health Study (QSkin).[6]

Multivariate linear regression analyses were used to assess the correlation between glaucoma PRSs and age at trabeculectomy. The covariates included self-reported sex and family history of glaucoma. Intraocular pressure was not included in the model because of the correlation between PRSs and IOP.[1,2] Based on their PRS values, the participants were stratified into the top decile, bottom decile, and intermediate group (10th–89th percentile). The stratifications were performed using internal normalization because of the skewed distribution of this data set. A secondary analysis correlated glaucoma PRSs with the length of time from diagnosis to trabeculectomy. The covariates included age at diagnosis, self-reported sex, and family history of glaucoma. The P value for statistical significance was set at 0.05.

Results

The surgical data of 903 genotyped participants with POAG were reviewed, of whom 187 had undergone at least 1 trabeculectomy and had the date of surgery recorded. The mean age at glaucoma

diagnosis was 64.1 ± 9.91 years, 57.3% were women, and the mean highest recorded IOP was 28.0 ± 8.66 mmHg (Table S1, available at www.ophthalmologyglaucoma.org).

Participants in the top decile were diagnosed with glaucoma at a younger age (mean difference, 5.19 years; 95% confidence interval [CI], 3.03−7.36; $P < 0.001$) (Table S1) and had a higher recorded IOP than participants in the bottom decile, although this did not reach statistical significance ($P = 0.052$; Table S1).

Linear regression correlated higher PRSs with younger age at the first trabeculectomy (β, −1.94 years/standard deviation; 95% CI, − 0.41 to −3.47; $P = 0.014$). Participants in the top decile underwent their first trabeculectomy approximately 7 years earlier than participants in the lowest decile (mean difference, −7.04 years; 95% CI, 2.82−11.26; multivariate $P = 0.002$; Fig 1A).

A secondary multivariate analysis was used to assess the correlation between glaucoma PRSs and time from diagnosis to first trabeculectomy. Participants in the top decile underwent trabeculectomy 5.8 years earlier than participants in the bottom decile (β, −5.84 years/standard deviation; 95% CI, −1.14 to − 10.55; $P = 0.022$). This association persisted after the inclusion of the highest IOP in the model (β, 5.85 years; 95% CI, 0.96−10.73; $P = 0.024$). The time from diagnosis to trabeculectomy was not associated with glaucoma PRSs in a univariate analysis ($P = 0.143$).

Finally, participants in the top decile were observed to be 1.41-fold more likely to require bilateral trabeculectomy than participants in the bottom decile (odds ratio, 1.41; 95% CI, 1.06−1.91; $P = 0.021$) (Fig 1B).

Discussion

This report correlated higher glaucoma PRSs with younger age at the first trabeculectomy, a shorter duration between diagnosis and the first trabeculectomy, and a greater need for bilateral trabeculectomy in patients with POAG.

Our findings extend previous studies that linked glaucoma PRSs with the outcomes of glaucoma treatment.[1] For those with a disease that may ultimately require surgery, this could mean that trabeculectomy is considered earlier in higher-risk individuals, potentially avoiding vision loss resulting from failed trials of more conservative options. It may also help prevent unnecessary surgery or delay surgery in those who are deemed to be at a low risk. The results of this work, combined with the observation that a majority of individuals with POAG are interested in PRS testing,[7] highlights the potential utility of genomic risk stratification of patients with this disease.

There are several limitations to our study design. It only included participants who had undergone at least 1 trabeculectomy and did not include individuals who had not undergone this procedure. The absence of a univariate association between time from diagnosis to trabeculectomy and glaucoma PRSs is potentially a reflection of a survivorship bias, in which patients diagnosed later in life are more likely to have lower glaucoma PRSs and, possibly, also less likely to survive long enough or be fit enough to require surgery. The exclusion of participants of non-European ancestry limits the application of these findings to other populations. The focus on trabeculectomies as a treatment intervention means that the association between PRSs and other incisional procedures (i.e., glaucoma drainage devices) or the timing of initiation or escalation of other interventions remains unknown. Because these treatment decisions have significant economic implications on quality of life and health, there is a clear need for further investigation in this area.

Footnotes and Disclosures

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All authors have completed and submitted the ICMJE disclosures form.

The authors have made the following disclosures: S.M.: Patent application; Equity owner − Seonix Pty Ltd.

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HUMAN SUBJECTS: Human subjects were included in this study. The study was approved by the Southern Adelaide Clinical Human Research Ethics Committee. This study adhered to the tenets of the Declaration of Helsinki and followed the National Health and Medical Research Council statement of ethical conduct in research involving humans. Informed written consent was obtained from all participants.

No animal subjects were used in this study.

Author Contributions:

Conception and design: Marshall, Hollitt, Wilckens, Kuruvilla, Craig, Siggs

Data collection: Marshall, Hollitt, Wilckens, Mullany, Kuruvilla, Souzeau, Landers, Han, MacGregor, Craig, Siggs

Analysis and interpretation: Marshall, Hollitt, Souzeau, Han, MacGregor, Craig, Siggs

Obtained funding: Craig

Overall responsibility: Marshall, Hollitt, Wilckens, Mullany, Kuruvilla, Landers, Souzeau, Han, MacGregor, Craig, Siggs

Abbreviations and Acronyms:
IOP = intraocular pressure; **POAG** = primary open-angle glaucoma; **PRS** = polygenic risk score.

Keywords:
Glaucoma, Open-angle glaucoma, Polygenic risk score, Risk stratification, Trabeculectomy.

