DISSECTING THE GENETICS OF AUTOIMMUNE DISEASES

Thesis submitted by

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STATEMENT

The research contained within this study was performed in the Medical Genomics Laboratory in the Comparative Genomics Centre at James Cook University, Townsville, under the supervision of Professor Alan Baxter. All research procedures reported in the thesis received the approval of James Cook's Animal Ethics Committee. The data presented is my own work, with all contributions from others clearly stated in the acknowledgements, methods and the body of the thesis.

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ABSTRACT

Autoimmune diseases occur when the immune system mistakenly attacks its own healthy tissue. There are more than 80 different types of autoimmune diseases and they are classified as either systemic or tissue specific depending on the antigen/s targeted and the effector mechanism involved. Although not necessarily pathological, autoimmunity may lead to clinically relevant tissue damage in some individuals. Three-point-five percent of Western populations are afflicted, causing a huge burden on the country's resources. Many of the diseases are related with members of a cluster commonly occurring in an individual or a family suggesting some commonality in inheritance. Uncovering genes involved in one autoimmune disease may therefore also be relevant in other autoimmune diseases as well as in their underlying mechanisms. The "candidate gene" approach was for many years the only option for tackling the genetics of complex diseases but although biologically sound it led to initial elation at discovering a gene being turned to disappointment when it didn't stand up to scrutiny and/or could not be replicated by independent researchers. As the effects of a single gene may be small and the disease or animal model often pleiotropic with overlapping phenotypes, family based linkage studies too have achieved only limited success, mainly due to limitations in identifying common variants with modest effects. In the course of this thesis, I have used mouse models of disease with previously identified linkage regions and the powerful tools of a positional cloning approach coupled with microarray gene expression analyses to identify genes contributing to an important immuno-regulatory cell type, NKT cell number, as well as genes contributing to experimental autoimmune gastritis. Candidate genes identified were subsequently validated by real time PCR and FACS analyses on new sample sets

and polymorphic differences in gene structure were identified between strains positive for the phenotype compared to those phenotypically negative for it as a possible explanation for the observed differential expression patterns. To this end, thirty-nine sub-congenic mouse lines of the gastritis linkage region on chromosome 4 were produced and microarray gene expression analyses were carried out on the most informative of these to reveal at least four chromosomal regions contributing to the gastritis phenotype. Two of these regions contain a single candidate gene: *Cap1* and *Apitd1* that are both involved in apoptosis. A subcongenic approach to identifying NKT cell genes revealed a minimum of four candidates, with at least one on chromosome 1 and three or more on chromosome 2. One of the candidate genes, *Slamf1*, was subsequently confirmed through transgenic complementation, while a previously unknown role for peroxisomes in NKT cell biology identified by microarray analyses and confirmed using a knock-out mouse system.

ACKNOWLEDGEMENTS

Like most of the PhD students I know, my years of study have been both rewarding and frustrating. When I first decided to embark on a doing a PhD I was quite prepared for the hard “slog” but could not have predicted the number of extra obstacles I would have to deal with. Not only was I juggling work, study and keeping a home, but I went through one of the most difficult times of my life due to my father’s illness. With him being thousands of kilometers away and there being many times that things were “touch and go” I felt helpless and upset, and when he died, I went through a re-evaluation of my own life. The roller coaster of my life as a PhD student continued in tandem with things at home. There were many “highs” with good results and theories coming to fruition, coupled with “lows” due to mice not breeding as expected, not finding mice with the crucial genotypes and/or sexes to set up the new breeding pairs, or needing to go back to heterozygous mice when the only homozygotes succumbed to diabetes and had to be culled. The genetic contamination of the *Pex* knock-out mice, discovered following a year of establishing experimental strains, was followed by a further year of backcrossing and intercrossing to re-estabish the experimental lines. However, it was all worth-while when results reflected differences in NKT cell number between knock-out and wild-type sib-pairs. There were many frustrating moments in making the construct for the Slam transgenics, but again, I was elated when the mouse lines were finally established and phenotyping revealed the good news that all had not been in vain.

