THE UNIVERSITY OF QUEENSLAND

AUSTRALIA

Immunogenetic studies of Guillain - Barré syndrome and chronic inflammatory demyelinating polyradiculoneuropathy

Stefan Blum

Dr. med., FRACP

A thesis submitted for the degree of Doctor of Philosophy at

The University of Queensland in 2014

School of Medicine

Abstract

Guillain-Barre syndrome (GBS) is an acute inflammatory neuropathy that is frequently triggered by an infectious illness. It is thought to be autoimmune in origin, but differs from other autoimmune diseases in being of male predominance and not showing a HLA-association.

Chronic inflammatory demyelinating polyradiculoneuropathy is a chronic inflammatory neuropathy, which shares some pathological features with GBS.

We investigated the clinical features of GBS in an Australian cohort of subjects by performing a retrospective chart review. We obtained subjects from several public hospitals and private neurologists in Queensland and New South Wales.

We found 335 patients, for 228 of whom electrophysiological data was available. Of these acute inflammatory demyelinating polyradiculoneuropathy (AIDP) with 168 cases was the most common form, whilst axonal and focal forms were rarer. We identified upper respiratory tract infections, occurring predominantly in winter months, as the most frequently named triggering factor for GBS. The illness severity in our cohort was similar to that in earlier Australian studies.

Also, we identified a cohort of 78 subjects fulfilling diagnostic criteria for CIDP from similar sources.

We investigated a possible underlying genetic susceptibility to develop GBS or CIDP by contacting members of this cohort and collecting their DNA. We embarked on both a candidate gene approach to investigate the killer immunoglobulin-like receptors (KIRs) and their HLA ligands, as well as performing a genome-wide association study (GWAS) of the subjects with GBS as a hypothesis-free method.

For the KIR/HLA system, whilst no significant differences were detected in the frequency of KIR genes, HLA-C2 and HLA-B Bw4-T were more frequent in subjects with GBS than controls ($p<0.001$). The inhibitory pairs of KIR-2DL2/HLA-C2 and KIR-3DL1/HLA-B Bw4-T were more frequent in GBS than controls ($p<0.005$). Whilst there were some differences between CIDP subjects and controls, interestingly, there were no difference between subjects with GBS and CIDP. This could point to similarities of pathogenesis between these two inflammatory neuropathies.

A GWAS was performed on a largely identical cohort of 190 subjects with GBS. Whilst this cohort is relatively small for a modern GWAS and no genome-wide significant results were expected, we were able to identify a number of regions of interest based on clusters of SNPs in linkage disequilibrium of increased significance. These included rs10519519 on Chromosome 2, which is associated with *MYT1L*, a transcription factor in neuronal development; rs7663689 on Chromosome 4, which is in the promotor region of *IL15*; rs11151180 on Chromosome 18 which is in a gene-free region; rs17095496 on Chromosome 14, which amongst other genes is associated with *DACT1*, which encodes a regulator of cell division involved in the NF-κB pathway; and rs2302524 on chromosome 19, associated among other genes with *NFKBIB*, which encodes an inhibitor of the NF-κB pathway. Relative negatives included the HLA region, as indeed were all other candidate genes previously studied in GBS.

Taken together, these findings could indicate a role of the innate immune system, particularly natural killer, cells in the pathogenesis of GBS. Downstream mechanisms, including dysregulation of the adaptive immune system also seem important. Finally, the finding of genes of neuronal development in GBS GWAS could indicate a role for target organ susceptibility in GBS.

Declaration by author

This thesis *is composed of my original work, and contains* no material previously published or written by another person except where due reference has been made in the text. I have clearly stated the contribution by others to jointly-authored works that I have included in my thesis.

I have clearly stated the contribution of others to my thesis as a whole, including statistical assistance, survey design, data analysis, significant technical procedures, professional editorial advice, and any other original research work used or reported in my thesis. The content of my thesis is the result of work I have carried out since the commencement of my research higher degree candidature and does not include a substantial part of work that has been submitted *to qualify for the award of any* other degree or diploma in any university or other tertiary institution. I have clearly stated which parts of my thesis, if any, have been submitted to qualify for another award.

I acknowledge that an electronic copy of my thesis must be lodged with the University Library and, subject to the General Award Rules of The University of Queensland, immediately made available for research and study in accordance with the *Copyright Act 1968*.

I acknowledge that copyright of all material contained in my thesis resides with the copyright holder(s) of that material. Where appropriate I have obtained copyright permission from the copyright holder to reproduce material in this thesis.

Publications during candidature

Publications in peer-reviewed journals:

Todd Hardy, Stefan Blum, Pamela McCombe, and Stephen Reddel, 'Guillain-Barré Syndrome: Modern Theories of Etiology', Current Allergy and Asthma Reports, Issue 11, pages 197-204, 2011

Stefan Blum, Stephen Reddel, Judy Spies, and Pamela McCombe, 'Clinical Features of Subjects with Guillain Barré Syndrome at Seven Hospitals on the East Coast of Australia', Journal of the Peripheral Nervous System, Volume 18, Issue 4, pages 316–320, December 2013.

Stefan Blum, Peter Csurhes, Stephen Reddel, Judy Spies, and Pamela McCombe, 'Killer Immunoglobulin-Like Receptor and their HLA Ligands in Guillain–Barré Syndrome', Journal of Neuroimmunology, Volume 267, Issues 1–2, 15 February 2014, Pages 92–96.

Stefan Blum, Pamela McCombe, ‘Genetics of Guillain-Barré Syndrome (GBS) and Chronic inflammatory demyelinating polyradiculoneuropathy: current knowledge and future directions’, Journal of the Peripheral Nervous System, *accepted for publication 10th April 2014*

Publications included in this thesis

Todd Hardy, Stefan Blum, Pamela McCombe, and Stephen Reddel, 'Guillain-Barré Syndrome: Modern Theories of Etiology', Current Allergy and Asthma Reports, Issue 11, pages 197-204, 2011. **Appendix A, also excerpts are included in chapters 1 + 2**

Contributor	Statement of contribution
Todd Hardy	Wrote paper (30 %)
Stefan Blum	Wrote paper (30 %)
Pamela McCombe	Edited paper (15 %)
Stephen Reddel	Wrote and edited paper (25 %)

Stefan Blum, Stephen Reddel, Judy Spies, and Pamela McCombe, 'Clinical Features of Subjects with Guillain Barré Syndrome at Seven Hospitals on the East Coast of Australia', Journal of the Peripheral Nervous System, Volume 18, Issue 4, pages 316–320, December 2013. **Incorporated as Chapter 3**

Contributor	Statement of contribution
Stefan Blum	Wrote paper and collected data (80%)
Stephen Reddel	Edited paper (5 %)
Judy Spies	Edited paper (5 %)
Pamela McCombe	Edited paper (10 %)

Stefan Blum, Peter Csurhes, Stephen Reddel, Judy Spies, and Pamela McCombe, 'Killer Immunoglobulin-Like Receptor and their HLA Ligands in Guillain–Barré Syndrome', Journal of Neuroimmunology, Volume 267, Issues 1–2, 15 February 2014, Pages 92–96. **Incorporated as chapter 4**

Contributor	Statement of contribution
Stefan Blum	Wrote paper and collected data (70 %)
Peter Csurhes	Collected data and edited paper (10 %)
Stephen Reddel	Edited paper (5 %)
Judy Spies	Edited paper (5 %)
Pamela McCombe	Edited paper (10 %)

Stefan Blum, Pamela McCombe. 'Genetics of Guillain-Barre Syndrome (GBS) and chronic inflammatory polyradiculoneuropathy (CIDP): current knowledge and future directions', Journal of the peripheral nervous system – accepted for publication April 2014. **Incorporated as Appendix B**

Contributor	Statement of contribution
Stefan Blum	Wrote paper (50 %)
Pamela McCombe	Wrote paper (50 %)

Contributions by others to the thesis

Genetic analysis was performed by the laboratory of Prof. Matt Brown, Diamantina Institute, University of Queensland. Prof. Brown and his staff assisted with analysis of the raw data obtained and assisted with selection of SNPs of increased significance.

Casey M Pfluger provided technical assistance with some of the experiments, specifically isolation of DNA and PCR analysis.

Statement of parts of the thesis submitted to qualify for the award of another degree

None.

Acknowledgements

I would like to thank my supervisor Prof. Pamela McCombe, who has been working in the field of inflammatory neuropathies for many years and has brought a great deal of valuable experience to this project. Prof. McCombe has been extremely helpful and dedicated during all stages of this thesis, and it has been a pleasure working with her and benefiting from her extensive knowledge. She is one of the few people I know who are able to combine excellent clinical acumen and skill with an impressive career in research. Her unceasing energy has kept me going through the more difficult parts of this project.

Also, I would like to thank my co-supervisor Dr. Judith Greer, who has been very helpful with her experience, support and advice, and gave very good suggestions and corrections to this thesis.

Prof. Michael Pender, Dr. Robert Henderson and A/Prof. David MacMillan have kindly proof-read this manuscript as well as my milestone documents and have I am grateful for their suggestions and corrections. I would particularly like to thank Dr. Henderson for his support as the former director of the Department of Neurology, RBWH, where I was lucky enough to train under his supervision.

Prof. Matt Brown and members of his team have been invaluable in analyzing the GWAS data and helping me to better understand this complex area. I am grateful for their assistance.

The Royal Brisbane and Women’s Hospital Research Foundation has enabled me to concentrate on this project for some time by their generous financial support of 2 years of scholarship.

Thank you to my parents Gabriele and Manfred Blum, for their continued help and support, which distance does not diminish.

Last, but not least, I am greatly indebted to my wife Dr. Kristina Klein, who has tolerated my absence on weekends and evenings for some time, especially during the final stages of this thesis. Thank you.

Keywords

Guillain-Barré Syndrome

Chronic inflammatory demyelinating polyradiculoneuropathy

Epidemiology

Killer immunoglobulin-like receptors

Human leukocyte antigen

Genome wide association study

Australian and New Zealand Standard Research Classifications (ANZSRC)

060406 Genetic immunology (40 %)

110905 Peripheral nervous system (40 %)

111706 Epidemiology (20 %)

Fields of Research (FoR) Classification

1103 Clinical sciences (40 %)

1107 Immunology (20 %)

1109 Neurosciences (40 %)

Table of Contents

Chapter 1 Guillain-Barré Syndrome – Overview of pathogenesis 22
1.1. Introduction 22
1.2. Diagnosis 23
1.3. Clinical subtypes 23
1.3.1. Acute inflammatory demyelinating polyradiculoneuropathy 25
1.3.2. Acute motor axonal neuropathy 25
1.3.3. Acute motor and sensory axonal neuropathy 26
1.3.4. Miller Fisher syndrome 26
1.3.5. Overlap syndromes 27
1.4. Pathogenesis of GBS 28
1.4.1. Triggering events 28
1.4.2. Self-tolerance and molecular mimicry 30
1.4.3. Autoinflammatory diseases and its relevance in GBS pathogenesis 33
1.4.4. Discussion of hypotheses of GBS pathogenesis 33
1.5. Assessment of severity 34
1.6. Treatment 35
1.7. Hypotheses and aims – Part 1 35
Chapter 2 Immunogenetic factors in Guillain-Barré Syndrome 36
2.1. Introduction 36
2.2. Evidence for a genetic tendency in GBS 37
2.2.1. Familial GBS 37
2.2.2. Interaction of GBS with hereditary neuropathy 37
2.2.3. Risk in relatives 37
2.2.4. Gender differences 38
2.3. Specific genes associated with GBS 38

2.3.1. HLA genes 38

2.3.2. T cell receptor genes 39

2.3.3. CD1 40

2.3.4. IL10 41

2.3.5. TNF alpha 41

2.3.6. MMP9 41

2.3.7. Fc Receptors 42

2.3.8. Lipopolysaccaride receptors 42

2.3.9. Glucocorticoid receptor 42

2.4. Killer immunoglobulin-like receptors 43

2.4.1. Genetics of KIR 43

2.4.2. KIR ligands 44

2.4.3. Role of KIR in human disease, focusing on autoimmune disease 45

2.5. Conclusions 46

2.6. Hypotheses and aims – Part 2 46

Chapter 3 Clinical features of Guillain-Barré Syndrome at seven hospitals on the East Coast of Australia 48

Chapter 4 Killer-immunoglobulin like receptors and their HLA ligands in Guillain-Barré Syndrome 55

Chapter 5 KIR and their HLA ligands in patients with chronic inflammatory demyelinating polyradiculoneuropathy 68

5.1. Chronic inflammatory demyelinating polyradiculoneuropathy 68

5.1.1. Diagnostic criteria and electrophysiology 69

5.1.2. Pathology 69

5.1.3. Immunogenetics 71

5.1.4. Comparison of CIDP and GBS 72

5.1.5. Natural killer cells in CIDP 73

5.1.6. Aim 74

5.2. Methods 74

5.2.1. Laboratory methods ... 74
5.2.2. Haplotypes and ligands ... 75
5.2.3. Statistical analysis ... 75
5.3. Results ... 76
5.3.1. Frequency of KIR genes ... 76
5.3.2. HLA frequencies in CIDP ... 77
5.3.3. Frequency of KIR/ligand carriage ... 78
5.4. Discussion ... 81
Chapter 6 A genome wide association study of Guillain-Barré Syndrome ... 83
6.1. Introduction ... 83
6.1.1. Overview of recent developments in genetic research ... 83
6.1.2. Genome wide association studies ... 84
6.2. Methods ... 89
6.2.1. Study population ... 89
6.2.2. DNA extraction ... 90
6.2.3. Genetic analysis assay ... 90
6.2.4. Quality control and population stratification ... 91
6.2.5. Choosing areas of interest ... 91
6.2.6. Relevant negatives ... 91
6.3. Results ... 92
6.3.1. Overview and Manhattan plot ... 92
6.3.2. Regions of interest ... 94
6.3.3. Short description of candidate genes ... 109
6.3.4. Relevant negatives ... 112
6.4. Discussion ... 116
6.4.1. IL-15 ... 116
6.4.2. NFKBIB ... 118
6.4.3. MYT1L ... 119

6.4.4. rs17095496 and rs11151180 119
6.4.5. Relevant negatives 120
6.4.6. Summary 120
Chapter 7 Summary and conclusions 122
7.1. Characteristics of GBS in Australia 122
7.2. A genetic basis of GBS pathogenesis? 123
7.2.1. KIRs and their HLA ligands in GBS and CIDP 124
7.2.2. A genome-wide association study of GBS 125
7.3. NK cells in the pathogenesis of GBS 127
7.4. Downstream mechanisms 130
7.5. GBS - an autoimmune disease? 131
7.6. GBS – an autoinflammatory disease? 132
7.7. Target organ susceptibility 132
7.8. Future directions 133
Appendix A – Guillain Barré Syndrome: Modern theories on Etiology 134
Appendix B - Genetics of Guillain Barré Syndrome (GBS) and Chronic Inflammatory Demyelinating Polyradiculoneuropathy (CIDP): current knowledge and future directions 143
Reference List 168

List of Figures

Figure 1.1 – The concept of molecular mimicry: Relation between infections, antiganglioside antibodies, and clinical course of GBS (van Doorn, Ruts et al. 2008) 32

Figure 2.1 - Haplotypic variation of 17 KIR genes: Relative distribution of gene frequencies for haplotype A and B (Dorak 2007) 44

Figure 2.2 –HLA-ligand binding specificities for KIR (Kulkarni, Martin et al. 2008) 45

Figure 6.1 - Blocks of LD in the human genome (after (Goldstein 2001)). 87

Figure 6.2 - Statistical power analysis of a case-control study using Quanto (Gauderman 2002) 88

Figure 6.3 – Manhattan blot of GBS GWAS using Haploview (Barrett, Fry et al. 2005). Chromosomes are arbitrarily colour-coded. P values of all SNPs are coded on the vertical axis as a logarithm of 10. The X axis represents position of the SNP on the human genome. 93

Figure 6.4 - Manhattan plot of entire Chromosome 2 using Haploview. A marker (red oval) defines the region surrounding rs10519519. P values of all SNPs are coded on the vertical axis as a logarithm of 10. The X axis represents position of the SNP on the human genome. 94

Figure 6.5 - Manhattan plot Chromosome 2: Zoom of region surrounding rs10519519 (purple marker). P values of all SNPs are coded on the vertical axis as a logarithm of 10. The X axis represents position of the SNP on the human genome. Neighbouring SNPs in linkage disequilibrium are colour-coded according to r^2 (see graph). 95

Figure 6.6 - LD plot of region 2284303 on Chromosome 2. MYT1L is the only gene found in the neighbourhood of rs10519519 (Haploview) 96

Figure 6.7 – Manhattan plot of entire Chromosome 4 using Haploview: The cluster of SNPs of increased significance around rs7663689 is marked by the red oval. P values of all SNPs are coded on the vertical axis as a logarithm of 10. The X axis represents position of the SNP on the human genome. 97

Figure 6.8 - Manhattan plot; Zoom to region surrounding rs7663689. P values of all SNPs are coded on the vertical axis as a logarithm of 10. The X axis represents position of the SNP on the human genome. Rs7663689 is marked in purple. Neighbouring SNPs in linkage disequilibrium are colour-coded according to r^2 (see graph). 98

Figure 6.9 - LD plot of region 142688981 on Chromosome 4 showing IL15 as the gene most closely linked to rs7663689 (Haploview) 99

Figure 6.10 - Manhattan plot of entire Chromosome 18 using Haploview: The cluster of SNPs of increased significance around rs11151180 is marked by the red oval. P values of all SNPs are coded on the vertical axis as a logarithm of 10. The X axis represents position of the SNP on the human genome. 100

Figure 6.11 - Manhattan plot; Zoom to region surrounding rs11151180. P values of all SNPs are coded on the vertical axis as a logarithm of 10. The X axis represents position of the SNP on the human genome. Rs11151180 is marked in purple. Neighbouring SNPs in linkage disequilibrium are colour-coded according to r^2 (see graph). 101

Figure 6.12 - LD plot rs1115180 region (Haploview); no genes in close neighbourhood to11151180 were found 102

Figure 6.13 - Manhattan plot of entire Chromosome 14 using Haploview. The cluster of SNPs of increased significance around rs17095496 is marked by the red oval. P values of all SNPs are coded on the vertical axis as a logarithm of 10. The X axis represents position of the SNP on the human genome. 103

Figure 6.14 - Manhattan plot Chromosome 14, zoom on region surrounding rs17095496 . P values of all SNPs are coded on the vertical axis as a logarithm of 10. The X axis represents position of the SNP on the human genome. Rs17095496 is marked in purple. Neighbouring SNPs in linkage disequilibrium are colour-coded according to r^2 (see graph). 104

Figure 6.15 - LD plot of Chromosome 14, region of rs17085496 (Haploview) 105

Figure 6.16 - Manhattan plot of entire chromosome 19 using Haploview. The cluster of SNPs of increased significance around rs2302524 is marked by the red oval. P values of all SNPs are coded on the vertical axis as a logarithm of 10. The X axis represents position of the SNP on the human genome. 106

Figure 6.17 - Manhattan plot Chromosome 19: Zoom to region surrounding rs2302524 using Haploview. P values of all SNPs are coded on the vertical axis as a logarithm of 10. The X axis represents position of the SNP on the human genome 107

Figure 6.18 - LD plot of region rs2302524 showing several genes closely linked to rs2302524 as detailed in the text (Haploview) 108

Figure 6.19 - Human IL-15 mRNA and protein structure (Fehniger and Caligiuri 2001). Green colours represent the encoding gene of the mature protein, yellow parts of the pre-proteins, purple are splice variants and red are parts of the mRNA not encoded into the precursor protein. 117

Figure 7.1 - Functional model of NK cell function in GBS pathogenesis (see Chapter 4) 129

List of Tables

Table 1.1 - Electrophysiological criteria for AIDP, AMSAN and AMAN (Hadden, Cornblath et al. 1998, Hughes, Wijdicks et al. 2005). LLN = Lower limit of normal; ULN = upper limit of normal 24

Table 5.1 - Electrodiagnostic criteria for CIDP as proposed by the American Academy of Neurology (1991) 70

Table 5.2 - Comparison of clinical and paraclinical features of CIDP and GBS 73

Table 5.3 - KIR genes in subjects with CIDP, compared with GBS subjects and healthy controls. Chi-Square test; Bonferonni correction for multiple tests (ND = not done) 76

Table 5.4 - HLA genes in subjects with CIDP, compared with GBS subjects and healthy controls Chi-Square test; Bonferonni correction for multiple tests with p<0.01 regarded as significant. * p<0.01 77

Table 5.5 – Genefrequency of KIRs and their specific HLA-Ligands CIDP vs. HC. Percentages given are for subjects with subjects (n=195) / Controls (n=225). Chi-Square test; Bonferonni correction for multiple tests with p<0.005(*) regarded as significant. 79

Table 5.6 – Genefrequency of KIRs and their specific HLA-Ligands CIDP vs. GBS subjects. Percentages given are for subjects with subjects (n=195) / Controls (n=225). Chi-Square test; Bonferonni correction for multiple tests with p<0.005 regarded as significant 80

Table 6.1 - Definition of haplotype frequencies for two loci with two alleles 85

Table 6.2 - Definition of allele frequencies based on haplotype frequencies 85

Table 6.3 - Patient characteristics 89

Table 6.4 - Neighbouring SNPs to rs10519519 of increased significance as compared to the sample of HC 95

Table 6.5 - Neighbouring SNPs to rs7663689 of increased significance as compared to the Australian and WTCCC sample of HC 98

Table 6.6 - Neighbouring SNPs to rs11151180 of increased significance as compared to the Australian and WTCCC cohort of HC 101

Table 6.7 - Neighbouring SNPs to rs17095496 of increased significance as compared to an Australian cohort and WTCCC cohort of healthy controls 104

Table 6.8 - Neighboring SNPs to rs2302524 of increased significance as compared to the Australian cohorts and WTCCC cohort of healthy controls 107

Table 6.9 - Previously studied genes in GBS and CIDP and p-values of associated SNPs 113

Abbreviation list

A-CIDP	Acute-onset CIDP
AIDP	Acute inflammatory demyelinating polyradiculoneuropathy
AID	Autoinflammatory diseases
AMAN	Acute motor axonal neuropathy
AMSAN	Acute motor and sensory axonal neuropathy
Anti-GQ1b	Autoantibodies against ganglioside GQ1b
APC	Antigen presenting cell
C. jejuni	Campylobacter jejuni
CD	Cluster of differentiation
CDH7	Cadherin 7
CIDP	Chronic inflammatory demyelinating polyradiculoneuropathy
CMAP	Compound muscle action potential
CMT	Charcot-Marie-Tooth neuropathy
CMV	Cytomegalovirus
CNS	Central nervous system
CNV	Copy number variation
CSF	Cerebrospinal fluid
DAAM1	Disheveled-associated activator of morphogenesis
DACT1	Dapper homolog 1
DHRS7	Dehydrogenase/reductase (SDR family) member 7

EAN	Experimental autoimmune neuritis
EBV	Epstein-Barr virus
ECH1	Peroxisomal enoyl CoA hydratase 1
EMG	Electromyography
ERK	Extracellular signal-regulated kinase
FBXO17	F-Box protein 17
GalNac-GD1a	N-acetylgalactosaminyl Disialoganglioside 1a
GM1	Monosialotetrahexosylganglioside 1
GM1b	Monosialotetrahexosylganglioside 1b
GBS	Guillain-Barré Syndrome
GD1a	Disialoganglioside 1a
GQ1b	Quatrosialogangioside 1b
GR	Glucocorticoid receptor
GWAS	Genome wide association studies
HC	Healthy controls
HLA	Human leukocyte antigen
HNRPL	Heterogeneous nuclear ribonucleoprotein L
ICU	Intensive care unit
IFNγ	Interferon gamma
IL	Interleukin
iNKT	Invariant natural killer T

IVIg	Intravenous immunoglobulin
IκB	Inhibitor of kappa B
kDa	Kilo-Dalton
KIR	Killer immunoglobulin-like receptor
LD	Linkage disequilibrium
LGALS4 and 7	Soluble galactoside-binding lectin 4 and 7
LLN	Lower limit of normal
LOS	Lipooligosacchacarides
LPS	Lipopolysaccaride
LSP	Long signal peptide
MAF	Minor allele frequency
MAPK	Mitogen-activated protein kinase
MFS	Miller Fisher Syndrome
MHC	Major histocompatibility complex
MMP9	Matrix metalloproteinase 9
MRPS2	Mitochondrial ribosomal protein S2
NCBI	National Centre of Biotechnology Informatics
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NFκBIB	NF-κB inhibitor beta
NINDS	National Institute of Neurological Disorders and Stroke
NK cell	Natural killer cell

PCR	Polymerase chain reaction
PE	Plasma exchange
PNS	Peripheral nervous system
POEMS	Polyneuropathy, organomegaly, endocrinopathy, monoclonal protein, skin changes
SARS2	Mitochondrial seryl-tRNA synthetase 2
SIRT2	Sirtuin 2
SNP	Single nucleotide polymorphism
SSP	Short signal peptide
TLR	Toll-like receptor
TNFα	Tumor necrosis factor alpha
TNFγ	Tumor necrosis factor gamma
ULN	Upper limit of normal
URTI	Upper respiratory tract infection
WTCCC	Welcome Trust Case Control Consortium
ZNF 300	Zinc finger protein 330

Chapter 1 Guillain-Barré Syndrome – Overview of pathogenesis

1.1. Introduction

Guillain-Barre Syndrome (GBS) was first described at the beginning of the 20th century by three French neurologists Georges Guillain, Jean-Alexandre Barré, and André Strohl, who reported two soldiers with acute areflexic paralysis, followed by recovery. They reported the combination of increased protein concentration with a normal cell count in the cerebrospinal fluid (CSF) - albuminocytological dissociation, which separated the condition from poliomyelitis. Despite the fact that Landry de Thezillat had already reported similar cases in 1859, the combination of these clinical and laboratory features became known as Guillain-Barré syndrome (GBS) (Guillain 1916, van Doorn, Ruts et al. 2008).

Today, Guillain-Barré Syndrome (GBS) is defined as an acute or subacute immune mediated polyradiculoneuropathy. The cardinal clinical features of GBS are progressive, usually symmetric, flaccid muscle weakness accompanied by absent or depressed deep tendon reflexes and ascending sensory changes. The course is usually monophasic, with clinical recovery following the rapid initial deterioration.

The annual incidence in Australia is estimated at about 1 per 100,000 (Storey, Cook et al. 1989), which is in keeping with a number of world-wide studies (Hughes and Rees 1997, Chio, Cocito et al. 2003). The incidence of GBS increases steadily with age from less than one per 100 000 in patients younger than 30 years to about four per 100 000 in those older than 75 years (Bogliun, Beghi et al. 2004).

1.2. Diagnosis

The diagnosis of GBS relies on the history and physical examination, especially early in the disease course, when ancillary testing can be negative. The clinical spectrum of GBS is broad, ranging from mild difficulty with walking or pure sensory symptoms to nearly complete paralysis, including facial, respiratory, and bulbar muscles.

Diagnostic criteria have been proposed by the National Institute of Neurological Disorders and Stroke (NINDS), and have been widely used both in research and clinical practice (Anonymous 1978). These require the presence of progressive muscle weakness in more than one limb combined with areflexia or hyporeflexia; maximum severity of the illness is reached by 4 weeks at the latest. Supportive features include progression of symptoms for up to four weeks, relative symmetry, mild sensory symptoms or signs, cranial nerve involvement, recovery starting two to four weeks after progression halts, autonomic dysfunction, lack of fever at the onset, elevated protein in CSF with a cell count $<10\ mm^3$ and electrodiagnostic abnormalities consistent with GBS.

1.3. Clinical subtypes

Guillain-Barré Syndrome is a heterogeneous condition, with several variants distinguishable by clinical picture, electrophysiological criteria, pathological features and serological markers. Based on electrophysiological criteria, several subtypes of GBS have been described, however some overlap exists. The most commonly encountered subtypes include Acute Inflammatory Demyelinating Polyradiculoneuropathy (AIDP), Acute Motor-Sensory Axonal Neuropathy (AMSAN), Acute Motor Axonal Neuropathy (AMAN) and Miller-Fisher Syndrome (MFS). Rarer variants include facial diplegia, acute autonomic neuropathy and the pharyngeal-cervical-brachial variant of GBS.

Neurophysiological testing is most important in subtype classification in the clinical and research setting. Neurophysiological criteria for AIDP, AMSAN and AMAN have been established (Table 1.1). In AIDP, the presence of patchy demyelination is characterized by delayed distal motor latency of the compound muscle action potential (CMAP), decreased motor conduction velocity, conduction block and delayed F wave latency. In AMAN, nerve conduction studies show decreased CMAP amplitudes, with no evidence of sensory

involvement or demyelination. AMSAN is characterized by decreased CMAP amplitudes and sensory involvement. In MFS conduction studies of peripheral nerves are usually normal.

Table 1.1 - Electrophysiological criteria for AIDP, AMSAN and AMAN (Hadden, Cornblath et al. 1998, Hughes, Wijdicks et al. 2005). LLN = Lower limit of normal; ULN = upper limit of normal

AIDP

At least one of the following in each of at least two nerves, or at least two of the following in one nerve if all others inexcitable and distal CMAP >10%:

Motor conduction velocity <90% LLN (85% if distal CMAP <50% LLN)

Distal motor latency >110% ULN (>120% if distal CMAP <100% LLN)

Proximal CMAP / distal CMAP ratio <0·5 and distal CMAP >20% LLN

F-response latency >120% ULN

AMSAN

None of the features of AIDP except one demyelinating feature allowed in one nerve if distal CMAP <10% LLN

Sensory action potential amplitudes <LLN

AMAN

None of the features of AIDP except one demyelinating feature allowed in one nerve if distal CMAP <10% LLN

Sensory action potential amplitudes normal

Inexcitable

Distal CMAP absent in all nerves or present in only one nerve with distal CMAP <10% LLN

1.3.1. Acute inflammatory demyelinating polyradiculoneuropathy

AIDP is the most common variant of GBS in developed countries, including Europe, North America and Australia, with more than 80% of cases suffering from this subtype of GBS.

1.3.1.1. *Pathology*

The pathological hallmark of GBS is a multifocal mononuclear cell infiltration throughout the peripheral nervous system, with the distribution of inflammation corresponding to the clinical deficit (Asbury, Arnason et al. 1969). Pathological alterations can be found at all regions of the peripheral nervous system, from the roots to the most distal intramuscular motor nerve endings. The lesions are pronounced along the ventral roots, proximal spinal nerves, and lower cranial nerves (Kieseier, Kiefer et al. 2004). Macrophages and T lymphocytes invade nerve tissue and strip off lamellae of myelin (Prineas 1981). Disruption of the blood-nerve barrier, allowing migration of T lymphocytes, could be partially due to increased secretion of metalloproteinase MMP9 (Creange, Sharshar et al. 1999).

1.3.1.2. *Electrophysiology*

Inflammation is thought to start at the level of the nerve roots, where the blood-nerve barrier is less well developed. Consistent with this, one of the earliest electrophysiological signs is prolonged or absent F waves and absent H reflexes, often with reduced recruitment on EMG of affected muscles (Gordon and Wilbourn 2001). Temporal dispersion of CMAP, decreased conduction velocity, conduction block and prolonged distal latency follows (Albers, Donofrio et al. 1985), combined with spontaneous activity on EMG. As well, sensory responses are slowed or absent, often with sparing of the sural nerve (Murray and Wade 1980). Axonal degeneration is often seen in subsequent studies, and is associated with a worse prognosis (Cornblath, Mellits et al. 1988).

1.3.2. Acute motor axonal neuropathy

Acute motor axonal neuropathy (AMAN) is relatively uncommon in developed countries, but is the predominant form of GBS in China (McKhann, Cornblath et al. 1991, McKhann, Cornblath et al. 1993). It has also been reported to occur in high frequencies in other parts of Asia and Mexico. In these regions, AMAN occurs more often in summer and is frequently

associated with antecedent *Campylobacter jejuni (C. jejuni)* infection (Blaser, Olivares et al. 1991). Affected patients are usually young (15 months to 37 years, median 7 years). Clinical signs of AMAN are similar to those of AIDP, however the electrophysiological features are those of an axonal neuropathy, without evidence of demyelination, and sparing of sensory fibers (McKhann, Cornblath et al. 1991, Vucic, Kiernan et al. 2009).

In contrast to AIDP, where demyelination is the pathological hallmark, in AMAN antibody- mediated attack on the nodes of Ranvier predominates. Particularly, antibodies against Monosialotetrahexosylganglioside 1 (GM1), Monosialotetrahexosylganglioside 1b (GM1b), N-acetylgalactosaminyl Disialoganglioside 1a (GalNac-GD1a) and Disialoganglioside 1a (GD1a), have been found (Ang, Yuki et al. 1999, Ho, Willison et al. 1999). GM1 occurs in high concentration at the node of Ranvier (Corbo, Quattrini et al. 1993). There is some evidence that this leads to reversible blockage of voltage-gated Na^+ channels at the node of Ranvier, resulting in conduction block (Weber, Rudel et al. 2000). As patients with AMAN usually recover with adequate supportive therapy, the resulting damage to the axon appears to be short-lived and may reflect a temporary blockade of conduction, although axonal degeneration distally is possible, leading to persistent disability.

1.3.3. Acute motor and sensory axonal neuropathy

Acute motor and sensory axonal neuropathy (AMSAN) is the name given to the motor axonal form of GBS when there is co-existent sensory involvement. Clinically, patients are often severely affected. The pathology is similar to that seen in AMAN with involvement of ventral nerve roots and peripheral motor axons targeted as a site of macrophage invasion periaxonally; however dorsal nerve roots and sensory fibres are also involved. As in AMAN, there appears to be a limited role for demyelination or lymphocytic inflammation (Griffin, Li et al. 1996). Immunological studies have shown that patients with AMAN and AMSAN share a common profile of anti-GM1, -GD1a, and -GM1b antibodies supporting an antibody-mediated pathogenesis in both of these conditions (Yuki, Kuwabara et al. 1999).

1.3.4. Miller Fisher syndrome

In 1956, the American neurologist Miller Fisher described a syndrome consisting of ataxia, areflexia and ophthalmoplegia, now known as Miller Fisher Syndrome (MFS), which has proved to be a variant of GBS (Fisher 1956, Mori, Kuwabara et al. 2001). Apart from the classical features of the original description, facial weakness, bulbar palsy, dysaesthesia and

limb weakness are also seen in some subjects (Mori, Kuwabara et al. 2001). Its incidence has been reported as 0.1 per 100000 in one Italian study (Bogliun, Beghi et al. 2004), and about 5% of GBS cases are estimated to suffer from this variant in developed countries. The incidence of MFS appears to be higher in Asia (Lyu, Tang et al. 1997). MFS overlaps with AIDP and Bickerstaff Encephalitis (Lo 2007).

MFS is strongly associated with IgG antibodies against quatrosialoganglioside 1b (GQ1b) (Chiba, Kusunoki et al. 1995, Koga, Yuki et al. 1998). The pathophysiology of this condition involves the paranode where clusters of GQ1b antigen have been localized using immunohistochemical techniques. Unsurprisingly, given the clinical features, cranial nerves innervating the extra-ocular muscles appear to be more densely replete with GQ1b antigen than other cranial nerves. Ataxia is thought to reflect Anti-GQ1b antibodies targeting antigens in the dorsal root ganglia and nerve terminal of the muscle spindle (Liu, Willison et al. 2009).

1.3.5. Overlap syndromes

The classification of AIDP into AMAN or AMSAN is largely reliant on neurophysiological studies, but this has limitations. For example, conduction block, a neurophysiological finding typical of demyelination, can be present in axonal disease if blocking of the nodes of Ranvier occurs (Uncini, Manzoli et al. 2010). This could lead to the misdiagnosis of AMAN as AIDP and may have some impact on the reported prevalence of the different forms of GBS in epidemiological studies (Uncini and Kuwabara 2012). Also, timing of electrophysiological studies during the illness course might be of importance, with axonal features being more prominent later in the disease course, when secondary degeneration of axons of demyelinated nerve fibres is not uncommon (Uncini, Manzoli et al. 2010).

This relates to the finding that axonal changes are frequently seen in more severe AIDP. This picture is referred to as ‘bystander’ damage. According to this hypothesis, axons are damaged by the inflammatory process and may become compromised by endoneurial oedema, immune attack on axon epitopes situated beneath myelin targets, ischaemia or intra-axonal accumulation of sodium and calcium due to voltage gated ion channel dysfunction (Madrid, Wi et al. 1977, Sobottka, Harrer et al. 2009).

1.4. Pathogenesis of GBS

Based on its pathological features, its response to immunomodulatory therapies and the presence of activated lymphocytes and antibodies against neural cell surface antigens, GBS is thought to be an immune-mediated disease. However, there are a number of features distinguishing GBS from other classical autoimmune diseases, like systemic lupus erythematosus or type 1 diabetes mellitus. In contrast to most autoimmune diseases, men are about 1.5 times more likely to be affected than are women. Other atypical features include a lack of associated autoimmune diseases and no confirmed HLA association. Finally, in contrast to autoimmune diseases, GBS is a monophasic disease in most instances, even though relapsing disease has been reported (Kuitwaard, van Koningsveld et al. 2009). These differences to autoimmune diseases are well recognized, and GBS is often labelled as post-infectious inflammatory disorder(van Doorn, Ruts et al. 2008). Within this thesis we propose the hypothesis that GBS is better thought of as an autoinflammatory disorder, a group of disorders characterized by dysfunction of the innate, rather than the adaptive immune system.

1.4.1. Triggering events

GBS can rapidly transfer a healthy, fit individual to a highly dependent state requiring intensive supportive care. The question as to how and why such a rapidly evolving disease is initiated has held the attention of researchers for some time. Of interest in this context is that about two thirds of GBS patients report an infection in the weeks preceding their illness, however other events have also been recorded (Winer 2001).