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References

1. Craig JE, Han X, Qassim A, et al. Multitrait analysis of glaucoma identifies new risk loci and enables polygenic prediction of disease susceptibility and progression. *Nat Genet*. 2020;52:160–166.
2. Qassim A, Souzeau E, Siggs OM, et al. An intraocular pressure polygenic risk score stratifies multiple primary open-angle glaucoma parameters including treatment intensity. *Ophthalmology*. 2020;127:901–907.
3. Siggs OM, Han X, Qassim A, et al. Association of monogenic and polygenic risk with the prevalence of open angle glaucoma. *JAMA Ophthalmol*. 2021;139:1023–1028.
4. Gedde SJ, Vinod K, Wright MM, et al. Primary open-angle glaucoma preferred practice pattern. *Ophthalmology*. 2021;128:P71–P150.
5. Souzeau E, Goldberg I, Healey PR, et al. Australian and New Zealand Registry of Advanced Glaucoma: methodology and recruitment. *Clin Exp Ophthalmol*. 2012;40:569–575.
6. Olsen CM, Green AC, Neale RE, et al. Cohort profile: the QSkin Sun and Health Study. *Int J Epidemiol*. 2012;41:929–929i.
7. Hollitt GL, Siggs OM, Ridge B, et al. Attitudes towards polygenic risk testing in individuals with glaucoma. *Ophthalmol Glaucoma*. 2021;5:436–446.

Publication 4: Genetic Risk Assessment of Degenerative Eye Disease (GRADE): study protocol of a prospective assessment of polygenic risk scores to predict diagnosis of glaucoma and age-related macular degeneration

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STUDY PROTOCOL Open Access

Genetic Risk Assessment of Degenerative Eye Disease (GRADE): study protocol of a prospective assessment of polygenic risk scores to predict diagnosis of glaucoma and age-related macular degeneration

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Abstract

Background Glaucoma and age-related macular degeneration (AMD) account for a substantial portion of global blindness. Both conditions are highly heritable, with recognised monogenic and polygenic inheritance patterns. Current screening guidelines lack decisive recommendations. Polygenic risk scores (PRS) allow for cost-effective broad population risk stratification for these conditions. The predictive potential of PRS could facilitate earlier diagnosis and treatment, and prevent unnecessary vision loss.

Methods The Genetic Risk Assessment of Degenerative Eye disease (GRADE) study is a prospective study designed to generate high-quality evidence about the feasibility of PRS to stratify individuals from the general population, enabling identification of those at highest risk of developing glaucoma or AMD. The targeted recruitment is 1000 individuals aged over 50 years, from which blood or saliva samples will be used for genotyping and an individual PRS for glaucoma and AMD will be derived. Individuals with PRS values in the bottom decile (n = 100), top decile (n = 100) and middle 80% (n = 100) for both glaucoma and AMD will undergo a detailed eye examination for glaucoma and/or AMD.

Discussion The primary objective will be to compare the prevalence of glaucoma and AMD cases between low, intermediate, and high PRS risk groups. We expect to find a higher prevalence of both diseases in the high PRS risk group, as compared to the middle and low risk groups. This prospective study will assess the clinical validity of a PRS for glaucoma and AMD in the general Australian population. Positive findings will support the implementation of PRS into clinical practice.

†Owen M Siggs, Emmanuelle Souzeau, Jamie E Craig Denotes authors who contributed equally.

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Keywords Glaucoma, Polygenic risk score, Genetic testing, Macular degeneration, POAG, AMD

Background

Glaucoma and age-related macular degeneration (AMD) are the two most common causes of irreversible vision loss among elderly people worldwide [1, 2]. With the ageing population, these diseases will pose an increasingly significant burden. Furthermore, sight is generally considered to be the most valued sense by the general public, so identifying cost-effective screening methods to facilitate early diagnosis, prevention, and timely intervention is important [3]. In Australia, vision impairment results in significant direct and indirect health care costs, ranking as the seventh most costly health condition [4]. It is important to also consider the impact of vision loss on an individual, which can result in poorer wellbeing outcomes through the impact on quality of life, lost income, and personal healthcare costs [4].

Glaucoma is predicted to affect up to 111.8 million people worldwide by 2040 [2]. The condition results in irreversible vision loss due to progressive optic nerve damage. Primary open angle glaucoma (POAG) is the most common form of the disease, characterised by a normal, open anterior chamber drainage angle [5]. Accepted risk factors for POAG are both genetic and non-genetic. Risk factors with a genetic basis include increasing age, African ancestry, a positive family history, and elevated intraocular pressure (IOP) [6, 7]. The only known modifiable risk factor is raised IOP which is often, but not always, associated with the development of POAG, and IOP-lowering treatment modalities are effective at preventing or slowing disease progression [8]. Glaucoma is usually asymptomatic in the early stages, although progressive vision loss can lead to blindness if left untreated. Current screening methods are inadequate as approximately half of those with glaucoma are undiagnosed [9]. Glaucoma is one of the most heritable common complex conditions, with heritability estimated at 80% [10]. Both highly penetrant rare variants and common variants with much smaller effect sizes, have been associated with POAG [10]. Rare variants in genes including *MYOC*, *TBK1* and *OPTN*, account for less than 5% of POAG cases, [11] with common variants therefore thought to explain the majority of POAG genetic risk.

Similar to glaucoma, AMD is a common eye condition, with a reported prevalence of 13% in those aged over 85 years [1] and is predicted to affect 288 million people by 2040 [12]. It is a progressive condition that causes degeneration of the macula, leading to central vision loss. AMD is asymptomatic in its early stages, with variable progression to visually significant advanced disease depending on clinical and environmental factors [13]. Recognised risk factors for AMD include increasing age, smoking and genetic predisposition [1]. Advanced AMD is classified as either non-neovascular (dry AMD) or neovascular (wet AMD) based on the presence or absence of choroidal neovascularisation. Currently, dry AMD management relies on lifestyle modifications such as smoking cessation and dietary supplementation, [14] while wet AMD is treated with intravitreal injections of vascular endothelial growth factor (VEGF) inhibitors, a key modulator of neovascularisation [15]. Importantly, treatment with VEGF inhibition must be implemented in a timely fashion from the onset of exudative disease. Although some environmental risk factors are well recognised, research indicates there is a strong genetic basis for AMD [1]. Genetic factors may explain variance in disease severity, with heritability estimated at 45–70% [17].

