In-depth characterisation of the Alms1$^{foz/foz}$ mouse model of Alström syndrome

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Thesis summary

Monogenic causes of obesity and type 2 diabetes mellitus (T2DM) include Alström syndrome (AS). AS belongs to an interesting new class of disorders called ciliopathies, which have a common origin in gene mutations causing dysfunction of an important cellular organelle known as the primary cilium. AS is inherited as an autosomal recessive disorder caused by a mutation in the ALMS1 gene that leads to an extensive clinical phenotype encompassing childhood metabolic disorders, retinal degeneration, sensorineural deafness, cardiomyopathy and infertility. Thus, research into ciliopathies including AS represents an exciting novel focus in a wide range of research fields including endocrinology and neurosciences.

The "Fat Aussie" (FA) mouse or Alms1$^{foz/foz}$ is a model for AS that carries a spontaneous deletion (*foz*) in the exon 8 of the ALMS1 gene. The Alms1$^{foz/foz}$ mouse recapitulates the disorders occurring in AS patients and represents a unique opportunity to further characterise the pathogenic mechanisms underlying ciliopathy-associated obesity, insulin resistance and T2DM. The metabolic phenotyping study of Alms1$^{foz/foz}$ mice has revealed that early peripheral insulin resistance is an inherent primary consequence of the ALMS1 gene disruption at a time that β-cell function isn't affected. Insulin resistance may thereby drive the subsequent metabolic complications in the Alms1$^{foz/foz}$ mouse model. Outcomes from this study also suggest that the defect leading to insulin resistance in Alms1$^{foz/foz}$ mice

must be downstream of AS160 phosphorylation in the insulin pathway and might concern either the translocation of GLUT4 or its recycling.

Female NOD/Alms1$^{foz/foz}$ mice were then used as a new model to investigate the intricate relationship between metabolic disturbances such as obesity and T2DM and the onset of type 1 diabetes mellitus (T1DM). Surprisingly, NOD/Alms1$^{foz/foz}$ mice were protected against T1DM. Data showed that β cell destruction was significantly suppressed in NOD/Alms1$^{foz/foz}$ mice which had intact hyperplastic β-islets, limited immune cell infiltration and unaltered insulin secretory capacity. Thus, metabolic disturbances in NOD/Alms1$^{foz/foz}$ mice may paradoxically inhibit the development of T1DM.

New features, which have not been described before in AS mouse models, have been highlighted in this project. Alms1$^{foz/foz}$ mice displayed early mild cognitive impairment worsening with age suggesting defective neuronal function. The axonal transport of Alms1 protein further suggests a possible involvement of Alms1 in neuronal protein trafficking. Neuroendocrine chromaffin cells from Alms1$^{foz/foz}$ mice showed a reduced exocytosis rate but unimpaired pore fusion kinetics. Together, these data suggest a possible involvement of the Alms1 protein in neuronal signalling and vesicle trafficking.

This project has helped to better characterise the underlying defects that drive the FA mouse model of AS to multi-organ pathology by finding clues

to Alms1 protein function. Research into unravelling Alms1 protein function should not only lead to improved treatments for patients with AS, but also provide a better understanding of cellular pathways involved in more common disorders such as obesity and T2DM.

Declaration

'I certify that this thesis does not incorporate without acknowledgement any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the test.'

Dorothée Angélique Gaëlle Girard

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Publications and Seminars

Review

1. **Dorothée Girard** and Nikolai Petrovsky. Alström syndrome: insights into the pathogenesis of metabolic disorders. Nature Reviews Endocrinology 7, 77–88 (2011).

Conference abstracts arising from this thesis

1. **Dorothée Girard,** Nikolai Petrovsky. Investigation of Alms1 protein function using the "Fat Aussie" mouse model of human Alström syndrome. Workshop: "France and South Australia – Current Joint Activities in Science & Technology and Future Directions for Cooperation". The South Australian Department of the Premier and Cabinet and the Embassy of France in Australia, Adelaide, 24th July 2009. Oral presentation.

2. **Dorothee Girard**, Nikolai Petrovsky. Increased insulin resistance compounded by reduced insulin sensitivity drives the Fat Aussie (Alms1$^{foz/foz}$) model of Alström syndrome towards obesity and type 2 diabetes mellitus. Cilia 2012 conference – Cilia in Development and Disease, London, 17-18th May 2012. Poster presentation.