1.4.1.1. *Infections*

Gastrointestinal or upper respiratory tract infections are commonly reported. Most frequently, evidence of *C. jejuni* infection or Mycoplasma pneumonia is found. Other organisms reported to precede GBS include cytomegaly virus (CMV), Epstein-Barr virus (EBV) and other viruses (Hadden, Karch et al. 2001). However, in the majority of cases, no specific agent can be identified.

One Japanese study found that the most frequent antecedent symptoms in GBS and related disorders were fever (52%), cough (48%), sore throat (39%), nasal discharge (30%), and diarrhea (27%) (Koga, Yuki et al. 2001).

1.4.1.2. *Vaccinations*

A variety of vaccinations have been reported to trigger GBS. Administration of vaccines prepared from suckling mouse brain or sheep brain led to an acute neuropathy resembling GBS in a number of patients (Hemachudha, Griffin et al. 1988). This was thought to be caused by cross-reactivity of brain antigens with peripheral nerve antigens. Also, the parenteral administration of gangliosides led to a GBS-like picture in some individuals (Raschetti, Maggini et al. 1995). This was thought to arise as an immune response to ganglioside antigens in peripheral nerve.

GBS has also been described in context of influenza vaccinations in the US (Schonberger, Bregman et al. 1979). During the 2009/10 H1N1 epidemic several cases of GBS were reported after H1N1 infection (Chaari, Bahloul et al. 2010) and after vaccinations. A preliminary report issued by the Centre for Disease Control and Prevention showed a rate of 0.8 cases of GBS per $1x10^6$ vaccinations which is in keeping with that seen following other seasonal influenza vaccines (Centers for Disease and Prevention 2010).

There are also case reports of GBS occurring following hepatitis and meningococcal vaccines (Blumenthal, Prais et al. 2004, Velentgas, Amato et al. 2012).

1.4.1.3. *Campylobacter jejuni and AMAN*

Epidemiologically, the association of GBS with preceding infections is best established for *C. jejuni* in the AMAN subtype of GBS. This has mainly been established in Asian countries. However, it was reported recently that GBS after *C. jejuni* infections in The Netherlands is also strongly, but not exclusively, associated with axonal changes (Drenthen, Yuki et al. 2011). Interestingly, the incidence of GBS has declined in New Zealand since the rate of *C. jejuni* infections has decreased (Baker, Kvalsvig et al. 2013).

C jejuni isolates from patients express lipo-oligosaccharides (LOS) that mimic the carbohydrates of gangliosides (Godschalk, Kuijf et al. 2007). *C. jejuni* strains that produce LOS resembling GM1 and GD1a were found in higher frequencies in patients with GBS than in patients with Campylobacter enteritis (Koga, Gilbert et al. 2006). Also, the LOS structure seems to determine ganglioside-antibody specificity and the clinical syndrome that develops in the host and ganglioside antibodies often cross-react with *C. jejuni* strains isolated from the same patient. *C jejuni* isolates from patients with pure motor or axonal GBS frequently express a GM1-like and GD1a-like LOS, whereas those isolated from patients with

ophthalmoplegia or MFS usually express a GD3-like, GT1a-like, or GD1c-like LOS (Kuijf, Godschalk et al. 2007).

1.4.1.4. *Other triggers*

Especially for the demyelinating forms of GBS, a range of other triggers have been described. GBS has been associated with trauma, including head injury but not peripheral nerve injury, surgical interventions (Shuert and Gamble 1972, Duncan and Kennedy 1987, Baldwin, Pierce et al. 1992, Hogan, Briggs et al. 1992), and even lightning strike (Theodorou, Limmroth et al. 2008), but the mechanism by which these events lead to GBS is unclear. These all lead to relatively stereotyped patterns of disease (see Chapter 3).

This indicates that a range of events that lead to activation of the immune system, could lead to an immune attack against myelin in a susceptible individual. Interesting in this context are the observations made by Kuitwaard et al. on recurrent GBS (Kuitwaard, van Koningsveld et al. 2009). In their case series of 32 patients, symptoms and signs of GBS were similar for different episodes of GBS, even though symptoms and signs of preceding infection (where present) were mostly different.

1.4.1.5. *Relationship between triggering event and GBS*

In summary, a range of triggering events has been described for GBS, which all lead to a relatively stereotyped immune response against nerve tissue. All these events appear to have in common the enteral, respiratory or parenteral exposure to a pathogen, which is usually easily controlled by the host. Some individuals, however, go on to develop a severe acute neuropathy. Why this exaggerated, pathological attack on peripheral nerves occurs is unclear, and several theories have been proposed.

1.4.2. Self-tolerance and molecular mimicry

In order for the immune system to protect the host from pathogens, it has a powerful array of effector mechanisms available. These mechanisms must not be used in an attack against self-structures; failure of doing so can lead to a range of sometimes severe diseases and is encompassed in the term autoimmunity (Matzinger 2002).

Several mechanisms prevent B and T lymphocytes from attacking self antigens. These include firstly the apotosis of autoreactive lymphocytes during development in bone marrow

and thymus. Secondly, autoreactive lymphocytes can become inactivated – this is called ‘anergy’. Finally, there is a complex array of regulatory and suppressive factors and cells, i.e. regulatory T cells, that can inhibit T and B cell effector functions.

Evolutionary pressure will lead to invading pathogens attempting to go unnoticed by innate and adaptive components of the immune system by presenting antigens resembling host proteins. This phenomenon is also termed ‘crypsis’ - being camouflaged to resemble part of the environment.

The term molecular mimicry was first used by Damian in 1964, even though he dismissed the idea (Damian 1964). The hypothesis states that antigens on invading organisms resemble host antigens, which leads to dual recognition of host and microbe antigens by T cell receptors (TCR) or antibodies. While the main evolutionary aim of the microorganism is evasion of immune recognition, the resemblance of microorganism and host antigens could lead to an erroneous recognition of auto-antigens and triggering of an immune response against host tissues. This has led to the concept that autoimmune disease can be a consequence of molecular mimicry. Molecular mimicry due to a range of infectious agents has been implicated in the pathogenesis of a range of autoimmune diseases, including rheumatic fever, multiple sclerosis, and diabetes mellitus. In GBS, antibodies found are predominantly of IgG type, implying T helper cell involvement (Sekiguchi, Uncini et al. 2012).

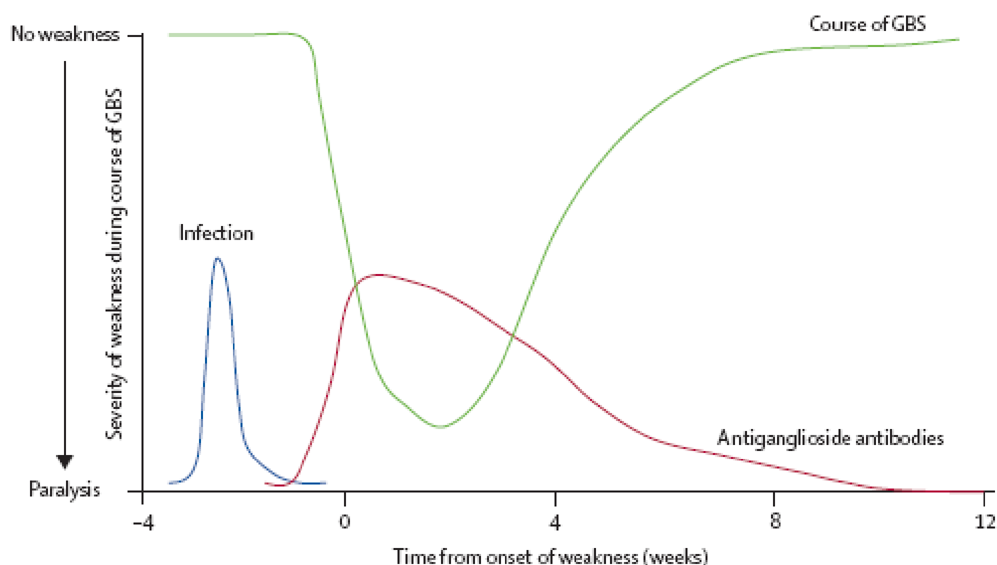


Figure 1.1 – The concept of molecular mimicry: Relation between infections, antiganglioside antibodies, and clinical course of GBS (van Doorn, Ruts et al. 2008)

Molecular mimicry is an attractive immunopathological mechanism to explain the relationship between trigger and immune response in GBS (Ang, Jacobs et al. 2004). Indicators of this relationship are: (1) the temporal relationship between infection and GBS (Figure 1.1), (2) identification of host T cells and antibodies against gangliosides (Csurhes, Sullivan et al. 2005), (3) structural similarities between bacterial LOS and gangliosides (Willison and Yuki 2002), and (4) reproduction of a neuritis and in an animal model after immunization with gangliosides (Yuki, Yamada et al. 2001, Yuki and Kuwabara 2007). However, while immunization with LOS does lead to production of anti-ganglioside antibodies in rabbits (Ang, De Klerk et al. 2001), no neurological sequelae have been observed in this model.

These concepts are relatively well established for AMAN, but less so in AIDP. Here, the relationship to *C. jejuni* as trigger is less definite, and no ganglioside antibodies are found in the majority of cases.

1.4.3. Autoinflammatory diseases and its relevance in GBS pathogenesis

Autoinflammatory diseases (AID) are a group of disorders of the immune system, characterized by seemingly unprovoked inflammation. They are predominantly hereditary, either inherited in a Mendelian or in a polygenic fashion. Examples include Familial Mediterranean fever and other periodic fever syndromes, pyoderma gangrenosum, Behcet's disease and Crohn's disease (Galeazzi, Gasbarrini et al. 2006).

AID are a relatively new group of disorders, and are distinct from classical autoimmune diseases. AID and autoimmune diseases share the common feature of an immune attack against self and chronic activation of the immune system. However, the specific effector mechanisms differ: in AID, the innate immune system causes tissue damage directly, whilst in autoimmune diseases the innate system acts via activation of the adaptive immune system. In both groups, inflammation can be triggered by preceding infections or vaccinations (Efthimiou, Flavell et al. 2008, Touitou and Kone-Paut 2008). Inflammatory markers, like CRP and ESR, are always increased in AID, but not necessarily in autoimmune diseases.

In contrast to autoimmune diseases, AID are not associated with MHC class II haplotypes (Theofilopoulos, Gonzalez-Quintial et al. 2010). Rather, AIDs have been linked to a range of genetic variations in different loci, including genes related to IL-1β activation (i.e. *NLRP3* in cryopyrinopathies) (Hoffman, Mueller et al. 2001), and activation of NF-κB (i.e. *NOD2* in Crohn's disease) (Hugot, Chamaillard et al. 2001).

1.4.4. Discussion of hypotheses of GBS pathogenesis

In summary, different pathogenic models have been proposed to explain GBS pathogenesis. The most widely accepted model at present describes GBS as an autoimmune disorder, characterized by activated B and T cell mediated responses against peripheral nerve proteins triggered by molecular mimicry.

However, as described in more detail in Chapter 2, GBS lacks both a MHC class II association and a link to other autoimmune diseases. Also, whilst auto-antibodies have been found in some subforms of GBS (i.e. Anti-GQ1b in MFS), these are usually not found in the more common demyelinating variants.

In this thesis, we will propose that GBS could be explained by an autoinflammatory, rather than autoimmune, process, highlighting the role of the innate immune system in GBS pathogenesis.Both autoimmune diseases and AIDs can be quite specific in their target tissue, as seen in GBS, or cause more wide-spread inflammation.

1.5. Assessment of severity

As outlined above, the severity of GBS is highly variable, ranging from barely noticeable symptoms and signs to a severe illness necessitating intensive support or even leading to death. Dysfunction of respiratory and bulbar muscles or severe autonomic dysfunction necessitates intensive care and ventilator support in 10 to 30% of cases (Alshekhlee, Hussain et al. 2008).

A simple, but very reliable, scale for disability in GBS has been developed by Hughes (Hughes, Newsom-Davis et al. 1978), and has subsequently been used in a large numbers of clinical trials. It consists of a 7 point scale as follows:

0 = Healthy

1 = Minor symptoms and signs of neuropathy, able to work and run

2 = Able to walk without support of a stick, unable to work or run

3 = Able to walk with a stick or other support

4 = Confined to chair or bed

5 = requiring assisted ventilation

6 = Death

1.6. Treatment

One of the features of GBS which strongly supports the concept of GBS being an immune mediated disease is its response to immunomodulatory therapy. However, treatment for GBS differs markedly from that of other autoimmune diseases. For example, GBS does not respond to oral or intravenous corticosteroids, and such therapy might even be harmful (Hughes, Newsom-Davis et al. 1978, Hughes, Swan et al. 2010).

The mainstay of therapy at present is either plasma exchange or intravenous immunoglobulins, which have been found to be effective in a number of trials (summarized in (Hughes, Wijdicks et al. 2005)). Early initiation of therapy appears important by preventing accumulation of damage. Also, spontaneous amelioration of the immune response as part of the natural history of the disease could make therapy given late in the disease course less effective (Hughes, Swan et al. 2007, Randall 2010).

1.7. Hypotheses and aims – Part 1

GBS is a rare, but potentially devastating disease of the peripheral nervous system. Its epidemiology differs between geographical regions, with axonal subsets being more frequent in the developing world, in contrast to developed countries, where demyelinating forms predominate. It is unclear at present whether genetic or environmental factors are responsible for this difference.

Based on epidemiological studies from Europe and North America, as well as older Australian studies, we hypothesized that AIDP is still the predominant form of GBS in Australia. The first aim of this study was to study a cohort of patients with a confirmed diagnosis of GBS, and describe in detail their clinical, para-clinical and electrophysiological characteristics.

Chapter 2 Immunogenetic factors in Guillain-Barré Syndrome

2.1. Introduction

The complexity of immune responses is partially determined by variation of the genetic background of an individual. After a suitable trigger, there is failure of self-tolerance, and self-antigens are attacked by the immune system.

Whilst a range of infections are often described as environmental triggers for GBS, the genetic basis of GBS is unknown. In other autoimmune diseases, such as MS and type 1 diabetes, where extensive genetic studies have been performed, there are associations of disease with many genes that have a small individual risk (Beecham, Patsopoulos et al. 2013).

It is likely that genetic variation in immune related genes could contribute to the risk of acquiring GBS, similar to more classical autoimmune diseases, or influence severity of disease. For autoimmune diseases it is also possible that genetic variation in the target organ could contribute to the susceptibility to disease.

This chapter provides a literature review of the genetics of GBS with particular emphasis on genes studied within this thesis.

2.2. Evidence for a genetic tendency in GBS

2.2.1. *Familial GBS*

If there were a genetically determined susceptibility to develop GBS, it might be expected that disease occurs in families. After an early report of familial GBS (Saunders and Rake 1965) there have been further reports. From Holland there is a report of 12 families with GBS (Geleijns, Brouwer et al. 2004). There is a report of a mother and son, both with HLA DR2, who both developed GBS (Wilmshurst, Pohl et al. 1999). In another family, 4 siblings born to a consanguineous marriage developed GBS (Aquil, Khan et al. 2011). Also, there is a report of GBS in father and son with strikingly similar HLA types (Davidson, O'Sullivan et al. 1992). There is also a report of familial GBS from India (Naik, Saroja et al. 2012). Some reports could be linked to concurrent infection with *C. jejuni (Yuki and Tsujino 1995, Tokuhara, Hirokawa et al. 2010)*, but is not likely for the other reports mentioned above, as the onset of GBS occurred many years apart, and frequently in subsequent generations. Preceding environmental influence cannot be excluded based on the data presented.

2.2.2. *Interaction of GBS with hereditary neuropathy*

There is a report of axonal GBS in a patient with Charcot Marie Tooth (CMT) disease (Odaka, Yuki et al. 2003). There have been reports of some types of inherited neuropathies that are responsive to corticosteroids (Dyck, Swanson et al. 1982).

2.2.3. *Risk in relatives*

Twin studies can be used to evaluate the risk of diseases that can be attributed to genetic predisposition, but there are no twin studies in GBS. A Dutch study of familial GBS found that siblings of people with GBS have a risk to develop GBS, which is increased by factor 2.6 compared to the general population (Geleijns, Brouwer et al. 2004). This could possibly be due to genetic similarities, but environmental factors cannot be excluded.

For comparison, in multiple sclerosis, a inflammatory disease of the central nervous system with well-established genetic influences, the risk of first degree relatives to develop the illness has been reported to be as high as 7.1 (Nielsen, Westergaard et al. 2005).

2.2.4. Gender differences

Another genetically determined risk factor is gender. Males have a significantly higher risk for developing GBS than females (Chapter 3), which is an unusual feature in autoimmune diseases (McCombe, Greer et al. 2009). Gender is determined by the Y chromosome, which carries few genes. Amongst these is the *Sry* gene, which is responsible for the formation of male sexual organs by controlling of gonadal hormone production. A second locus is the *Yaa* gene cluster, which predisposes to autoimmunity in mice after translocation of TLR7 from the X chromosome (Subramanian, Tus et al. 2006). Dosage effects of X-chromosome genes (e.g. CD40L) are important in many female-predominated autoimmune diseases (i.e. SLE).

2.3. Specific genes associated with GBS

There have been studies of HLA associations, of genes to do with antigen processing, of T cell receptors and Fc receptors, and of immune mediators and cytokines (particularly in the promoter regions) which have been associated with many diseases, including autoimmune disease (Vandenbroeck 2012).

Below, we outline studies that have investigated candidate genes to do with GBS. For a more exhaustive review on GBS genetics, the reader is referred to Appendix B.

2.3.1. HLA genes

The MHC locus on chromosome 6 contains a range of genes related to the immune system functions, including genes encoding cell-surface antigen-presenting proteins, the HLA molecules. HLA molecules have paramount importance for the specific immune system in both health and disease due to their role in antigen presentation to T cells. Also, class I HLA molecules are important for the innate immune system, by providing binding sites for Killer immunoglobulin-like receptors (see Chapter 2.4). Certain genetic variants have been associated with a range of autoimmune diseases, and, indeed, the presence of an HLA association is among the criteria used to define an autoimmune disease (Rose and Bona 1993).

In GBS, a number of studies in different populations have been conducted over the last 30 years, without consistent positive results. Early studies using serological methods generally

found no HLA association (Latovitzki, Suciu-Foca et al. 1979, Kaslow, Sullivan-Bolyai et al. 1984, Hafez, Nagaty et al. 1985), although one study in Mexico found that GBS was associated with carriage of HLA-DR3 (Gorodezky, Varela et al. 1983). The majority of these studies have not found any associations between GBS and carriage of class I or class II HLA molecules (Winer, Briggs et al. 1988, Piradov, Dolbin et al. 1995). However, when GBS patients have been grouped according to whether they have AIDP or axonal neuropathy, it has been found that certain DQB1 molecules are associated with AIDP but not axonal neuropathy (Magira, Papaioakim et al. 2003). A study from Japan found no HLA association with GBS but a tendency for increased HLA-DRB1*08:03 in subjects with GBS associated with *C. jejuni* infection (Ma, Nishimura et al. 1998). A study from Holland found no association of HLA class II alleles with GBS (Geleijns, Schreuder et al. 2005). An Australian study found no HLA association in GBS, except for a slight negative association of HLA-DR5 (McCombe, Csurhes et al. 2006).

In summary, in spite of numerous investigations, no reproducible HLA association has been found for GBS. This lack of association of HLA with GBS could partly be due to the lack of differentiation between AIDP and AMAN, or insufficient patient numbers. Alternatively, the lack of strong HLA class II association with inflammatory neuropathy could suggest that class II restricted antigen presentation is not of prime importance in susceptibility to GBS. This could be of relevance, especially if components of the innate immune system are of more importance in GBS pathogenesis than hitherto acknowledged.

2.3.2. T cell receptor genes

There are a number of indications that T cells play an important role in GBS pathogenesis. These include the finding of T cell infiltrates in nerve biopsies of patients with GBS (Schmidt, Toyka et al. 1996) as well as the presence of cross-reactive IgG1 and IgG3 antibodies to LOS and gangliosides. Formation of these antibodies typically requires T lymphocyte help in their production (Yuki, Handa et al. 1995, Yuki, Ichihashi et al. 1995). T cell reactivity to peripheral nerve antigens has been reported in GBS and CIDP (Csurhes, Sullivan et al. 2005).

When T cell receptor genes were studied in GBS, there was no association with GBS development, although there was a trend to an influence on the severity of disease (Ma, Nishimura et al. 1998).

2.3.3. CD1

CD1 is part of a conserved family of transmembrane glycoproteins, resembling MHC. It has been shown to be crucial for capturing and presenting a variety of both microbial and self-glycolipids (Porcelli 1995). The human CD1 family consists of five glycosylated proteins, CD1A, CD1B, CD1C, CD1D and CD1E, which have limited polymorphisms and are all expressed by professional antigen-presenting cells (Porcelli 1995).

T cells that recognize lipids in association with CD1 molecules can be broadly divided into two groups: those that are restricted to CD1A, CD1B and CD1C molecules and have similar properties to peptide-specific T cells, and those that are restricted by CD1D, which share many of the properties of innate immune cells, labelled invariant natural killer T (iNKT) cells (Godfrey and Kronenberg 2004, De Libero and Mori 2005). In fact, lipid antigens must be presented by CD1 antigen-presenting molecules to be recognized by T cells (Shamshiev, Donda et al. 1999, Shamshiev, Donda et al. 2000). This implies that only lipids that can bind to CD1 are immunogenic for T cells (De Libero and Mori 2005).

CD1A and CD1B have been observed on CD68+ cells and macrophages in the endoneurium in nerve biopsies of GBS and CIDP patients (Khalili-Shirazi, Gregson et al. 1998, Van Rhijn, Van den Berg et al. 2000).

The second exons of both CD1A and CD1E are allelic, possibly affecting function. These alleles have been investigated in GBS, but with conflicting results. An Italian study found that subjects with CD1E*01/01 genotype are 2.5 times more likely to develop GBS whereas subjects with CD1A*01/02 or CD1E*01/02 have a reduced relative risk (Caporale, Papola et al. 2006). This was, however, not confirmed in a larger Dutch study using similar techniques (Kuijf, Geleijns et al. 2008), and a subsequent metaanalysis found no difference (Wu, Zhou et al. 2012). Interestingly, several reports showed polymorphisms of CD1 as a susceptibility factor in multiple sclerosis, a demyelinating disease of the central nervous system (Jamshidian, Nikseresht et al. 2010, Caporale, Notturno et al. 2011).

2.3.4. IL10

IL-10 is a cytokine which is secreted by both macrophages and T lymphocytes. It is involved in the immune response of both infectious and autoimmune diseases and can have both pro-inflammatory and anti-inflammatory effects depending on the milieu.

One study found that polymorphisms in the promoter region of IL10 were associated with disease susceptibility but not disease severity (Myhr, Vagnes et al. 2003).

2.3.5. TNF alpha

TNFα is a cytokine produced mainly by macrophages and monocytes. It has an important role in early phase of immunity and inflammation by stimulation of the acute phase reaction, acting particularly on the innate immune system. Circulating levels of TNFα were found to be increased in GBS (Radhakrishnan, Sumi et al. 2003). GBS and MFS has been associated with administration of TNFα inhibitors for rheumatological disorders (Shin, Baer et al. 2006).

A study of polymorphisms of TNFα in 263 subjects with GBS found that these were not associated with the disease susceptibility but were associated with disease severity, as measured as degree of weakness at the peak of illness and long-term functional outcome (Geleijns, Emonts et al. 2007). A study from China showed that TNFα polymorphisms were associated with the AMAN and AMSAN forms of GBS (Jiao, Wang et al. 2012). An association with GBS was found in a Japanese study (Zhang, Dong et al. 2007). A meta-analysis of data from 1590 cases of GBS found that polymorphisms of TNFα 308G/A were significantly associated with risk of GBS (Wu, Zhou et al. 2012). This makes TNFα the gene with the most evidence for a role in GBS pathogenesis. TNFα inhibition (either by genetically determined polymorphisms or iatrogenic administration of antagonists) could lead to the development of Guillain-Barre syndrome by augmenting the number of activated peripheral T cells or by disturbing the intrinsic balance of TNF and its receptors in the local peripheral nervous system compartment (Shin, Baer et al. 2006).

2.3.6. MMP9

Matrix metalloproteinases can play a role in inflammation, and possibly are involved in opening the blood-nerve barrier. MMP9 is expressed in the peripheral nerve in GBS (Kieseier, Clements et al. 1998), and is upregulated in peripheral blood (Nyati, Prasad et al. 2010). A study of polymorphisms of MMP9 in 269 subjects with GBS found that these were

not associated with the disease susceptibility but were associated with disease severity, as measured as degree of weakness at the peak of illness and long-term functional outcome (Geleijns, Emonts et al. 2007).

2.3.7. Fc Receptors

Fc receptors bind immunoglobulins in immune complexes. Polymorphisms in these receptors have been associated with infectious diseases and autoimmunity (Zidan, Sabbah et al. 2013). Genotyping of 80 GBS patients and 80 healthy controls from North India found that polymorphisms in genes encoding FcγRIIA and FCγRIIIA, but not FcγRIIIB, were associated with GBS (Geleijns, Emonts et al. 2007). The FcγRIIIa gene variants were also associated with severity of GBS (van Sorge, van der Pol et al. 2005). However, this could not be confirmed in a larger metaanalysis (Wu, Zhou et al. 2012). All these receptors bind IgG, but differ in their distribution on immune cells, with FCγRIIIA being found predominantly on NK cells, whereas FCγRIIA and FCγRIIIB are predominantly found on macrophages and neutrophils.

2.3.8. Lipopolysaccaride receptors

Several receptors are involved in detection of microbial antigens by the innate immune system, including Toll-like receptor 4 (TLR) which binds to lipopolysaccharides (LPS) and CD14 (Zanoni and Granucci 2013). The nature of GBS as a post-infectious illness makes an involvement of these proteins in pathogenesis seem possible.

In a Dutch study of 242 GBS subjects polymorphisms of CD14 and TLR4 were not associated with GBS (Geleijns, Jacobs et al. 2004).

2.3.9. Glucocorticoid receptor

Glucocorticoid receptors (GR) act directly in the nucleus to regulate gene transcription. GR also interact with other transcription factors to modulate their ability to regulate transcription. In GBS, polymorphisms of the glucocorticoid receptor have not been found to be linked to susceptibility, but are related to outcome (Dekker, van den Akker et al. 2009).

2.4. Killer immunoglobulin-like receptors

NK cells use a wide variety of surface receptors to recognize infected and malignantly transformed cells and initiate an early immune response. These receptors belong to two distinct families, the C-type lectins-like group (CD94:NKG2) which map to chromosome 12q1.3–13.4, and the immunoglobulin-like super family which consists of the killer immunoglobulin-like receptors (KIRs), leukocyte immunoglobulin-like receptors and the leukocyte-associated immunoglobulin-like receptors and map to chromosome 19q13.4.3. Of those, KIRs constitute the largest family of human NK receptors, with multiple inhibitory and activating members which show extensive polymorphism. They are found on both NK cells and on subgroups of both CD8+ αβ and γδ T lymphocytes (Snyder, Weyand et al. 2004). Interaction of KIR with their Class I HLA ligands can produce both inhibitory and activating signals, that regulate NK cell function (Middleton and Gonzelez 2010).

2.4.1. Genetics of KIR

KIR receptors are encoded by a gene cluster on chromosome 19q13.4 within the leukocyte receptor complex (Kulkarni, Martin et al. 2008). To date, 15 KIR genes and 2 pseudogenes have been described. There is significant variability in the gene content at the KIR locus, which has been attributed to gene duplication and non-allelic homologous recombination (Martin, Bashirova et al. 2003, Martin, Kulski et al. 2004).

The different KIRs are named according to the number of extracellular Ig domains (2D or 3D) and the length of the cytoplasmic tail (L for long, S for short). Long cytoplasmic tails are associated with inhibitory receptors, while short tails are found on activating receptors (Dorak 2007). So, while the intracellular parts of activating and inhibitory KIRs differ significantly, the extracellular parts of activating KIRs are almost identical in sequence to the inhibitory KIRs (Stewart, Laugier-Anfossi et al. 2005).

Two basic haplotypes have been described, termed haplotype A and B. Haplotype A is composed of six inhibitory genes (KIR2DL1, 2DL3, 3DL1, 3DL1, 3DL3, 2DL4) and one activating gene (KIR2DS4), together with two pseudogenes (2DP1 and 3DP1) (see Figure 2.1). Haplotype B is more variable, and consists of a number of both activating and inhibitory genes. KIR genes do not undergo somatic rearrangement, but the number of genes on each haplotype is variable.

Figure 2.1 - Haplotypic variation of 17 KIR genes: Relative distribution of gene frequencies for haplotype A and B (Dorak 2007)

More than 100 highly homologous KIR variant sequences have been deposited in databases (Trowsdale 2001). Consequently, unrelated individuals rarely share identical KIR genotypes (Shilling, Guethlein et al. 2002), and ethnic populations differ markedly in the distribution of KIR genotype frequencies (Yawata, Yawata et al. 2002).

2.4.2. KIR ligands

HLA class I molecules HLA-A, HLA-B and HLA-Cw are ligands for KIRs, allowing NK cells to discriminate between normal cells and cells that have lost the expression of MHC class I (e.g., tumour cells or virally infected cells). HLA genes are located on chromosome 6, and are therefore unlinked to KIR genes on chromosome 19. Thus, inheritance and expression of receptor and ligands are independent of each other. Either receptor or ligand can be absent in individuals, resulting in a functional null situation (Kulkarni, Martin et al. 2008).

The interaction of KIRs with their HLA ligands is complex and not understood in all details. It is likely that multiple interactions with numerous different receptors and ligands are possible simultaneously and can influence each other.

HLA-C is recognized by the KIR 2DL1, 2DL2 and 2DL3, and probably by some of their activating counterparts. Each HLA-C haplotype belongs to two ligand groups, distinguished by a dimorphism at position 80. HLA-C1 has asparagine at this position, whereas HLA-C2

contains lysine. To date, the relationship of the inhibitory KIRs 2DL2 and 2DL3 with HLA-C1 and 2DL1 with HLA-C2 are best understood. It is likely that the activating KIR 2DS2 and 2DS1, whose extracellular domains are very similar to their inhibitory counterparts, can interact with the same HLA molecules.

KIR3DL1, and possibly 3DS1, are known to interact with HLA-Bw4. A polymorphism at position 80 affects this interaction, with isoleucine containing molecules (HLA-Bw4I) displaying a stronger interaction (Carr, Pando et al. 2005).

Other specific interactions of KIR and HLA molecules are likely, but less well described. Specifically, interaction of activating KIR with HLA-C1, –C2 and –Bw4 is likely, even though direct binding has not been shown for all (Fauriat, Ivarsson et al. 2010).

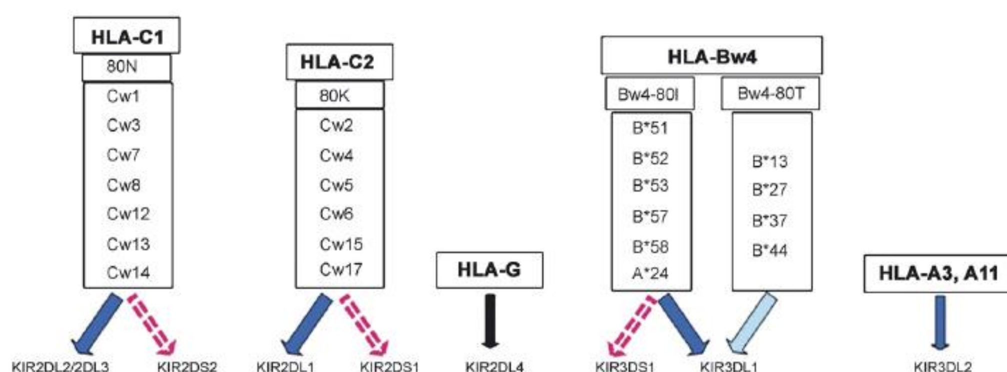


Figure 2.2 –HLA-ligand binding specificities for KIR (Kulkarni, Martin et al. 2008)

2.4.3. *Role of KIR in human disease, focusing on autoimmune disease*

KIRs have been implicated in numerous human diseases, e.g. viral infections like HIV and hepatitis, malignancies (melanoma, lymphoma), pre-eclampsia, graft-versus-host-disease (Venstrom, Gooley et al. 2010) and autoimmune diseases (summarized in (Kulkarni, Martin et al. 2008)).

Specific KIRs have been linked to susceptibility to a variety of other autoimmune diseases, including multiple sclerosis (Fusco, Guerini et al. 2010), Crohn’s disease (Hollenbach, Ladner et al. 2009), Sjögren’s syndrome, systemic lupus erythematosus (Hou, Zhang et al. 2010) and scleroderma (Pellett, Siannis et al. 2007). No studies of KIR in GBS or CIDP have been undertaken so far.

2.5. Conclusions

Whilst incompletely studied, there is some evidence to support clustering of GBS in families and an increased risk for family members, supporting a role for genetic susceptibility in GBS.

A range of candidate genes have been investigated in GBS; however most of these have only been studied in a relatively low number of subjects, and most studies have not been replicated.

Many of the candidate genes studied have been previously linked to other autoimmune diseases, where there is considerable evidence for genetic susceptibility. However, as mentioned, GBS does differ in a number of aspects from autoimmune diseases. Of interest is the lack of an unequivocal Class II HLA association, which is unusual for an autoimmune disease.

Further studies could involve investigation of other, so far untested, candidate genes or a whole genome approach with genome-wide association studies. In this thesis, both approaches have been pursued independently.

2.6. Hypotheses and aims – Part 2

A number of candidate genes have been studied in recent years, but few genes have consistently been found to be associated with GBS. That there is genetic susceptibility to develop GBS seems likely based on the reports of familial forms of this disease, and the links to other inherited neuropathies.

The second hypothesis of this study was that there are genes that predispose to GBS. To test this, the aims were twofold:

Firstly, to investigate the KIR and HLA ligands associated with GBS and to compare this to healthy controls and subjects with CIDP. As outlined above, the KIR-HLA interaction provides an interesting topic for research because of its role in the early innate immune response to infection. Moreover, because of the complex gene-gene interaction, this gene region is not amenable to study using smaller scale genome wide-association studies. It was expected that, if KIR/HLA interactions are important in GBS, there would be genetic

differences between subjects with GBS and healthy controls. These might or might not be seen comparing subjects with CIDP to the GBS cohort.

The second aim was to use the hypothesis-free approach of a genome-wide association study in order to identify regions of interest on the human genome that could contribute to susceptibility to GBS.

Chapter 3 Clinical features of Guillain-Barré Syndrome at seven hospitals on the East Coast of Australia

Rationale

Guillain-Barré syndrome has a wide range of clinical presentations, from mild presentations to severely affected patients who require long periods of intensive supportive therapy. Moreover, GBS is heterogeneous in its electrophysiological properties and associated autoimmune markers.

In the past, AIDP has been thought of as the most common form of GBS in Australia, but this has not been systematically studied.

In order to describe in sufficient detail the patient population studied, the first aim of this thesis was an overview of the clinical features of the population. The population described in this chapter served as basis for recruitment for the genetic studies outlined in the subsequent chapters.

Using hospital records, we collected and analyzed clinical data from 335 patients admitted to seven hospitals along the East Coast of Australia. We found that the predominant form of GBS is AIDP. Axonal and focal variants were less frequently encountered. There was a male predominance, with a male to female ratio of 1.6 : 1.

URTIs were the most commonly identified trigger, with other infections, including those causing diarrhea being relatively uncommon. Consistent with this was the higher incidence of GBS in winter months. However, a wide variety of triggers was identified.

Severity of illness was high, with most patients being bedbound or requiring intensive support during some part of their illness.

GBS in Australia seems to have similar characteristics to other countries of the developed world. In comparison to studies performed 30 years ago, before the wide-spread use of IVIg and plasma exchange for GBS, the illness severity has not significantly decreased, indicating a need for more effective therapies for GBS.

Journal of the Peripheral Nervous System 18:316–320 (2013)

RESEARCH REPORT

Clinical features of patients with Guillain-Barré syndrome at seven hospitals on the East Coast of Australia

Stefan Blum[1,2], Stephen Reddel[3], Judy Spies[4], and Pamela McCombe[1,2]

[1]Department of Neurology, Royal Brisbane and Women's Hospital, Herston, Australia; [2]Centre of Clinical Research, University of Queensland, Herston Campus, Herston, Australia; [3]Department of Neurology, Concord Repatriation General Hospital, Concord, Australia; and [4]Department of Neurology, Royal Prince Alfred Hospital, Camperdown, Australia

Abstract To document the clinical features of Guillain-Barré syndrome (GBS) in Australia, we performed a retrospective analysis of all patients admitted to several hospitals along the East Coast of Australia from 2000 to 2012. Using hospital records, we reviewed all patients with a diagnosis of GBS admitted to seven hospitals. From these, we report information of subjects who fulfilled standard diagnostic criteria. We excluded patients where inadequate information was available or who were under the age of 18. We report the features of 335 patients, in 228 of whom neurophysiological data were available. There were 168 cases of acute inflammatory demyelinating polyneuropathy (AIDP), 17 of acute motor axonal neuropathy (AMAN), 4 of acute motor and sensory axonal neuropathy (AMSAN), and 35 of Miller-Fisher syndrome (MFS). The median age at onset was 52.5 years (18–89 years) with a male : female ratio of 1.61 : 1. Upper respiratory tract infections were the most frequently identified trigger (151 subjects, 44.5%). Most patients were severely affected, with 42.7% of subjects bedbound, and an additional 24% requiring ventilatory support. GBS affects adults of all ages and usually follows a severe clinical course. In contrast to other autoimmune diseases, males are more frequently affected. A wide variety of triggering factors leads to a relatively stereotypical clinical syndrome. The most common variant of GBS in Australia is AIDP. This study shows that the clinical features of GBS in Australia are similar to that previously reported and confirms the male predominance, increased incidence with age, and frequent evidence of peripheral nerve demyelination as features of GBS.