Screening for glaucoma and AMD is largely opportunistic, and broad community screening has not been demonstrated to be cost-effective [16, 17]. For this reason, identifying cost-effective screening methods to facilitate early diagnosis and timely intervention is important. The National Health and Medical Research Council (NHMRC) in Australia currently recommends screening with a clinical examination for first-degree relatives of patients with glaucoma, commencing 5–10 years earlier than the age of glaucoma onset in their affected relative. Additionally, screening from the age of 40 years is recommended in people of African ancestry, compared to from 50 years of age in people of European ancestry [16]. There are no similar recommendations for AMD.

Polygenic risk scores (PRS) are an emerging clinical tool which offer a unique opportunity to improve disease risk prediction for complex heterogeneous diseases, such as glaucoma and AMD [18]. Genome-wide association studies (GWAS) have led to the identification of genetic variants, in the form of single nucleotide polymorphisms (SNPs), which are associated with a disease phenotype. Each SNP confers a different effect on disease risk, with the effect size of each SNP derived from its strength of association with a disease or disease trait in large cohort studies. A PRS summarises this genetic information into an accessible tool to quantify the genetic risk for complex genetic diseases. A PRS is the sum of independent risk alleles an individual carries, weighted by the effect size of each variant [19]. The normal distribution of a PRS, allows risk to be classified into equal groups of frequency distribution [19]. Clinically, quantiles allow for easy assessment of where an individual lies on the population distribution. Ultimately, this score can be used in addition to conventional risk factors to estimate overall disease risk rather than diagnose diseases.

Large GWAS have identified a significant number of common genetic variants associated with POAG or its endophenotypes [20]–[24]. The collective impact of these common variants on glaucoma risk, in the form of a glaucoma PRS, has been effective in stratifying risk within the general population, as well as predicting structural progression and the likelihood of requiring surgical intervention in those with already diagnosed glaucoma [20].

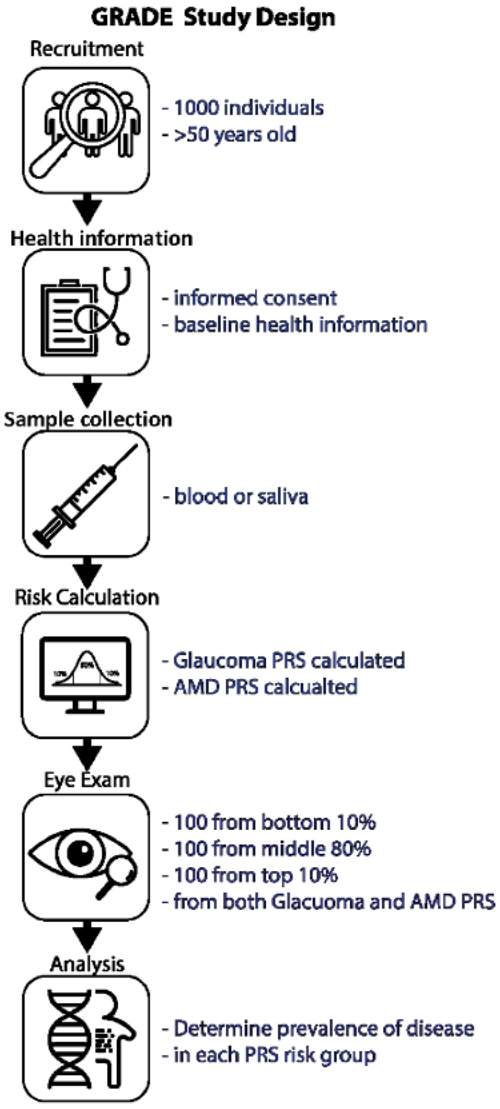

Fig. 1 Study design

In the subset of patients with monogenic variants associated with glaucoma (*MYOC* variant (p.Gln368Ter), the PRS can further stratify individuals into high versus low risk groups [20, 10]. Common and rare variants have also been implicated in AMD risk through GWAS [25, 26]. An AMD PRS using 52 variants showed a 44-fold increased risk of developing AMD for those in the top decile compared to the bottom decile [25]. Furthermore, this PRS was associated with more rapid disease progression [27, 28]. The discovery of genetic associations has also helped to reveal underlying pathophysiologic mechanisms of AMD, exposing potential new treatment targets [29].

With the ability to identify those at highest risk of disease, as well as estimating disease severity and treatment response, there is potential to offer personalised care for glaucoma and AMD patients. This predictive approach could facilitate an exciting change in disease screening and treatment, and ultimately lead to a reduction in vision loss caused by these common conditions. Here we present a prospective population-based study which will assess the prevalence of both glaucoma and AMD across their relative PRS spectra. This will be the first study to assess the clinical validity of a PRS for glaucoma and AMD for clinical implementation in a real-world setting.

Study design

This prospective cohort study was approved by the Southern Adelaide Clinical Human Research Ethics Committee (SAC HREC) and adheres to the Revised Declaration of Helsinki. The study design is summarised in Fig. 1. The research is being conducted at the Department of Ophthalmology at Flinders University, the QIMR Berghofer Medical Research Institute and Seonix Bio under separate ethics approvals and agreements.

Methods

Study objectives and hypotheses

The study will apply PRS testing in 1000 individuals over the age of 50 years from the general population, and then examine a subset of individuals across the PRS spectrum with the aim of ascertaining all cases of glaucoma and AMD. We will prospectively assess the clinical validity of a PRS in stratifying high and low risk individuals, and hypothesise that there will be a higher prevalence of glaucoma and AMD in the high risk PRS groups compared to the middle and low risk groups.