There are many people whom I need to acknowledge and thank for their efforts in ensuring that my project ran as smoothly as possible. Firstly, I would like to thank

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PUBLICATIONS ARISING FROM THIS STUDY

Primary papers:

1 **Jordan MA**, Fletcher JM, Jose R, Chowdhury S, Gerlach N, Allison J and Baxter AG. Role of SLAM in NKT cell development revealed by transgenic complementation in NOD mice. J Immunol. (2011) 186 (7)

2. Fletcher JM***, Jordan MA***, Snelgrove SL, Slattery RM, Pellicci D, Besra GS, Godfrey DI and Baxter AG. Congenic Analysis of the NKT Cell Control Gene *Nkt2* Implicates the Peroxisomal Protein *Pxmp4*. J. Immunol. (2008)181:3400-3412. (*** similar contribution**).

3. **Jordan MA***, Fletcher JM*, Pellici D and Baxter AG. *Slamf1* the NKT cell control gene *Nkt1*. J Immunol. (2007) 178(3):1618-27. (*** similar contribution**).

4. Ang DKY*, Brodnicki TC*, **Jordan MA**, Wilson WE, Gliddon BL, Baxter AG and van Driel IR. Two genetic loci independently confer susceptibility to autoimmune gastritis. Int Immunol. (2007)19(9):1135-44. Epub 2007 Aug 13. (* similar contribution).

5.Fletcher JM*, **Jordan MA***, Hawke C, Poulton L and Baxter AG. Genetic control of NK cell phenotype in autoimmune prone NOD mice associated with *Cd247* expression. In preparation. (*** similar contribution**).

Review articles:

1. **Jordan MA** and Baxter AG. The genetics of immunoregulatory T cells. J Autoimmun. (2008) 31:237-244. PMID: 18550334

2. **Jordan MA** and Baxter AG. Quantitative and Qualitative Approaches to GOD: The first ten years of the clonal selection theory. Immunology and Cell Biology (2008) 86:72-79. E-Pub 2007; doi:10.1038/sj.icb.7100140

3. Biros E, **Jordan MA**, Baxter AG. Genes mediating environment interactions in type 1 diabetes. Diabetes Studies (2006) 2(4):192-207.

4. Baxter AG, Silveira PA, Wilson WE, **Jordan MA** and van Driel IR. Genetic control of susceptibility to autoimmune gastritis. Int Rev Immunol (2005) 24(1-2):55-62.

Other publications:

1. Baune BT , Dannlowski U, Domschke K, Janssen DGA, **Jordan MA**, Ohrmann P, Bauer J, Biros E, Arolt V, Kugel H, Baxter AG, Suslow T. The *Interleukin 1 Beta* (*IL1B*) Gene Is Associated with Failure to Achieve Remission and Impaired Emotion Processing in Major Depression. Biol Psychiatry (2009) Dec 29. [Epub ahead of print] PMID: 20044070.

2. **Jordan MA**, Poulton LD, Fletcher JM and Baxter AG. Allelic variation of *Ets1* does not contribute to NK and NKT cell deficiencies in type 1 diabetes susceptible NOD mice. Rev Diabetic Stud (2009) 6: 104-116.

ABBREVIATIONS

α-GalCer	-	α-Galactosylceramide
β2M	-	β2 microglobulin
[γ^{32}P]ATP	-	[γ^{32}P] Adenosine 5' –triphosphate
^{3}H	-	tritiated thymidine
^{51}Cr	-	^{51}Chromium
Ab	-	Antibody
Abs	-	Antibodies
ACR	-	American College of Rheumatology
ADCC	-	Antibody-dependent cellular cytotoxicity
AHA	-	Autoimmune haemolytic anaemia
AICD	-	Activation induced cell death
AIH	-	Autoimmune Hepatitis
Aire	-	Autoimmune regulator
ALPS	-	Autoimmune lymphoproliferative syndrome
ANA	-	Antinuclear antibodies
AOD	-	Autoimmune Ovarian Dysgenesis
APC	-	Antigen Presenting Cell
APECED	-	Autoimmune polyendocrinopathy-candidiasis ectodermal dystrophy
APS-1	-	Autoimmune Polyendocrinopathy Syndrome type 1
Aq	-	Aqueous solution
Asp	-	Aspartic Acid
ATP	-	Adenosine triphosphate
ATPase	-	Adenosine Triphosphatase