Other conference abstracts

1. **Dorothée Girard**, Linyan Wu, Christian Schoenbach, Nikolai Petrovsky. Novel translational frameshift mechanism contributing to genome complexity. Adelaide Immunology Retreat, Annual event of the Australasian Society for Immunology (ASI), Normanville, 12th-13th Sept 2008. Oral presentation.

2. **Dorothée Girard**, Michelle Lui, Nikolai Petrovsky. Obesity-associated impaired vaccine responsiveness in Alms1$^{foz/foz}$ mice is compensated by a novel polysaccharide adjuvant. Medical research week, SA scientific meeting, Australian Society for Medical Research (ASMR), Adelaide, 2nd June 2009. Oral presentation.

Awards

1. Immune Strategies Area of Strategic Research Investment (ASRI) Postgraduate Scholarship (1st July 2008-30th June 20).

2. Seeding Grant 2008, Faculty of Health Sciences Research Grants 2008, Flinders University.

3. Finalist of the 2009 AusBiotech-GSK student excellent awards South Australia with the project "Obesity-associated impaired vaccine responsiveness in Alms1$^{foz/foz}$ mice is compensated by a novel polysaccharide adjuvant."

Abbreviations

5HT6	5-hydroxytryptamine receptor 6
ABR	Auditory brain response
AGRP	Agouti-related protein
AS	Alström syndrome
AS160 (or TBC1D4)	Akt Substrate of 160 kDa
ARC	Arcuate nucleus
AU	Arbitrary unit
BBS	Bardet-Biedl syndrome
BDNF	Brain-Derived Neurotrophic Factor
BMI	Body mass index
bp	Base pair
CART	Cocaine and amphetamine related transcript
C/EBP-α	CCAAT/enhancer-binding protein alpha
CNS	Central nervous system
CPE	Carboxypeptidase E
CY	Cyclophosphamide
DAPI	Diamidino-2-phenylindole
DCM/CHF	Dilated cardiomyopathy with congestive heart failure
EGF	Epidermal growth factor
ER	Endoplasmic reticulum
ERG	Electroretinogram
FA	Fat Aussie mouse strain

GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
GH	Growth hormone
GLP	Glucagon like peptide
Glut 4	Glucose transporters 4
H&E	Hematoxylin and eosin
HFD	High fat diet
HOMA	Homeostatic model assessment
IFT	Intraflagellar transport
IGF	Insulin-like growth factor
INPP5E	Inositol polyphosphate-5-phosphatase E
IPGTT	Intraperitoneal glucose tolerance test
IR	Insulin resistance
IRAP	Insulin-regulated aminopeptidase
IRS1	Insulin receptor substrate 1
ITT	Insulin tolerance test
JS	Joubert syndrome
KLH	Keyhole Limpet Hemocyanin
KRPH	Krebs Ringer Phosphate Hepes
Mb	Megabase
MCHR1	Melanin concentrating hormone receptor 1
MCP	Macrophage chemoattractant proteins
MDCK	Madin-Darby canine kidney
MJ	Megajoule

MORM	Mental retardation, truncal obesity, retinal dystrophy and micropenis
NA	Not applicable
ND	Normal diet
NOD	Non obese diabetic
NPY	Neuropeptide Y
NS	Not significant
Nt	Nucleotide
OMIM	Online Mendelian Inheritance in Man
p75NTR	p75 neurotrophin receptor
PEI	Polyethylenimine
PBS	Phosphate buffered saline
PBST	Phosphate buffered saline with Tween 20
PKD	Polycystic kidney disease
POMC	Propiomelanocortin
RD	Restricted diet
RFX	Regulatory factor X
RNA	Ribonucleic acid
RP	Retinitis pigmentosa
RT	Room temperature
SC	Subcutaneous
SEM	Standard error of the mean
Shh	Sonic hedgehog
SNAP	Synaptosomal associated protein

SSTR3	Somatostatin receptor 3
T1DM	Type 1 diabetes mellitus
T2DM	Type 2 diabetes mellitus
UTI	Urinary tract infection
VR2	Vasopressin receptor 2
WAT	White adipose tissue
WT	Wildtype