Participants

Participant recruitment methods are compliant with the Health Care Act 2008. A minimum of 1000 individuals over the age of 50 years will be invited to participate. Glaucoma and AMD prevalence increases with age, with

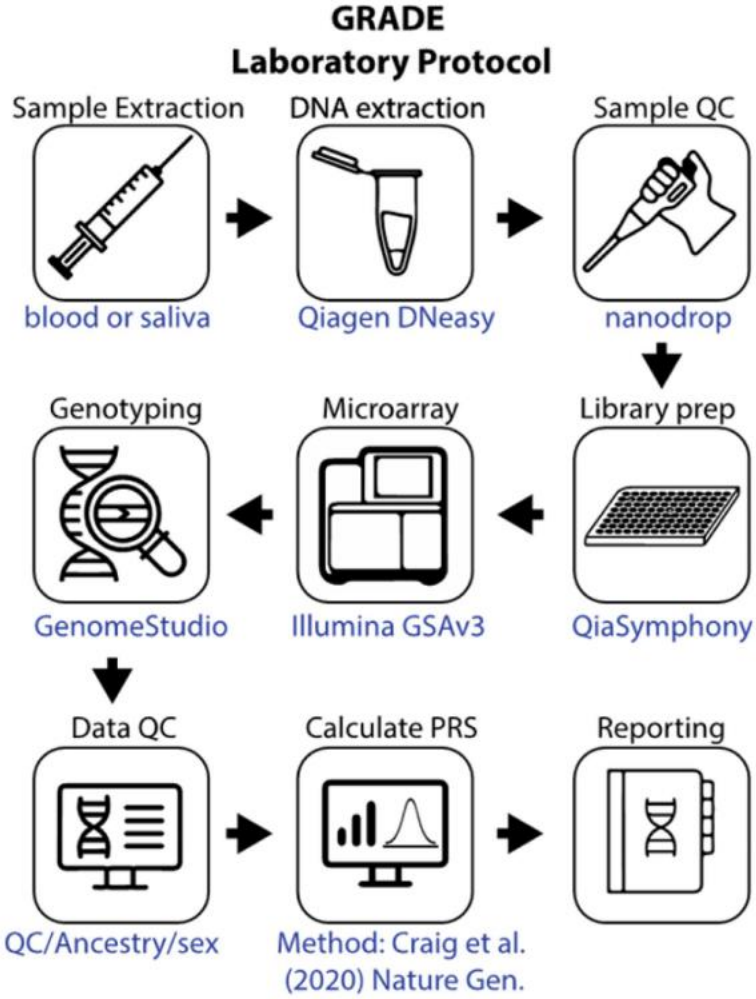

Fig. 2 Flowchart of PRS calculation framework

prevalence rates commonly reported from 50 years of age [1, 2, 12]. Consequently, identifying early or established disease in individuals across the risk spectrum will be easier for individuals within this age range. Exclusion criteria include age under 50 years, or an inability to provide written informed consent. Individuals already diagnosed with glaucoma and/or AMD will not be excluded, nor will they be targeted. Recruitment will be unselected to include individuals of any ethnicity.

Potential participants will be identified using several approaches. All eligible individuals who participated in a questionnaire-based study of individuals without glaucoma assessing attitudes towards polygenic risk testing for glaucoma will be invited to participate in this study [30]. A flyer advertising the project will be displayed in public and private outpatient clinics, and sporting venues and community clubs, provided to social/community organisations and distributed via email to these groups. Presentations about degenerative eye disease will be given to community organisations to promote interest and stimulate recruitment from the general population. Individuals in outpatient clinics will be approached in person and invited to participate if the inclusion criteria are met. Demographic and health information recorded

for each participant will include past medical, ocular and medication history. Individuals with a personal or family history of glaucoma or AMD may be more likely to respond to advertisements, however selection bias will largely be mitigated by wide and non-selective recruitment from all other avenues.

Participation requirements

Participation requires individuals to provide a blood sample (2×9ml EDTA tubes) or a saliva sample (Oragene OG-500 collection tube, DNA Genotek, Ottawa, Ontario, Canada). A subset of participants will be invited to undergo a detailed eye examination for glaucoma and/or AMD. Eye examinations will be performed on 100 individuals each in the bottom 10%, top 10%, and middle 80% of the PRS distributions for glaucoma and AMD. Individuals undergoing eye examinations will be randomly selected within their respective PRS grouping. In total 300 participants will be examined for each disease, with a maximum of 600 participants being examined. In practice, some participants will be selected to be examined for both their glaucoma and AMD PRS results, so the number of participants undergoing eye examinations will be less than 600.

Genetic studies

The laboratory protocol is summarised in Fig. 2. Genomic DNA will be extracted using column-based DNA purification protocols (Qiagen DNeasy) from either blood or saliva samples. Both blood and saliva will be considered viable alternatives for DNA extraction. De-identified samples of extracted DNA will be provided to a genotyping provider for array-based genotyping. Samples will be genotyped on Illumina GSA v3 arrays, with genotype imputation performed locally with Minimac3 using the 1000 Genomes data as a reference panel. Imputation and derivation of glaucoma PRS values will be performed in the laboratory of S.M. using the multitrait analysis of GWAS (MTAG) glaucoma PRS described in detail elsewhere, [20] and the pipelines developed by Seonix Bio. All individuals will have their PRS percentile determined from the relevant 1000 Genomes population, [31] with individual ancestry based on estimates from principal components derived from the genome-wide genetic data. Depending on the distribution of ancestries within the cohort, a sub-analysis may then be performed comparing outcomes between European and non-European groups. Imputation and derivation of AMD PRS values will also be performed by S.M. using a MTAG AMD PRS described in detail elsewhere [25, 26].

Eye examinations

Clinical eye examinations will be performed on 100 individuals from each of the bottom decile, top decile, and the middle 80% of the PRS distributions for glaucoma and AMD. Individuals will be selected using random sampling methods. Examinations will include best-corrected visual acuity, IOP (as measured by Goldmann applanation tonometry), corneal pachymetry, 24−2 Humphrey automated perimetry, spectral domain optical coherence tomography (OCT) of the optic disc and macula, fundus autofluorescence, anterior segment OCT, stereo-disc and fundus photography [32]. All clinical investigation results will be reviewed by independent clinicians who will determine their glaucoma or AMD classification by consensus. Examiners and clinicians reviewing results will be blinded to individuals' PRS results. Glaucoma diagnostic classification will follow previous definitions used in the PROGRESSA study [33]. Each eye will be classified as either normal examination, glaucoma suspect, open-angle glaucoma (OAG) or non-OAG (e.g. primary closed angle glaucoma). For AMD, each eye will be classified as either no AMD or normal ageing changes, early AMD, intermediate AMD, or late AMD.