BB	-	Bio-Breeding
BBB	-	Blood-brain barrier
BC1	-	Backcross 1 generation
BCG	-	Bacille Calmette-Guèrin
BMC	-	Bone-marrow chimera
bp	-	Base-pair
BrdU-5	-	Bromo-2'-deoxy-uridine
BSA	-	Bovine Serum Albumin
Ca	-	Calcium
CB4	-	Coxsackievirus B4 strain
Cbl	-	Casitas B-lineage lymphoma protein
CBP	-	CREB-binding protein
CDR	-	Complementary determining region
CEAT	-	Chronic experimental autoimmune thyroiditis
CFA	-	Complete Freund's adjuvant
CFSE	-	carboxyfluorescein succinimydyl
CIA	-	Collagen Induced Arthritis
CLT	-	Chronic lymphocyte thyroiditis
cM	-	CentiMorgan
CNS	-	Central nervous system
CO_2	-	Carbon dioxide
Con A	-	Concanavalin A
CPM	-	Counts per minute
CTLA-4	-	Cytotoxic T lymphocyte antigen 4
Cyclo.	-	Cyclophoshamide
d3Tx	-	Day 3 Thymectomy
dATP	-	Deoxyadenosine Triphosphate

DC	-	Dendritic cell
dCTP	-	Deoxycytidine Triphosphate
dGTP	-	Deoxyguanosine Triphosphate
DN	-	Double negative ($CD4^-CD8^-$) thymocyte
DNA	-	Deoxyribonucleic Acid
dNTP	-	Deoxyribonucleotide triphosphate
DP	-	Double positive ($CD4^+CD8^+$) thymocyte
DR	-	Diabetes-resistant
dsDNA	-	Double Stranded Deoxyribonucleic Acid
DTH	-	Delayed type hypersensitivity
dTTP	-	deoxythymidine triphosphate
EAE	-	Experimental Autoimmune Encephalomyelitis
EAG	-	Experimental Autoimmune Gastritis
EAT	-	Experimental Autoimmune Thyroiditis
ELISA	-	Enzyme Linked Immunosorbent Assay
EOA	-	Experimental Allergic Orchitis
Erk	-	Extracellular signal-regulated kinase
$FADH_2$	-	Flavin adenine dinucleotide
FasL	-	Fas Ligand
FCS	-	Foetal calf serum
FcγR	-	Fc gamma receptor
FITC	-	Fluoroscein isothiocyanate
GAD	-	Glutamic Acid Decarboxylase
GBM	-	Glomerular basement membrane
GD	-	Graves' Disease
GEF	-	Guanine exchange factor
gld	-	Generalized Lymphoproliferative Disorder

GN	-	Glomerulonephritis
GRR	-	Genotype Risk Ratio
H2	-	Histocompatibilty 2
HA	-	Haemolytic Anaemia
HEL	-	Hen egg lysozyme
HgCl2	-	Mercuric chloride
HLA	-	Human Leukocyte Antigen
i.p.	-	Intraperitoneal
i.v.	-	Intravascular
IC	-	Immune complex
IC1	-	Intercross 1
ICA	-	Islet cell antibodies
Idd	-	Murine insulin-dependent diabetes
IDDM	-	Human insulin-dependent diabetes mellitus
IFA	-	Incomplete Freund's adjuvant
IFN	-	Interferon
Ig	-	Immunoglobulin
IL	-	Interleukin
Ins	-	Insulin
IPEX	-	Immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome
IRPB	-	Interphotoreceptor retinoid-binding protein
ITAM	-	Immuno-receptor tyrosine-based activation motifs
ITIM	-	Immuno-receptor tyrosine-based inhibitory motifs
IVT	-	In vitro transcription
Kb	-	Kilobasepairs
kD	-	Kilo Dalton

