

Some devastating childhood diseases are inherited within families. Of potentially blinding inherited eye disorders, paediatric cataract and primary congenital glaucoma (PCG) are 2 major causes of childhood blindness.

Cataract is an opacity of the ocular lens that impairs vision. Paediatric cataract is a genetically and clinically heterogeneous condition with an incidence of 2.2 per 10,000 live births in Australia, with 8-25% of cases being hereditary. PCG is the most common type of childhood glaucoma. The condition is caused by a developmental defect of the trabecular meshwork and anterior chamber angle resulting in disruption in drainage of the aqueous humor and an increased intraocular pressure (IOP) resulting in damage to the optic nerve and subsequent visual loss. In Australia the incidence of PCG is 1 per 30000.

The overall aim of this project was to use Massively Parallel sequencing (MPS) technologies to mine our paediatric cataract and PCG DNA repository for genetic mutations in known candidate genes, and identify new paediatric cataract and PCG causing genes. Furthermore, this project aimed at providing a molecular genetic diagnosis to the affected family members in our repository.

Phase 1 aimed to screen our world leading repository of DNA samples from patients with familial or sporadic paediatric cataracts or PCG for mutations in candidate genes. We screened a novel PCG candidate gene, *TEK* (a recently identified gene), in our cohort of 53 Australian PCG cases, aiming to evaluate the association between variations in *TEK* and the disease in Australia. Five heterozygous protein changing variants in *TEK* were detected and this gene showed significant enrichment for mutations in PCG patients. In conjunction with functional data generated by our collaborators, these data indicate that mutations in the *TEK* gene are likely associated with PCG.

A total of 98 samples with paediatric cataract (65 from Australia and 33 from Asia) were screened for mutations in 51 previously reported paediatric cataract genes. The study was able to detect the genetic cause behind 42% of Australian patients with familial paediatric cataracts, 40% of sporadic Australian cases and in 23% of the Asian cohort.

In phase 2 we undertook gene discovery in families (paediatric cataract or PCG) with no identifiable causes in known genes using next generation whole exome sequencing. Two novel candidate genes for paediatric cataract (*HTRIF* and *NOL9*) and one for PCG (*GREB1*) were identified. In addition, for the first time, we identified a copy number variant (CNV) of a crystallin gene in an Australian family with paediatric cataract, also identified through whole exome sequencing.

This study demonstrated the feasibility of using next generation sequencing technologies to screen genes panels in a heterogeneous condition like paediatric cataract and the potential for this technology in novel gene discovery. Furthermore, for the first time it showed the possibility of the involvement of copy number variation in isolated paediatric cataract pathogenesis. In addition a higher mutation rate in the *TEK* gene was detected in PCG cohort than in the general population which supports its association with PCG development. Further investigation is required to determine the role of the novel candidate genes identified in this study in PCG and paediatric cataract pathogenicity.