Sample size and power calculations

Using data from the UK Biobank (age at ICD-10 or self-reported glaucoma diagnosis), we estimate that ~3% of individuals will have a glaucoma diagnosis by the age of 64 years (Fig. 3D in reference [20]). Assuming an equal representation of subjects across all age groups, and assuming that 50% of glaucoma is undiagnosed in the community, [9] we expect ~10% of individuals in the top decile will have glaucoma, compared to ~3% in the bottom decile. The proportion of glaucoma suspects is expected to be more than 2 times the glaucoma cases based on the same preliminary analyses [9]. Based on the combined estimated incidence of glaucoma plus glaucoma suspect cases in each group (i.e. 30% in the top decile vs. 9% in the bottom decile), the current sample size will yield >95% power (α=0.05) to detect a significant difference between the top and bottom deciles of the PRS distribution (logistic regression of glaucoma status on PRS decile).

Similar analyses for AMD suggest a disease prevalence of 0.7% in the bottom decile, and 22.7% in the top decile, [25] within a general population above 75 years of age and a disease prevalence of 5%. Australian epidemiological studies have estimated an AMD population prevalence of 14.3% in individuals aged 49 years and over, [34] so we expect to be sufficiently powered to detect a significant difference between the top and bottom PRS deciles at >80% power (α=0.05). Based on the same published analyses, [25] we are also sufficiently powered to detect a difference between the top PRS decile (AMD prevalence of 22.7%) and the bottom PRS decile (AMD prevalence of 0.7%). While these analyses were used for the purpose of

a power calculation, we acknowledge that the study population may be younger.

Statistical analyses

For all cases, family history of glaucoma and AMD, gender, and ancestry will be self-reported. Genetic ancestry and biological sex will also be determined from genotyping array data. Statistical analyses will be performed in *R* (RCore Team, Austria). Missing information will be treated as missing data in analyses. For association analysis, logistic or linear regression will be used, including age, genetic sex and genetic ancestry as covariates. Other confounding variables will be added when clinically and statistically appropriate. Appropriate regressions will be performed to investigate the rate of each glaucoma or AMD classification across the risk spectrum of the PRS, and to identify any additional factors which were associated with these outcomes. An individual will be defined as a glaucoma or AMD case regardless of whether one or both eyes meet diagnostic criteria.

Study outcomes

The primary outcome will be assessing the prevalence of glaucoma and AMD between the bottom decile, middle 80% and top decile of both respective PRS spectra. We will assess the clinical sensitivity and specificity, as well as the positive and negative predictive values of each of the glaucoma and AMD PRS. Secondary outcomes will compare glaucoma suspect cases to their PRS results, compare disease prevalence with the presence or absence of various comorbid conditions, treatment intensity requirements including the number of cases with actionable disease, the rate of diagnosed versus undiagnosed disease, and the prevalence of family history. Additionally, glaucoma and AMD cases may be graded by severity, and compared to their PRS results.

Discussion

Glaucoma and AMD are the most common causes of irreversible blindness worldwide [2]. Both conditions are highly heritable, with recognised Mendelian and complex inheritance [35, 36, 37]. There is a paucity of screening protocols for both diseases and current guidelines are not cost-effective, in part due to poor sensitivity or specificity. To our knowledge this is the first prospective study to apply PRS testing for glaucoma and AMD in individuals from the general population, specifically recruited for this purpose.

The current NHMRC screening guidelines in Australia lack specific guidance, and are mainly relevant to those with a family history of glaucoma [16]. PRS testing for glaucoma is likely to be useful for those who do not have a known family history and have an unrecognised underlying risk. These individuals are less likely to be identified early by current screening guidelines given screening at an earlier age is only recommended for those with a family history and people of African ancestry [16]. There are no current screening guidelines for AMD in Australia. Detection is reliant on an individual experiencing symptoms and seeking ophthalmic review, or opportunistic recognition of disease during a routine assessment. The findings from this study will assist in the development of better screening guidelines for glaucoma and AMD.

Currently, risk estimation for developing glaucoma and AMD is based on a combination of demographic and clinical factors. The predictive ability of polygenic risk models for POAG and AMD are well established, particularly in European populations, and are summarised elsewhere [38]. For glaucoma, risk factors include increasing age, family history of glaucoma, African ancestry, and elevated IOP [6, 7]. Genetic risk has been largely estimated through family history alone. A positive family history carried a 9-fold risk for first-degree relatives compared to controls in one study, but this required full examination of all first degree relatives rather than self-report [39]. The accuracy of self-reported family history for glaucoma has been studied and found to be an unreliable measure as many patients are unaware of family members with diagnosed glaucoma, or have erroneous views as to what caused vision loss in relatives [40]. More recent data indicates that PRS provides a more accurate representation of risk with family history in an Australian population based study [20]. Several risk calculators have been developed to aid clinicians in screening and treatment decisions, however there remains no consensus regarding optimal timing and frequency of population screening for glaucoma [41, 42]. PRS provides a more accurate estimation of risk than traditional methods alone, with risk prediction optimised when all factors are combined [20]. AMD risk involves an interplay of genetic and environmental factors. There are several recognised environmental risk factors including age and smoking, with sex, ancestry, cardiovascular disease, and diet also suggested to be implicated [29]. A prediction model incorporating genetic, demographic and environmental risk factors was independently associated with incidence and prevalence of advanced AMD, all with strong predictive power [43]. Effective risk algorithms incorporating environmental, clinical and genetic risk factors will need to be developed. While environmental and clinical risk factors may change over time, the genetic contribution to overall risk will remain constant given genetic disease liability is fixed from conception. Therefore, an important benefit of polygenic risk testing is that PRS can be calculated at any stage of life and may be useful to inform disease prognosis and response to treatment before individuals exhibit vision loss.