KI	-	Knock-in
KIR	-	Killer immunoglobulin-like receptor
KLH	-	Keyhole limpet hemocyanin
KO	-	Knock-out
Lck	-	Lymphocyte-specific protein tyrosine kinase
LCMV	-	Lymphocytic choriomeningitis virus
LD	-	Linkage Disequilibrium
LN	-	Lupus nephritis
LOD	-	Logarithm of odds
lpr	-	Lymphoproliferation
LPS	-	Lipopolysaccharide
LRC	-	Leukocyte receptor complex
LRS	-	Likelihood Ratio Statistic
LT	-	Lymphotoxin
MAb	-	Monoclonal antibody
MASPs	-	Marker assisted selection protocols
Mb	-	Mega-basepairs
MBP	-	Myelin Basic Protein
MCMV	-	Murine cytomegalovirus
$MgCl_2$	-	Magnesium chloride
MGD	-	Mouse Genome Database
MHC	-	Major histocompatability complex
MIT	-	Massachusetts Institute of Technology
MOG	-	Myelin oligodendrocyte glycoprotein
mOVA	-	Membrane ovalbumin
mRNA	-	Messenger RNA
MS	-	Multiple Sclerosis

NAD	-	Nicotinamide adenine dinucleotide
NADPH	-	Nicotinamide adenine dinucleotide phosphate-oxidase
Neo	-	Neomycin resistance cassette
NK	-	Natural Killer
NKC	-	Natural killer complex
NKT cell	-	Natural Killer T cell
NLS	-	Nuclear localisation signal
NOD	-	Nonobese diabetic
NON	-	Non-Obese non-diabetic
NPC	-	Niemann-Pick type C disease
NS	-	Not Significant
NZB	-	New Zealand Black
NZBW	-	(NZB x NZW) F1
NZW	-	New Zealand White
OVA	-	Ovalbumin
OxLDL	-	Oxidized low-density lipoproteins
PAGE	-	Polyacrylamide Gel Electrophoresis
PBL	-	Peripheral blood lymphocyte
PBMC	-	Peripheral blood mononuclear cell
PBS	-	Phosphate Buffered Saline
PCR	-	Polymerase Chain Reaction
PE	-	Phycoerythrin
PI	-	Propidium iodide
PLP	-	Proteolipid protein
PMP	-	Peroxisomal membrane protein
PNK	-	Polynucleotide kinase
poly I:C	-	Polyinosine-polycytodylic acid

PPAR		Peroxisome proliferator-activated receptors
Pro	-	Proline
PTEN	-	Phosphatase and tensin homolog
QRTPCR	-	Quantitative real-time PCR
QTL	-	Quantitative Trait Locus
RA	-	Rheumatoid Arthritis
RARα	-	Retinoic acid receptor alpha
RAG	-	Recombination-activity gene
RBC	-	Red Blood Cell
RPM	-	Revolutions per minute
RT	-	Room temperature
SD	-	Standard Deviation
SDS	-	Sodium dodecyl sulphate
SEB	-	Staphylococcal enterotoxin β superantigen
SEM	-	Standard error of mean
Ser	-	Serine
SHP-1	-	Haemopoietic cell phosphatase
SLE	-	Systemic Lupus Erythematosus
SNP	-	Single Nucleotide Polymorphism
SP	-	Single positive ($CD4^+CD8^-$ / $CD4^-CD8^+$) thymocyte
ssDNA	-	Single Stranded Deoxyribonucleic Acid
SSLP	-	Simple sequence length polymorphism
ssRNA	-	Single Stranded Ribonucleic Acid
SV40	-	Simian virus 40
T1D	-	Type 1 diabetes
TCR	-	T cell receptor
TEC	-	Thymic epithelial cell

TGFβ	-	Transforming growth factor β
Th	-	T helper
Th1	-	T helper type 1
Th2	-	T helper type 2
Thr	-	Threonine
TNF	-	Tumour Necrosis Factor
TP	-	Total protein
Treg	-	$CD4^+CD25^+$ $FoxP3^+$ regulatory T cell
Ts	-	'Suppressor' T cell
TUNEL	-	TdT-mediated dUTP-biotin nick end labelling
UK	-	United Kingdom
UV	-	Ultraviolet
WT	-	Wild-type

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