Key words: acute inflammatory demyelinating polyradiculoneuropathy, Australia, epidemiology, Guillain-Barré syndrome, seasonal variation

Introduction

Guillain-Barré syndrome (GBS) is an acute, acquired, immune-mediated peripheral neuropathy that usually has a monophasic course. Several subtypes have been characterized, based on electrophysiological, pathological, and clinical criteria *(Asbury and Cornblath, 1990*; *Hadden et al., 1998)*. Frequently, the diagnosis can be made on clinical grounds and treatment is commenced before electrophysiological data are obtained.

To document the clinical features of GBS in Australia, we performed a retrospective analysis of all patients with this diagnosis admitted to several

Address correspondence to: Stefan Blum, Department of Neurology, Royal Brisbane and Women's Hospital, Butterfield St, Herston QLD 4029, Australia. Tel: +61 7 36468111; Fax: +61 7 36467675, E-mail: stefan_blum@health.qld.gov.au

© 2013 Peripheral Nerve Society

hospitals along the East Coast of Australia from 2000 to 2012.

Patients and Methods

Patient ascertainment

By using computerised records of discharge diagnoses for GBS (ICD Code G61.0), we identified patients admitted to seven hospitals along the East Coast of Australia (Royal Brisbane and Women's Hospital, Princess Alexandra Hospital, Gold Coast Hospital, Nambour Hospital, Townsville Hospital, Royal Prince Alfred Hospital, Concord Hospital) between 2000 and 2012. Both written and electronic clinical records were assessed by a single neurologist (SB). We excluded patients under the age of 18 and patients for whom there was insufficient clinical information to meet standard diagnostic criteria of GBS *(Asbury and Cornblath, 1990)*.

For age adjusted rate of incidence, 2011 data for Queensland and New South Wales was obtained from the Australian Bureau of Statistics (http://www.abs.gov.au).

Clinical data

The severity of disease at the peak of the illness was graded using the GBS Disability Scale (0 = normal, 1 = minimal signs and symptoms, 2 = able to walk without assistance, 3 = able to walk with assistance for 5 m, 4 = bed or chair bound, 5 = requiring ventilatory support, 6 = death) *(Hughes et al., 1978)*. We assessed the charts for details of antecedent events, CSF protein and CSF cell counts and results of electrophysiological studies.

When possible, patients were classified as having acute inflammatory demyelinating polyneuropathy (AIDP), acute motor axonal neuropathy (AMAN), or acute motor and sensory axonal neuropathy (AMSAN) based on standard electrophysiological criteria *(Ho et al., 1995; Hadden et al., 1998; Van der Meche et al., 2001)*. A diagnosis of Miller-Fisher syndrome (MFS) was made in patients with acute onset ataxia, areflexia, and ophthalmoplegia *(Fisher, 1956)*, usually in the context of positive ganglioside GQ1b antibodies.

Statistical analysis

Relationship between age and sex of patients was analysed using ANOVA. Seasonal variation of incidence was analysed using the chi-square test, with the null-hypothesis of no seasonal variation being rejected when $p < 0.05$. Chi-square test was used for other tests.

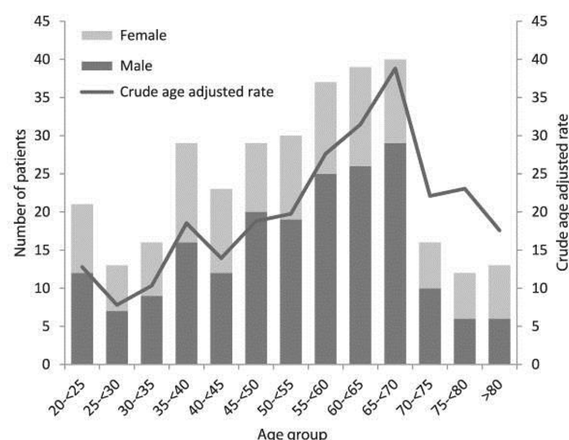


Figure 1. Age distribution in years with and without adjustment for age group. Crude age adjusted rate was calculated using information obtained from the Australian Bureau of Statistics, by dividing the number of subjects per age group by the number of individuals living in the geographical area studied, times 100,000. Patients under the age of 20 were not included in this graph for reasons of clarity (age cut-off for this study was 18).

Ethical approval

This study was reviewed by ethic committees in Queensland and New South Wales. As a retrospective chart review no informed consent was required.

Results

Details of patients

We identified a total of 335 patients. As we did not survey all hospitals servicing the area of study and not all patients with GBS are hospitalized, a population incidence could not be determined.

Gender and age of onset

The incidence of GBS at these hospitals was found to increase from age 25 to age 65, with peak incidence between ages 55 and 65. The lowest incidence was between ages 25 and 34; numbers were as low in the elderly but incidence was higher after adjustment for age (Fig. 1). There was a minor peak of incidence for young adults. The age of onset was not different between the two sexes ($p = 0.499$). Male gender predominated in our cohort with 230 males (61.8%) vs. 142 (38.2%) females (M : F = 1.61 : 1).

Preceding illness

A preceding illness was identified by 253 patients. Upper respiratory tract infections (URTI) were most frequently encountered (151 patients, 45.1%), followed by diarrhea (64 patients, 19.1%) and herpes

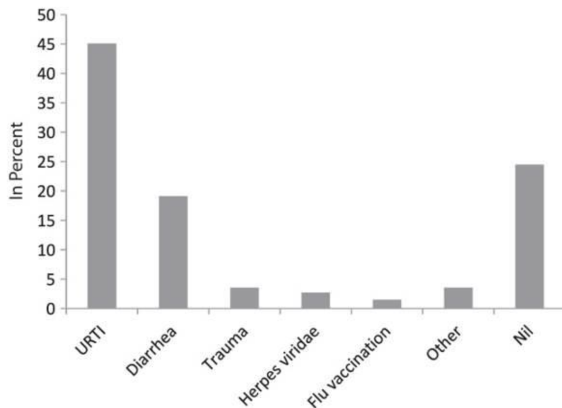


Figure 2. Triggers for developing Guillain-Barré syndrome. Subjects identifying events in the preceding 4 weeks prior to presentation to hospital in percent. Upper respiratory tract infection and diarrhea were most commonly named, with a number of less frequent events.

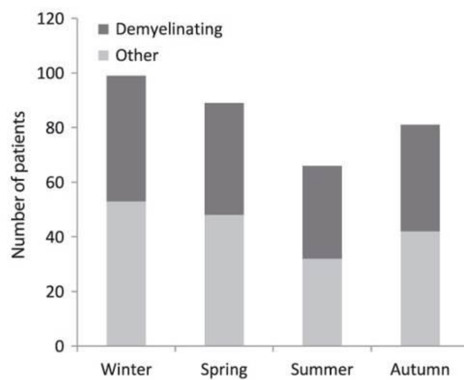


Figure 3. Seasonal variation of Guillain-Barré syndrome incidence. Confirmed demyelinating forms compared with all others (axonal, Miller-Fisher syndrome, no data). Incidence was lowest in summer months, and highest in winter months.

group viral infections (9 patients, 2.7%). Influenza vaccination was named in five patients and preceding trauma or surgery in 12 patients. Other preceding events included facial rash (3), urinary tract infections (2), recurrent seizures (1), acute myocardial infarction (1), conjunctivitis and pregnancy (1). No trigger could be identified in 82 patients (Fig. 2).

Seasonal variation

The incidence of GBS was lowest in the southern hemisphere summer months (December, January, February), and highest in winter (May, June, July) ($p = 0.009$). The lowest incidence was seen in February and the highest in June and July (Fig. 3).

The incidence of GBS triggered by URTIs was significantly higher in the winter months, whereas other triggers were evenly distributed throughout the seasons ($p = 0.02$) (Fig. 4).

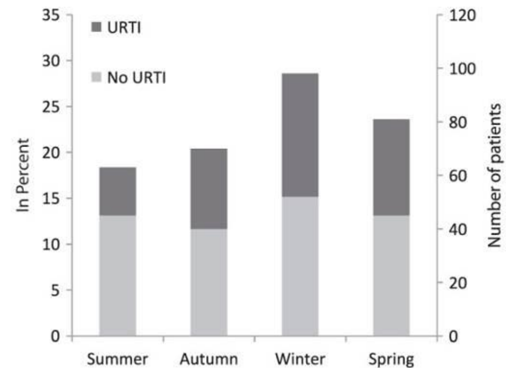


Figure 4. Seasonal variation of Guillain-Barré syndrome by trigger. Highest incidence was seen in the Southern hemisphere winter with more subjects presenting after an upper respiratory tract infection.

Disease subtype

The hospitals studied were distributed over a wide geographical area and included both tertiary referral centres and smaller peripheral hospitals, where electrophysiological studies are not easily available, which explains the incompleteness of electrophysiological data.

For 228 patients, it was possible to assign a disease subtype. For AIDP and AMAN this was based on nerve conduction studies, but in the case of MFS the diagnosis was based on the clinical picture, usually combined with ganglioside GQ1b antibodies. In 107 patients, no definite classification could be reached, usually because nerve conduction studies had not been performed or were unavailable. A diagnosis of AIDP could be made in 168 patients (70.5%), AMAN in 17 patients, and AMSAN in 4 patients (axonal combined: 11.4%). MFS was diagnosed in 35 patients, facial diplegia in 4 patients and the PCB variant of GBS in 2 patients (focal forms combined: 18%) (Fig. 5).

AMAN was significantly associated with diarrhea as the precipitating event (9 patients with diarrhea vs. 8 with other events, $p < 0.001$). This was not observed in AIDP; also, AIDP was not related to URTI as a triggering event ($p = 0.348$).

Illness severity and ICU admissions

Most patients in this series were severely affected at the peak of their illness, with 143 patients (42.7%) being bed- or wheelchair-bound, and an additional 82 patients (24%) requiring ventilatory support (Fig. 6). ICU admission was required in 99 patients (38.2%), with length of stay varying from 2 to 180 days (mean 26.5 days, median: 16 days). Six patients, all of whom

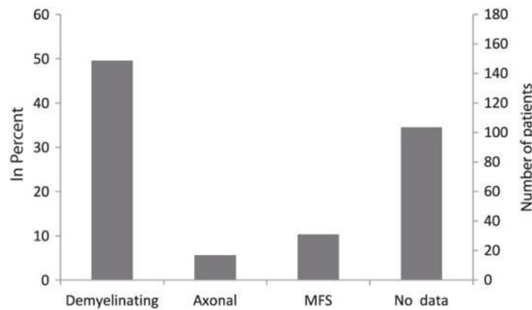


Figure 5. Frequency of disease subtypes, including demyelinating, axonal forms of Guillain-Barré syndrome and Miller-Fisher syndrome. "No data" = no electrophysiological data was obtainable.

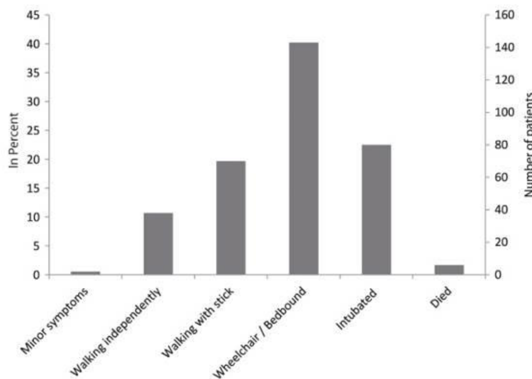


Figure 6. Maximum severity of disability at peak of illness.

were older than 50 years, died of their illness. 302 patients (90.7%) experienced motor weakness of the limbs, with 298 patients (89.3%) having sensory changes. Upper cranial nerves were involved in 175 patients (52.8%), whereas bulbar dysfunction was documented in 126 patients (37.9%). Autonomic dysfunction was present in 70 patients (24%).

Therapy

Intravenous immunoglobulin (IVIg) was given as first line therapy in 281 patients (83.9%), with plasma exchange (PE) used initially in 15 patients (4.5%). A combination of IVIg and PE was used in 17 patients (5.1%). No treatment was given in 22 patients (6.5%), usually when disease severity was mild. For MFS, 24 out 35 patients (68%) were given IVIg; 11 patients had supportive care only.

Discussion

We report the clinical features of a large cohort of Australian patients with GBS. GBS is thought to have an autoimmune pathogenesis, but, in contrast to other autoimmune diseases, GBS has been known to be more common in males than females *(McCombe et al., 2009)*, with a ratio of around 1.25 : 1 *(Sedano et al., 1994; Hughes and Rees, 1997; Bogliun et al., 2004)*. We found a slightly higher male to female ratio in this series (M : F = 1.61 : 1).

GBS has previously been recognized to have a bimodal distribution of age of onset *(Storey et al., 1989)*, and this was evident in our cohort. The bimodal age distribution could be explained by differing exposure to environmental factors at different ages or by immunosenescence *(Prelog, 2006; Le Saux et al., 2012)*. In the very old, lack of recognition of the disease in the context of confounders might explain some of the decrease in incidence seen *(McKhann et al., 1993)*.

As a hospital based series, the severity of illness was high, with two-thirds of patients being unable to walk at the peak of disease. Six patients died during their acute admission. The rate of patients requiring ventilatory support was high and was similar to that reported prior to the widespread use of IVIg, despite almost all patients in this study receiving IVIG or PE *(Larsen et al., 1985; Hankey, 1987; Storey et al., 1989)*.

The geographical distribution differs for axonal and demyelinating subtypes, which has been related to the triggering infectious agent *(Bogliun et al., 2004; Islam et al., 2010; Baker et al., 2013)*. Our study is incomplete in regard to electrophysiological data and subtype classification, largely because a proportion of the patients were treated in smaller hospitals, where this test is unavailable. Where data was obtainable, demyelinating forms predominated, with both focal and axonal forms of GBS seen in less than 15% of patients.

We found a significantly increased incidence of GBS occurring in the southern hemisphere winter months, often after URTIs. Increased incidence in winter months has been noted previously in an Australian series *(Storey et al., 1989)*, and is also seen in some studies from Asia *(McKhann et al., 1993; Islam et al., 2010)*, but not in Europe *(Larsen et al., 1985; Sedano et al., 1994; Van Koningsveld et al., 2000)*.

GBS frequently is preceded by infections, which lead to the concept of molecular mimicry causing the disease *(Damian, 1989; Ang et al., 2004; Hardy et al., 2011)*. This has been best demonstrated with Campylobacter intestinal infections causing the axonal motor variant of GBS *(Islam et al., 2012)*. Axonal GBS was associated with diarrhea in our series. It has been suggested that genetic differences between countries determine GBS subtype and we note that the Australian population is predominantly (85%) of European descent *(Australian Bureau of Statistics, 2012)*. An alternative determinant could be the relative frequencies of antecedent infections, and in particular

Campylobacter jejuni. The incidence of GBS has been reduced with control of Campylobacter infections in New Zealand *(Baker et al., 2013)*.

We saw a wide variety of events being named as preceding GBS, including trauma and surgery which has previously been reported *(Tan et al., 2010)*, status epilepticus, conjunctivitis, facial rash, pregnancy, and herpes viridae infections *(Kang et al., 2010)*. Whilst influenza vaccinations as possible trigger have been extensively studied in recent years *(McGrogan et al., 2009; Centers for Disease Control and Prevention, 2010)*, this has been rarely identified in our series. The wide range of triggering events, including non-infectious triggers, leading to a relatively stereotyped clinical and pathological picture, raises questions about the validity of molecular mimicry as a pathomechanism for all forms of GBS *(Ang et al., 2004)*.

In conclusion, this recent study shows similar clinical features of GBS in Australia compared with previous reports and confirms the male predominance, increased incidence with age, and frequent evidence of peripheral nerve demyelination as features of GBS.

References

Ang CW, Jacobs BC, Laman JD (2004). The Guillain-Barre syndrome: a true case of molecular mimicry. Trends Immunol 25:61–66.

Asbury AK, Cornblath DR (1990). Assessment of current diagnostic criteria for Guillain-Barre syndrome. Ann Neurol 27:S21–S24.

Australian Bureau of Statistics (2012). Cultural diversity of Australia. 20710 – reflecting a nation: stories from the 2011 census, 2012–2013.

Baker MG, Kvalsvig A, Zhang J, Lake R, Sears A, Wilson N (2013). Declining Guillain-Barre syndrome after campylobacteriosis control, New Zealand, 1988–2010. Emerg Infect Dis 18:226–233.

Bogliun G, Beghi E, Italian GBS Registry Study Group (2004). Incidence and clinical features of acute inflammatory polyradiculoneuropathy in Lombardy, Italy, 1996. Acta Neurol Scand 110:100–106.

Centers for Disease Control and Prevention (CDC) (2010). Preliminary results: surveillance for Guillain-Barre syndrome after receipt of influenza A (H1N1) 2009 monovalent vaccine – United States, 2009–2010. MMWR Morb Mortal Wkly Rep 59:657–661.

Damian RT (1989). Molecular mimicry: parasite evasion and host defense. Curr Topics Microbiol Immunol 145:101–115.

Fisher M (1956). Syndrome of ophthalmoplegia, ataxia and areflexia. N Engl J Med 255:57–65.

Hadden RD, Cornblath DR, Hughes RA, Zielasek J, Hartung HP, Toyka KV, Swan AV (1998). Electrophysiological classification of Guillain-Barre syndrome: clinical associations and outcome. Plasma Exchange/Sandoglobulin Guillain-Barre Syndrome Trial Group. Ann Neurol 44:780–788.

Hankey GJ (1987). Guillain-Barre syndrome in Western Australia, 1980–1985. Med J Aust 146:130–133.

Hardy T, Blum S, McCombe P, Reddel S (2011). Guillain-Barré syndrome: modern theories of etiology. Curr Allergy Asthma Rep 11:197–204.

Ho TW, Mishu B, Li CY, Gao CY, Cornblath DR, Griffin JW, Asbury AK, Blaser MJ, McKhann GM (1995). Guillain-Barre syndrome in northern China. Relationship to Campylobacter jejuni infection and anti-glycolipid antibodies. Brain 118:597–605.

Hughes RA, Rees JH (1997). Clinical and epidemiologic features of Guillain-Barre syndrome. J Infect Dis 176:S92–S98.

Hughes RA, Newsom-Davis JM, Perkin GD, Pierce JM (1978). Controlled trial prednisolone in acute polyneuropathy. Lancet 2:750–753.

Islam Z, Jacobs BC, van Belkum A, Mohammad QD, Islam MB, Herbrink P, Diorditsa S, Luby SP, Talukder KA, Endtz HP (2010). Axonal variant of Guillain-Barre syndrome associated with Campylobacter infection in Bangladesh. Neurology 74:581–587.

Islam Z, Gilbert M, Mohammad QD, Klaij K, Li J, van Rijs W, Tio-Gillen AP, Talukder KA, Willison HJ, van Belkum A, Endtz HP, Jacobs BC (2012). Guillain-Barré syndrome-related Campylobacter jejuni in Bangladesh: ganglioside mimicry and cross-reactive antibodies. PLoS ONE 7:e43976.

Kang J-H, Sheu J-J, Lin H-C (2010). Increased risk of Guillain-Barre syndrome following recent herpes zoster: a population-based study across Taiwan. Clin Infect Dis 51:525–530.

Larsen JP, Kvale G, Nyland H (1985). Epidemiology of the Guillain-Barre syndrome in the county of Hordaland, Western Norway. Acta Neurol Scand 71:43–47.

Le Saux S, Weyand CM, Goronzy JJ (2012). Mechanisms of immunosenescence: lessons from models of accelerated immune aging. Ann N Y Acad Sci 1247:69–82.

McCombe PA, Greer JM, Mackay IR (2009). Sexual dimorphism in autoimmune disease. Curr Mol Med 9:1058–1079.

McGrogan A, Madle GC, Seaman HE, de Vries CS (2009). The epidemiology of Guillain-Barre syndrome worldwide. A systematic literature review. Neuroepidemiology 32:150–163.

McKhann GM, Cornblath DR, Griffin JW, Ho TW, Li CY, Jiang Z, Wu HS, Zhaori G, Liu Y, Jou LP, Liu TC, Cy G, Mao JY, Blaser MJ, Mishu B, Asbury AK (1993). Acute motor axonal neuropathy: a frequent cause of acute flaccid paralysis in China. Ann Neurol 33:333–342.

Prelog M (2006). Aging of the immune system: a risk factor for autoimmunity? Autoimmun Rev 5:136–139.

Sedano MJ, Calleja J, Canga E, Berciano J (1994). Guillain-Barre syndrome in Cantabria, Spain. An epidemiologic and clinical study. Acta Neurol Scand 89:287–292.

Storey E, Cook M, Peppard R, Newton-John H, Byrne E (1989). Guillain-Barre syndrome and related conditions in Victorian teaching hospitals 1980–84. Aust N Z J Med 19:687–693.

Tan IL, Ng T, Vucic S (2010). Severe Guillain-Barre syndrome following head trauma. J Clin Neurosci 17:1452–1454.

Van der Meche FG, van Doorn PA, Meulstee J, Jennekens FG, GBS-consensus group of the Dutch Neuromuscular Research Support Centre (2001). Diagnostic and classification criteria for the Guillain-Barre syndrome. Eur Neurol 45: 133–139.

Van Koningsveld R, van Doorn PA, Schmitz PIM, Ang CW, Van der Meche FG (2000). Mild forms of Guillain-Barre syndrome in an epidemiologic survey in the Netherlands. Neurology 54:620–625.

Chapter 4 Killer-immunoglobulin like receptors and their HLA ligands in Guillain-Barré Syndrome

Rationale

In this thesis two different approaches to the study of genetic susceptibility of GBS were used. At first we used a candidate gene approach to study KIRs and their HLA ligands.

NK cells have a range of receptors on their surface controlling their activation status and effector function. Of these, the KIR/HLA system is of paramount interest, not only because of its central role in innate immune system activation, but also because of the wide number of polymorphisms described, which are intricately linked to their function. Both receptor and ligand need to be present for a functional interaction. As such, the KIR/HLA system is not amenable to GWAS.

NK cell function has not been studied in depth in GBS, but there are several links making NK cells a potential key factor in GBS pathogenesis. It appears clear that within days after the triggering event the immune system becomes dysregulated, with this rapid response pointing to dysfunction of the innate immune system, as adaptive responses usually take longer to develop. Also, the fact that a range of triggering events can cause a relatively stereotyped illness points to involvement of the early immune response, which does not have the range of the adaptive immune response.

We recruited subjects with a confirmed diagnosis of GBS based on Asbury criteria from the cohort described in more detail in Chapter 3. Recruitment was incomplete because of the retrospective nature of patient identification (patients moved, deceased, refused consent, etc.). The control subjects were recruited from staff from the Royal Brisbane and Women's Hospital and UQCCR, students, partners of subjects with GBS and other healthy volunteers.

We found that KIR receptor gene frequencies were not different between subjects with GB and healthy controls (HC). In contrast, HLA-C2 and HLA-B Bw4-T were more frequent

in GBS patients than HC. Also, the inhibitory KIR/HLA pairs of KIR-2DL2/HLA-C2 and KIR-3DL1/HLA-B Bw4-T were more common in GBS than healthy controls.

These findings suggest a role for NK cells in the early pathogenesis of GBS. We hypothesize that decreased NK cell activity in the context of an infection could lead to dysregulation of the adaptive immune system with a subsequent immune attack on myelin.

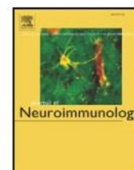
Supplementary data for this publication is included on pages 61-66.

Journal of Neuroimmunology 267 (2014) 92–96

Contents lists available at ScienceDirect

Journal of Neuroimmunology

journal homepage: www.elsevier.com/locate/jneuroim



Killer immunoglobulin-like receptor and their HLA ligands in Guillain–Barré Syndrome

Stefan Blum [a,b,*], Peter Csurhes [b], Stephen Reddel [c], Judy Spies [d], Pamela McCombe [a,b]

[a] Royal Brisbane and Women's Hospital, Department of Neurology, Herston, QLD 4029, Australia
[b] University of Queensland, Centre of Clinical Research, Herston Campus, Herston, QLD 4029, Australia
[c] Concord Repatriation General Hospital, Concord, NSW 2139, Australia
[d] Royal Prince Alfred Hospital, Camperdown, NSW 2050, Australia

ARTICLE INFO

Article history:
Received 28 July 2013
Received in revised form 1 November 2013
Accepted 4 December 2013

Keywords:
Guillain-Barré syndrome
Receptors, KIR
Histocompatibility antigens, Class I
Killer cells, Natural
Gene frequency

ABSTRACT

Guillain–Barré Syndrome (GBS) is an inflammatory neuropathy that occurs in some individuals after exposure to an infectious illness. We investigated the role of Killer-immunoglobulin-like receptors (KIR) and their HLA ligands as potential genetic factors in the pathogenesis of GBS. These receptors are involved in the innate immune response to infections.

Whilst no significant differences in the frequencies KIR genes were found, HLA-C2 and HLA-B Bw4-T were more frequent in subjects with GBS than controls ($p < 0.001$). The inhibitory pairs KIR-2DL2/HLA-C2 and KIR-3DL1/HLA-B Bw4-T were more frequent in GBS than controls (all $p < 0.005$).

We propose that NK cell inhibition is an important factor in the pathogenesis of GBS.

© 2013 Elsevier B.V. All rights reserved.

1. Introduction

Guillain–Barré syndrome (GBS) is an acquired neuropathy characterised by inflammation of peripheral nerve. It has clinical subgroups, including Acute Motor Axonal Neuropathy (AMAN), which is an antibody mediated disease, and Acute Inflammatory Demyelinating Polyradiculopathy (AIDP) where there is inflammation in the peripheral nerve with macrophages and T cells (Asbury et al., 1969). Whilst increased T cell reactivity against myelin antigens has been found in some studies, the specific target antigen in GBS remains unknown (Csurhes et al., 2005a,b). GBS frequently occurs after infections (Winer, 2001). This has led to the suggestion that GBS is due to molecular mimicry, where there is cross-reactivity between antigens on pathogens and those expressed on peripheral nerve (Ang et al., 2004). The best evidence for molecular mimicry is found with the AMAN variant where there is an immune response to *Campylobacter jejuni*. However, a wide variety of infectious agents and physical stressors have been reported to trigger GBS. As well as Campylobacter, these include, *Haemophilus influenza*, *Helicobacter pylori*, *Herpes viridae*, vaccinations and mechanical injury, like surgery or trauma. All these triggers lead to a relatively stereotyped illness course and pathological picture (van Doorn et al., 2008; Blum et al., in press). Particularly for AIDP, this suggests that GBS could be due to a non-specific activation of the immune system by infection or other triggers. Such a non-specific activation could stimulate APCs to present antigen to pre-existing autoreactive T cells (Ichikawa et al., 2002).

The genetic predisposition to GBS is not known. HLA associations are frequently described in human autoimmune diseases (Rose and Bona, 1993). However, in GBS, HLA has been studied in a number of cohorts of different genetic backgrounds with conflicting results and no confirmed association (Gorodezky et al., 1983; Piradov et al., 1995; Koga et al., 1998; Li et al., 2000; Geleijns et al., 2005). These results might have been hampered by imprecise definitions of GBS, the conflation of subjects with different subtypes of disease and the limited numbers of subjects studied.

Natural killer cells (NK cells) are lymphocytes that share common progenitor cells with T lymphocytes (Vivier et al., 2011). NK cells are crucial components of the early innate immune response, due to their ability to produce cytokines and chemokines and lyse target cells (Middleton and Gonzelez, 2010). They also have important roles in the regulation of the subsequent immune response against the pathogen via production of cytokines and chemokines (Vivier et al., 2011).

Killer immunoglobulin-like receptors (KIRs) constitute the largest family of human NK receptors, with multiple inhibitory and activating members showing extensive polymorphisms (Middleton and Gonzelez, 2010). Generally, KIR with a long cytoplasmic tail (i.e. KIR 2DL1) are inhibitory, whereas KIR with a short tail are activating (i.e. KIR 2DS1). KIRs are found on NK cells, but also on subgroups of CD4+ and CD8+ T lymphocytes (van Bergen et al., 2004). For a functional interaction

* Corresponding author at: Royal Brisbane and Women's Hospital, Department of Neurology, Butterfield St., Herston, QLD 4029, Australia. Tel.: +61 7 36468111; fax: +61 7 36467675.
E-mail address: stefan_blum@health.qld.gov.au (S. Blum).

0165-5728/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jneuroim.2013.12.007

both KIR and its specific Class 1HLA-ligand need to be present (Carrington, 2009). The importance of these interactions has been shown in a number of diseases, e.g. in multiple sclerosis, where the KIR 2DS1/HLA-C2 pair appears to be a risk factor (Fusco et al., 2010). KIR and their HLA ligands have also been found to have a role in susceptibility to a range of viruses, but have not been studied for the infective agents more commonly associated with GBS (i.e. Campylobacter, upper respiratory tract infections) (Kulkarni et al., 2008). The intricacies of the KIR/HLA interaction and their role in human diseases are complex and have been reviewed elsewhere (Kulkarni et al., 2008; Rajalingam, 2012).

HLA molecules are down-regulated on the cell surface of virally infected cells and tumour cells (Boyton and Altmann, 2007). Activation of NK cells occurs in the context of 'missing self', i.e. the absence of corresponding HLA molecules on the target cell (Ljunggren and Karre, 1990).

By the time of appearance of symptoms, auto-inflammation in GBS is well established, arguing for an early divergence of the normal immune response to infection and suggesting a role of the innate immune system in GBS pathogenesis (Hardy et al., 2011). NK cells have an important role in the early immune response against pathogens and in the regulation of the adaptive immune system activation, which could make them key players in the aberrant immune response leading to GBS. NK cell activity, but not NK cell frequency, has been found to be decreased in peripheral blood in early GBS, with subsequent recovery of function after plasmapheresis (Yoshii and Shinohara, 1998). As genetic variability can lead to variability in cell function, we have used a genetic approach and have investigated the presence of KIR and their HLA-ligands in subjects with GBS and compared these with healthy controls (HC).

2. Methods

2.1. Subjects

We recruited a cohort of subjects ($n = 195$) with a confirmed diagnosis of GBS from several hospitals on the East Coast of Australia. All subjects fulfilled standard diagnostic criteria for GBS (Asbury and Cornblath, 1990). The clinical characteristics are summarized in Table 1. As a control group healthy volunteers were recruited (79 male, 145 female), and matched for ethnicity. Severity of disease at the peak of the illness was graded using the GBS Disability Scale (Hughes et al., 1978).

Ethical approval was obtained; all subjects gave informed consent.

2.2. Laboratory methods

Highly pure genomic DNA was extracted either from peripheral blood (Nucleospin, Machery Nagel, Dueren, Germany) or saliva (Oragene, DNAGenothek, Kanata, Canada).

For KIR, typing the KIR Genotyping SSP Kit (Invitrogen, Carlsbad, USA) was used. For typing specific KIR associated HLA ligands the KIR-HLA Ligand kit (Olerup, Vienna, Austria) was used. This kit distinguishes HLA-CwAsparagin80 (HLA-C1) from HLA-CwLysine80 (HLA-C2) and HLA-BBw4-Threonine80 (HLA-BBw4-T) from HLA-BBw4+ Isoleucine80 (HLA-BBw4-I), and tests for presence of HLA-ABw4. These kits do not provide complete HLA typing. The kits were used according to the manufacturer's instructions.

PCR was performed followed by analysis with gel electrophoresis using a 2% agarose gel at 100 V and photographed using a ultraviolet analysing system (Biorad).

2.3. Haplotypes and ligands

HLA-C ligands for KIRs are classified as C1 or C2 KIR ligand groups, based on the amino acid at position 80 (Aspargine as C1; Lysine as C2). KIRs interact with specific MHC class 1 ligands on the target cell: KIR 2DL1 and KIR 2DS1 interact with HLA-C2, KIR 2DL2, KIR 2DL3 and KIR 2DS2 have as their ligand the C1 epitope; KIR 3DL1 and KIR 3DS1 recognize the HLA-Bw4 motif (Jamil and Khakoo, 2011).

Genotypes can be resolved into two broad haplotypes termed A and B based on KIR gene content (Middleton and Gonzelez, 2010). Group B KIR haplotypes were characterised by presence of one or more of the following genes: KIR 2DL2, KIR 2DL5, KIR 2DS1, KIR 2DS2, KIR 2DS3, KIR 2DS5 and KIR 3DS1. Conversely, group A haplotypes were characterised by the absence of all these genes. It is difficult to determine with the methods used whether, in the presence of a B haplotype, the other allele is A or B. Therefore, for KIR haplotype analysis AA and BX were used, where X can stand for either an A or B haplotype.

Table 1
Clinical characteristics of subjects with GBS. Severity of illness was assessed using the GBS disability scale (1 = minimal signs and symptoms, 2 = able to walk without assistance, 3 = able to walk with assistance for 5 m, 4 = bed or chair bound, 5 = requiring ventilatory support, 6 = death); URTI = Upper respiratory tract infection; AMSAN = Acute motor and sensory axonal neuropathy; MFS = Miller Fisher Syndrome.

Clinical variables		Values
Sex (M/F)		124/69
Median age at onset (minimum–maximum age)		54.45 years (15.3–89.1 years)
Maximal severity of Illness	1	1
	2	18
	3	30
	4	81
	5	41
	6	2
Subtype	AIDP	98
	AMAN	9
	AMSAN	3
	MFS	16
	No electrophysiological data	69
Triggers	URTI	85
	Diarrhoea	29
	No trigger identified	45
	Other triggers/no information	34

2.4. Statistical analysis

Data analysis was performed using Excel (Microsoft) and GraphPad Prism. Statistical analysis was performed using Chi-Square, with $p < 0.05$ regarded as statistically significant; Bonferroni correction was used for multiple tests, with adjustment of p divided by the number of tests regarded as significant (For KIR genes $p < 0.003$; For HLA ligands $p < 0.01$; for KIR/HLA-ligand interactions $p < 0.005$). The association of each polymorphism with the disease was measured by Odds Ratio (OR) and its 95% confidence interval (95%CI).

3. Results

3.1. KIR and KIR haplotypes

The frequencies of the KIR genes are shown in Table 2. Whilst there were some differences between subjects and HC in the frequency of several KIR genes (KIR 2DL2, KIR 2DL3), these did not reach statistical significance after Bonferroni correction. Also, there was no statistically significant difference between the A haplotype (containing mainly inhibitory KIRs) and BX haplotype (containing both inhibitory and activating KIRs).

3.2. HLA-ligand

The frequency of the HLA genes is shown in Table 3. For HLA genotypes no significant differences were found for HLA-C1, HLA-B Bw4-I+ and HLA-A BW4+. In contrast, HLA-C2 ($p < 0.001$; OR: 1.98, 95% CI: 1.98 (1.61–2.43)), HLA-B Bw4+ T+ ($p < 0.002$; OR: 1.93, 95% CI:

Table 2
Gene frequency of 14 KIR genes and KIR haplotypes in subjects with GBS and controls (ND —not done). Bonferroni correction was performed for multiple tests.

KIR gene	Subjects with GBS (n = 195) N (%)	Controls (n = 225) N (%)	P value
2DL1	182 (93.3)	214 (95.1)	0.613
2DL2	114 (58.5)	110 (48.9%)	0.05
2DL3	160 (82.1)	200 (88.9)	0.046
2DL4	192 (98.5)	224 (99.6)	ND
2DL5A	67 (34.4)	84 (37.3)	0.527
2DL5B	47 (24.1)	47 (20.9)	0.431
2DS1	83 (42.6)	92 (40.9)	0.728
2DS2	106 (54.4)	111 (49.3)	0.304
2DS3	59 (30.3)	56 (24.9)	0.219
2DS4	74 (37.9)	95 (42.2)	0.373
3DL1	174 (89.2)	212 (94.2)	0.061
3DL2	195 (100)	223 (99.1)	ND
3DL3	194 (99.5)	224 (99.5)	ND
3DS1	75 (38.5)	88 (39.1)	0.89
Haplotype			
AA	53 (27.1)	73 (32.4)	0.24
BX	142 (72.9)	152 (67.6)	0.24

1.58–2.38) was seen significantly more frequently in subjects with GBS than in HC.

Also, the presence of two homozygous HLA-C2 alleles (C2/C2) was observed more frequently in subjects with GBS than HC ($p = 0.003$, OR: 2.31, 95% CI: 1.73–3.07), whereas the C1/C1 homozygosity was less frequent in the GBS cohort ($p = 0.001$, OR: 0.52, 95% CI: 0.42–0.63).

3.3. KIR/HLA ligand combinations

In order to assess whether subjects and HC carried both molecules required for the interaction of KIR with its corresponding HLA ligand, or lacked one or both of these molecules, we compared presence and absence of the HLA ligand in those subjects that carried the KIR gene (see Table 4). The inhibitory combinations KIR 2DL1/HLA-C2 ($p < 0.001$, OR: 1.98, 95% CI: 1.32–2.93), KIR 3DL1/HLA-B Bw4-T ($p = 0.0015$, OR: 1.97, 95% CI: 1.29–2.99) were more frequent in subjects with GBS than HC, with KIR 3DL1/HLA-B Bw4+I not reaching statistical significance after Bonferroni correction ($p < 0.021$; OR: 1.66, 95% CI: 1.08–2.56). A reverse situation was seen for KIR 2DL2/HLA-C1 ($p = 0.0016$, OR: 0.29, 95% CI: 0.13–0.64) and KIR 2DS2/HLA-C1 ($p < 0.001$, OR: 0.27, 95% CI: 0.12–0.61), with a small number only seen in the missing HLA ligand situation (Table 4). Other KIR/HLA combinations did not reach statistical significance after Bonferroni correction.

3.4. Gender differences

As KIR and HLA are both autosomal co-dominantly inherited no gender differences were expected. We performed an independent analysis

Table 3
Gene frequency of HLA ligands in subjects with GBS and controls (Bonferroni correction for multiple tests was performed; $p < 0.01$ was regarded as significant).