Glaucoma genetic testing is currently limited to Mendelian genes (e.g. *MYOC)* which explain less than 5% of adult onset glaucoma [10, 11]. PRS testing, however, captures a much larger component of glaucoma genetic risk. Those with high polygenic risk had a comparable glaucoma risk to those with the most common Mendelian variant (OR 2.77 vs. OR 4.19), as well as being ~15 times more prevalent [10]. At present, genetic testing for AMD is not recommended and exists predominantly in research contexts [29, 44, 45] . Direct to consumer tests incorporating various PRS tests for both diseases are available, however these lack prospective evidence demonstrating their effectiveness [46, 47]. This study will assess the clinical validity of PRS testing in a sample representative of the general population in Australia in order to determine its application in the community.

We have previously demonstrated strong interest in polygenic risk testing for glaucoma among various groups, including those with diagnosed glaucoma, those with a first-degree relative with glaucoma, and those without any personal or family history of the condition [30, 48]. Although PRS testing for glaucoma was theoretically accepted, we identified a number of concerns and potential barriers to implementation, including residing in a rural location and unwillingness to pay for testing. There are a number of additional questions which must first be addressed before PRSs can be integrated into clinical practice.

Firstly, results must lead to actionable and cost-effective measures. Guidelines will be needed to clarify which PRS classifications warrant intervention. Those identified to be at high risk for developing glaucoma or AMD may receive more regular follow-up with an optometrist or ophthalmologist, allowing for timely treatment initiation. Treatment may be commenced before the disease becomes symptomatic. Early interventions for glaucoma may include topical IOP-lowering medication or laser therapy. Earlier surgical intervention may be considered for those with a PRS indicating a likelihood to progress rapidly or to advanced disease. While treatment options for early AMD are lacking, there are a large number of treatments under research including various pharmaceutical agents, gene therapies and surgical interventions [49]. Antioxidant supplements based on the Age-Related Eye Disease Studies (AREDS) may have benefit in those with intermediate disease in one or both eyes to reduce the risk of progressing to late AMD, or in those with late stage disease in only one eye to reduce the risk of developing it in the other eye [50]. Smoking is the only established modifiable risk factor for AMD, with the risk of progression to neovascular AMD shown to be double for those who had ever smoked [51]. Despite there being few treatment options for AMD, risk factor modification and antioxidant supplementation may still be valuable interventions in high-risk individuals. Progression from early to advanced AMD may occur rapidly and result in severe vision loss if treatment is delayed. Using tools such as an Amsler grid, individuals who are recognised to be at higher risk of this occurring could be educated to self-monitor for progression, with a pathway to access rapid assessment if symptomatic. Conversely, PRS may prevent unnecessary follow-up or treatment in those presumed to be at higher risk based on traditional risk prediction models. This may improve the cost-effectiveness of the PRS.

Secondly, it will be critical to develop frameworks which allow PRS results to be reported and communicated in a meaningful manner. Pilot reports need to be developed and tested to assess communication preferences and understanding of reported results among different stakeholders, including patients and healthcare professionals. We have previously demonstrated that the preferred method of receiving results may depend on the result itself, so report content and structure will likely vary depending on risk classification [30, 48]. This study will form the foundations of future research to develop our understanding of the clinical implementation of PRS testing for glaucoma and AMD.

Finally, there are a number of health economic elements which need to be considered before implementing PRS into clinical practice. Population-based screening for glaucoma or AMD is not currently cost-effective, so public health frameworks need to be developed which allow identification of those at increased risk while also ensuring adequate access to further treatment. Disease prevention is at the forefront of public health policy, and polygenic risk stratification has the potential to enhance primary, secondary and tertiary facets of this. Ultimately, enhanced disease screening will minimise the personal and economic costs of significant vision loss. Improved risk stratification will alleviate workload created by over investigation and treatment of those at high risk calculated using traditional risk factors, but at low genetic risk. However, it will be important to integrate genetic risk with clinical or environmental risk factors. Individuals with a strong family history would still be recommended to have regular clinical testing, even if shown to have a low PRS, due to the influence of Mendelian variants or other factors not covered by the PRS. We have shown that financial implications appear to be important to people and while some are unwilling to pay for testing the majority of individuals would be prepared to pay varying amounts [30]. Subsidisation may improve uptake, however will only be an option if it is cost-effective for the healthcare system which remains to be demonstrated.

Current PRSs for glaucoma or AMD are based on predominantly European populations and have not yet been comprehensively tested across other ethnicities.

Individuals of non-European ancestry are not excluded from the study, although the accuracy of their risk predictions may be reduced. Better validation of a single pan-ancestry PRS, or ancestry-specific scores covering all ethnicities, are a major unmet need to avoid future health disparities.

In conclusion, this prospective study aims to demonstrate the clinical validity of PRS to stratify individuals from the general population and identify those who are at high risk of developing glaucoma or AMD. This will help to move towards the implementation of PRS into clinical practice and provide an objective screening tool for glaucoma and AMD. The ability to identify at-risk individuals will allow for closer monitoring and timely intervention, and ultimately reduce irreversible vision loss. Further studies will need to look into how PRS testing could alleviate some of the socioeconomic burden resulting from vision loss. The outcomes from this study will form the basis for future interventional studies to further enable a shift in the detection, treatment and prevention of diseases with complex inheritance.

List of abbreviations

POAG	Primary open-angle glaucoma
AMD	Age-related macular degeneration
PRS	Polygenic risk score
IOP	Intra-ocular pressure
VEGF	Vascular endothelial growth factor
GWAS	Genome-wide association studies
SNP	Single nucleotide polymorphism
MTAG	Multitrait analysis of GWAS
OCT	Ocular coherence tomography
OAG	Open-angle glaucoma

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Authors' contributions
AQ, TN, JL, SM, DE, ES and JC contributed to the study conception and design. GH recruited participants and collected data. DT, JS and SM developed the data analysis tools (PRS pipeline). DT prepared Figs. 1 and 2. GH drafted the manuscript. All authors reviewed and approved the submitted manuscript.