HLA Gene	Subjects with GBS (n = 195) N (%)	Controls (n = 225) N (%)	P value	OR (95% CI)
HLA-C1	156 (80)	201 (89.3)	0.008	0.48 (0.36–0.63)
HLA-C2	130 (66.6)	113 (50.2)	<0.001**	1.98 (1.61–2.43)
HLA-B Bw4-T	88 (45.1)	67 (29.8)	0.0011*	1.93 (1.58–2.38)
HLA-B Bw4-I	70 (35.9)	61 (27.1)	0.053	1.50 (1.21–1.86)
HLA-A Bw4+	69 (35.4)	63 (28)	0.104	1.41 (1.21–1.86)
C1/C1	65 (33.3)	110 (48.9)	0.0013*	0.52 (0.42–0.63)
C1/C2	91 (46.7)	93 (41.3)	0.272	1.24 (1.01–1.51)
C2/C2	39 (20)	22 (9.8)	0.003*	2.31 (1.73–3.07)

* <0.005.
** <0.001.

of the difference between GBS patients and HC in males and females (see Supplemental Tables 1–3) and the difference in the carriage of KIR and HLA in male and female GBS patients and HC (see Supplemental Tables 4–6). No differences were found between genders.

4. Discussion

GBS is thought to be immune mediated, but differs from other autoimmune diseases, like myasthenia gravis or systemic lupus erythematosus, in a range of important aspects. These include its clinical course, which is monophasic with usually good recovery and no recurrence (Kuitwaard et al., 2009); the male predominance (McCombe et al., 2006); the lack of associated autoimmune diseases and lack of a clear HLA association. Furthermore, GBS usually occurs after a trigger, typically an infection, with the aberrant immune response occurring weeks after the triggering illness. The occurrence of GBS after infection suggests a role for the innate immune system which is involved in the response to infection and which has important links to the adaptive immune system via antigen presentation, co-stimulation and soluble factors (i.e. chemokines, cytokines) (Ichikawa et al., 2002; Vivier et al., 2011).

In GBS, the role of the innate immune system has not been extensively investigated. NK cells are important cells in the innate immune response and are among the first cells to arrive at the site of inflammation. NK cells have important functions in regulating the adaptive immune response (Vivier et al., 2008; Shi et al., 2011). NK cells have been reported to be protective in a range of autoimmune diseases, including Type 1 diabetes and multiple sclerosis (Johansson et al., 2005). NK cell activity was found to be decreased in the early phases of GBS, with subsequent recovery after plasma exchange (Yoshii and Shinohara, 1998). There are no prior studies of receptors regulating NK function in this setting. In this study, we examined the gene frequency of KIRs and their HLA ligands in subjects with GBS.

KIRs have been found to be relevant in a range of autoimmune diseases, including multiple sclerosis, Crohn's disease and a range of rheumatologic disorders (Pellett et al., 2007; Jiao et al., 2008; Hollenbach et al., 2009; Fusco et al., 2010). In order to mask themselves from detection by T lymphocytes many malignant or virally infected cells downregulate the expression of HLA molecules on their cell surface, thereby preventing presentation of peptide fragments for recognition by T lymphocytes. In contrast, NK cells are active in the absence of MHC, enabling detection of non-presenting target cells with subsequent killing of the target cell. The specific receptor–ligand combination is thought to be important for recognizing an infected host cell, with individuals with a large repertoire of functional combinations having a better immune response (Jamil and Khakoo, 2011).

In our cohort, there were no significant differences between subjects with GBS and HC in the frequency of the KIR genes and haplotypes studied. However, HLA ligands HLA-C2 and HLA Bw4+Thr80 were more frequently seen in subjects with GBS than HC. Also, homozygosity for C2 was more frequently seen in the GBS group, whereas HC were more likely to be homozygous for C1 (Table 3).

For a functional interaction both KIR and HLA ligand genes need to be present. This situation was more frequently seen in subjects with GBS for the inhibitory KIR-HLA combinations KIR 3DL1/HLA-B Bw4-T and KIR 2DL1/HLA-C2, when compared with HC (Table 4). A reversed relationship was seen with KIR 2DL1/HLA-C2 and KIR 2DS1/HLA-C2, albeit with small numbers.

In summary, we found that specific combinations of KIR and their HLA ligands can be either risk factors or protective for immune responses triggering GBS. With the exception of KIR 2DL2/HLA-C1 all statistically significant KIR/HLA combinations were inhibitory in nature.

NK cells play a key role in linking the innate immune response and the subsequent adaptive immune response. We hypothesize that decreased NK cell activity, caused by aberrant KIR signalling or other means, could lead to dysregulation of the adaptive immune response,

Table 4
Gene frequency of KIRs and their specific HLA-ligands. Percentages given are for subjects with subjects (n = 195) / controls (n = 225). Chi-square test; Bonferonni correction for multiple tests with p < 0.005 regarded as significant. The likely effect of the population imbalances on degree of NK cell activation or inhibition is indicated in the last column.

KIR gene/HLA ligand gene	GBS n (% of group)	HC n (% of group)	P	OR (95% CI)	Likely NK effect of population imbalance in GBS
2DL1 +/HLA-C2 +	121 (62.1)	107 (47.6)	<0.001**	1.98 (1.32–2.93)	Inhibition
2DL1 +/HLA-C2 −	61 (31.3)	107 (47.6)			
2DS1 +/HLA-C2 +	57 (29.3)	50 (22.2)	0.052	1.84 (0.99–3.42)	
2DS1 +/HLA-C2 −	26 (13.3)	42 (18.7)			
2DL2 +/HLA-C1 +	87 (44.6)	101 (44.9)	0.0016*	0.29 (0.13–0.64)	Activation
2DL2 +/HLA-C1 −	27 (13.9)	9 (4)			
2DS2 +/HLA-C1 +	80 (41.0)	102 (45.3)	0.001*	0.27 (0.12–0.61)	Inhibition
2DS2 +/HLA-C1 −	26 (13.3)	9 (4)			
2DL3 +/HLA-C1 +	130 (66.7)	179 (79.5)	0.025	0.51 (0.28–0.93)	
2DL3 +/HLA-C1 −	30 (15.4)	21 (9.3)			
2DS3 +/HLA-C1 +	49 (25.1)	51 (22.6)	0.20	0.48 (0.15–1.51)	
2DS3 +/HLA-C1 −	10 (5.1)	5 (2.2)			
3DL1 +/HLA-B Bw4-T +	79 (40.5)	63 (28.0)	0.0015*	1.97 (1.29–2.99)	Inhibition
3DL1 +/HLA-B Bw4-T −	95 (48.7)	149 (66.2)			
3DS1 +/HLA-B Bw4-T +	33 (16.9)	28 (12.4)	0.1	1.68 (0.89–3.19)	
3DS1 +/HLA-B Bw4-T −	42 (21.5)	60 (26.7)			
3DL1 +/HLA-B Bw4-I +	65 (33.3)	56 (24.9)	0.021	1.66 (1.08–2.56)	
3DL1 +/HLA-B Bw4-I −	109 (55.9)	156 (69.3)			
3DS1 +/HLA-B Bw4-I +	33 (16.9)	28 (12.4)	0.10	1.68 (0.89–3.19)	
3DS1 +/HLA-B Bw4-I −	42 (21.5)	60 (26.7)			

* $p < 0.005$.
** $P < 0.001$.

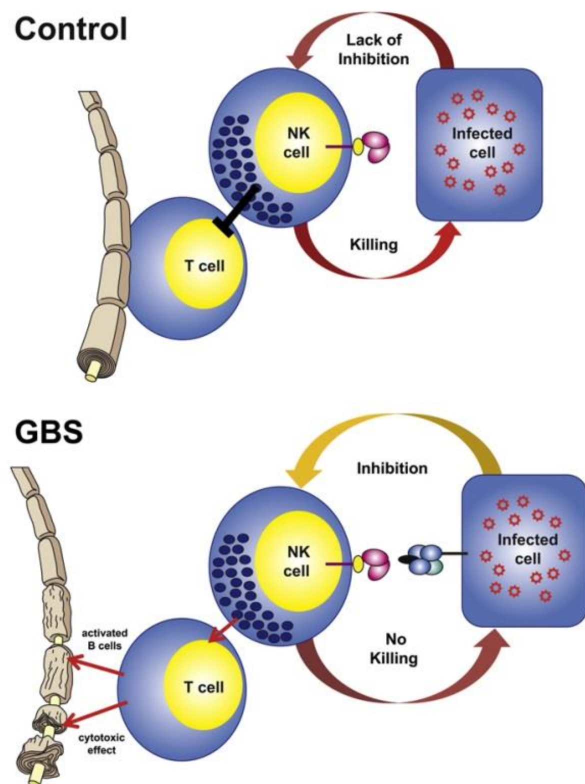


Fig. 1. Functional model of NK cell function in GBS pathogenesis. Recognition of HLA by NK cell KIR inhibits NK cells activity, and thereby protects the target cell from NK cell attack. Decreased NK cell activity can alter the subsequent activation of the adaptive immune system, either by a change in chemokine and cytokine production or by a direct co-stimulatory signal or lack of inhibition of autoreactive T cells. Alternatively, an insufficient NK cell response could necessitate a more active response of the adaptive immune system. This could facilitate the abnormal T cell or B cell mediated attack of nerve tissue in GBS.

with a subsequent aberrant attack against peripheral nerve tissue in GBS (Yoshii and Shinohara, 1998) (Fig. 1). Mechanisms for this could include direct modulation of cytotoxic T cell activation by co-stimulatory signals, e.g. via OX40 (CD134); lack of secretion of inhibitory cytokines like TNF, TGF-β and IL-10 (Zingoni et al., 2005; Vivier et al., 2008; Zingoni et al., 2012) or direct cytotoxic effects of NK cells on proliferating autoreactive T cells as were detected in an EAE mouse model (Xu et al., 2005). Another pathway would be inhibition of activated autoreactive B cells (Takeda and Dennert, 1993). This could be impacted by CD4+ T cells or NKT cells expressing KIR (Chi et al., 2007; Hardy et al., 2011; Soghoian and Streeck, 2010). CD4+ T cells are thought to have a role in GBS pathogenesis (Harness and McCombe, 2008).

Whilst these mechanisms are speculative, we think that our finding of genetic differences of KIR indicates an involvement of the innate immune system in GBS. Whilst our results suggest a novel pathway for the development of disease, it is known that GBS is monophasic, and other regulatory mechanisms, such as regulatory T cells could be required to explain why the disease does not recur (Hardy et al., 2011; Harness and McCombe, 2008; Kuitwaard et al., 2009). Nevertheless, the role of the innate immune system appears to be important in the development of disease.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jneuroim.2013.12.007.

Acknowledgements

We would like to thank the Royal Brisbane and Women's Hospital Foundation for their support of this work with several research grants. We also would like to thank the Herston Multimedia Unit for their assistance with graphic design.

References

Ang, C.W., Jacobs, B.C., Laman, J.D., 2004. The Guillain–Barre; syndrome: a true case of molecular mimicry. Trends Immunol. 25, 61–66.
Asbury, A.K., Cornblath, D.R., 1990. Assessment of current diagnostic criteria for Guillain–Barre syndrome. Ann. Neurol. 27 (Suppl.), S21–S24.
Asbury, A., Arnason, B., Adams, R., 1969. The inflammatory lesion in idiopathic polyneuritis. Medicine 48, 173–215.

Blum, S., Reddel, S., Spies, J., McCombe, P., 2013. Clinical features of patients with Guillain-Barre syndrome at seven hospitals on the East Coast of Australia. J Peripher Nerv Syst. (Oct 31). http://dx.doi.org/10.1111/jns5.12045. (in press) (Epub ahead of print).

Boyton, R.J., Altmann, D.M., 2007. Natural killer cells, killer immunoglobulin-like receptors and human leucocyte antigen class I in disease. Clin. Exp. Immunol. 149, 1–8.

Carrington, M., 2009. The KIR Gene Cluster. NCBI.

Chi, L.-J., Wang, H.-B., Zhang, Y., Wang, W.-Z., 2007. Abnormality of circulating CD4(+) CD25(+) regulatory T cell in patients with Guillain–Barre syndrome. J. Neuroimmunol. 192, 206–214.

Csurhes, P.A., Sullivan, A.-A., Green, K., Greer, J.M., Pender, M.P., McCombe, P.A., 2005a. Increased circulating T cell reactivity to GM1 ganglioside in patients with Guillain–Barré syndrome. J. Clin. Neurosci. 12, 409–415.

Csurhes, P.A., Sullivan, A.A., Green, K., Pender, M.P., McCombe, P.A., 2005b. T cell reactivity to P0, P2, PMP-22, and myelin basic protein in patients with Guillain–Barre syndrome and chronic inflammatory demyelinating polyradiculoneuropathy. J. Neurol. Neurosurg. Psychiatry 76, 1431–1439.

Fusco, C., Guerini, F.R., Nocera, G., Ventrella, G., Caputo, D., Valentino, M.A., et al., 2010. KIRs and their HLA ligands in remitting–relapsing Multiple Sclerosis. J. Neuroimmunol. 229 (1-2), 232–237.

Geleijns, K., Schreuder, G.M.T., Jacobs, B.C., Sintnicolaas, K., van Koningsveld, R., Meulstee, J., et al., 2005. HLA class II alleles are not a general susceptibility factor in Guillain–Barre syndrome. Neurology 64, 44–49.

Gorodezky, C., Varela, B., Castro-Escobar, L.E., Chavez-Negrete, A., Escobar-Gutierrez, A., Martinez-Mata, J., 1983. HLA-DR antigens in Mexican patients with Guillain–Barre syndrome. J. Neuroimmunol. 4, 1–7.

Hardy, T., Blum, S., McCombe, P., Reddel, S., 2011. Guillain–Barré syndrome: modern theories of etiology. Curr. Allergy Asthma Rep. 11, 197–204.

Harness, J., McCombe, P.A., 2008. Increased levels of activated T-cells and reduced levels of CD4/CD25+ cells in peripheral blood of Guillain–Barré syndrome patients compared to controls. J. Clin. Neurosci. 15, 1031–1035.

Hollenbach, J.A., Ladner, M.B., Saeteurn, K., Taylor, K.D., Mei, L., Haritunians, T., et al., 2009. Susceptibility to Crohn's disease is mediated by KIR2DL2/KIR2DL3 heterozygosity and the HLA-C ligand. Immunogenetics 61, 663–671.

Hughes, R.A., Newsom-Davis, J.M., Perkin, G.D., Pierce, J.M., 1978. Controlled trial prednisolone in acute polyneuropathy. Lancet 2, 750–753.

Ichikawa, H.T., Williams, L.P., Segal, B.M., 2002. Activation of APCs through CD40 or Toll-like receptor 9 Overcomes tolerance and precipitates autoimmune disease. J. Immunol. 169, 2781–2787.

Jamil, K.M., Khakoo, S.I., 2011. KIR/HLA interactions and pathogen immunity. J. Biomed. Biotechnol. 2011.

Jiao, Y.L., Ma, C.Y., Wang, L.C., Cui, B., Zhang, J., You, L., et al., 2008. Polymorphisms of KIRs gene and HLA-C alleles in patients with ankylosing spondylitis: possible association with susceptibility to the disease. J. Clin. Immunol. 28, 343–349.

Johansson, S., Berg, L., Hall, H., Höglund, P., 2005. NK cells: elusive players in autoimmunity. Trends Immunol. 26, 613–618.

Koga, M., Yuki, N., Kashiwase, K., Tadokoro, K., Juji, T., Hirata, K., 1998. Guillain–Barre and Fisher's syndromes subsequent to *Campylobacter jejuni* enteritis are associated with HLA-B54 and Cw1 independent of anti-ganglioside antibodies. J. Neuroimmunol. 88, 62–66.

Kuitwaard, K., van Koningsveld, R., Ruts, L., Jacobs, B.C., van Doorn, P.A., 2009. Recurrent Guillain Barre syndrome. J. Neurol. Neurosurg. Psychiatry 80, 56–59.

Kulkarni, S., Martin, M.P., Carrington, M., 2008. The Yin and Yang of HLA and KIR in human disease. Semin. Immunol. 20, 343–352.

Li, H., Yuan, J., Hao, H., Yan, Z., Wang, S., 2000. HLA alleles in patients with Guillain–Barre syndrome. Chin Med J (Engl) 113, 429–432.

Ljunggren, H.G., Karre, K., 1990. In search of the 'missing self': MHC molecules and NK cell recognition. Immunol. Today 11, 237–244.

McCombe, P.A., Csurhes, P.A., Greer, J.M., 2006. Studies of HLA associations in male and female patients with Guillain–Barre syndrome (GBS) and chronic inflammatory demyelinating polyradiculoneuropathy (CIDP). J. Neuroimmunol. 180, 172–177.

Middleton, D., Gonzelez, F., 2010. The extensive polymorphism of KIR genes. Immunology 129, 8–19.

Pellett, F., Siannis, F., Vukin, I., Lee, P., Urowitz, M.B., Gladman, D.D., 2007. KIRs and autoimmune disease: studies in systemic lupus erythematosus and scleroderma. Tissue Antigens 69 (Suppl. 1), 106–108.

Piradov, M.A., Dolbin, A.G., Voylokova, R., Yazdovsky, V.V., 1995. HLA antigens in severe forms of Guillain–Barre syndrome. Neurology 45, 1419–1420.

Rajalingam, R., 2012. Overview of the killer cell immunoglobulin-like receptor system. Methods Mol. Biol. 882, 391–414.

Rose, N.R., Bona, C., 1993. Defining criteria for autoimmune diseases (Witebsky's postulates revisited). Immunol. Today 14, 426–430.

Shi, F.D., Ljunggren, H.G., La Cava, A., Van Kaer, L., 2011. Organ-specific features of natural killer cells. Nat. Rev. Immunol. 11, 658–671.

Soghoian, D.Z., Streeck, H., 2010. Cytolytic CD4(+) T cells in viral immunity. Exp. Rev. Vaccin. 9, 1453–1463.

Takeda, K., Dennert, G., 1993. The development of autoimmunity in C57BL/6 lpr mice correlates with the disappearance of natural killer type 1-positive cells: evidence for their suppressive action on bone marrow stem cell proliferation, B cell immunoglobulin secretion, and autoimmune symptoms. J. Exp. Med. 177, 155–164.

van Bergen, J., Thompson, A., van der Slik, A., Ottenhoff, T.H.M., Gussekloo, J., Koning, F., 2004. Phenotypic and functional characterization of CD4 T cells expressing killer Ig-like receptors. J. Immunol. 173, 6719–6726.

van Doorn, P.A., Ruts, L., Jacobs, B.C., 2008. Clinical features, pathogenesis, and treatment of Guillain–Barré syndrome. Lancet Neurol. 7, 939–950.

Vivier, E., Tomasello, E., Baratin, M., Walzer, T., Ugolini, S., 2008. Functions of natural killer cells. Nat. Immunol. 9, 503–510.

Vivier, E., Raulet, D.H., Moretta, A., Caligiuri, M.A., Zitvogel, L., Lanier, L.L., et al., 2011. Innate or adaptive immunity? The example of natural killer cells. Science 331, 44–49.

Winer, J.B., 2001. Guillain Barre syndrome. Mol. Pathol. 54, 381–385.

Xu, W., Fazekas, G., Hara, H., Tabira, T., 2005. Mechanism of natural killer (NK) cell regulatory role in experimental autoimmune encephalomyelitis. J. Neuroimmunol. 163, 24–30.

Yoshii, F., Shinohara, Y., 1998. Natural killer cells in patients with Guillain–Barre syndrome. J. Neurol. Sci. 157, 175–178.

Zingoni, A., Sornasse, T., Cocks, B.G., Tanaka, Y., Santoni, A., Lanier, L.L., 2005. NK cell regulation of T cell-mediated responses. Mol. Immunol. 42, 451–454.

Zingoni, A., Ardolino, M., Santoni, A., Cerboni, C., 2012. NKG2D and DNAM-1 activating receptors and their ligands in NK-T cell interactions: role in the NK cell-mediated negative regulation of T cell responses. Front. Immunol. 3, 408.

Supplemental Table 1 – Genefrequency of HLA ligands in subjects with GBS and healthy controls by gender

	Total				**Male**				**Female**			
HLA Gene	GBS (n=195) N (%)	Controls (n=225) N (%)	P	OR (95% CI)	GBS (n=126) N (%)	Controls (n=79) N(%)	P	OR (95% CI)	GBS (n=69) N(%)	Controls (n=146) N(%)	P	OR (95% CI)
HLA-C1	156 (80)	201 (89.3)	0.008	0.48 (0.36 – 0.63)	103 (81.7)	68 (86.1)	0.42	0.72 (0.33 – 1.58)	53 (76.8)	133 (91.1)	0.01	0.32 (0.14 – 0.72)
HLA-C2	130 (66.6)	113 (50.2)	<0.001	1.98 (1.61 – 2.43)	86 (68.3)	41 (51.9)	0.02	1.99 (1.11 – 3.56)	44 (63.7)	72 (49.3)	0.05	1.80 (1.0 – 3.26)
HLA-B Bw4-T	88 (45.1)	67 (29.8)	0.0011	1.93 (1.58 – 2.38)	57 (45.2)	22 (27.8)	0.01	2.14 (1.17-3.91)	31 (44.9)	45 (30.8)	0.04	1.83 (1.02 – 3.3)
HLA-B Bw4-I	70 (35.9)	61 (27.1)	0.053	1.50 (1.21 – 1.86)	44 (34.9)	21 (26.6)	0.21	1.48 (0.8 – 2.75)	26 (37.7)	40 (27.4)	0.13	1.60 (0.87 – 2.94)
HLA-A Bw4+	69 (35.4)	63 (28)	0.104	1.41 (1.21 – 1.86)	44 (34.9)	26 (32.9)	0.77	1.09 (0.6 – 1.98)	25 (36.2)	37 (25.3)	0.1	1.67 (0.9 – 3.1)
C1/C1	65 (33.3)	110 (48.9)	0.0013	0.52 (0.42 – 0.63)	40 (31.7)	38(48.1)	0.03	0.05 (0.28 – 0.9)	25 (36.2)	72 (50)	0.08	0.58 (0.32 – 1.05)
C1/C2	91 (46.7)	93 (41.3)	0.272	1.24 (1.01 – 1.51)	63 (50)	31 (39.2)	0.15	1.55 (0.87 – 2.74)	28 (40.6)	61 (42.4)	0.88	0.95 (0.53 – 1.7)
C2/C2	39 (20)	22 (9.8)	0.003	2.31 (1.73 – 3.07)	23 (18.3)	10 (12.7)	0.69	1.29 (0.58 – 2.87)	16 (23.2)	11 (7.6)	0.002	3.7 (1.61 – 8.5)

Supplemental Table 2 – Gene frequency of 14 KIR genes and KIR haplotypes of GBS patients and healthy controls by gender.

	Total				**Male**				**Female**			
KIR gene	**GBS (n = 195)**	**Controls (n=225)**	**P**	**OR (95% CI)**	**GBS (n = 126)**	**Controls (n=79)**	**P**	**OR (95% CI)**	**GBS (n = 69)**	**Controls (n=146)**	**P**	**OR (95% CI)**
2DL1	182 (93.3)	214 (95.1)	0.43	0.72 (0.31 – 1.65)	118 (93.6)	75 (94.9)	0.7	0.78 (0.23 – 2.7)	64 (92.7)	139 (95.2)	0.46	0.64 (0.19 – 2.11)
2DL2	114 (58.5)	110 (48.9%)	0.05	1.47 (1.0 – 2.17)	79 (62.7)	42 (53.1)	0.17	1.48 (0.83 – 2.62)	35 (50.7)	68 (46.6)	0.57	1.18 (0.66 – 2.1)
2DL3	160 (82.1)	200 (88.9)	0.05	0.57 (0.33 – 0.99)	105 (83.3)	69 (87.3)	0.44	0.72 (0.32 – 1.63)	55 (79.7)	131 (89.7)	0.045	0.45 (0.2 – 0.99)
2DL4	192 (98.5)	224 (99.6)	ND		124 (98.4)	78 (98.7)	ND		68 (98.6)	146 (100)	ND	
2DL5A	67 (34.4)	84 (37.3)	0.52	0.88 (0.59 – 1.31)	48 (38.1)	25 (31.6)	0.34	1.30 (0.73 – 2.4)	19 (27.5)	59 (40.4)	0.07	0.56 (0.3 – 1.05)
2DL5B	47 (24.1)	47 (20.9)	0.43	1.2 (0.76 – 1.9)	33 (26.2)	16 (20.2)	0.33	1.4 (0.71 – 2.75)	14 (20.3)	31 (21.2)	0.87	0.94 (0.47 – 1.91)
2DS1	83 (42.6)	92 (40.9)	0.72	1.07 (0.72 – 1.58)	54 (42.8)	27 (34.2)	0.22	1.44 (0.8 – 2.59)	29 (42.0)	65 (44.5)	0.73	0.9 (0.5 – 1.61)
2DS2	106 (54.4)	111 (49.3)	0.30	1.22 (0.83 – 1.8)	73 (57.9)	44 (55.7)	0.75	1.1 (0.62 – 1.93)	33 (47.8)	67 (45.9)	0.79	1.08 (0.61 – 1.91)
2DS3	59 (30.3)	56 (24.9)	0.22	1.31 (0.85 – 2.01)	42 (33.3)	19 (24.0)	0.15	1.58 (0.84 – 2.98)	17 (24.6)	37 (25.3)	0.91	0.96 (0.46 – 1.87)
2DS4	74 (37.9)	95 (42.2)	0.37	0.84 (0.57 – 1.24)	46 (36.5)	36 (45.5)	0.2	0.68 (0.39 – 1.22)	28 (40.6)	59 (40.4)	0.98	1.0 (0.56 – 1.8)
3DL1	174 (89.2)	212 (94.2)	0.061	0.51 (0.25 – 1.04)	111 (88.1)	76 (96.2)	0.05	0.29 (0.08 – 1.04)	63 (91.3)	136 (93.1)	0.63	0.77 (0.27 – 2.21)
3DL2	195 (100)	223 (99.1)	ND		126 (100)	78 (98.7)	ND		69 (100)	145 (99.3)	ND	
3DL3	194 (99.5)	224 (99.5)	ND		125 (99.2)	78 (98.7)	ND		69 (100)	146 (100)	ND	
3DS1	75 (38.5)	88 (39.1)	0.89	0.97 (0.66 – 1.44)	57 (45.2)	27 (34.2)	0.11	1.59 (0.89 – 2.85)	23 (33.3)	61 (38.4)	0.23	0.69 (0.38 – 1.27)

Supplemental Table 3 – Genefrequency of KIRs and their specific HLA-Ligands, analysed by gender. Chi-Square test with Bonferonni correction for multiple tests; $p<0.005$ was regarded as significant.

	Total				**Male**				**Female**			
KIR gene / HLA ligand gene	**GBS n (%)**	**HC n (%)**	**P**	**OR (95% CI)**	**GBS n (%)**	**HC n (%)**	**P**	**OR (95% CI)**	**GBS n (%)**	**HC n (%)**	**P**	**OR (95% CI)**
2DL1 + / HLA-C2 +	121 (62.1)	107 (47.6)	<0.001	1.98 (1.32 – 2.93)	81 (64.3)	40 (50.6)	0.01	2.07 (1.13 – 3.79)	40 (58.0)	67 (45.9)	0.06	1.8 (0.98 – 2.29)
2DL1 + / HLA-C2 -	61 (31.3)	107 (47.6)			37 (29.3)	35 (44.3)			24 (34.8)	72 (49.3)		
2DS1 + / HLA-C2 +	57 (29.3)	50 (22.2)	0.052	1.84 (0.99 – 3.42)	37 (29.3)	17 (13.5)	0.25	1.74 (0.67 – 4.51)	20 (29.0)	35 (24.0)	0.17	1.91 (0.75 – 4.80)
2DS1 + / HLA-C2 -	26 (13.3)	42 (18.7)			15 (18.9)	12 (15.2)			9 (13.0)	30 (20.5)		
2DL2 + / HLA-C1 +	87 (44.6)	101 (44.9)	0.0016	0.29 (0.13 – 0.64)	62 (49.2)	17 (13.5)	0.52	0.64 (0.16 – 2.46)	25 (36.2)	62 (42.5)	<0.001	0.08 (0.01 – 0.39)
2DL2 + / HLA-C1 -	27 (13.9)	9 (4)			39 (49.3)	3 (3.8)			10 (14.5)	6 (4)		
2DS2 + / HLA-C1 +	80 (41.0)	102 (45.3)	0.001	0.27 (0.12 – 0.61)	56 (44.4)	41 (51.9)	0.02	0.24 (0.06 – 0.87)	24 (34.8)	61 (41.8)	0.02	0.26 (0.08 – 0.8)
2DS2 + / HLA-C1 -	26 (13.3)	9 (4)			17 (13.5)	3 (3.8)			9 (13.0)	6 (4.1)		
2DL3 + / HLA-C1 +	130 (66.7)	179 (79.5)	0.025	0.51 (0.28 – 0.93)	85 (67.4)	59 (74.7)	0.44	0.72 (0.31 – 1.65)	45 (65.2)	120 (82.2)	0.05	0.41 (0.16 – 1.04)
2DL3 + / HLA-C1 -	30 (15.4)	21 (9.3)			20 (15.9)	10 (12.7)			10 (14.5)	11 (7.5)		
2DS3 + / HLA-C1 +	49 (25.1)	51 (22.6)	0.20	0.48 (0.15 – 1.51)	36 (28.5)	17 (21.5)	0.26	0.44 (0.1 – 1.88)	13 (18.8)	34 (23.3)	0.11	0.29 (0.05 – 1.46)
2DS3 + / HLA-C1 -	10 (5.1)	5 (2.2)			6 (4.7)	2 (2.5)			4 (5.8)	3(2.1)		
3DL1 + / HLA-B Bw4-T +	79 (40.5)	63 (28.0)	0.0015	1.97 (1.29 – 2.99)	50 (39.6)	21 (26.6)	0.01	2.15 (1.15 – 4.0)	29 (42.0)	42 (28.7)	0.04	1.91 (1.03 – 3.53)
3DL1 + / HLA-B Bw4-T -	95 (48.7)	149 (66.2)			61 (48.4)	55 (69.6)			34 (49.2)	94 (64.4)		
3DS1 + / HLA-B Bw4-T +	33 (16.9)	28 (12.4)	0.1	1.68 (0.89 – 3.19)	20 (15.9)	9 (11.4)	0.87	1.08 (0.41 – 2.85)	13 (18.8)	19 (13.0)	0.001	5.75 (1.79 – 18.4)
3DS1 + / HLA-B Bw4-T -	42 (21.5)	60 (26.7)			37 (29.4)	18 (22.8)			5 (7.24)	42 (28.7)		
3DL1 + / HLA-B Bw4-I +	65 (33.3)	56 (24.9)	0.021	1.66 (1.08 – 2.56)	40 (31.7)	20 (25.3)	0.16	1.57 (0.83 – 2.99)	25 (36.2)	36 (24.6)	0.06	1.82 (0.97 – 3.44)
3DL1 + / HLA-B Bw4-I -	109 (55.9)	156 (69.3)			71 (56.3)	56 (70.9)			38 (55.0)	100 (68.5)		
3DS1 + / HLA-B Bw4-I +	33 (16.9)	28 (12.4)	0.10	1.68 (0.89 – 3.19)	23 (18.3)	8 (9.1)	0.17	1.95 (0.74 – 5.01)	10 (13.3)	20 (13.7)	0.12	2.31 (0.78 – 6.79)
3DS1 + / HLA-B Bw4-I -	42 (21.5)	60 (26.7)			34 (27.0)	23 (29.1)			8 (10.6)	37 (49.3)		

Supplemental Table 4 – Differences in genefrequency of HLA ligands between gender for both GBS subjects and controls

		GBS				**Controls**		
HLA Gene		Males (n=126)	Females (n=69)	P		Males (n=79)	Females (n=146)	P
HLA-C1		103	53	0.41		68	133	0.24
HLA-C2		86	44	0.53		41	72	0.71
HLA-B Bw4-T		57	31	1		22	45	0.76
HLA-B Bw4-I		44	26	0.76		21	40	0.89
HLA-A Bw4+		44	25	0.88		26	37	0.23
C1/C1		40	25	0.53		38	72	0.89
C1/C2		63	28	0.23		31	61	0.78
C2/C2		23	16	0.46		10	11	0.23

Supplemental Table 5 – Differences in genefrequency of KIR between gender for both GBS subjects and controls

		GBS				Controls		
KIR gene		**Males (n = 126)**	**Females (n=69)**	**P**		**Males (n = 79)**	**Females (n=146)**	**P**
2DL1		118	64	0.81		75	139	0.93
2DL2		79	35	0.1		42	68	0.35
2DL3		105	55	0.53		69	131	0.59
2DL4		124	68	ND		78	146	ND
2DL5A		48	19	0.14		25	59	0.2
2DL5B		33	14	0.36		16	31	0.86
2DS1		54	29	0.91		27	65	0.13
2DS2		73	33	0.18		44	67	0.16
2DS3		42	17	0.21		19	37	0.83
2DS4		46	28	0.57		36	59	0.46
3DL1		111	63	0.49		76	136	0.35
3DL2		126	69	ND		78	145	ND
3DL3		125	69	ND		78	146	ND
3DS1		57	23	0.11		27	61	0.26

Supplemental Table 6 – Differences in genefrequency of KIRs and their specific HLA-Ligands between gender.

		GBS				Controls		
KIR gene / HLA ligand gene		**Males**	**Females**	**P**		**Males**	**Females**	P
2DL1 + / HLA-C2 +		81	40	0.4		40	67	0.47
2DL1 + / HLA-C2 -		37	24			35	72	
2DS1 + / HLA-C2 +		37	20	0.84		17	35	0.67
2DS1 + / HLA-C2 -		15	9			12	30	
2DL2 + / HLA-C1 +		62	25	0.29		17	25	0.25
2DL2 + / HLA-C1 -		39	10			3	10	
2DS2 + / HLA-C1 +		56	24	0.66		41	61	0.69
2DS2 + / HLA-C1 -		17	9			3	6	
2DL3 + / HLA-C1 +		85	45	0.89		59	120	0.18
2DL3 + / HLA-C1 -		20	10			10	11	
2DS3 + / HLA-C1 +		36	13	0.39		17	34	0.76
2DS3 + / HLA-C1 -		6	4			2	3	
3DL1 + / HLA-B Bw4-T +		50	29	0.9		21	42	0.62
3DL1 + / HLA-B Bw4-T -		61	34			55	94	
3DS1 + / HLA-B Bw4-T +		20	13	0.57		9	19	0.84
3DS1 + / HLA-B Bw4-T -		37	5			18	42	
3DL1 + / HLA-B Bw4-I +		40	25	0.63		20	36	0.98
3DL1 + / HLA-B Bw4-I -		71	38			56	100	
3DS1 + / HLA-B Bw4-I +		23	10	0.26		8	20	0.37
3DS1 + / HLA-B Bw4-I -		34	8			23	37	

Chapter 5 KIR and their HLA ligands in patients with chronic inflammatory demyelinating polyradiculoneuropathy

To compare our findings of KIR and the HLA ligands in GBS with other inflammatory neuropathies, we proceeded to investigate the same genetic markers in chronic inflammatory demyelinating polyradiculoneuropathy.

5.1. Chronic inflammatory demyelinating polyradiculoneuropathy

Chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) is an acquired disorder of peripheral nerves and nerve roots leading to progressive or relapsing weakness and sensory loss over years. While patients with typical signs and symptoms have been reported for more than 75 years, the concept of CIDP as a separate entity was introduced in 1975 (Dyck, Lais et al. 1975). The autoimmune nature of CIDP was previously suggested by the response of patients with certain types of neuropathies to treatment with glucocorticoids (DeVivo and Engel 1970). Since then multiple therapies, including plasma exchange, intravenous immunoglobulins and a range of immunosuppressive drugs have been successfully used in CIDP (Koller, Schroeter et al. 2005).

Whether CIDP is a single disease or a syndrome is still controversial. Many other neuropathies have chronicity, demyelination, inflammation on nerve biopsy or other evidence of immune mediation in common with CIDP. These include multifocal motor neuropathy (MMN), Lewis-Sumner syndrome, anti-MAG neuropathy and POEMS (polyneuropathy, organomegaly, endocrinopathy, monoclonal protein, skin changes) syndrome. Moreover, a range of systemic disorders can be associated with an inflammatory demyelinating neuropathy, including Hepatitis B and C, HIV, lymphoma, diabetes mellitus and renal disease (Csurhes, Sullivan et al. 2005, Dalakas 2011). However, CIDP can also be triggered by

infection. Concurrent central nervous system and peripheral nervous system demyelination is sometimes found, meeting diagnostic criteria of both CIDP and MS, and in such cases there could be an immune attack on antigens that are common to the peripheral and central nervous systems (Sharma, Saadia et al. 2008). Onset of disease is either subacute or chronic, with the nadir of disease being reached after 8 weeks, differentiating it from AIDP. The course of CIDP is either progressive or relapsing-remitting.

5.1.1. Diagnostic criteria and electrophysiology

Numerous diagnostic criteria have been proposed for CIDP since its first description in 1975 (Van den Bergh and Pieret 2004, Koller, Schroeter et al. 2005, Koski, Baumgarten et al. 2009, Rajabally, Nicolas et al. 2009). These criteria differ in a number of aspects, but usually rely heavily on electrophysiological studies.

For the purposes of this study, the electrodiagnostic criteria proposed by the American Academy of Neurology were used (see Table 5.1).

5.1.2. Pathology

Characteristically, segmental demyelination and remyelination with onion bulb formation as well as lymphocytic and macrophage infiltration are found (Bouchard, Lacroix et al. 1999). Nerve enlargements are thought to be due to oedema, onion bulb formation and the deposition of acid mucopolysaccharides.

CD3+ T cells can primarily be localized to perivascular infiltrates in the epineurium and perineurium, whereas CD68+ immunoreactive macrophages are localized within the endoneurium (Sharma, Saadia et al. 2008). Increased numbers of IFN-γ and IL-5 secreting cells in the peripheral blood of patients with CIDP were found in one study (Csurhes, Sullivan et al. 2005). Also, increased T cell mediated response against PMP-$22_{51\text{-}64}$ was found (Csurhes, Sullivan et al. 2005). Circulating CD4+CD25+ regulatory T cells showed both decreased numbers and suppressive function in CIDP (Chi, Wang et al. 2008). Often axonal degeneration is superimposed on this demyelinating process, especially with advancing disease. Demyelination occurs by active unravelling and degrading of myelin (Prineas 1971).

Table 5.1 - Electrodiagnostic criteria for CIDP as proposed by the American Academy of Neurology (1991)

At least three of the following four to be fulfilled:

1. Significant reduction in motor nerve conduction velocity in two or more motor nerves:

a. ,80% of LLN if CMAP >80% of LLN
b. ,70% of LLN if CMAP <80% of LLN

2. Partial conduction block or abnormal temporal dispersion in one or more motor nerves: either peroneal nerve between ankle and below fibular head, median nerve between wrist and elbow, or ulnar nerve between wrist and below elbow.

Criteria suggestive of partial conduction block:

>15% change in duration between proximal and distal sites and >20% drop in negative peak (–p) area or peak-to-peak (pp) amplitude between proximal and distal sites.

Criteria for abnormal temporal dispersion and possible conduction block:

>15% change in duration between proximal and distal sites and .20% drop in –p area or p-p amplitude between proximal and distal sites

3. Significant prolongation of distal motor latency in two or more motor nerves:

a. .125% of ULN if CMAP >80% of LLN
b. .150% of ULN if CMAP <80% of LLN

4. Significant prolongation or absence of F-waves in two or more motor nerves:

a. .120% of ULN if CMAP >80% of LLN
b. .150% of ULN if CMAP <80% of LLN

Moreover, antibodies against P0 and P2 were detected in patients with CIDP (Yan, Archelos et al. 2001, Inglis, Csurhes et al. 2007). Deposition of immunoglobulin IgM and complement or its membrane attack complex on the myelin surface has also been reported,

arguing for both antibody-mediated and cell-based attack on peripheral nerve (Ben-Smith, Gaston et al. 1996, Sanvito, Makowska et al. 2009).

Studies of NK cells in CIDP are limited in number. Decreased numbers of circulating NK were detected in one study (Sanvito, Makowska et al. 2009).

5.1.3. Immunogenetics

There are few recent studies of immunogenetics in CIDP. Most previous studies were performed at a time when the heterogeneity of CIDP was not fully appreciated and it is possible that subjects with other inflammatory demyelinating neuropathies, such as the neuropathy associated with anti-MAG antibodies or multifocal motor neuropathy, were included. The study of CIDP within families is impacted by the similarities between CIDP and inherited neuropathies like Charcot-Marie Tooth disease (CMT). Interestingly, there are a number of reports of genetically proven CMT1A with inflammatory changes of nerve biopsy, responsive to treatment with corticosteroids (Dyck, Swanson et al. 1982, Vital, Vital et al. 2003, Ginsberg, Malik et al. 2004).

Weak HLA associations with HLA-8, CW7, and DR3 were detected in several small early studies, but could not be confirmed in others (Adams, Festenstein et al. 1979, Feeney, Pollard et al. 1990, van, Schreuder et al. 1991, McCombe, Csurhes et al. 2006). The frequency of the M3 allele of α1 anti-trypsin was found to be increased from 11.5% in controls to 29% in CIDP patients (McCombe, Clark et al. 1985).

Differential gene expression in sural biopsies and skin biopsies in patients with CIDP compared with healthy controls showed genes predominantly involved in immunity and signal transduction (Renaud, Hays et al. 2005). Consistent with the pathological features, these include macrophage genes (Macrophage scavenger receptor 1, Allograft Inflammatory factor) and T cell genes (T cell receptor beta locus, class II HLA). Also, Stearoyl CoA desaturase, the rate limiting enzyme in the biosynthesis of monounsaturated fatty acid (including oleic acid, a major fatty acid in PNS myelin) was upregulated.

Upregulation of genes involved in inflammatory processes were also detected in skin biopsies of patients with CIDP (Lee and Shin). Again, these genes were associated with macrophage activation and differentiation. Upregulation of these genes in skin biopsies were thought to be indicative of a systemic heightened immune state or an inflammatory reaction to myelinated fibres in the skin.

Also, genetic differences were found in the V24JQ invariant TCR chain in NKT cells originating from peripheral blood and sural nerve biopsies; NKT cells have important roles in IL-4 production and regulation of the immune response to a Th2 profile (Illes, Kondo et al. 2000, Hughes, Allen et al. 2006) and in the response to lipid antigens (Bondarenko, Catapano et al. 2013).

5.1.4. Comparison of CIDP and GBS

CIDP shares a number of clinical, pathological and electrophysiological features with GBS, whilst differing in others (summarized in Table 5.2). Common features include male predominance, and the relatively symmetrical, predominantly motor neuropathy, with frequent involvement of other nerve fibres (sensory, autonomic) (McCombe, Pollard et al. 1987, Hughes, Allen et al. 2006, Dionne, Nicolle et al. 2010). CSF protein is increased in both conditions, indicating breakdown of the blood-nerve barrier at a root level with subsequent leakage of albumin into the CSF (Kanda 2013). There is a response to immune therapy in both illnesses, with a generally favourable prognosis (Kuwabara, Misawa et al. 2006)

CIDP differs from GBS in a range of aspects, including length of disease and treatment options (see Table 5.2). By definition, CIDP is a chronic illness with a time-course of more than 2 months, in contrast to GBS where the nadir of illness is reached within 4 weeks of symptom onset (McCombe, Pollard et al. 1987, Dionne, Nicolle et al. 2010). Treatment for GBS relies on IVIg and plasma exchange combined with supportive care (Hughes, Swan et al. 2007). These agents have also been proven effective for CIDP (Hahn, Bolton et al. 1996, Mehndiratta and Hughes 2012, Nobile-Orazio, Cocito et al. 2012) . Chronic steroid therapy, which is detrimental in GBS, has been shown to be beneficial in CIDP (Dyck, O'Brien et al. 1982, Nobile-Orazio, Cocito et al. 2012). In CIDP, this is frequently combined with steroid sparing agents like azathioprine or methotrexate, but evidence for this is less clear, with only case series being available (Dyck, O'Brien et al. 1985, Gladstone, Prestrud et al. 2005).

Table 5.2 - Comparison of clinical and paraclinical features of CIDP and GBS

	GBS	CIDP
Time course	Less than 4 weeks to nadir, followed by recover	More than 8 weeks to nadir, relapsing or progressive course
Gender	Male predominant	Male predominant (Hughes, Allen et al. 2006)
Clinical picture	Symmetric sensory-motor peripheral neuropathy Bulbar involvement in more than 30% (our data)(Dionne, Nicolle et al. 2010)	Bulbar dysfunction in 6 to 15 % (McCombe, Pollard et al. 1987, Dionne, Nicolle et al. 2010)
CSF	High protein, normal to mildly elevated cell count	High protein, normal cell count
Electrophysiology	Axonal or demyelinating, often patchy polyradiculoneuropathy, sural nerve sparing	Demyelinating, symmetrical polyradiculoneuropathy
Treatment	IVIg, Plasma exchange, supportive	Steroids, IVIg, Plasma exchange immunosuppressive therapy

Confusingly, GBS can have a relapsing course, which can be very difficult to distinguish from CIDP (Kuitwaard, van Koningsveld et al. 2009, Olivier, Laribi et al. 2010). Also, during the acute episode of GBS, treatment related fluctuations can occasionally be observed (Ruts, van Koningsveld et al. 2005, Ruts, Drenthen et al. 2010). Electrophysiological and clinical criteria have been proposed to distinguish the two entities (Dionne, Nicolle et al. 2010, Ruts, Drenthen et al. 2010) Finally, CIDP can sometimes present with an acute onset, resembling GBS with partial recovery; only subsequent fluctuations or deterioration makes a diagnosis of CIDP possible. This form has been called acute onset CIDP (A-CIDP) (Ruts, van Koningsveld et al. 2005).

5.1.5. Natural killer cells in CIDP

Natural killer cells (NK cells) are lymphocytes that share common progenitor cells with T lymphocytes. NK cells are crucial components of the early innate immune response, due to their ability to produce cytokines and chemokines and lyse target cells (Middleton and Gonzelez 2010). They also have important roles in the regulation of the subsequent immune response against the pathogen.

In CIDP, NK cells have been found in decreased numbers in blood of patients compared with controls (Sanvito, Makowska et al. 2009). Also, IVIg infusions have been found to lead

to a substantial decline in the number of peripheral NK cells and a suppression of NK-cell-mediated cytotoxicity (Bohn, Nederby et al. 2011). In nerve biopsies of patients with CIDP, NK cells play a less dominant role, with the histological picture being predominated by T lymphocytes and macrophages (Dalakas 2011).

5.1.6. Aim

We investigated the possible role of NK cells in the pathogenesis of CIDP using a gene based approach. We investigated gene frequencies of KIR and their HLA ligands. We compared KIR and HLA ligands to HC and patients with GBS.

5.2. Methods

A cohort of patients (n = 78) with a confirmed diagnosis of CIDP was recruited from several hospitals in Queensland and from private neurologists. All patients fulfilled standard diagnostic criteria for CIDP (Koski, Baumgarten et al. 2009).

This was compared with a cohort of patients (n = 195) with a confirmed diagnosis of GBS from several hospitals on the East Coast of Australia. All subjects fulfilled standard diagnostic criteria for GBS (Asbury and Cornblath 1990). As a control group healthy volunteers were recruited (n = 225), and matched for ethnicity.

Ethical approval was obtained; all subjects gave informed consent.

5.2.1. Laboratory methods

Highly pure genomic DNA was extracted either from peripheral blood (Nucleospin, Machery Nagel, Dueren, Germany) or saliva (Oragene, DNAGenothek, Kanata, Canada).

For KIR, typing the KIR Genotyping SSP Kit (Invitrogen, Carlsbad, USA) was used. For typing specific KIR associated HLA ligands the KIR-HLA Ligand kit (Olerup, Vienna, Austria) was used. This kit distinguishes HLA-CwAsparagine80 (HLA-C1) from HLA-CwLysine80 (HLA-C2) and HLA-BBw4-Threonine80 (HLA-BBw4-T) from HLA-BBw4+ Isoleucine80 (HLA-BBw4-I), and tests for presence of HLA-ABw4. These kits do not provide complete HLA typing. The kits were used according to the manufacturers' instructions.

PCR was performed followed by analysis with gel electrophoresis using a 2% agarose gel at 100 volts and photographed using an ultraviolet analyzing system (Biorad).

5.2.2. *Haplotypes and ligands*

HLA-C ligands for KIRs are classified as C1 or C2 KIR ligand groups, based on the amino acid at position 80 (Aspargine as C1; Lysine as C2). KIRs interact with specific MHC class 1 ligands on the target cell: KIR 2DL1 and KIR 2DS1 interact with HLA-C2, KIR 2DL2, KIR 2DL3 and KIR 2DS2 have as their ligand the C1 epitope; KIR 3DL1 and KIR 3DS1 recognize the HLA-Bw4 motif (Jamil and Khakoo).

Genotypes can be resolved into two broad haplotypes termed A and B based on KIR gene content (Middleton and Gonzelez 2010). Group B KIR haplotypes are characterized by presence of one or more of the following genes: KIR 2DL2, KIR 2DL5, KIR 2DS1, KIR 2DS2, KIR 2DS3, KIR 2DS5 and KIR 3DS1. Conversely, group A haplotypes are characterized by the absence of all these genes. It is difficult to determine with the methods used whether, in the presence of a B haplotype, the other allele is A or B. Therefore, for KIR haplotype analysis AA and BX were used, where X can stand for either an A or B haplotype.

5.2.3. *Statistical analysis*

Data analysis was performed using Excel (Microsoft) and GraphPad Prism. Chi Square test was used, with $p<0.05$ regarded as statistically significant. Bonferroni correction was used for multiple tests, with adjustment of p divided by the number of tests regarded as significant (For KIR genes $p<0.003$; For HLA ligands $p<0.01$; for KIR / HLA-ligand interactions $p<0.005$). The association of each polymorphism with the disease was measured by Odds Ratio (OR) and its 95% confidence interval (95%CI).

5.3. Results

5.3.1. *Frequency of KIR genes*

The frequency of the KIR genes is shown in Table 5.3. For the KIR genes studied no statistically significant differences were found both compared with HC and GBS. Also, the haplotypes AA and Bx were evenly distributed.

Table 5.3 - KIR genes in subjects with CIDP, compared with GBS subjects and healthy controls. Chi-Square test; Bonferonni correction for multiple tests (ND = not done)

KIR gene	CIDP (n = 78) N (%)	Controls (n = 225) N (%)	P value (Fisher)	GBS (n=195) N (%)	P value (Fisher)
2DL1	74 (94.8)	214 (95.1)	0.95	182 (93.3)	0.78
2DL2	48 (61.5)	110 (48.9)	0.06	114 (58.5)	0.68
2DL3	70 (89.7)	200 (88.9)	1	160 (82.1)	0.14
2DL4	78 (100)	224 (99.6)	ND	192 (98.5)	ND
2DL5A	30 (38.5)	84 (37.3)	0.89	67 (34.4)	0.57
2DL5B	20 (25.6)	47 (20.9)	0.43	47 (24.1)	0.88
2DS1	35 (44.8)	92 (40.9)	0.59	83 (42.6)	0.78
2DS2	45 (57.7)	111 (49.3)	0.24	106 (54.4)	0.69
2DS3	23 (29.4)	56 (24.9)	0.45	59 (30.3)	1
2DS4	33 (42.3)	95 (42.2)	1	74 (37.9)	0.58
2DS5	26 (33.3)	80 (34.7)	0.78	63 (32.0)	0.49
3DL1	74 (94.8)	212 (94.2)	1	174 (89.2)	0.17
3DL2	78 (100)	223 (99.1)	ND	195 (100)	ND
3DL3	78 (100)	224 (99.5)	ND	194 (99.5)	ND
3DS1	31 (39.7)	88 (39.1)	1	75 (38.5)	0.89
Haplotype					
AA	20 (25.6)	73 (32.4)	0.31	53 (27.1)	0.88
BX	58 (74.4)	152 (67.6)		142 (72.9)	

5.3.2. HLA frequencies in CIDP

The HLA frequencies are shown in Table 5.4. For the HLA types HLA-B Bw4T and Bw4I significant differences were found between CIDP and HC, but not between CIDP and GBS. Whilst the frequency of C1 and C2 was similar in all 3 groups, the heterozygous situation of C1/C2 was significantly more frequently found in CIDP than HC; no differences in the frequency of C1/C2 were detected between CIDP and GBS.

*Table 5.4 - HLA genes in subjects with CIDP, compared with GBS subjects and healthy controls Chi-Square test; Bonferonni correction for multiple tests with p<0.01 regarded as significant. * p<0.01*

HLA Gene	CIDP (n=78) N (%)	Controls (n=225) N (%)	P value (Fisher)	OR (95% CI)	GBS (n=195) N (%)	P value (Fisher)	OR (95% CI)
HLA-C1	67 (85.9)	201 (89.3)	0.41	0.72 (0.33 – 1.56)	156 (80)	0.30	1.52 (0.73 - 3.15)
HLA-C2	50 (64.1)	113 (50.2)	0.03	1.77 (1.04 – 3.01)	130 (66.6)	0.78	0.89 (0.52 – 1.55)
HLA-B Bw4-T	36 (46.1)	67 (29.8)	0.01*	2.02 (1.19 – 3.43)	88 (45.1)	0.89	1.04 (0.61 – 1.766)
HLA-B Bw4-I	35 (44.8)	61 (27.1)	0.0047*	2.18 (1.28 – 3.74)	70 (35.9)	0.17	1.45 (0.85 – 2.48)
HLA-A Bw4+	30 (38.4)	63 (28)	0.09	1.61 (0.93 – 2.76)	69 (35.4)	0.67	1.14 (0.66 – 1.96)
C1/C1	27 (34.6)	110 (48.9)	0.03	0.55 (0.32 – 0.94)	65 (33.3)	0.88	1.06 (0.61 – 1.84)
C1/C2	42 (53.8)	93 (41.3)	0.01*	1.99 (1.16 – 3.41)	91 (46.7)	0.1	1.6 (0.93 – 2.76)
C2/C2	9 (12.5)	22 (9.8)	0.67	1.2 (0.53 – 2.74)	39 (20)	0.11	0.52 (0.24 – 1.12)

5.3.3. Frequency of KIR/ligand carriage

The comparison of the CIDP and HC subjects carrying different KIR/HLA ligands is shown in Table 5.5. The KIR/HLA combinations 3DL1/Bw4T and 3DL1/Bw4I were more frequent in CIDP than HC, this was statistically significant for 3DL1/Bw4I even after Bonferroni correction.

No significant differences in KIR/HLA combinations were found between CIDP and GBS (see Table 5.6).

Table 5.5 – Genefrequency of KIRs and their specific HLA-Ligands CIDP vs. HC. Percentages given are for subjects with subjects (n=195) / Controls (n=225). Chi-Square test; Bonferonni correction for multiple tests with p<0.005(*) regarded as significant.

KIR gene / HLA ligand gene	CIDP n (% of group)	HC n (% of group)	P	OR (95% CI)	Effect on NK cells
2DL1 + / HLA-C2 +	47 (60.3)	107 (47.6)	0.06	1.74 (1.01 – 3)	Inhibition
2DL1 + / HLA-C2 -	27 (34.6)	107 (47.6)			
2DS1 + / HLA-C2 +	23 (29.4)	50 (22.2)	0.31	1.61 (0.71 – 3.62)	Activation
2DS1 + / HLA-C2 -	12 (15.4)	42 (18.6)			
2DL2 + / HLA-C1 +	43 (55.1)	101 (44.9)	0.76	0.77 (0.24 – 2.42)	Inhibition
2DL2 + / HLA-C1 -	5 (6.4)	9 (4)			
2DS2 + / HLA-C1 +	41 (52.6)	102 (45.3)	1	0.9 (0.26 – 3.1)	Activation
2DS2 + / HLA-C1 -	4 (5.1)	9 (4)			
2DL3 + / HLA-C1 +	60 (76.9)	179 (79.6)	0.39	0.7 (0.3 – 1.58)	Inhibition
2DL3 + / HLA-C1 -	10 (12.8)	21 (9.3)			
2DS3 + / HLA-C1 +	20 (25.6)	51 (22.7)	0.69	0.65 (0.14 – 3)	Activation
2DS3 + / HLA-C1 -	3 (3.8)	5 (2.2)			
3DL1 + / HLA-B Bw4-T +	34 (43.6)	63 (28)	0.015	2.01 (1.16 – 3.46)	Inhibition
3DL1 + / HLA-B Bw4-T -	40 (51.3)	149 (66.2)			
3DS1 + / HLA-B Bw4-T +	15 (19.2)	28 (12.4)	0.12	2 (0.87 – 4.63)	Activation
3DS1 + / HLA-B Bw4-T -	16 (20.5)	60(26.6)			
3DL1 + / HLA-B Bw4-I +	33 (42.3)	56 (24.9)	0.005*	2.24 (1.29 – 3.89)	Inhibition
3DL1 + / HLA-B Bw4-I -	41 (52.6)	156 (69.3)			
3DS1 + / HLA-B Bw4-I +	13 (16.7)	24 (10.6)	0.17	1.93 (0.82 – 4.52)	Activation
3DS1 + / HLA-B Bw4-I -	18 (23.1)	64 (28.4)			

Table 5.6 – Genefrequency of KIRs and their specific HLA-Ligands CIDP vs. GBS subjects. Percentages given are for subjects with subjects (n=195) / Controls (n=225). Chi-Square test; Bonferonni correction for multiple tests with p<0.005 regarded as significant

KIR gene / HLA ligand gene	CIDP N (% of group)	GBS N (% of group)	P	OR (95% CI)	Effect on NK cell function
2DL1 + / HLA-C2 +	47 (60.3)	121 (62.1)	0.66	0.88 (0.5 – 1.54)	Inhibition
2DL1 + / HLA-C2 -	27 (34.6)	61 (31.3)			
2DS1 + / HLA-C2 +	23 (29.4)	57 (29.2)	0.83	0.87 (0.37 – 2.02)	Activation
2DS1 + / HLA-C2 -	12 (15.4)	26 (13.3)			
2DL2 + / HLA-C1 +	43 (55.1)	87 (44.6)	0.06	2.67 (0.96 – 7.4)	Inhibition
2DL2 + / HLA-C1 -	5 (6.4)	27 (13.8)			
2DS2 + / HLA-C1 +	41 (52.6)	80 (41.0)	0.027	3.33 (1.09 – 10.2)	Activation
2DS2 + / HLA-C1 -	4 (5.1)	26 (13.3)			
2DL3 + / HLA-C1 +	60 (76.9)	130 (66.7)	0.46	1.39 (0.64 – 3)	Inhibition
2DL3 + / HLA-C1 -	10 (12.8)	30 (15.4)			
2DS3 + / HLA-C1 +	20 (25.6)	49 (25.1)	1	1.36 (0.34 – 5.47)	Activation
2DS3 + / HLA-C1 -	3 (3.8)	10 (5.1)			
3DL1 + / HLA-B Bw4-T +	34 (43.6)	79 (40.5)	1	1.02 (0.59 – 1.76)	Inhibition
3DL1 + / HLA-B Bw4-T -	40 (51.3)	95 (48.7)			
3DS1 + / HLA-B Bw4-T +	15 (19.2)	33 (16.9)	0.83	1.19 (0.51 – 2.76)	Activation
3DS1 + / HLA-B Bw4-T -	16 (20.5)	42 (21.5)			
3DL1 + / HLA-B Bw4-I +	33 (42.3)	65 (33.3)	0.32	1.35 (0.77 – 2.34)	Inhibition
3DL1 + / HLA-B Bw4-I -	41 (52.6)	109 (55.9)			
3DS1 + / HLA-B Bw4-I +	13 (16.7)	31 (15.9)	1	1.03 (0.44 – 2.37)	Activation
3DS1 + / HLA-B Bw4-I -	18 (23.1)	44 (22.6)			

5.4. Discussion

GBS and CIDP share a number of clinical, pathological and electrophysiological features, whilst being distinctly different in others. Whilst both GBS and CIDP are thought to be autoimmune, they are different from most other autoimmune disorders by their male predominance. The onset of the illness is always subacute in GBS, but can be more variable in CIDP. However, CIDP can also initially present as a GBS-like illness, and then only becomes evident as CIDP in retrospect when the disease progresses or fluctuates long-term (termed Acute-CIDP or A-CIDP) (Ruts, van Koningsveld et al. 2005, Ruts, Drenthen et al. 2010). Also, there is overlap in the treatments employed, i.e. IVIg and plasma exchange. This points to both differences and similarities in pathogenesis in these two disorders.

In this study the frequency of KIR/HLA gene carriage was assessed in subjects with CIDP and compared to both HC and to subjects with GBS. The number of subjects was relatively low for a study of this kind, and so the statistical power is limited. However, differences in gene frequencies were found between CIDP and HC, but not between CIDP and GBS.

No differences were found between the KIR genes in any of the 3 groups. In contrast, HLA were different in several aspects. Whilst in GBS HLA-C2/HLA-B BwT4 was more frequent than HC (see Chapter 4), HLA-B Bw4I was more frequent in CIDP than HC, with a trend to significance for HLA-B Bw4T. The heterozygous situation HLA-C1/C2 was more frequently seen in CIDP than HC.

However there were some differences in the carriage of combinations of KIR/HLA. Interestingly, all differences found in HLA and KIR/HLA frequencies were between CIDP and HC or GBS and HC. In contrast, no differences between CIDP and GBS could be detected for any of the genes and gene combinations studied. This could possibly indicate underlying genetic similarities of CIDP and GBS patients causing the autoimmune attack on the peripheral nervous system.

The similarities in KIR/HLA gene frequencies in GBS and CIDP could indicate similarities in the early pathogenesis of these disorders. Indeed, it could be speculated that the initial attack against the peripheral nervous system is identical in both disorders, but the subsequent immune regulation is more effective in GBS, leading to complete cessation of

autoimmunity against myelin (see Appendix A). This does not occur in CIDP. If these were correct, one would anticipate similarities in the innate immune system response, but differences in genes responsible for immune regulation of the specific immune system.

The association of certain KIR/HLA ligands with CIDP and GBS could suggest that NK cells are involved in the pathogenesis. NK cells are a group of lymphocytes, which are related but distinct from T lymphocytes. They were originally described and named due to their cytotoxic properties against tumour cells. In recent years our understanding of the role of NK cells has expanded, and now encompasses detection and killing of virally infected cells, secretion of chemokines and cytokines, antigen presentation and direct cell-cell interactions with T cells and dendritic cells (Vivier, Raulet et al. 2011). NK cells are part of the innate immune system and are able to respond rapidly to infection without any prior sensitization (Rajalingam 2012). NK cell function is regulated by multiple inhibitory and activating receptors, of which the functionally most important is the KIR/HLA system. Alternatively, the KIR/HLA system is also of relevance in a range of T cell subsets, including CD8 T cells.

The role of NK cells in the pathogenesis of CIDP has not been studied in depth. One study showed decreased numbers of circulating NK cells (Sanvito, Makowska et al. 2009). IVIg administration, which is a standard therapy for CIDP (Eftimov, Winer et al. 2009), is associated with a decreased number of circulating NK cells and reduced NK-cell-mediated cytotoxicity (Bohn, Nederby et al. 2011). In summary, in spite of a relatively small number of studied subjects with CIDP, we could detect differences of KIR/HLA ligand combinations between CIDP and HC, but not CIDP and GBS. This could indicate a role for NK cells in the pathogenesis of both GBS and CIDP and suggests similarities in the early pathogenesis of these disorders.

Chapter 6 A genome wide association study of Guillain-Barré Syndrome

6.1. Introduction

6.1.1. Overview of recent developments in genetic research

Around the turn of the century, the first human genome sequence was published, promising a new era of understanding of human development and disease (Lander, Linton et al. 2001, Venter, Adams et al. 2001). However, the initial DNA sequence of a single individual has raised questions rather than providing answers. In recent years, studies have focused on the variability of the human genome, particularly focusing on genetic differences in healthy and diseased individuals and between different populations.

In parallel, a collaborative effort on the part of scientists, clinicians, lawyers and the community at-large has been initiated to safeguard individuals and direct the usage of genomic data with the promise of bettering the health of mankind in what has become the genomic era (Collins, Green et al. 2003).

The studies of the human genome sequence have indicated that the number of human genes is lower than previously thought, with recent estimates quoting a number of approximately 22000 genes (Claverie 2005, Consortium, Bernstein et al. 2012). These protein-coding sequences make up 1-2% of the genome, and are interspersed by long stretches of non-coding sequences. The role of these non-coding parts of the human genome in gene regulation and cell biology is becoming increasingly clear (Carninci and Hayashizaki 2007).

The intense research in genomics has led to a rapid development in techniques available to interrogate the genome. Concurrently costs of genomic studies have rapidly decreased. The sequencing of the first human genome was an international effort, involving numerous private and public institutions around the globe in an 11 year project and a price tag of around US$3 billion dollars. In 2008 the “1000 genomes project” was started, with an expected price tag of US$30 – 50 million dollars. The data was published 3 years later (Abecasis, Altshuler et al. 2010). The “$1000

dollar genome", a catchphrase first devised in 2001 seems to become goal achievable in the not-too-distant future.

6.1.2. Genome wide association studies

In recent years, genome wide association studies (GWAS) have allowed the detection of alleles associated with disease phenotypes. The first successful GWAS, which was on age-related macular degeneration was published in 2005 (Klein, Zeiss et al. 2005). Since then, substantial progress has been made in a number of diseases. For example, massive efforts have been put into multiple sclerosis (MS), a demyelinating disease of the central nervous system, where a recent GWAS on 50000 healthy controls and 26000 MS patients found a total of 110 loci associated with MS (Beecham, Patsopoulos et al. 2013).

A prerequisite for human disease gene mapping is a reasonably high heritable contribution to the overall risk for the disease of interest. GWAS are made possible by a dense set of genetic markers, known as single nucleotide polymorphisms (SNPs) across the human genome. SNPs are sites in the DNA sequence where a single nucleotide varies between members of a species. Depending on the site of the mutation, some SNPs can affect the final protein sequence, whereas other do not. For *homo sapiens*, by April 2013 almost 10 million SNPs have been validated across the human genome (dbSNP 2013). This equates to roughly one SNP per 350 base pairs throughout the human genome.

GWAS rely on investigations of common genetic variants (SNPs) of different individuals with or without a certain trait. GWAS use hundreds of thousands to millions of SNPs distributed throughout the genome to determine regions of the genome associated with the trait under investigations. To date 1200 GWAS have been published (Pearson and Manolio 2008, Johnson and O'Donnell 2009, Genome.gov 2012). GWAS are usually not able to elicit the causative mutation. The most widely used nomenclature of SNPs is according to the National Centre of Biotechnology Informatics (NCBI) database, which involves a reference number of reported SNPs with the prefix "rs".

Associations found in a GWAS do not necessarily imply causation. Confounders include difference in ethnic ancestry, DNA quality, techniques used at genotyping centres and environmental exposures, all of which can affect the same causal pathway. These factors need to be corrected for, as much as possible, as part of data cleaning and vigorous quality control (Pearson and Manolio 2008).

6.1.2.1. *Linkage disequilibrium*

SNPs tested during GWAS might or might not be directly responsible for the phenotype studied. Most frequently, the SNPs studied in GWAS are in non-coding regions and have an indirect association with functional SNPs (Hirschhorn and Daly 2005).

Genetic association testing is based on linkage disequilibrium (LD) between different SNPs. LD measures the degree to which alleles at two loci are associated, using the frequencies of each haplotype. Two or more polymorphic loci, e.g. SNPs, are in LD when certain alleles of these loci are observed together more often than would be expected by chance in a given population (Zondervan and Cardon 2004).

To calculate LD, the haplotype frequencies for two loci and two alleles is examined (see Table 6.1).

Table 6.1 - Definition of haplotype frequencies for two loci with two alleles

Haplotype	Frequency
A_1B_1	X_{11}
A_1B_2	X_{12}
A_2B_1	X_{21}
A_2B_2	X_{22}

From this table the frequency of each allele at each locus can be calculated as below (see Table 6.2).

Table 6.2 - Definition of allele frequencies based on haplotype frequencies

Allele	Frequency	Population nomenclature
A_1	$X_{11} + X_{12}$	p_1
A_2	$X_{21} + X_{22}$	p_2
B_1	$X_{11} + X_{21}$	q_1
B_2	$X_{12} + X_{22}$	q_2

Standard measure of LD is calculated as

$$D = \left((x_{11}) \times (x_{22})\right) - \left((x_{12}) \times (x_{21})\right)$$

If two loci are in linkage equilibrium, then, $D = 0$, if they are in linkage disequilibrium, $D \neq 0$.
.

A more frequently used measure of LD is r^2, which is defined as

$$r^2 = \frac{D^2}{p_1 \times p_2 \times q_1 \times q_2} = \frac{\left(\left((x_{11}) \times (x_{22})\right) - \left((x_{12}) \times (x_{21})\right)\right)^2}{(x_{11} + x_{12}) \times (x_{21} + x_{22}) \times (x_{11} + x_{21}) \times (x_{12} + x_{22})}$$

The range of r^2 is from $r^2 = 0$, signifying complete linkage equilibrium, to $r^2 = 1$, which represents complete linkage disequilibrium. There is no defined statistical test that states when two loci are in LD, and a wide variety of choices have been used in the literature. Frequently, r^2 is represented graphically.

LD is influenced by recombination, genetic drift, population size and, to a lesser extent, mutations.

6.1.2.2. *Blocks*

Because of chromosomal segregation during meiosis, LD is dependent on physical co-occurrence of both SNPs on the same chromosome. The amount of LD between any two SNPs is then influenced by recombination, natural selection, mutation, genetic drift, ancestral population demographics and mating patterns (Wall and Pritchard 2003, Zondervan and Cardon 2004).

Patterns of LD are well known for being noisy and unpredictable. Many studies have shown that although the background LD is significantly related to genetic distance, small physical distance does not guarantee high level of LD (Abecasis, Noguchi et al. 2001, Gabriel, Schaffner et al. 2002). For example, pairs of sites that are tens of kilobases apart might be in complete LD, whereas nearby pairs of sites from the same region might be in weak LD (Patil, Berno et al. 2001, Wall and Pritchard 2003).

Moreover, the genetic inheritance is not uniform across chromosomes; it rather follows formation of blocks, which are in close LD, interspersed by hot spots of separation (Goldstein 2001). Within these blocks, LD decays only very gradually with distance, or not at all. However, within hotspots LD falls away rapidly with distance, leading to increased LD between blocks, up to the extreme of only random association between neighbouring blocks. Therefore, a single SNPs

within a block could be representative of genetic variation located at some distance, but might be unrelated to closely neighbouring SNPs in a different block (see Figure *6.1*).

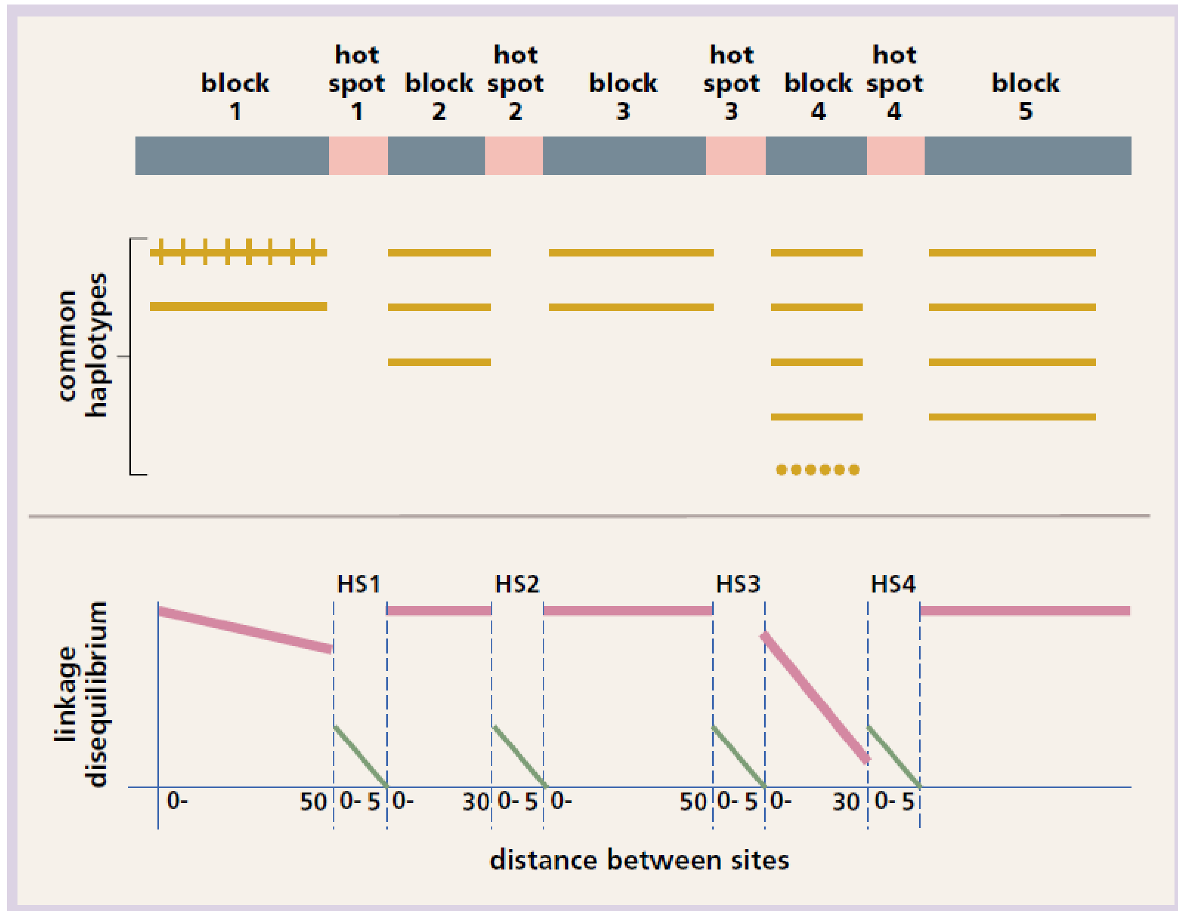


Figure 6.1 - *Blocks of LD in the human genome (after (Goldstein 2001)).*

6.1.2.3. *Power calculations*

Complex or multifactorial diseases are characterized by the interplay of multiple genetic and environmental risk factors usually with low effect sizes, which might interact in a complex manner (Zondervan and Cardon 2004) (see Figure 6.2). Also, as mentioned above, the SNPs observed in GWAS might not be causative, but only in LD with the causative gene.

Associations between SNPs and causal variants are expected to show low odds ratios, frequently below 1.5. In order to obtain a reliable signal, given the very large number of tests performed, associations must show a high level of significance to survive correction for multiple testing. This therefore requires study of large cohorts of subjects and controls.

GWAS are limited by complex statistical analysis and power calculations due to the high numbers of tests performed whilst testing more than 500,000 SNPs (Dudbridge and Gusnanto 2008,

Pe'er, Yelensky et al. 2008). Because of the high numbers of tests performed, using Bonferroni correction for multiple tests would give a value for genome-wide significance for a $p < 0.05$:

$$p < \frac{0.05}{500000} = 10^{-7}$$

SNPs with a lower p value can be considered for follow-up, but need to be confirmed, usually in a replication study.