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Data Availability
Data sharing is not applicable to this article as no datasets were generated or analysed during the current study.

Declarations

Competing interests
OM Siggs, S MacGregor and JE Craig report holding equity in Seonix Bio. S MacGregor, and JE Craig are listed as co-inventors on a patent application for the use of genetic risk scores to determine risk and guide treatment for glaucoma. Georgina L Hollitt, Ayub Qassim, Daniel Thomson, Joshua M Schmidt, Thi Thi Nguyen, John Landers, Emmanuelle Souzeau – None.

Ethics approval and consent to participate
This prospective cohort study was approved by the Southern Adelaide Clinical Human Research Ethics Committee (SAC HREC) and adheres to the Revised Declaration of Helsinki. HREC reference number: HREC/20/SAC/138. Informed consent was obtained from all individual participants included in the study.

Consent for publication
Not applicable.

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References
1. Smith W, Assink J, Klein R, et al. Risk factors for age-related macular degeneration: pooled findings from three continents. Ophthalmology. 2001;108(4):697–704.
2. Tham YC, Li X, Wong TY, Quigley HA, Aung T, Cheng CY. Global prevalence of glaucoma and projections of glaucoma burden through 2040: a systematic review and meta-analysis. Ophthalmology. 2014;121(11):2081–90.
3. Enoch J, McDonald L, Jones L, Jones PR, Crabb DP. Evaluating whether Sight is the most valued sense. JAMA Ophthalmol. 2019;137(11):1317–20.
4. Taylor HR, Pezzullo ML, Keeffe JE. The economic impact and cost of visual impairment in Australia. Br J Ophthalmol. 2006;90(3):272–5.
5. Kwon YH, Fingert JH, Kuehn MH, Alward WLM. Primary open-angle glaucoma. N Engl J Med. 2009;360(11):1113–24.
6. Gordon MO, Beiser JA, Brandt JD, et al. The ocular hypertension treatment study: baseline factors that predict the onset of primary open-angle glaucoma. Arch Ophthalmol. 2002;120(6):714–20. discussion 829–830.
7. Weih LM, Nanjan M, McCarty CA, Taylor HR. Prevalence and predictors of open-angle glaucoma: results from the visual impairment project. Ophthalmology. 2001;108(11):1966–72.
8. Weinreb RN, Khaw PT. Primary open-angle glaucoma. Lancet. 2004;363(9422):1711–20.
9. Mitchell P, Smith W, Attebo K, Healey PR. Prevalence of open-angle glaucoma in Australia. The Blue Mountains Eye Study. Ophthalmology. 1996;103(10):1661–9.
10. Charlesworth J, Kramer PL, Dyer T, et al. The path to open-angle glaucoma gene discovery: endophenotypic status of intraocular pressure, cup-to-disc ratio, and central corneal thickness. Invest Ophthalmol Vis Sci. 2010;51(7):3509–14.
11. Siggs OM, Han X, Qassim A, et al. Association of monogenic and polygenic risk with the prevalence of Open-Angle Glaucoma. JAMA Ophthalmol. July. 2021.
12. Khawaja AP, Viswanathan AC. Are we ready for genetic testing for primary open-angle glaucoma? Eye. 2018;32(5):877–83.
13. Wong WL, Su X, Li X, et al. Global prevalence of age-related macular degeneration and disease burden projection for 2020 and 2040: a systematic review and meta-analysis. Lancet Glob Health. 2014;2(2):e106–16.
14. Klaver CC, Assink JJ, van Leeuwen R, et al. Incidence and progression rates of age-related maculopathy: the Rotterdam Study. Invest Ophthalmol Vis Sci. 2001;42(10):2237–41.
15. Age-Related Eye Disease Study Research Group. A randomized, placebo-controlled, clinical trial of high-dose supplementation with vitamins C and E, beta carotene, and zinc for age-related macular degeneration and vision loss: AREDS report no. 8. Arch Ophthalmol. 2001;119(10):1417–36.
16. Rosenfeld PJ, Brown DM, Heier JS, et al. Ranibizumab for neovascular age-related macular degeneration. N Engl J Med. 2006;355(14):1419–31.
17. Seddon JM, Cote J, Page WF, Aggen SH, Neale MC. The US twin study of age-related macular degeneration: relative roles of genetic and environmental influences. Arch Ophthalmol. 2005;123(3):321–7.
18. National Health and Medical Research Council. NHMRC Guidelines for the Screening, Prognosis, Diagnosis, Management and Prevention of Glaucoma.