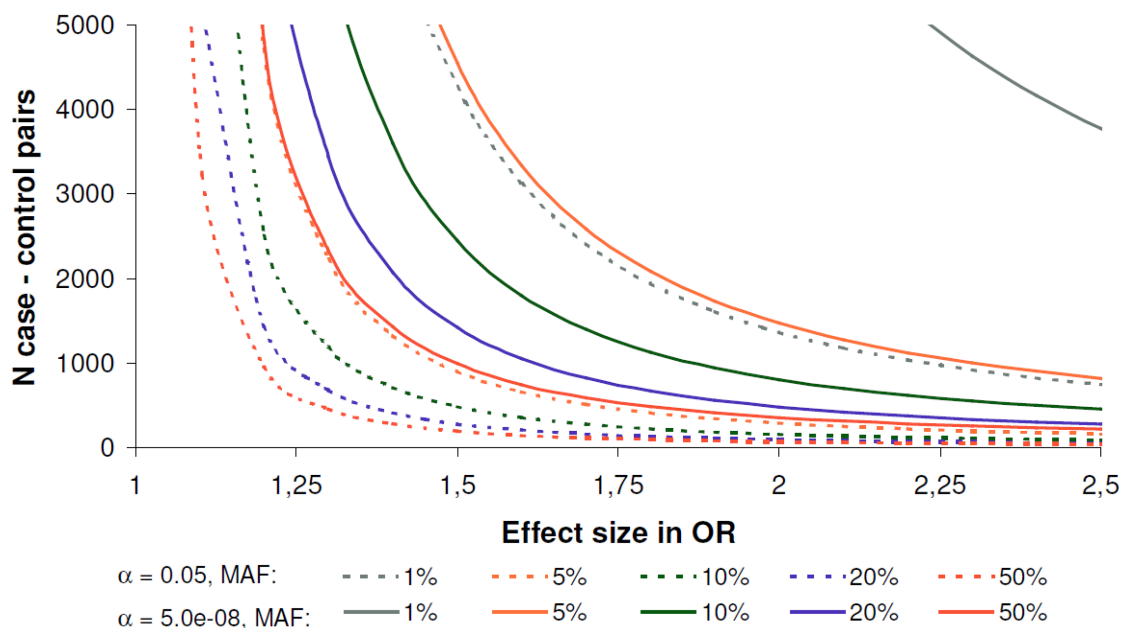


Figure 6.2 - Statistical power analysis of a case-control study using Quanto (Gauderman 2002)

As shown in Figure 6.2, especially with smaller effect sizes, large numbers of case-control pairs are needed to derive adequate statistical power. In a small study, only effects of high significance were detectable, and the risk of a false-positive result is relatively high. This can partially be off-set by a relatively large number of control subjects.

6.2. Methods

6.2.1. *Study population*

6.2.1.1. *Patients*

We obtained DNA of 230 patients with a confirmed diagnosis of GBS. The patient characteristics are summarized in Table 6.3. A more detailed overview is provided in previous chapters (Chapters 3 and 4).

Table 6.3 - Patient characteristics

Patient characteristic		**Number**
Gender	Male	144
	Female	86
Hughes scale	1	3
	2	27
	3	39
	4	105
	5	51
	6	5
Subtype	AIDP	104
	AMAN	11
	AMSAN	3
	MFS	21
	Other focal variants	7
	GBS, unspecified	84

6.2.1.2. *Controls*

Two control groups were used for two independent analyses:

a) A group of around 3000 samples from healthy Australians collected for the OATS (Lee, Mosing et al. 2012) and RAINE (http://www.rainestudy.org.au) studies. These were typed on an identical chip with the same methods as for the GBS subjects of this study (OmniExpress, Illumina).

b) Samples from more than 15000 individuals available through the WTCCC consortium in UK, obtained from the National Blood Transfusion service and the 1958 British Birth Cohort. These

samples were typed using Illumina 660W, leading to only a subset of SNPs being typed both in this group and the patient group.

As the WTCCC cohort is a larger sample than the Australian cohort p values for SNPs of interest were expected to be more significant in the WTCCC group, but any SNPs of interest was investigated in both groups.

6.2.2. DNA extraction

Blood or saliva samples were obtained from the donors. DNA was isolated from full blood using the Nucleospin® Blood XL kit (Macherey-Nagel) according to manufacturer instructions. In short, 10 mls of blood was treated with proteinase K, followed by cell lysis at 56ºC. DNA was precipitated using 100% Ethanol and separated using a membrane. DNA was eluted with elution buffer.

For saliva, collection was performed using a commercial collection kit, which can be posted to patients' homes (Oragene, DNAGenothek, Kanata, Canada). DNA was extracted using prepIT-L2P, according to manufacturer's instructions.

DNA concentrations were usually higher than 100 ng/mcl and of acceptable purity as determined by spectroscopy using ultraviolet light. Samples were stored at -20ºC.

6.2.3. Genetic analysis assay

GWAS was performed at the Laboratory of Prof. Matt Brown, Diamantina Institute, University of Queensland. Analysis was performed with the Omni-Express Bead Chip system using HiScan, Illumina (San Diego, USA). This chip is able to detect more than 715,000 individual SNPs with a call frequency of more than 99%.

Analysis was performed according to manufacturer's instructions. In short, 200 ng of original DNA was denatured and neutralized. A whole-genome amplification of the denatured DNA was performed overnight to increase the amount of the DNA sample. After this, DNA was fragmented enzymatically, using end-point fragmentation to avoid over-fragmentation. DNA was then precipitated with isopropanol and centrifuged at 4° C to collect fragmented DNA. After resuspensation in hybridization buffer, the samples were applied to a Bead Chip and incubated overnight to allow binding to the BeadChip. After washing away unhybridized and non-specifically hybridized DNA, single-base extensions were marked using fluorophore labels, followed by detection using a laser.

6.2.4. Quality control and population stratification

The capability of GWAS to identify true genetic associations depends on the overall quality of the data used (Turner, Armstrong et al. 2011). A total of 230 samples were used for primary analysis. We excluded related individuals, by excluding samples with >20% of identical genomic markers, from further analysis.

We excluded samples showing a deviation from Hardy-Weinberg equilibrium (HWE) at a significance level of $<5 * 10^{-5}$. Also, samples with a genotyping rate of less than 0.95 and a minimum sample completion rate of less than 0.97 were excluded. SNPs with a minor allele frequency (MAF) of less than 5% were ignored due to insufficient power.

The risk of confounding by population stratification increases with sample size. Eigenstrat analysis uses principal component analysis to detect and adjust for population stratification on a genome-wide scale in large samples (Patterson, Price et al. 2006, Price, Patterson et al. 2006). To exclude samples from a different genetic background we excluded all cases with an Eigenvector <0.02, leaving 190 samples used for final analysis. The quotient termed lambda was 1.276 pre-stratification, and reduced to 1.097 after exclusion of these samples.

6.2.5. Choosing areas of interest

Because the number of patients studied was relatively low for a GWAS, it was anticipated that no genome-wide significant results would be obtained. A Manhattan plot was drawn using Haploview (Barrett, Fry et al. 2005). Each chromosome was visually screened for clusters of SNPs of increased significance compared to background SNPs. Areas of interest were enlarged, and an LD plot obtained. Areas of interest were defined as clusters of SNPs in linkage disequilibrium, with at least one SNP reaching genome-wide significance ($p<10^{-5}$). All genes within a 300kB radius of the SNP of highest significance or lying in the same LD block were identified and subsequently characterized (candidate genes).

6.2.6. Relevant negatives

We performed a comprehensive review for all genetic markers previously studied in both GBS and CIDP. SNPs associated with these known genes were investigated for linkage disequilibrium.

6.3. Results

6.3.1. Overview and Manhattan plot

A total of 633875 SNPs were analyzed on the 190 samples passing quality control. These were compared with two cohorts of healthy controls as outlined above (Australian group and WTCCC). Likely due to the low number of cases, there were no SNPs meeting criteria for genome-wide significance. However, 5 regions of interest could be identified on chromosomes 2, 4, 14, 18 and 19, which are subsequently described in more detail.

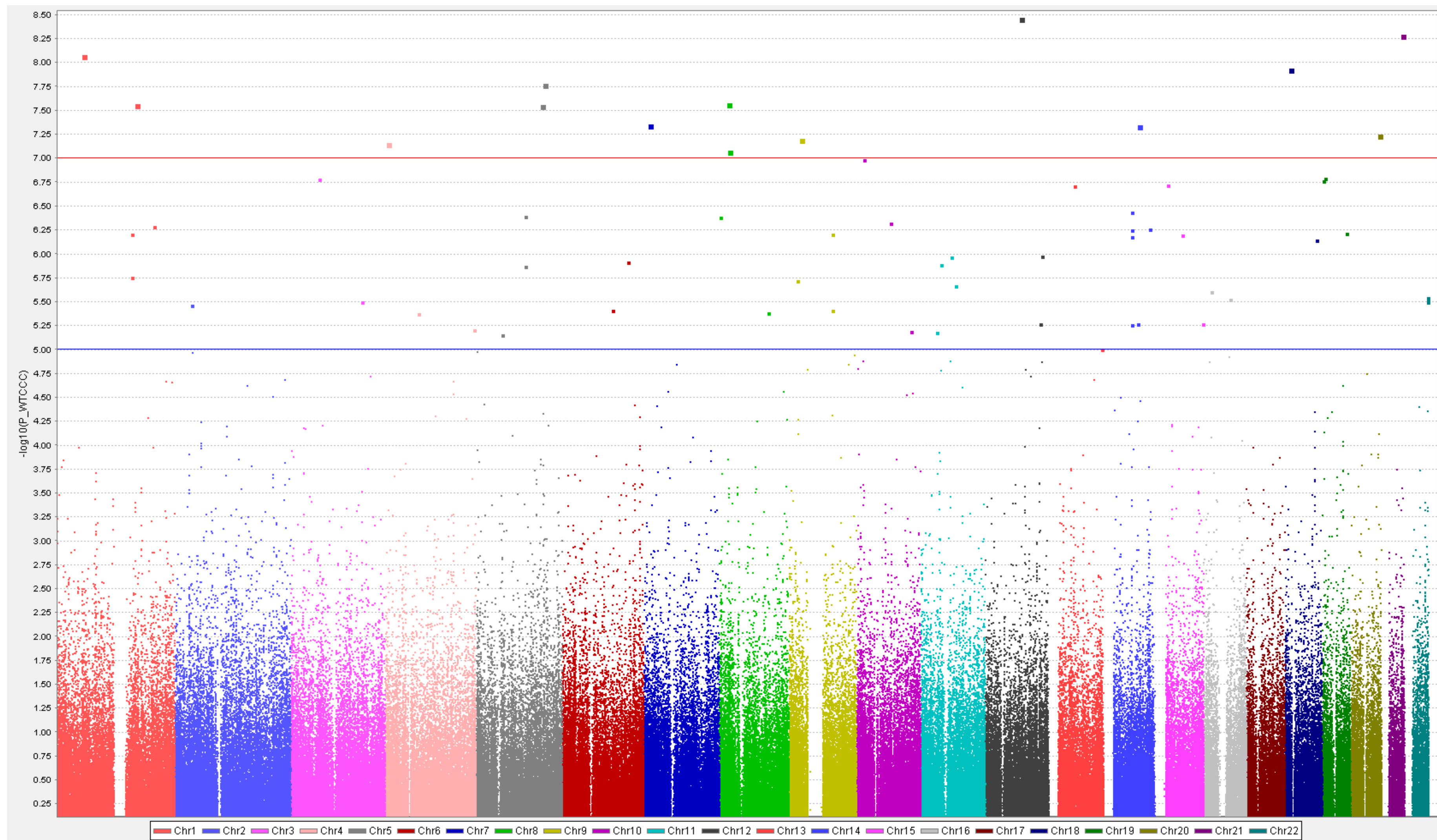


Figure 6.3 – Manhattan blot of GBS GWAS using Haploview (Barrett, Fry et al. 2005). Chromosomes are arbitrarily colour-coded. P values of all SNPs are coded on the vertical axis as a logarithm of 10. The X axis represents position of the SNP on the human genome.

6.3.2. Regions of interest

For each region of interest the Manhattan plot is shown, in low and high power followed by the LD for the region shown in Haploview. The LD plot shows the blocks in the region of interest and the location of nearby genes.

6.3.2.1. *Rs10519519*

Rs10519519 is at position 2284303 on chromosome 2, at locus 2p25.3 (see Figure 6.4 and Figure 6.5). Neighbouring SNPs of increased significance are listed in Table 6.4.

rs10519519 is located in an intron of a gene labeled Myelin Transcription Factor 1 - Like (*MYT1L*) (see Figure 6.6). SNPs rs10519519 and rs10495485 were not studied in the WTCCC sample.

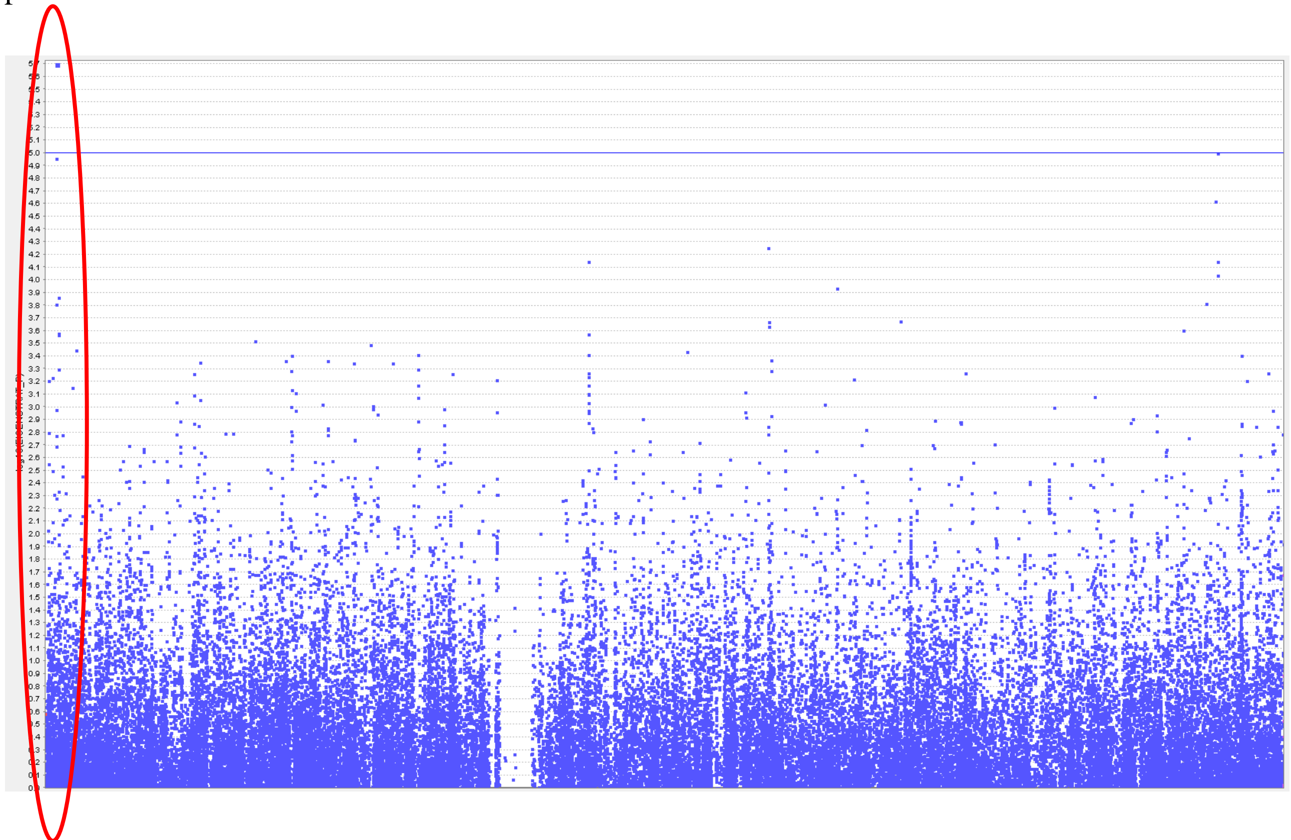


Figure 6.4 - Manhattan plot of entire Chromosome 2 using Haploview. A marker (red oval) defines the region surrounding rs10519519. P values of all SNPs are coded on the vertical axis as a logarithm of 10. The X axis represents position of the SNP on the human genome.

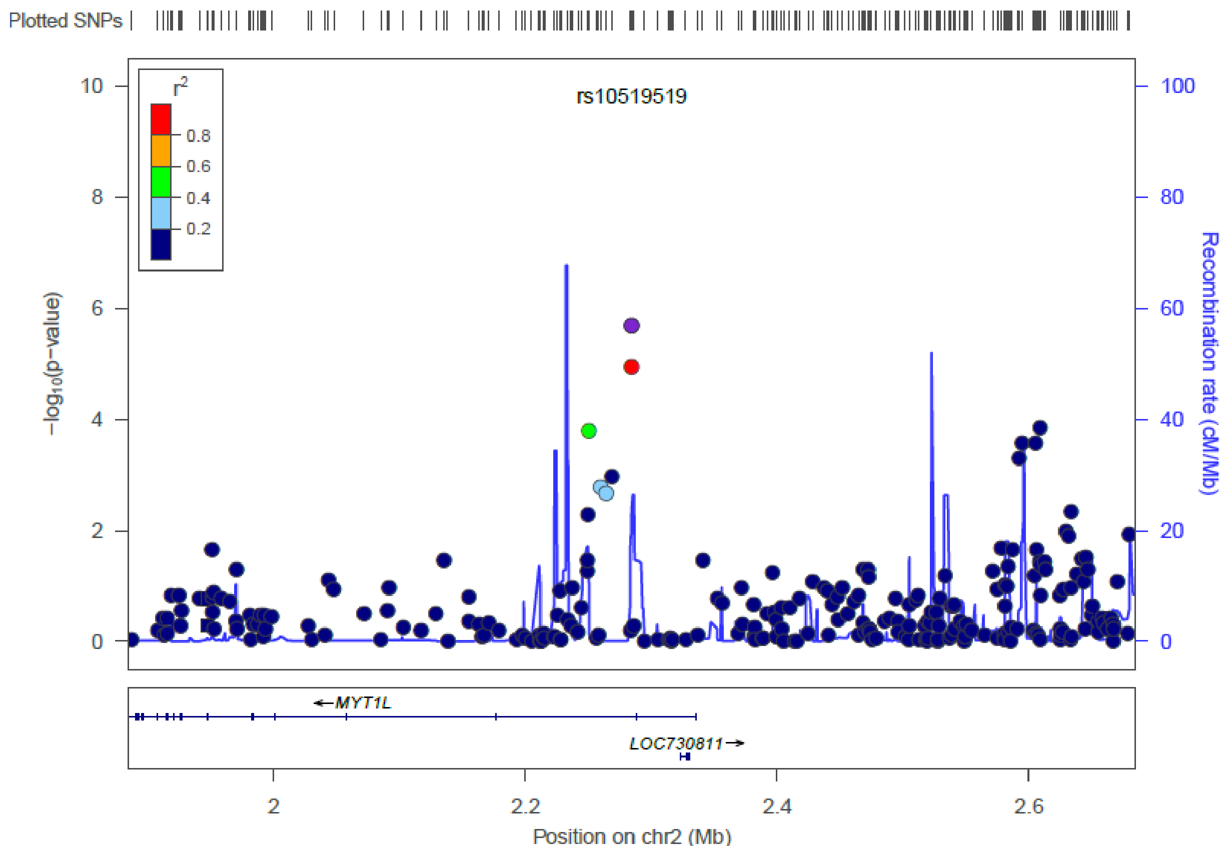


Figure 6.5 - Manhattan plot Chromosome 2: Zoom of region surrounding rs10519519 (purple marker). P values of all SNPs are coded on the vertical axis as a logarithm of 10. The X axis represents position of the SNP on the human genome. Neighbouring SNPs in linkage disequilibrium are colour-coded according to r^2 (see graph).

Table 6.4 - Neighbouring SNPs to rs10519519 of increased significance as compared to the sample of HC

SNP	Mutation	P Aust	P WTCCC	OR
Rs10519519	A/G	2.0E-6	N/A	
Rs10495485	C/T	1.1E-5	N/A	
Rs7598374	A/G	1.37E-4	0.027	1.446
Rs1421612	G/A	1.55E-4	0.008	1.567

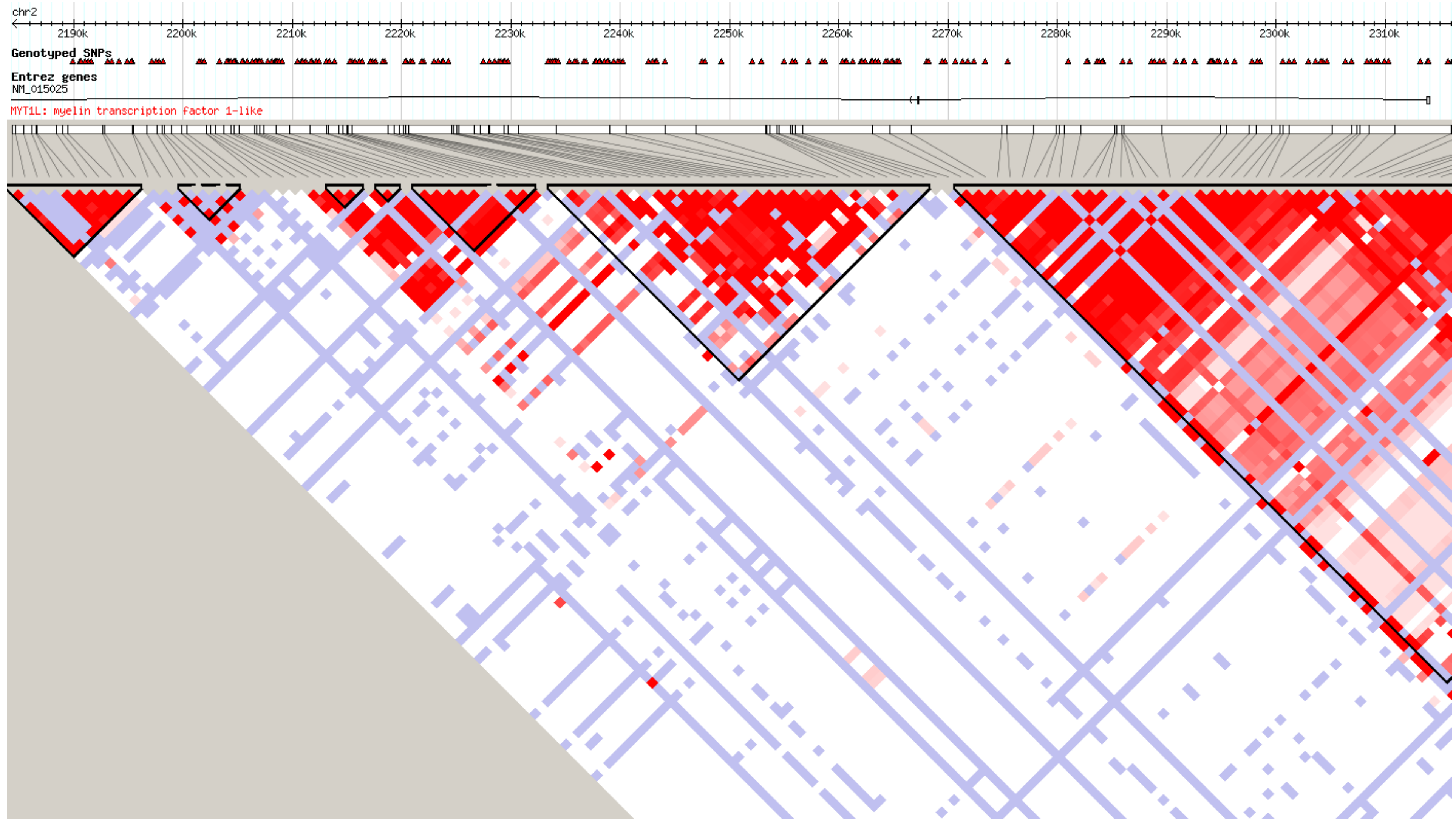


Figure 6.6 - LD plot of region 2284303 on Chromosome 2. MYT1L is the only gene found in the neighbourhood of rs10519519 (Haploview)

6.3.2.2. *rs7663689*

rs7663689 is found at position 142688981 on Chromosome 4, at locus 4q31.21 (Figure 6.7 and Figure 6.8). Neighbouring SNPs and their significance levels are listed in *Table 6.5*. rs7663689 localizes to the intergenic region between Zinc finger protein 330 (*ZNF330*) and the IL-15 pre-protein (*IL15*) as shown in Figure 6.9. It is part of the promoter region of *IL15*.

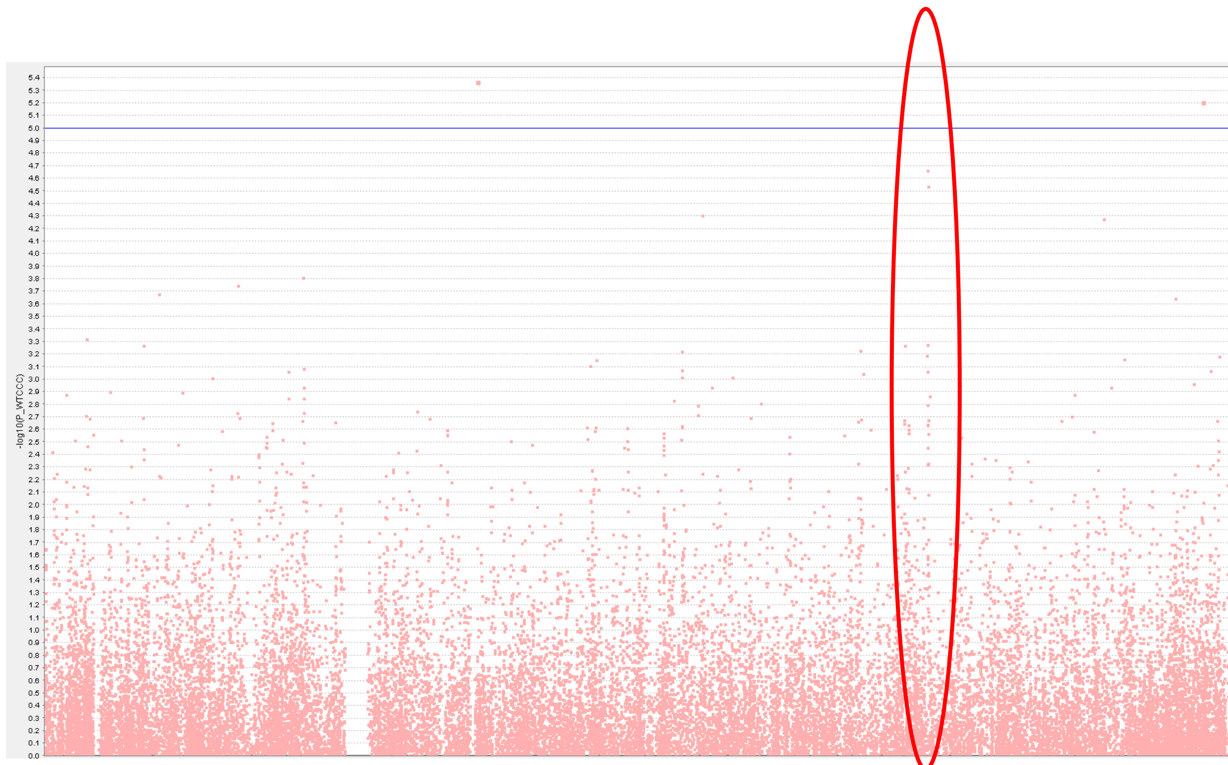


Figure 6.7 – Manhattan plot of entire Chromosome 4 using Haploview: The cluster of SNPs of increased significance around rs7663689 is marked by the red oval. P values of all SNPs are coded on the vertical axis as a logarithm of 10. The X axis represents position of the SNP on the human genome.

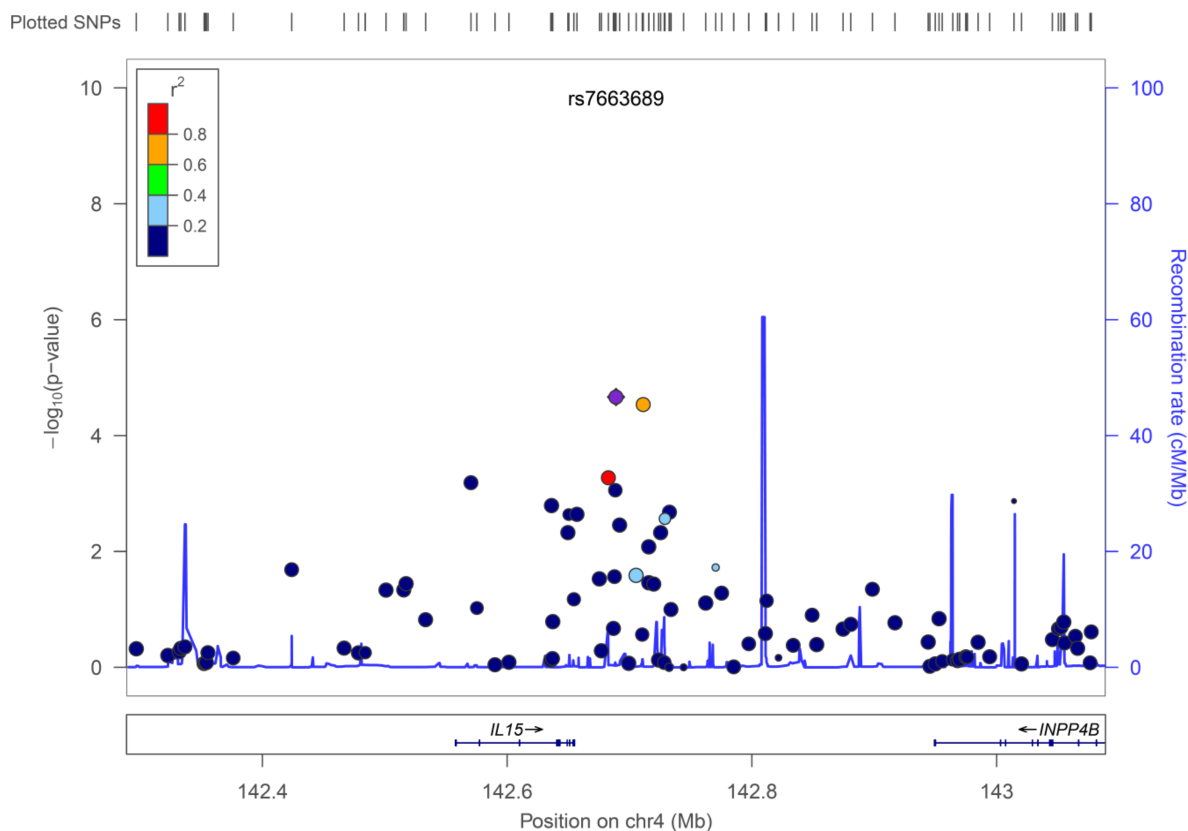


Figure 6.8 - Manhattan plot; Zoom to region surrounding rs7663689. P values of all SNPs are coded on the vertical axis as a logarithm of 10. The X axis represents position of the SNP on the human genome. Rs7663689 is marked in purple. Neighbouring SNPs in linkage disequilibrium are colour-coded according to r^2 (see graph).

Table 6.5 - Neighbouring SNPs to rs7663689 of increased significance as compared to the Australian and WTCCC sample of HC

SNP	Mutation	P Aust	P WTCCC	OR WTCCC
Rs7663689	T/C	1.7E-4	2.16E-5	1.707
Rs17701271	A/C	2.28E-4	2.90E-5	1.63
Rs10519629	C/T	0.005	5.30E-4	1.59
Rs1519551	G/A	4.04E-4	6.48E-4	0.69
Rs4254850	T/C	1.55E-4	8.73E-4	1.43

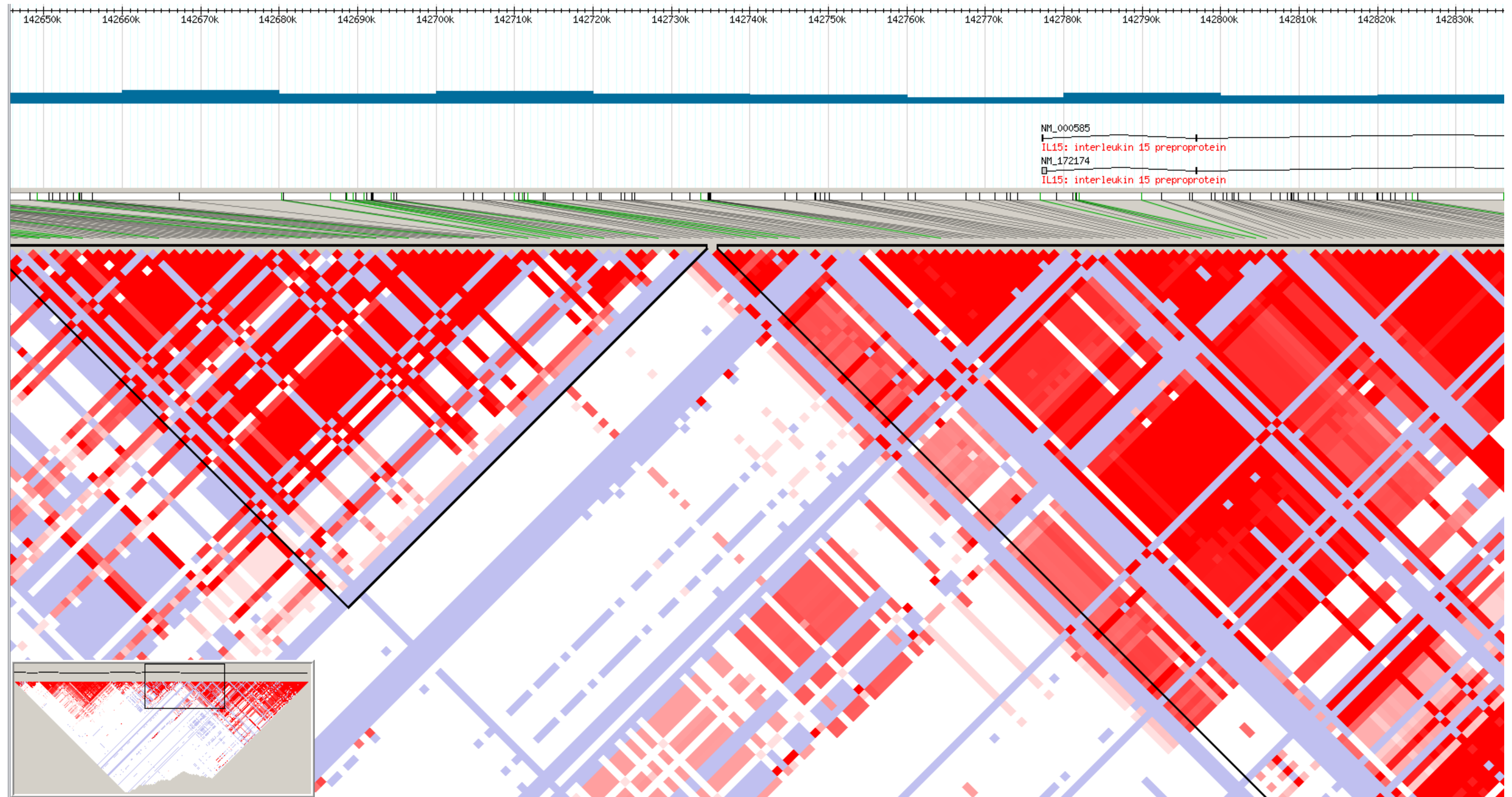


Figure 6.9 - LD plot of region 142688981 on Chromosome 4 showing IL15 as the gene most closely linked to rs7663689 (Haploview)

6.3.2.3. *rs11151180*

Another region of interest could be identified on Chromosome 18 by SNP 1151180 at position 62845961, locus 18q22.1 (see Figure 6.11). Neighbouring SNPs in linkage disequilibrium are summarized in Table 6.6. There were no genes in close vicinity to rs11151180 (see Figure 6.12). The closest known in gene is cadherin 7 (*CDH7*), which starts at 250kB from rs11151180.

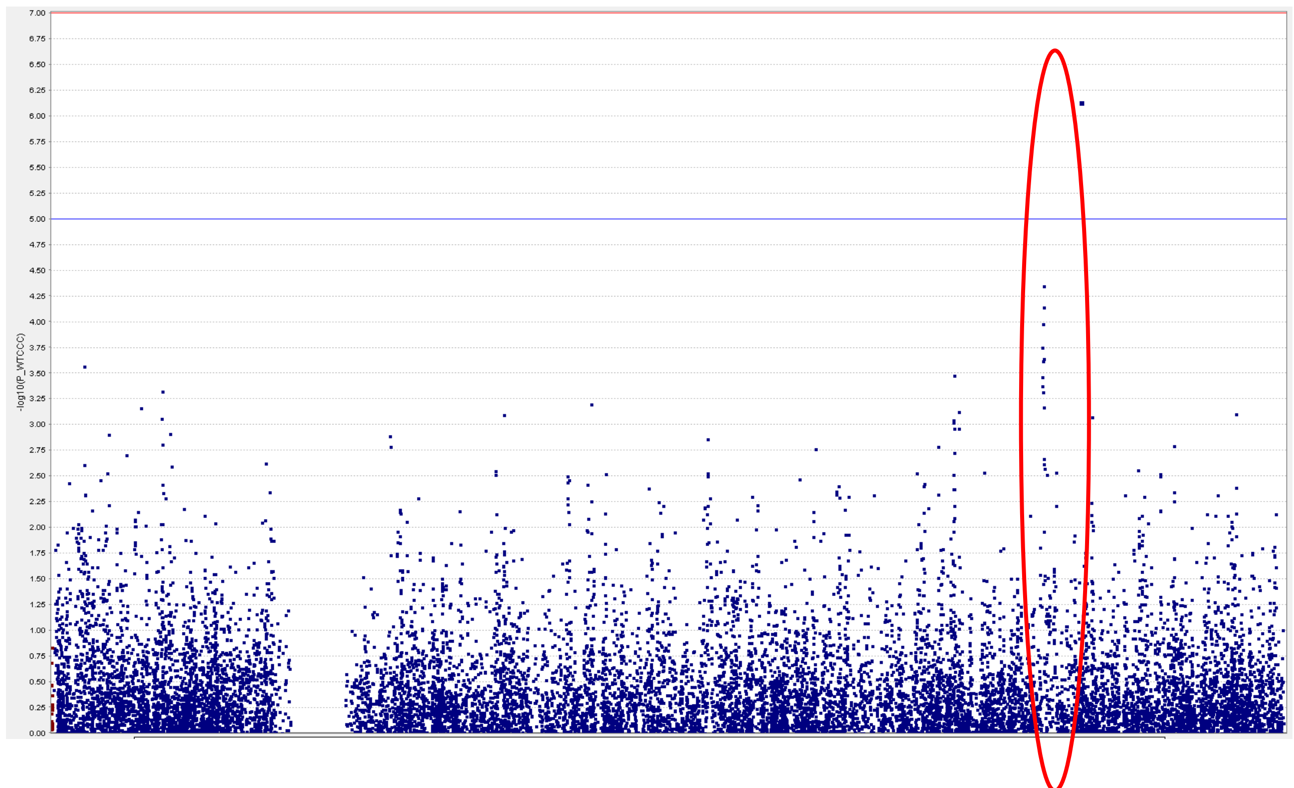


Figure 6.10 - Manhattan plot of entire Chromosome 18 using Haploview: The cluster of SNPs of increased significance around rs11151180 is marked by the red oval. P values of all SNPs are coded on the vertical axis as a logarithm of 10. The X axis represents position of the SNP on the human genome.