2010:1-181. https://www.nhmrc.gov.au/about-us/publications/guidelines-screening-prognosis-diagnosis-management-and-prevention-glaucoma
19. Tamura H, Akune Y, Hiratsuka Y, et al. Real-world effectiveness of screening programs for age-related macular degeneration: amended Japanese specific health checkups and augmented screening programs with OCT or AI. Jpn J Ophthalmol. 2022;66(1):19–32.
20. Lambert SA, Abraham G, Inouye M. Towards clinical utility of polygenic risk scores. Hum Mol Genet. 2019;28(R2):R133–42.
21. Lewis CM, Vassos E. Prospects for using risk scores in polygenic medicine. Genome Med. 2017;9(1):96.
22. Craig JE, Han X, Qassim A, et al. Multitrait analysis of glaucoma identifies new risk loci and enables polygenic prediction of disease susceptibility and progression. Nat Genet. 2020;52(2):160–6.
23. MacGregor S, Ong JS, An J, et al. Genome-wide association study of intraocular pressure uncovers new pathways to glaucoma. Nat Genet. 2018;50(8):1067–71.
24. Qassim A, Souzeau E, Siggs OM, et al. An intraocular pressure polygenic risk score stratifies multiple primary Open-Angle Glaucoma parameters including treatment intensity. Ophthalmology. 2020;127(7):901–7.
25. Springelkamp H, Iglesias AI, Mishra A, et al. New insights into the genetics of primary open-angle glaucoma based on meta-analyses of intraocular pressure and optic disc characteristics. Hum Mol Genet. 2017;26(2):438–53.
26. Gao XR, Huang H, Kim H. Polygenic risk score is Associated with intraocular pressure and improves Glaucoma prediction in the UK Biobank Cohort. Transl Vis Sci Technol. 2019;8(2):10.
27. Fritsche LG, Igl W, Bailey JNC, et al. A large genome-wide association study of age-related macular degeneration highlights contributions of rare and common variants. Nat Genet. 2016;48(2):134–43.
28. Han X, Gharahkhani P, Mitchell P, Liew G, Hewitt AW, MacGregor S. Genome-wide meta-analysis identifies novel loci associated with age-related macular degeneration. J Hum Genet. 2020;65(8):657–65.
29. Ding Y, Liu Y, Yan Q, et al. Bivariate analysis of age-related Macular Degeneration Progression using genetic risk scores. Genetics. 2017;206(1):119–33.
30. Heesterbeek TJ, de Jong EK, Acar IE, et al. Genetic risk score has added value over initial clinical grading stage in predicting disease progression in age-related macular degeneration. Sci Rep. 2019;9(1):1–8.
31. Warwick A, Lotery A. Genetics and genetic testing for age-related macular degeneration. Eye. 2018;32(5):849–57.
32. Hollitt GL, Siggs OM, Ridge B, et al. Attitudes towards polygenic risk testing in individuals with Glaucoma. Ophthalmol Glaucoma November. 2021.
33. 1000 Genomes Project Consortium, Auton A, Brooks LD, et al. A global reference for human genetic variation. Nature. 2015;526(7571):68–74.
34. Qassim A, Mullany S, Abedi F, et al. Corneal stiffness parameters are predictive of structural and functional progression in Glaucoma suspect eyes. Ophthalmology. 2021;128(7):993–1004.
35. Souzeau E, Goldberg I, Healey PR, et al. Australian and New Zealand Registry of Advanced Glaucoma: methodology and recruitment. Clin Exp Ophthalmol. 2012;40(6):569–75.
36. Mitchell P, Smith W, Attebo K, Wang JJ. Prevalence of age-related maculopathy in Australia. The Blue Mountains Eye Study. Ophthalmology. 1995;102(10):1450–60.
37. Wang R, Wiggs JL. Common and rare genetic risk factors for glaucoma. Cold Spring Harb Perspect Med. 2014;4(12):a017244.
38. Wang K, Gaitsch H, Poon H, Cox NJ, Rzhetsky A. Classification of common human diseases derived from shared genetic and environmental determinants. Nat Genet. 2017;49(9):1319–25.
39. Qassim A, Souzeau E, Hollitt G, Hassall MM, Siggs OM, Craig JE. Risk stratification and clinical utility of polygenic risk scores in Ophthalmology. Transl Vis Sci Technol. 2021;10(8):14.
40. Wolfs RC, Klaver CC, Ramrattan RS, van Duijn CM, Hofman A, de Jong PT. Genetic risk of primary open-angle glaucoma. Population-based familial aggregation study. Arch Ophthalmol. 1998;116(12):1640–5.
41. McNaught AI, Allen JG, Healey DL, et al. Accuracy and implications of a reported family history of glaucoma: experience from the Glaucoma inheritance study in Tasmania. Arch Ophthalmol. 2000;118(7):900–4.
42. Ocular Hypertension Treatment Study Group, European Glaucoma Prevention Study Group, Gordon MO, et al. Validated prediction model for the development of primary open-angle glaucoma in individuals with ocular hypertension. Ophthalmology. 2007;114(1):10–9.
43. Mansberger SL, Cioffi GA. The probability of glaucoma from ocular hypertension determined by ophthalmologists in comparison to a risk calculator. J Glaucoma. 2006;15(5):426–31.
44. Seddon JM, Reynolds R, Maller J, Fagerness JA, Daly MJ, Rosner B. Prediction model for prevalence and incidence of advanced age-related macular degeneration based on genetic, demographic, and environmental variables. Invest Ophthalmol Vis Sci. 2009;50(5):2044–53.
45. Stone EM, Aldave AJ, Drack AV, et al. Recommendations for genetic testing of inherited eye diseases: report of the American Academy of Ophthalmology task force on genetic testing. Ophthalmology. 2012;119(11):2408–10.
46. Csaky KG, Schachat AP, Kaiser PK, Small KW, Heier JS. The use of genetic testing in the management of patients with age-related Macular Degeneration: american society of retina specialists Genetics Task Force Special Report. J VitreoRetinal Dis. 2017;1(1):75–8.
47. Tandy-Connor S, Guiltinan J, Krempely K, et al. False-positive results released by direct-to-consumer genetic tests highlight the importance of clinical confirmation testing for appropriate patient care. Genet Med. 2018;20(12):1515–21.
48. Sanfilippo PG, Kearns LS, Wright P, Mackey DA, Hewitt AW. Current landscape of direct-to-consumer genetic testing and its role in ophthalmology: a review. Clin Exp Ophthalmol. 2015;43(6):578–90.
49. Hollitt GL, Siggs OM, Ridge B, et al. Attitudes towards glaucoma genetic risk assessment in unaffected individuals. bioRxiv Oct. 2021.
50. Hernández-Zimbrón LF, Zamora-Alvarado R, Ochoa-De la Paz L, et al. Age-related Macular Degeneration: New Paradigms for Treatment and Management of AMD. Oxid Med Cell Longev. 2018;2018:8374647.
51. Age-Related Eye Disease Study Research Group, SanGiovanni JP, Chew EY, et al. The relationship of dietary carotenoid and vitamin A, E, and C intake with age-related macular degeneration in a case-control study: AREDS Report No. 22. Arch Ophthalmol. 2007;125(9):1225–32.
52. Age-Related Eye Disease Study Research Group. Risk factors associated with age-related macular degeneration. A case-control study in the age-related eye disease study: Age-Related Eye Disease Study Report Number 3. Ophthalmology. 2000;107(12):2224–32.

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