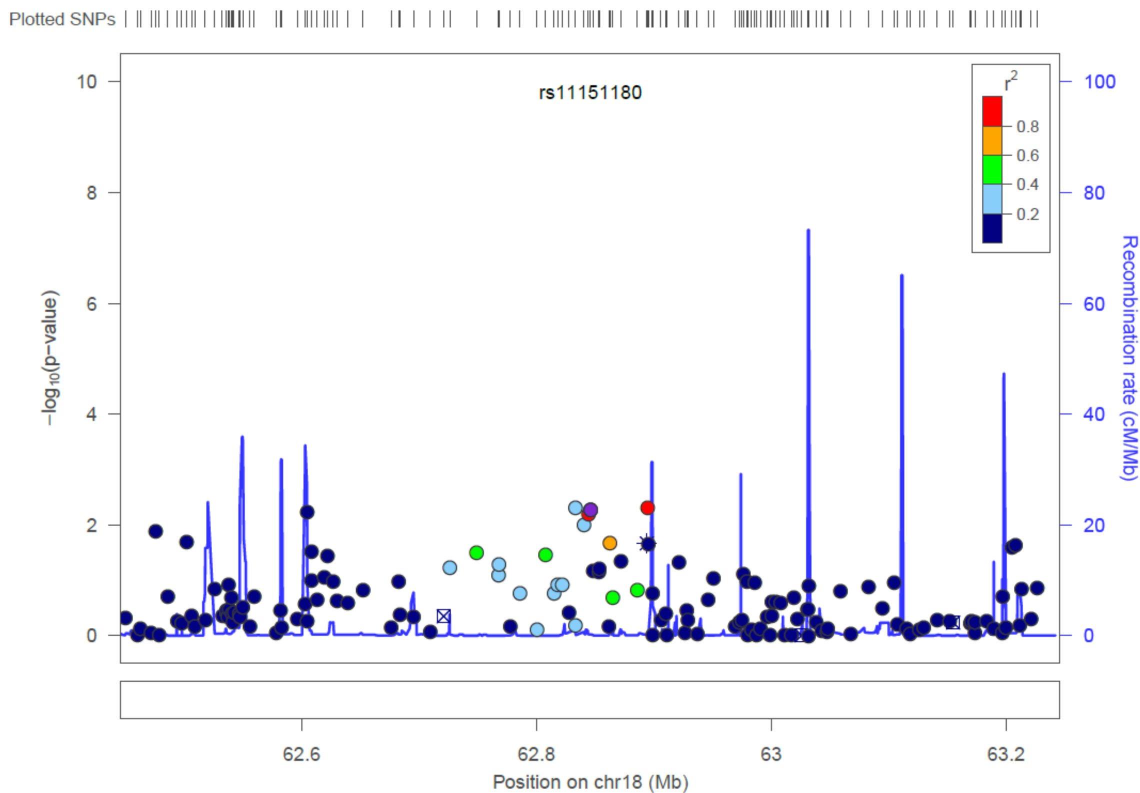


Figure 6.11 - Manhattan plot; Zoom to region surrounding rs11151180. P values of all SNPs are coded on the vertical axis as a logarithm of 10. The X axis represents position of the SNP on the human genome. Rs11151180 is marked in purple. Neighbouring SNPs in linkage disequilibrium are colour-coded according to r^2 (see graph).

Table 6.6 - Neighbouring SNPs to rs11151180 of increased significance as compared to the Australian and WTCCC cohort of HC

SNP	Mutation	P Aust	P WTCCC	OR WTCCC
rs11151180	A/G	0.005	4.46E-5	0.6294
rS4048109	G/A	0.006	7.153E-5	0.636
rs1108775	A/G	0.05	1.763E-4	0.6616
rs1035208	T/C	0.118	2.299E-4	0.6692
rs1183856	A/G	0.118	2.395E-4	0.0699
rs11662608	T/G	0.059	3.425E-4	0.6742

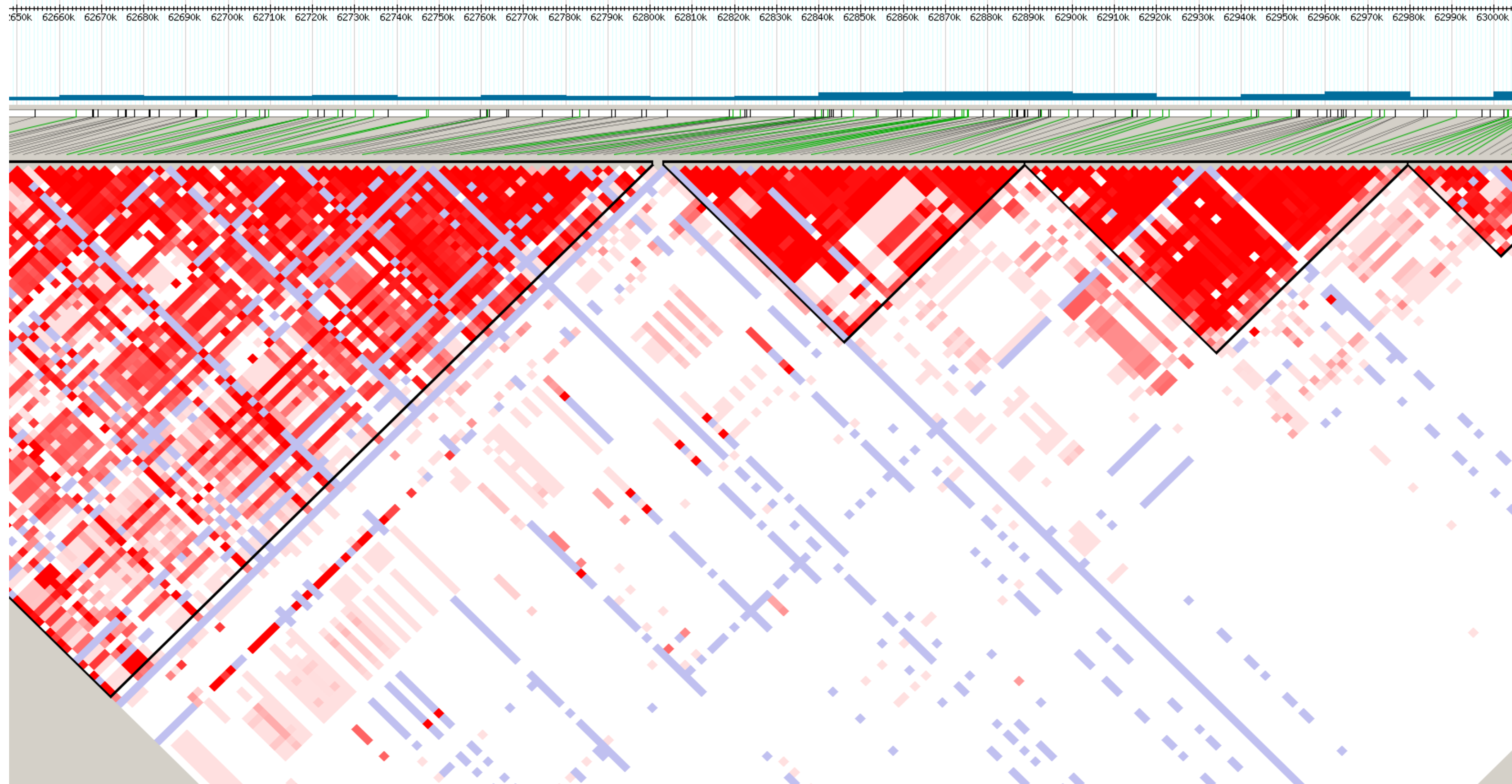


Figure 6.12 - LD plot rs1115180 region (Haploview); no genes in close neighbourhood to11151180 were found

6.3.2.4. *rs17095496*

rs17095496 is an intergenic SNP located on Chromosome 14, position 59447241, locus 14q23.1 (see Figure 6.13 and Figure 6.14). This region had the strongest linkage disequilibrium in our study (see Table 6.7), with four SNPs having OR above 2.

It is located in a large block, which encompasses two proteins, hypothetical protein *C14orf135* and Dehydrogenase/reductase (SDR family) member 7 (*DHRS7*), and is also associated with Disheveled-Binding Antagonist Of Beta-Catenin 1 gene (*DACT1*) and dishevelled associated activator of morphogenesis 1 (*DAAM1)* (Figure 6.15).

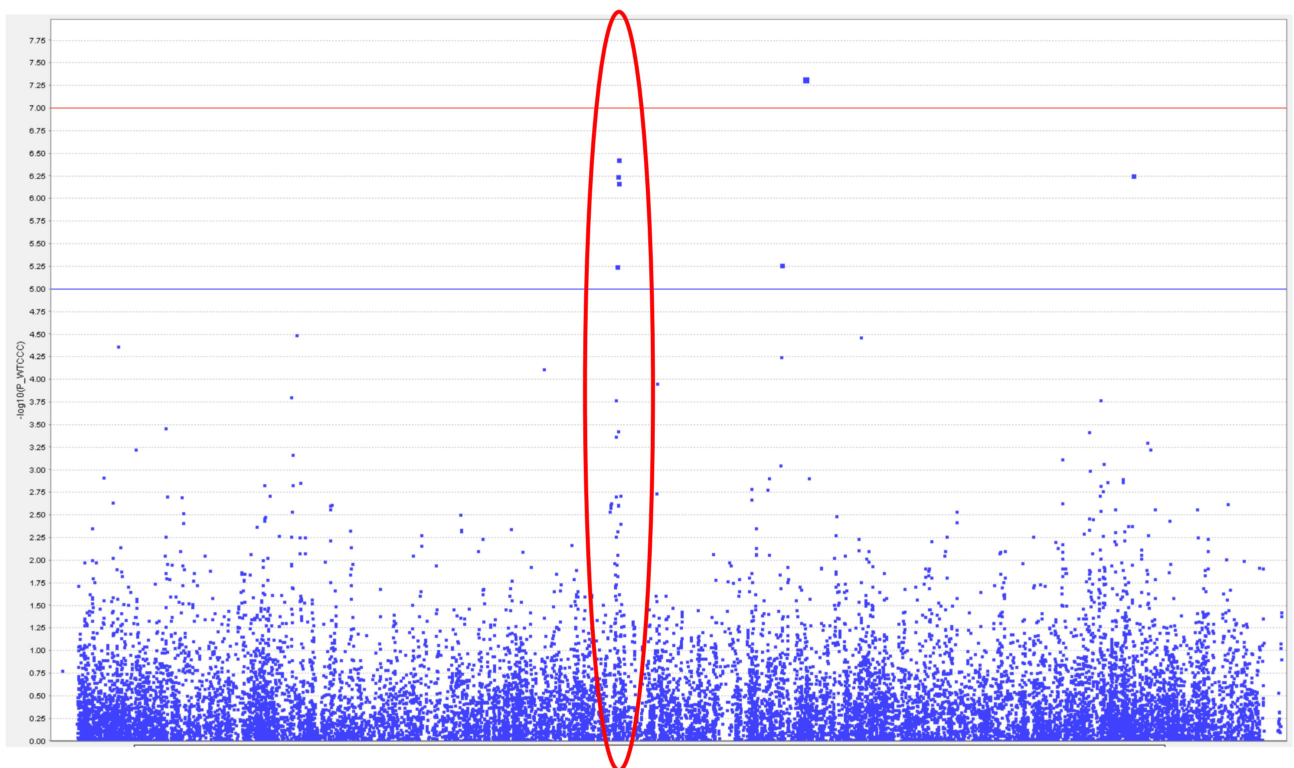


Figure 6.13 - Manhattan plot of entire Chromosome 14 using Haploview. The cluster of SNPs of increased significance around rs17095496 is marked by the red oval. P values of all SNPs are coded on the vertical axis as a logarithm of 10. The X axis represents position of the SNP on the human genome.

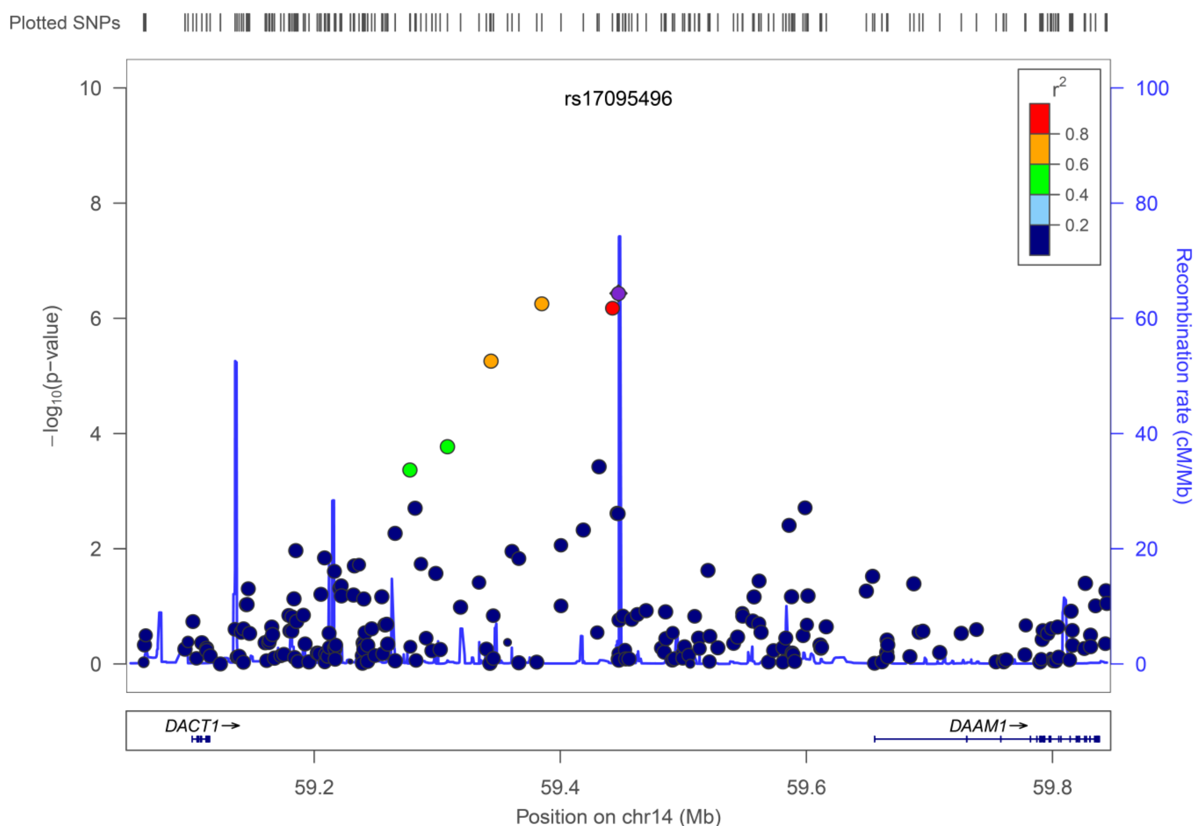


Figure 6.14 - Manhattan plot Chromosome 14, zoom on region surrounding rs17095496 . P values of all SNPs are coded on the vertical axis as a logarithm of 10. The X axis represents position of the SNP on the human genome. Rs17095496 is marked in purple. Neighbouring SNPs in linkage disequilibrium are colour-coded according to r^2 *(see graph).*

Table 6.7 - Neighbouring SNPs to rs17095496 of increased significance as compared to an Australian cohort and WTCCC cohort of healthy controls

SNP	Mutation	P Aust	P WTCCC	OR WTCCC
rs17095496	G/A	6.47E-4	3.681E-7	2.125
rs85425	T/C	5.24E-4	5.569E-7	2.15
rs17254989	A/G	5.79E-4	6.624E-7	2.106
rs1427327	T/G	1.69E-4	5.514E-6	2.247
rs380375	A/G	0.002	1.688E-4	1.899
rs7155136	A/G	0.048	3.748E-4	1.486
rs7159883	A/C	0.002	4.27E-4	1.762

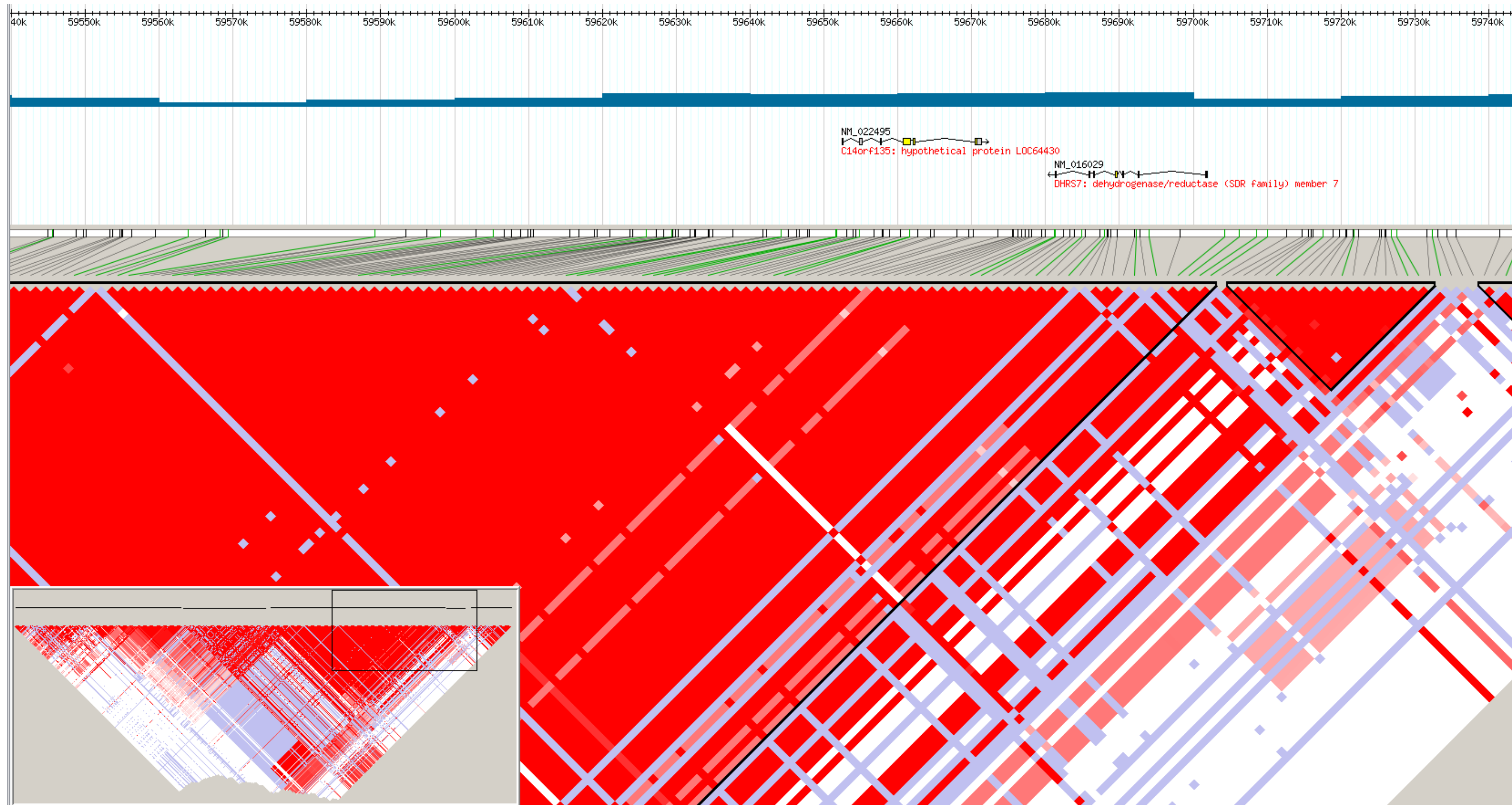


Figure 6.15 - LD plot of Chromosome 14, region of rs17085496 (Haploview)

6.3.2.5. *rs2302524*

rs2302524 is found on Chromosome, locus 19q13.2 (see Figure 6.16 and Figure 6.17). The SNPs of highest significance are summarized in Table 6.8. This region features a range of genes, which names and functions are delineated below (see Figure 6.18).

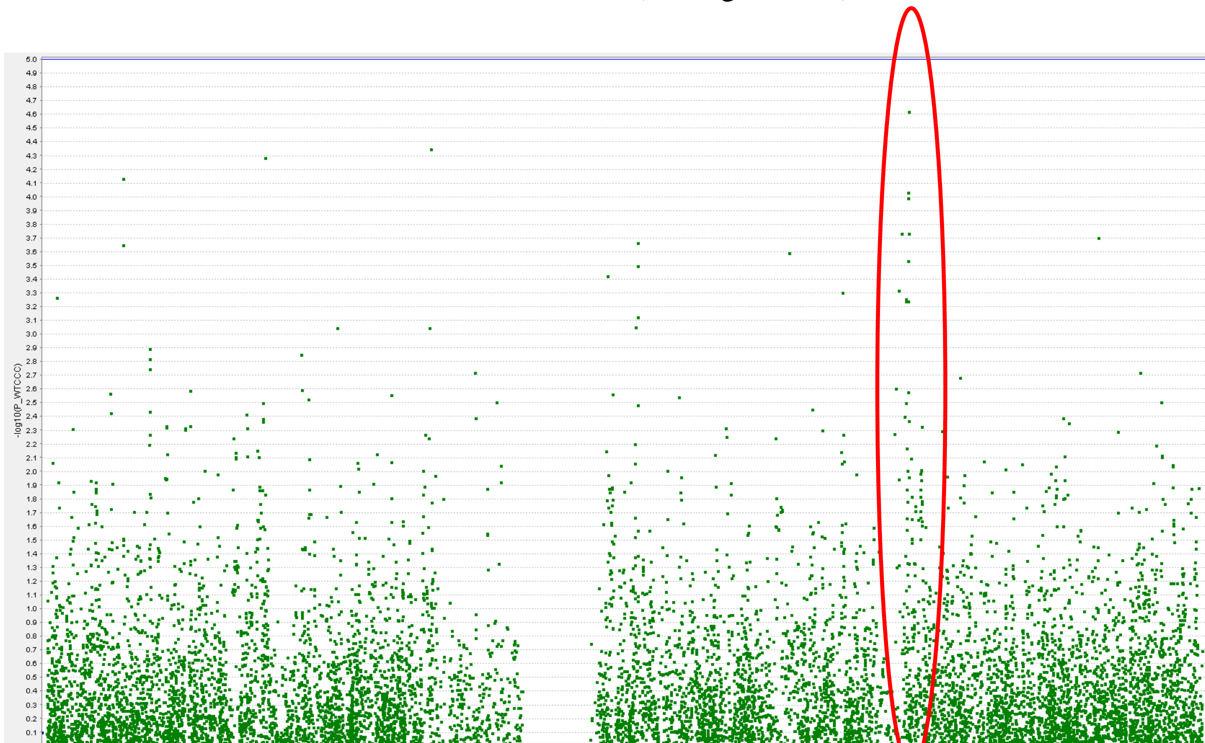


Figure 6.16 - Manhattan plot of entire chromosome 19 using Haploview. The cluster of SNPs of increased significance around rs2302524 is marked by the red oval. P values of all SNPs are coded on the vertical axis as a logarithm of 10. The X axis represents position of the SNP on the human genome.

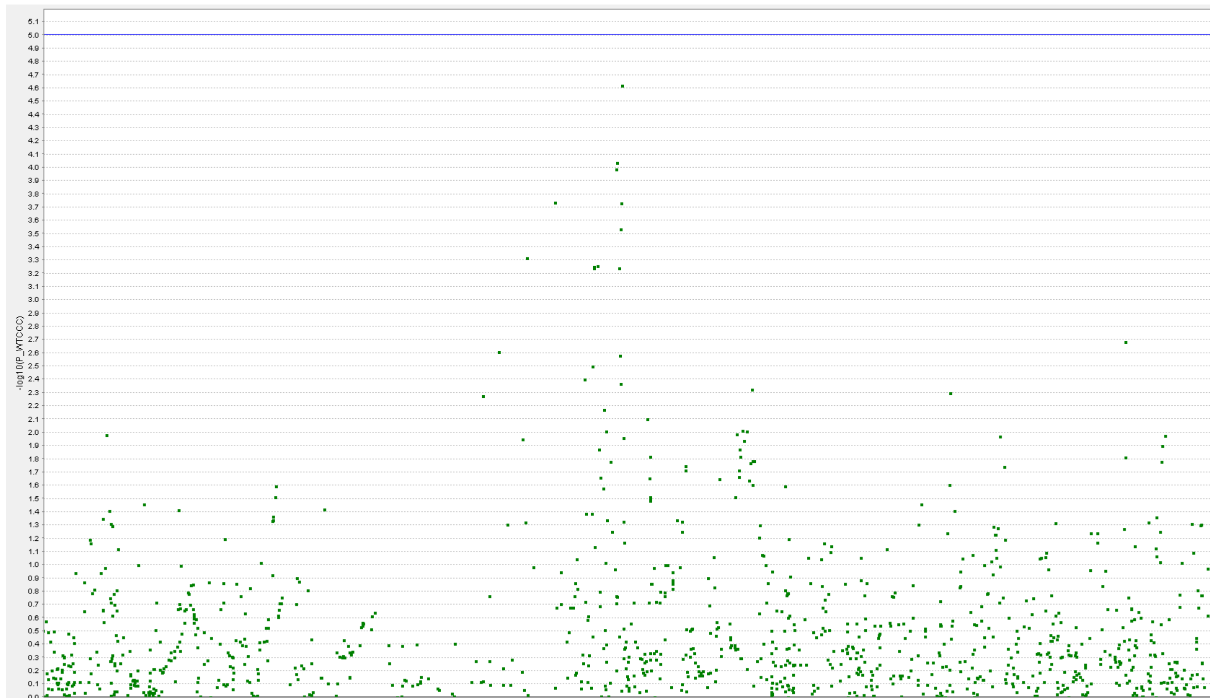


Figure 6.17 - Manhattan plot Chromosome 19: Zoom to region surrounding rs2302524 using Haploview. P values of all SNPs are coded on the vertical axis as a logarithm of 10. The X axis represents position of the SNP on the human genome.

Table 6.8 - Neighboring SNPs to rs2302524 of increased significance as compared to the Australian cohorts and WTCCC cohort of healthy controls

SNP	Mutation	P Aust	P WTCCC	OR WTCCC
rs2302524	C/T	1.9E-4	2.394E-5	1.693
rs3746003	A/G	0.015	9.24E-5	1.64
rs3746002	A/G	0.01	1.023E-4	1.635
rs4251938	C/T	0.003	1.85E-4	1.663
rs5009916	T/G	0.002	1.84E-4	1.488
rs4802189	A/C	0.002	2.914E-4	1.558

Figure 6.18 - LD plot of region rs2302524 showing several genes closely linked to rs2302524 as detailed in the text (Haploview)

6.3.3. *Short description of candidate genes*

6.3.3.1. *rs10519519*

MYT1L encodes a pan-neural transcription factor associated with neuronal differentiation and is expressed in the developing brain (Kim, Armstrong et al. 1997). Moreover, it has been found to be of paramount importance in the induction of neurons out of both human and mouse fibroblast cultures, together with other growth factors (Vierbuchen, Ostermeier et al. 2010, Ambasudhan, Talantova et al. 2011, Pang, Yang et al. 2011, Yoo, Sun et al. 2011).

6.3.3.2. *rs7663689*

Zinc finger protein 330 (*ZNF330*) is a protein-coding gene. The function of the encoding protein is unclear. It has been associated with conduct disorder in a recent GWA study (Dick, Aliev et al. 2011).

The *IL15* gene encodes a 14-15 kDa glycoprotein, and consists of nine exons and eight introns. Four exons (5 to 8) encode for the mature protein (Steel, Waldmann et al. 2012). It is expressed by a large number of cells, including monocytes, macrophages, dendritic cells, fibroblasts and nerve cells (Grabstein, Eisenman et al. 1994). Two transcript variants are encoded by the same protein, but differ in their splicing: The long signal peptide (IL-15-LSP) consists of a 316 bp 5'-untranslated region (UTR), 486 bp coding sequence and the C-terminus 400 bp 3'-UTR region. The short signal peptide (IL-15-SSP) has a short signal peptide encoded by exons 4A and 5. These isoforms differ in their cellular trafficking, but produce the same mature IL-15 protein (Bamford, DeFilippis et al. 1998).

6.3.3.3. *rs11151180*

CDH7 encodes a membrane protein, which is a calcium dependent cell-cell adhesion glycoprotein. It is comprised of five extracellular repeats, a transmembrane region and a cytoplasmic tail (Kools, Van Imschoot et al. 2000). It has been related to migration of malignant cells (Winklmeier, Contreras-Shannon et al. 2009).

6.3.3.4. *rs17085496*

C14orf135 is a hypothetical protein, alternative name: pecanex-like protein 4, is related to the pecanex family in Drosophila. There is no functional information about this protein. Pecanex has been linked to neuronal development and sterility in Drosophila (Yamakawa, Yamada et al. 2012).

Short-chain dehydrogenases/reductases (SDRs), such as *DHRS7*, catalyze the oxidation/reduction of a wide range of substrates, including retinoids and steroids (Haeseleer and Palczewski 2000). It has recently been better characterized and has been found to have an important role in metabolism of xenobiotics within the endoplasmic reticulum (Skarydova and Wsol 2012, Skarka, Skarydova et al. 2013, Stambergova, Skarydova et al. 2014). It is expressed in retinal tissue, and also in B- and T-lymphocytes and liver cells.

DAAM1 is a formin, a class of actin nucelators, required for centrosome re-orientation during cell proliferation and migration (Ang, Zhao et al. 2010, Nishimura, Honda et al. 2012). It localizes to the actin-myosin system, evident in the sub-nuclear system during cell division. It has recently been found to be overexpressed in acute myeloid leukemia (Bock, Mochmann et al. 2013).

Dapper homolog 1 (*DACT1*), a member of DACT family, is an important regulator in the planar cell polarity (PCP) pathway (Zhang, Gao et al. 2006). It has been found to be a tumor suppressor gene in gastric cancer by inhibition of the NF-κB pathway (Wang, Kang et al. 2012). DACT1 is widely expressed throughout the body, including a wide variety of immune cells and neural cell types. It has been linked to neuronal development with several rare mutations being linked to neural tube defects in humans (Shi, Ding et al. 2012).

6.3.3.5. *rs2302524*

Soluble galactoside-binding lectin 4 and 7 (*LGALS4* and *LGALS7*) are members of the galectin family. The galectins are beta-galactoside-binding proteins implicated in modulating cell-cell and cell-matrix interactions. Differential and in situ hybridization studies indicate that this lectin is specifically expressed in keratinocytes and found mainly in stratified squamous epithelium (Barondes, Cooper et al. 1994). Galectin 4 has been found to have decreased expression in colorectal cancer (Huflejt, Jordan et al. 1997, Rechreche, Mallo et al. 1997).

Peroxisomal Enoyl CoA Hydratase 1 (*ECH1*) encodes a member of the hydratase/isomerase superfamily. The gene product shows high sequence similarity to enoyl-coenzyme A (CoA) hydratases of several species, particularly within a conserved domain characteristic of these proteins. The encoded protein, which contains a C-terminal peroxisomal targeting sequence, localizes to the peroxisome (FitzPatrick, Germain-Lee et al. 1995). It has been found to be associated with myotonic dystrophy (Rusconi, Mancinelli et al. 2010).

Heterogeneous Nuclear Ribonucleoprotein L (*HNRPL*) encodes one of the heterogeneous nuclear RNAs (hnRNAs), which include mRNA precursors and mature mRNAs, are associated with specific proteins to form heterogeneous ribonucleoprotein (hnRNP) complexes. Heterogeneous

nuclear ribonucleoprotein L is among the proteins that are stably associated with hnRNP complexes and along with other hnRNP proteins is likely to play a major role in the formation, packaging, processing, and function of mRNA. Heterogeneous nuclear ribonucleoprotein L is present in the nucleoplasm as part of the HNRP complex (Pinol-Roma, Swanson et al. 1989).

Sirtuin 2 (*SIRT2*) encodes a member of the sirtuin family of proteins, homologs to the yeast *SIR2* protein. Members of the sirtuin family are characterized by a sirtuin core domain and grouped into four classes. The functions of human sirtuins have not yet been determined; however, yeast sirtuin proteins are known to regulate epigenetic gene silencing and suppress recombination of rDNA. Studies suggest that the human sirtuins may function as intracellular regulatory proteins with mono-ADP-ribosyltransferase activity. The protein encoded by this gene is included in class I of the sirtuin family (Afshar and Murnane 1999, Dryden, Nahhas et al. 2003, Voelter-Mahlknecht, Ho et al. 2005).

NF-κB inhibitor beta (*NFKBIB*) is a member of the IκB kinase family. The IκB Kinase family of proteins binds to NF-κB in holds it inactive in the cytoplasm. Cytokine-induced phosphorylation, ubiquitination and degradation of IκB leads to release and translocation of NF-κB into the cell nucleus (Mercurio, Zhu et al. 1997). IκB family consist of NKκBI alpha and beta, with close structural homology, but have distinct protein-protein interactions (Woronicz, Gao et al. 1997).

Mitochondrial Seryl-tRNA Synthetase 2 (*SARS2*) encodes the mitochondrial seryl-tRNA synthethase precursor, a member of the class II tRNA synthetase family. The mature enzyme catalyzes the ligation of Serine to tRNA(Ser) and participates in the biosynthesis of selenocysteinyl-tRNA(sec) in mitochondria. The enzyme contains an N-terminal tRNA binding domain and a core catalytic domain. It functions in a homodimeric form, which is stabilized by tRNA binding (Yokogawa, Shimada et al. 2000).

Mammalian mitochondrial ribosomal proteins are encoded by nuclear genes and help in protein synthesis within the mitochondrion (Cavdar Koc, Burkhart et al. 2001).

F-Box Protein 17 (FBXO17) encodes a member of the F-box protein family which is characterized by the F-box motif. The F-box proteins constitute one of the four subunits of the ubiquitin protein ligase complex called SCFs (SKP1-cullin-F-box), which function in phosphorylation-dependent ubiquitination (Ilyin, Serandour et al. 2002).

6.3.4. Relevant negatives

A number of genes have been studied in subjects with GBS, some of which have been associated with disease susceptibility (Myhr, Vagnes et al. 2003, Geleijns, Emonts et al. 2007, Prasad, Nyati et al. 2010, Jiao, Wang et al. 2012). The best evidence is of TNFα polymorphisms, which have been described in several studies (Wu, Zhou et al. 2012). For some of these genes, namely HLA and CD1, there are conflicting results between studies (Gorodezky, Varela et al. 1983, Winer, Briggs et al. 1988, Ma, Nishimura et al. 1998, Magira, Papaioakim et al. 2003, Caporale, Papola et al. 2006, Kuijf, Geleijns et al. 2008). Other genes have been studied, but no association has been found. Others again are associated with disease severity, but not susceptibility, e.g. MMP9 (Geleijns, Emonts et al. 2007). Some of these genes have also been studied in CIDP. We undertook to investigate SNPs associated with these previously studied genes in our GWAS sample.

None of the genes studied previously was associated with significant linkage disequilibrium in our cohort (see Table 6.9).

Table 6.9 - Previously studied genes in GBS and CIDP and p-values of associated SNPs

Protein	Gene	Chromosomal location	Link to GBS	Link to CIDP	SNP	P WTCCC	OR WTCCC
Alpha1 antitrypsin	*SERPINA 1*	14q32.1	No association (McCombe, Clark et al. 1985)	No association (McCombe, Clark et al. 1985)	Rs6647	0.335	1.01
Apolipoprotein E	*APOE*	19q13.2	No association (Pritchard, Hughes et al. 2003)		Rs1031309	0.3291	0.8809
CD1A	*CD1A*		Conflicting evidence (Caporale, Papola et al. 2006, Caporale, Uncini et al. 2008, Kuijf, Geleijns et al. 2008, Uncini, Notturno et al. 2011)		Rs16840041	0.329	0.7658
CD1D	*CD1D*		No association (Caporale, Papola et al. 2006)		Rs859008	0.202	0.8055
CD1E	*CD1E*		Conflicting evidence (Caporale, Papola et al. 2006, Caporale, Uncini et al. 2008, Kuijf, Geleijns et al. 2008, Uncini, Notturno et al. 2011)		Rs1065457	0.885	0.9515
CD14	*CD14*	5q31.1	No association (Geleijns, Jacobs et al. 2004)		Rs2563298	0.548	0.9252
Contactin 2 (axonal) (Transient	*CNTN2*	1q32.1		Conflicting evidence (Iijima, Koike et al. 2011,	Rs2275697	0.865	1.031

axonal glycoprotein-1)				Pang, Chan et al. 2012)			
CD59 (complement regulatory molecule)	*CD59*	11p13	Anti-CD59 as treatment for EAN (Halstead, Zitman et al. 2008)	Case report of CD59 deficiency (Nevo, Ben-Zeev et al. 2013)	Rs7046	0.3947	0.9109
Fas (CD95)	*FAS*	10q24.1	Association with ganglioside Ab (Geleijns, Laman et al. 2005)		Rs9658702	0.363	1.019
Fcgamma receptor IIa	*FCGR2A*		Conflicting evidence (Vedeler, Raknes et al. 2000, van Sorge, van der Pol et al. 2005, Sinha, Prasad et al. 2010)		Rs7529425	0.139	1.214
Fcgamma receptor IIIb	*FCGR2B*	1q23	Less severe disease (Vedeler, Raknes et al. 2000)		Rs11799952	0.552	1.037
Fcgammma receptor IIIa	*FCGR3A*	1q23	Conflicting evidence (Vedeler, Raknes et al. 2000, van Sorge, van der Pol et al. 2005, Sinha, Prasad et al. 2010)		Rs17411858	0.139	1.332
FcR- like 3	*FCRL3*	1q21	Association in Han Chinese (Sang, Chen et al. 2012)		Rs2282284	0.805	0.7972
Glucocorticoid receptor	*NR3C1*	5q31.3	No association, but linked to clinical course (Dekker, van den Akker et al. 2009)		Rs6190	0.777	0.898
HLA-A	HLA-B	6p21.3	No association (Ma,		Rs9404952	0.797	

			Nishimura et al. 1998)				
HLA-B	HLA-B	6p21.3			Rs3819299	0.664	1.336
HLA-C	HLA-C	6p21.3			Rs7750641	0.209	
HLA-DQ	HLA-DQ	6p21.3		Association (Mrad, Fekih-Mrissa et al. 2013)	Rs2854275	0.669	0.9692
HLA-DR	HLA-DR	6p21.3	Conflicting results (Gorodezky, Varela et al. 1983, Magira, Papaioakim et al. 2003, Sinha, Prasad et al. 2010)	Association (Mrad, Fekih-Mrissa et al. 2013)	Rs9286790	0.779	
Interleukin 10	*IL10*	1q31	Association (Myhr, Vagnes et al. 2003)		Rs3024492	0.09	0.8736
MMP9	*MMP9*	20q11.2			Rs2250889	0.216	1.398
Peripheral myelin protein 22	*PMP22*	17p12	Case reports (Odaka, Yuki et al. 2003)	Case reports (Korn-Lubetzki, Argov et al. 2002, Remiche, Abramowicz et al. 2013)	Rs16951239	0.262	1.022
T cell specific adaptor protein	*SH2D2a*	1q21		Association (Notturno, Pace et al. 2008)	Rs21150906	0.1111	1.376
TNF alpha	*TNF*	6p21.3	Association with axonal GBS (Jiao, Wang et al. 2012)		Rs3093662	0.437	0.5922
Toll-like receptor 4	*TLR4*	9q33.1	No association (Geleijns, Jacobs et al. 2004)		Rs1927906	0.22	0.805

6.4. Discussion

We performed a genome-wide association study of a cohort of patients with a confirmed diagnosis of GBS and a control group of subjects available from an Australian (OATS, RAINE) and UK cohort (WTCCC).

The patient characteristics of our case cohort were similar to previously described cohorts of Australian subjects with GBS (Hankey 1987, Storey, Cook et al. 1989, Blum, Reddel et al. 2013). Our controls were derived from a previously collected cohort of individuals with Caucasian heritage from an Australian (OATS, RAINE) and UK background (WTCCC). Individuals with non-Caucasian heritage were excluded from analysis.

Due to the small number of cases, it was anticipated that no genome-wide significance could be achieved in our study. The main aim, therefore, was to identify regions to enable more focused study in the future.

We found 5 regions of interest. Of these, 2 regions were in the area of genes whose corresponding proteins had previously been studied and are thought important in GBS pathogenesis – *IL15* and *NFKBIB*. Another region was associated with a transcription factor of importance in neuronal development – *MYT1L*. Finally, two other sites had less clear associations with known factors of GBS pathogenesis. We will subsequently review candidate genes identified by this method.

6.4.1. IL-15

rs7663689 is located in the promotor region of *IL15*, the gene encoding for the cytokine IL-15. It is structurally and functionally related to IL-2, and indeed the two proteins share subunits of their receptors (Di, Calarota et al. 2011, Steel, Waldmann et al. 2012). It has multiple functions in cell development, including both innate and adaptive lymphoid cells and neural cells (Lodolce, Burkett et al. 2002). IL-15 is widely expressed in the human body and expressed by both immune and non-immune cells (Fehniger and Caligiuri 2001) in development and adult individuals.

IL-15 is an important cytokine in the innate and adaptive immune system (Lodolce, Burkett et al. 2002). IL-15 has important roles in immunity in both health and disease. It has been implicated in the pathogenesis of autoimmune diseases, i.e. celiac disease and type I

diabetes mellitus (Yokoyama, Watanabe et al. 2009, DePaolo, Abadie et al. 2011, Di, Calarota et al. 2011, Bobbala, Chen et al. 2012), but also in immune response to malignancies (Steel, Waldmann et al. 2012).

IL-15 has its most important role in γ/δ+ lymphocytes and NK cells (de Rham, Ferrari-Lacraz et al. 2007). It is critical in NK cell development, as evidenced by the absence of NK cells in IL-15 knockout-mice (Kennedy, Glaccum et al. 2000) and the defect of NK cell development out of CD34+ hematopoietic progenitor cells in the absence of IL-15 (Mrozek, Anderson et al. 1996). Moreover, IL-15 plays an important role in innate immune system cross-talk, with activated macrophages secreting IL-15 in a paracrine fashion, stimulating NK cells (Carson, Ross et al. 1995). IL-15 primes NK cell killing function, which has been implicated in human disease (Tang, Sally et al. 2013).

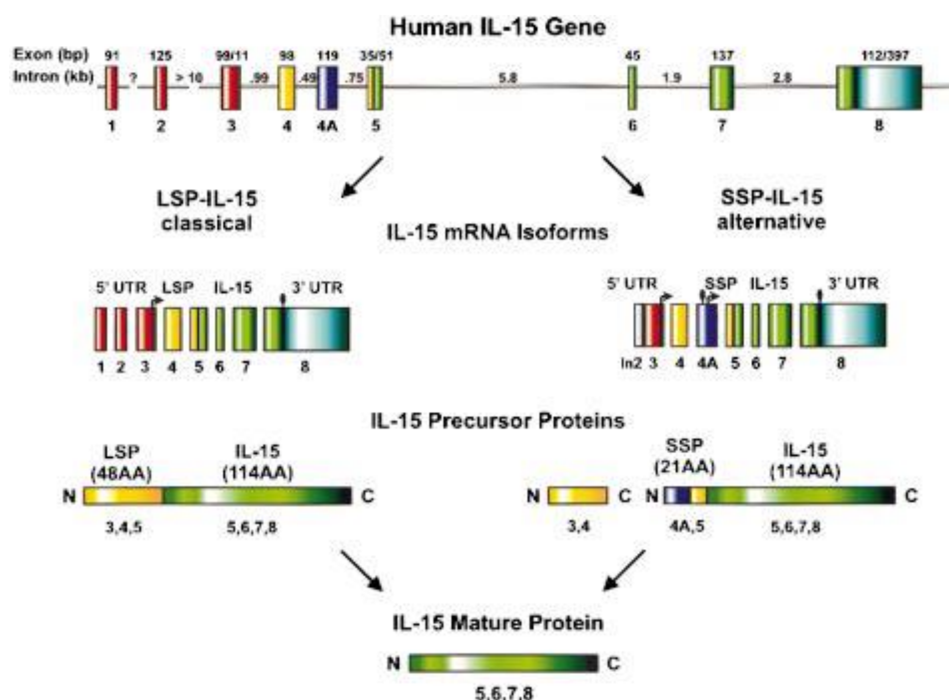


Figure 6.19 - Human IL-15 mRNA and protein structure (Fehniger and Caligiuri 2001). Green colours represent the encoding gene of the mature protein, yellow parts of the pre-proteins, purple are splice variants and red are parts of the mRNA not encoded into the precursor protein.

It is of interest, that the SNP found (rs7663689) is not directly located in the *IL15*, but rather in the promotor region of this gene. Common functional polymorphisms in cytokine genes, which are widely distributed in the population, may affect gene transcription and

subsequently individual variations in cytokine levels. Polymorphisms in the promotor regions of IL-2, IL-4, IL-6, IL-10 and TNFα have been described (Kurzawski, Pawlik et al. 2005, Amirzargar, Khosravi et al. 2007). These genetic differences can result in variations of cytokine levels, leading to susceptibility to a range of different diseases (Ollier 2004). Also, a number of SNPs have been described in the *IL15*, and have been linked to smoking, juvenile idiopathic arthritis, allergies and left ventricular hypertrophy (Bierbaum, Sengler et al. 2006, Christensen, Haagerup et al. 2006, Arnett, Devereux et al. 2009, Fujisaki, Kakuda et al. 2009, Liu, Pei et al. 2009). Polymorphisms of IL-15 have also been described to be associated with resistance to certain infections (Kalani, Rasouli et al. 2011).

A report by Van Rhijn et al. investigated the role of *C. jejuni* reactive T lymphocytes in patients with GBS. They found that γδ T cell proliferative responses were suppressed in patients with GBS due to Campylobacter infection, but could be restored by supplementing IL-2 or IL-15 (Van, Logtenberg et al. 2003). They suggested that host factors, rather than properties of the invading pathogen, are responsible for this aberrant response. Genetic changes in IL15 expression could therefore be a risk factor for developing GBS.

6.4.2. NFKBIB

Whilst other genes are in close vicinity to rs2302524, *NFKBIB* is the only gene in the region that can be easily associated with the immune system. *NFKBIB* is a member of the NF-κB inhibitor family IκB. NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) is a protein complex that controls transcription of DNA. NF-κB plays a key role in regulating the immune response to infection and other stressors (Gilmore 2006). The NF-κB complex is inhibited by IκB proteins, which inactivate NF-κB by trapping it in the cytoplasm (Woronicz, Gao et al. 1997). Under the influence of cytokine-induced kinases, phosphorylation of serine residues on these proteins marks them for destruction via the ubiquitination pathway, thereby allowing activation of NF-kappa-B, which translocates to the nucleus to function as a transcription factor. NF-κB is thereby intimately involved in cell responses to stress, cytokines, free radicals and bacterial and viral antigens (Gilmore 2006). NF-κB has been linked to cancer, inflammatory and autoimmune diseases, septic shock and viral infections.

In GBS and CIDP, NF-κB has been found to be increased in macrophages obtained from sural nerve biopsies, whereas IκB was upregulated in both macrophages and T cells

(Andorfer, Kieseier et al. 2001). In non-inflammatory neuropathies this upregulation of NF-κB and IκB in immune cells was not found. Subsequently, it could be demonstrated that

NF-κB translocates into the cell nucleus of inflammatory cells in both CIDP and GBS patients, but not in patients with other neuropathies (Mazzeo, Aguennouz et al. 2004).

6.4.3. *MYT1L*

Rs10519519 is located in an intron of *MYT1L*, with no other genes in close vicinity. The protein encoded by this gene is thought to be a pan-neural transcription factor associated with neuronal differentiation and is expressed in the developing brain (Kim, Armstrong et al. 1997). Moreover, it has been found to be of paramount importance in the induction of neurons out of both human and mouse fibroblast cultures, together with other growth factors (Vierbuchen, Ostermeier et al. 2010, Ambasudhan, Talantova et al. 2011, Pang, Yang et al. 2011, Yoo, Sun et al. 2011). *MYT1L* mutations and copy number variations (CNV) have been associated with a number of psychiatric disorders, including depression, autism and schizophrenia (Wang, Zeng et al. 2010, Lee, Mattai et al. 2012, Li, Wang et al. 2012, Meyer, Axelsen et al. 2012).

The role of *MYT1L* in the peripheral nervous system has not been elucidated. However, this finding does raise the possibility of differences in neuronal development of the nervous system being a susceptibility factor for development of GBS. Target organ susceptibility has been raised as a factor in development of autoimmune diseases, but not studied in depth (Kuhr, Hala et al. 1994).

6.4.4. *rs17095496 and rs11151180*

The situation is unclear for these two SNPs. rs11151180 on chromosome 18 is in a genetic desert, with no previously identified genes. rs17095496 is in the vicinity of 4 genes (*DACT1*, *DAAM1*, *C14orf135* and *DHRS7*), none of which has a known role which could be easily linked to GBS. Of some interest is the association of *DACT1* with NF-κB in tumour cells (Wang, Kang et al. 2012), but the relevance of this gene in the immune system is unclear at present.

6.4.5. Relevant negatives

We investigated SNPs on all previously studied genes in GBS. None of these was associated with a significant result in our cohort.

Of interest is the absence of any signal in the HLA region on chromosome 6. The HLA region is highly polymorphic and variations are strongly associated with virtually every autoimmune disease (Handunnetthi, Ramagopalan et al. 2010, Cho and Gregersen 2011, Thorsby 2011). Indeed, for many autoimmune diseases, the HLA locus hosts the strongest and most consistent susceptibility alleles (Rai and Wakeland 2011). In GBS, HLA has been extensively studied, with some conflicting results, but no clear and reproducible link has been established so far (Gorodezky, Varela et al. 1983, Winer, Briggs et al. 1988, Ma, Nishimura et al. 1998, Magira, Papaioakim et al. 2003, Geleijns, Schreuder et al. 2005). Whilst not conclusive, this argues against a dominant role of the adaptive immune system in GBS pathogenesis. Alternatively, this absence of signal could be a false-negative finding, given sample size.

6.4.6. Summary

In spite of the relatively small number of cases in our study, we were able to identify a number of loci of interest. Two of these (*IL15* and *NFKBIB*) can easily be linked to proteins of importance in the immune system in general and GBS in particular. The role of IL-15 in NK cell development is of particular interest in the context of this thesis, seeing the previously mentioned role of NK cell activation proteins in GBS pathogenesis (see Chapters 4 and 5). One locus (*MYT1L*) appears to be related to neuronal development; target organ susceptibility, rather than immune dysfunction, as cause for GBS is a novel idea and has not been studied so far. This is in contrast to the large GWAS performed in MS, where a number of immune genes have been identified, but no genes associated with neuronal function were detected (Beecham, Patsopoulos et al. 2013). For the two other loci no genes which could easily be related to GBS pathogenesis could be identified; one of these genes (*DACT1*) has been associated with NF-κB regulation in the context of gastric cancer. We found no indication that the HLA locus is in LD, which would make GBS very unusual compared with most other autoimmune diseases (Handunnetthi, Ramagopalan et al. 2010).

In summary, our study provided several loci and genes of interest in a GWAS of subjects with GBS. It would be worthwhile to further study these sites, either using a replication

cohort or alternatively using the data as basis for a candidate gene approach as well as functional studies.

Chapter 7 Summary and conclusions

This thesis studied the epidemiology of GBS in Australia and investigated a possible genetic basis of GBS pathogenesis in a cohort of Australian subjects with GBS.

GBS is an inflammatory disease of the peripheral nervous system, which affects subjects of virtually any age, but is particularly common in the 5th and 6th decade of life. It is most frequently seen in as a post-infectious illness in individuals of otherwise good health. All functions of peripheral nervous system can be affected, with weakness, sensory disturbance, pain and autonomic dysfunction being common. Onset of the disease is subacute, with a frequently severe illness course, but usually good recovery. Nevertheless, the cost of treatment per patient is substantial (Frenzen 2008), mainly related to the need for lengthy and intensive supportive care.

7.1. Characteristics of GBS in Australia

GBS is a heterogeneous illness, and a range of subtypes have been characterized. These include AIDP, AMAN, AMSAN, MFS and several focal variants of GBS. These subtypes are defined predominantly on the basis of clinical and electrophysiological criteria, but have also pathogenetic differences. It was therefore of importance to study in detail the clinical characteristics of GBS in a contemporary Australian cohort (see Chapter 3).

Using a retrospective chart review in seven hospitals along the East Coast of Australia, we identified a sizeable cohort of 335 subjects with a confirmed diagnosis of GBS. We found that incidence of GBS increased with age, peaking between the ages of 60 to 70 years, with a possible smaller peak in the early twenties. The predominant gender was male, with a ratio of M : F = 1.61 : 1. As expected with an inpatient based cohort, most patients were severely affected: 43% were bed- or wheelchair bound, and an additional 24% required ventilatory support. Mortality was low at 2%. Surprisingly, a comparison of our data with historical data collected before the wide-spread use of plasma exchange or IVIg showed that the illness peak severity and ICU admission rates have not been significantly altered by the use of these treatments (Hankey 1987, Storey, Cook et al. 1989). This highlights the need for more effective therapies of GBS.

Also of interest was the finding of the seasonal variation of GBS and its relationship to triggering factors. Based on historical data and the evidence for *C. jejuni* triggering axonal variants of GBS by molecular mimicry, diarrhea caused by this pathogen is still frequently thought to be the most common triggering factor. However, in our cohort the incidence of diarrhea as triggering factor was low (19%), compared with the finding that 45% of patients had preceding URTIs. This finding was congruent with the higher incidence of GBS during the Southern hemisphere winter months as compared to summer months. It could be speculated that changes in hygiene and wide-spread use of refrigeration has decreased the incidence of both *C. jejuni* and GBS triggered by this agent in recent decades, especially in developed countries (Baker, Kvalsvig et al. 2013). Axonal variants of GBS, possibly triggered by *C. jejuni*, still appear to be much more frequent in developing countries (McKhann, Cornblath et al. 1991, McKhann, Cornblath et al. 1993, Ye, Zhu et al. 2010, Ye, Wang et al. 2013). Diarrhea as trigger was significantly associated with AMAN in our cohort.

We found that patients named a surprising variety of factors other than URTIs and diarrhea preceding GBS. These included trauma and surgery, herpes viridae infections, urinary tract infections and other traumatic events (myocardial infarction, delivery, status epilepticus) accounting for 11% of the total. In 25% of patients no trigger could be identified. This heterogeneity of triggering factors leading to a relatively stereotyped clinical picture and electrophysiology is difficult to explain solely on the basis of molecular mimicry (Ang, Jacobs et al. 2004). Whilst the evidence for molecular mimicry is reasonably strong for AMAN triggered by *C. jejuni*, the data is much less strong for AIDP. We propose that factors other than molecular mimicry could be important in GBS pathogenesis.

7.2. A genetic basis of GBS pathogenesis?

That genetics could play a role in GBS pathogenesis has been suggested by several reports of familial cases of GBS from different geographic areas (Wilmshurst, Pohl et al. 1999, Geleijns, Brouwer et al. 2004, Aquil, Khan et al. 2011). Also, a link between GBS, CIDP and CMT disease has been suggested, based on reports of GBS and CIDP in the context of CMT (Dyck, Swanson et al. 1982, Odaka, Yuki et al. 2003). With the advent of large-scale GWAS it has also become clear that even diseases which do not show Mendelian inheritance patterns can have multiple genetic susceptibility markers scattered throughout the genome.

In the past, a number of genes have been studied in subjects with GBS, but genes reproducibly linked to GBS are scarce. Polymorphisms in HLA and CD1 have both been found in GBS cohorts, but these findings could not be confirmed in a larger sample (Gorodezky, Varela et al. 1983, Winer, Briggs et al. 1988, Wu, Zhou et al. 2012). A range of other genes, including IL-10, TNF-α, MMP9, Fc receptors and LPS receptors have been studied in single, un-replicated studies (see Chapter 2 and 6).

Analysis of global gene expression of peripheral blood leukocytes in a Taiwanese cohort with GBS showed up- or downregulation of a range of genes (Chang, Chuang et al. 2012). Network analysis of these genes identified biological functions associated with inflammatory response, infectious disease, cell death, cellular development, haematological cell function and immune cell trafficking. These included MMP9 and ERK/MAPK signaling pathway genes, which are involved in the process of demyelination and cytokine production. This would be expected as it is generally accepted that macrophages and lymphocytes are activated and involved in the pathogenesis of GBS.

Our approach to identify possible genetic factors for GBS pathogenesis was twofold:

Firstly, we employed a candidate gene approach investigating KIRs and their HLA-ligands in a cohort of GBS patients and also in patients with CIDP (see Chapters 2, 4 and 5). Because of the complex interactions at the protein level, it is to be anticipated that these loci would not be identifiable on a GWAS.

Secondly, we used a hypothesis-free approach by performing a GWAS on the same cohort of GBS patients, although the total number of subjects in the final analysis was reduced through quality control rejection of some samples (see Chapter 6).

7.2.1. KIRs and their HLA ligands in GBS and CIDP

KIRs are important receptors on NK cells, controlling their activation. KIRs show considerable inter-individual polymorphism. We investigated the role of KIRs and their HLA ligands in cohorts of patients with GBS, CIDP and healthy controls.

NK cells are important cells of the innate immune system and are of paramount importance in the early immune response against infection. Moreover, they are important in

linking the innate immune response to the adaptive immune system, predominantly by cytokine-mediated communication with T cells. NK cells could therefore have an important role in early GBS pathogenesis, but – rather surprisingly – have been only very incompletely studied.

NK cell activation is regulated by a complex range of different receptors, the most important of which include the C-type lectin-like group CD94:NKG2 and the KIR group and their HLA ligands. Of these, KIRs constitute a large family of receptors with extensive polymorphisms and a large number of inhibitory and activating members. For a functional interaction both KIR and the specific HLA ligand needs to be present. In short, KIRs with a long cytoplasmic tail are inhibitory, whereas KIRs with a short tail are activating. KIRs are found on NK cells, but also on some subgroups of T lymphocytes, including γδ lymphocytes.

Whilst no significant differences were found for the KIR gene frequencies themselves, we found significant difference when studying the HLA receptors of KIR and KIR/HLA functional pairs. We found that the inhibitory combinations KIR 3DL1/HLA-B Bw4-T and KIR 2DL1/HLA-C2 were significantly more frequently seen in GBS than in HC. Also, the activating combination 2DS2/HLA-C1 was less frequent in the GBS group, also producing a likely inhibitory effect on the NK cell population in GBS. The situation was reversed for 2DL2/HLA-C1, where this inhibitory combination was less frequently seen in GBS patients.

In CIDP patients, significant differences in KIR/HLA combinations were found for 3DL1/HLA-B Bw4-I compared with healthy controls, but interestingly no such difference existed between the CIDP and GBS group for any of the KIR and HLA genes, nor their functional combinations.

In summary, specific combinations of KIR and their HLA ligands can be either protective or risk factors for the development of GBS. We found some genetic evidence for NK cell involvement in the pathogenesis of GBS. Also of interest are the similarities in KIR/HLA gene frequencies found in GBS and CIDP groups, in contrast to healthy controls.

7.2.2. A genome-wide association study of GBS

Genome-wide association studies represent a hypothesis free method of studying genetic susceptibility of diseases. We performed a genetic analysis using the Omni-Express Bead Chip system, Illumina which tests for 650000 common polymorphisms. GWAS using SNP

data assume that common SNPs contribute to the risk of disease. Rare SNPs could also contribute to disease in the broad population. Because of the reliance on a large number of SNPs studied by this approach, successful GWASs need a large number of subjects and controls to give adequate statistical power.

Our study used a relatively small sample of 230 patients, predominantly due to difficulties in identification and recruitment of subjects with this relatively rare disease. However, this was partially balanced by obtaining access to data from two large cohorts of controls (Australian OATS and RAINE, UK data from WTCCC). Likely due to the low number of cases we did not find any SNPs with genome-wide significance. However, we were able to identify 5 regions of interest with clusters of SNPs in linkage disequilibrium. All these regions showed SNPs with some evidence for LD in both control groups. The SNPs with highest significance in these regions were rs10519519, rs7663689, rs11151180, rs17095496 and rs2302524. Some, but not all of these SNPs corresponded to known genes coding for proteins previously implicated in GBS pathogenesis.

rs10519519 is located within the *MYT1L* gene, with no other closely linked genes. *MYT1L* is a pan-neuronal transcription factor, which has been linked to neuronal development in the CNS, but has not been studied so far in the PNS. Mutations and copy number variants in this gene have been seen in a range of psychiatric disorders.

rs7663689 is in the promotor area of the *IL15* gene, coding for the cytokine IL-15. This location raises the possibility of polymorphisms in the promotor region influencing expression of *IL15* and therefore concentration of the final cytokine. IL-15 has structural and functional relationships with IL-2 and has multiple functions in cell development and in both the innate and adaptive immune system (Lodolce, Burkett et al. 2002).

rs2302524 is associated with a number of genes, including *LGALS7* and *LGALS4*, *ECH1*, *HNRPL*, *SIRT2*, *NFKBIB*, *SARS2*, *MRPS2* and *FBXO17*. Of particular interest in this region is *NFKBIB*, which is a member of the NF-κB inhibitor family IκB (Woronicz, Gao et al. 1997). NF-κB is a key transcription factor in immune regulation and is under complex control by a range of different factors (Gilmore 2006).

rs17095496 and rs11151180 are not linked to any genes known to have a role in the immune system. rs17095496 is in the vicinity of 4 genes (*DACT1*, *DAAM1*, *C14orf135* and

DHRS7), whereas the gene most closely linked with rs11151180 is *CDH7*, a gene related to migration of malignant cells (Winklmeier, Contreras-Shannon et al. 2009).

Of interest were a number of relevant negative results: We found no SNPs in linkage disequilibrium in the HLA region, which has been found to be strongly associated with a range of other autoimmune diseases. Furthermore, we did not find associations with candidate genes which had been previously studied in GBS. The lack of HLA association is a clear difference between GBS and other autoimmune diseases. This opens the possibility of other pathogenic mechanisms to be explored.

7.3. NK cells in the pathogenesis of GBS

The absence of a specific linkage disequilibrium in the HLA region in our GWAS is of interest as this region is closely linked to genetic susceptibility in virtually every autoimmune disease studied so far. This raises the possibility of the innate immune system playing a substantial role in GBS pathogenesis. This has been incompletely studied so far.

The finding of KIR/HLA ligand polymorphisms as well as *IL15* as region of interest in the GWAS points towards a possible role of NK cells in the pathogenesis of GBS.

NK cells as key factors in GBS would be consistent with the known role of NK in the early immune response to infection. NK cells are of paramount importance in linking an initial response to infections with subsequent adaptive immune system activation. We have suggested that the aberrant response of the adaptive immune system that causes GBS occurs early in the disease course, with rapid reconstitution of normal tolerance once this error has been “recognized” (see Appendix A).

In spite of this theoretical link, the role of the innate immune system in GBS pathogenesis has not been studied in depth. Studies so far include the finding of macrophage infiltration of peripheral nerves mediating demyelination (Prineas 1981). Yoshii et al. found decreased NK cell activity early in the disease course, with recovery of normal function after plasma exchange (Yoshii and Shinohara 1998). The cytokine TNFα has been studied in both EAN animals and in human subjects with GBS. Zhu et al. found TNFα production to be upregulated in rats with EAN (Zhu, Mix et al. 2003, Zhu, Mix et al. 2004). Moreover, TNFα has been found to be upregulated in the acute phase of AMAN (Deng, Yang et al. 2008).

Finally, TNFα gene polymorphisms have been consistently found to be associated with GBS (Wu, Zhou et al. 2012), but could not be seen as a region of interest in our GWAS (see Chapter 6).

Based on our findings, we propose that an aberrant response of NK cells is of paramount importance in the early development of GBS. Namely, we suggest that inhibition of normal NK cell response against infected cells (i.e. by KIR/HLA interaction, but likely also by other factors) leads to subsequent aberrant adaptive immune response to peripheral nervous system tissue in GBS (see Figure 7.1). There are well established links of NK cells to the adaptive immune system, namely T cells (Zingoni, Sornasse et al. 2005, Zingoni, Ardolino et al. 2012).

An interesting alternative explanation of the role of IL-15 in the pathogenesis of GBS – especially in the context of this thesis – is its link to the NF-κB pathway (Giron-Michel, Caignard et al. 2003). IL-15 has been shown to induce activation of NF-κB in lymphoid progenitor cells and other cell types. Also, macrophage mediated stimulation of CD4+ T lymphocytes has been described in rheumatoid arthritis (Ruckert, Brandt et al. 2009). This is speculative at present, but further work in this area could be of interest.

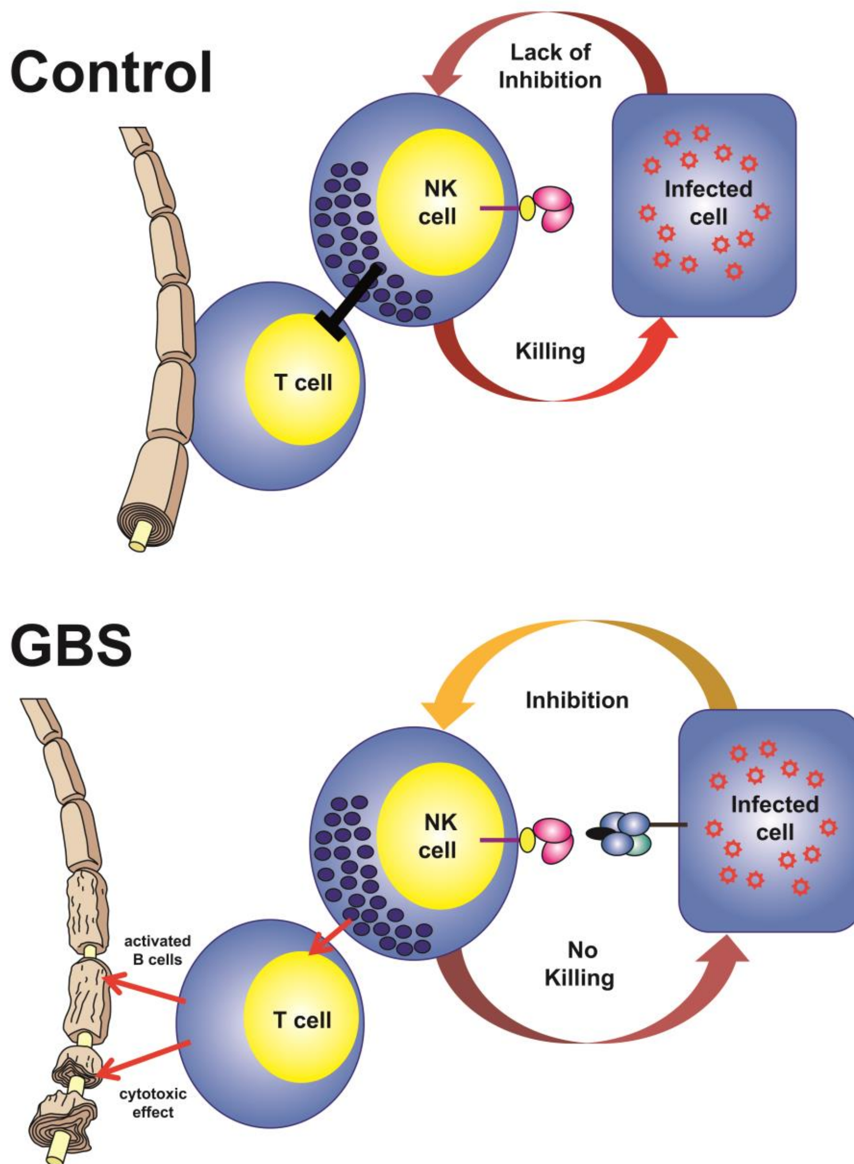


Figure 7.1 - Functional model of NK cell function in GBS pathogenesis (see Chapter 4)

7.4. Downstream mechanisms

Pathological studies and functional evidence clearly point towards a role of the adaptive immune system in the active demyelination found in the acute phase of GBS (Prineas 1981, Scelsa, Ghali et al. 2004, Csurhes, Sullivan et al. 2005, Csurhes, Sullivan et al. 2005, Chi, Wang et al. 2007, Cencioni, Notturno et al. 2009). Indeed, we found genetic evidence of factors intricately involved in activation of the adaptive immune system, mainly in relation to genes regulating function of NF-κB in our GWAS.

Rs2302524 is associated, amongst other genes, with *NFKBIB*, a gene coding for IκB. IκB is of paramount importance in cell activation, as it releases NF-κB upon cytokine-induced phosphorylation, allowing NF-κB to translocate into the nucleus (Mercurio, Zhu et al. 1997).

Interestingly, *DACT1*, which is associated with rs17095496, has been associated with gastric cancer by inhibition of the NF-κB pathway (Wang, Kang et al. 2012). However, whilst expressed by immune cells, it has not been studied in the context of immune function.

NF-κB has been found to be increased in macrophages in sural nerve biopsies, whereas IκB was upregulated in both macrophages and T cells (Andorfer, Kieseier et al. 2001). Moreover, translocation of NF-κB into the cell nucleus of inflammatory cells has been demonstrated in both CIDP and GBS, but was not seen in other neuropathies (Mazzeo, Aguennouz et al. 2004).

We propose that once the dysregulated immune response to the peripheral nervous system has been triggered by the innate immune system, further protective safety mechanisms on the level of the adaptive immune system are usually put in place. To enable the specific attack against myelin protein, further lack of inhibition, most likely of T lymphocytes, needs to take place before GBS can develop. There have been reports of reduced regulatory T cells in GBS (Harness and McCombe 2008). This two-tiered dysfunctional response would explain the relative rarity of recurrent GBS (Kuitwaard, van Koningsveld et al. 2009, Olivier, Laribi et al. 2010).

7.5. GBS - an autoimmune disease?

GBS is thought to be immune mediated, but there must be some questions as to whether it represents a classical autoimmune disease. Evidence for an autoimmune process is predominantly based on the findings of an inflammatory pattern on nerve biopsies in patients with acute GBS and the finding of auto-antibodies in some subforms of GBS. Also, there is some evidence of activation of the immune system at disease onset. Finally, a disease with a range of clinical and pathological similarities to GBS - Experimental Autoimmune Neuritis (EAN) - can be induced in animals by vaccination with nerve proteins. Interestingly, an illness indistinguishable from GBS has been involuntarily induced in humans after vaccination with substances derived from animal brain tissue and vaccination against influenza virus (Hemachudha, Griffin et al. 1988, Salemi and D'Amelio 2010, Baxter, Lewis et al.).

However, whilst having some similarities with autoimmune diseases like SLE or myasthenia gravis, GBS differs in a number of important aspects. These include the male predominance, self-limited monophasic course and being commonly triggered by infection. Also, GBS does not respond to treatment with steroids (Hughes, Newsom-Davis et al. 1978, Hughes, Swan et al. 2010). Finally, most autoimmune diseases show a clear genetic link to immune genes – most commonly HLA - which has not been consistently demonstrated in GBS (see Chapter 7.2).

This has led to a number of alternative hypotheses for the pathogenesis of GBS:

a) Steiner et al. proposed that GBS is caused by a transient immune deficiency induced by the pathogens involved in the triggering infection (Steiner, Rosenberg et al. 2010). His hypothesis would argue against an individual, genetically determined susceptibility for GBS.
b) Hardy et al. argued that GBS is caused by the activation of pre-existing auto-reactive lymphocytes getting activated by potent danger signals and antigen presentation of APCs, thus causing an attack on peripheral nerve. This is followed by rapid reconstitution of normal immune tolerance subsequently, explaining why GBS rarely recurs (see Appendix A).

7.6. GBS – an autoinflammatory disease?

An alternative approach to interpreting our data is to assess whether GBS could be an autoinflammatory disease, rather than an autoimmune disease.

As discussed previously, GBS does not meet all criteria for autoimmune diseases (see Chapter 7.5). As discussed in Chapter 1, autoinflammatory diseases differ from autoimmune diseases in a number of important aspects, and several of these aspects could be linked to GBS in our study:

- The inflammatory response is largely mediated by the innate immune system. We found two independent links of genetic determinants of NK cell function, the KIR/HLA system and IL-15, to be important in GBS.
- NF-κB is an important factor in both autoimmunity and AIDs. We found 2 genes, *NFKBIB* and *DACT1*, within areas of interest in our GWAS, which are regulators of NF-κB function.

On the other hand several facts argue against GBS being an autoinflammatory disease. These include the findings of anti-ganglioside antibodies in some subforms of GBS, the presence of T lymphocytes on biopsies in patients with GBS and the absence of raised inflammatory markers, like ESR and CRP, in the serum of patients with acute GBS.

In summary, GBS does not neatly fit into either the group of autoimmune diseases or into autoinflammatory diseases, and might indeed be in a class of its own.

7.7. Target organ susceptibility

The finding of *MYT1L*, a gene related to neuronal development, in this GWAS of GBS patients raises the possibility of target organ susceptibility being an important factor in GBS pathogenesis. Target organ susceptibility has been raised in the past as a possible factor in a range of autoimmune diseases, but has never been consistently proven. Indeed, the recent large GWAS of MS patients did not identify a single gene related to neuronal function or development (Beecham, Patsopoulos et al. 2013).

However, both GBS and CIDP have been described in the context of disorders of peripheral nerve myelination, namely CMT. Inflammatory changes on nerve biopsy and

response to immune therapy have all been seen in proven CMT1A (Dyck, Swanson et al. 1982, Vital, Vital et al. 2003, Ginsberg, Malik et al. 2004). Moreover, GBS has been described in several patients with both axonal and demyelinating forms of CMT and hereditary spastic paraparesis (Odaka, Yuki et al. 2003, Alhashel, Alshubaili et al. 2004, Münch, Epplen et al. 2008).

Target organ susceptibility could be the final step in the development of GBS, possibly by exposure of antigens to activated immune cells, or by enabling penetration of immune cells through the blood-nerve barrier.

7.8. Future directions

There are several avenues for further research into the pathogenesis based on the findings presented in this study.

The increased incidence of subjects with GBS with age is intriguing. It would be interesting to investigate the role of immunosenescence and its role in the innate and adaptive immune response to infection and its link to GBS (Solana, Tarazona et al. 2012, Ma and Fang 2013). Furthermore, there are gender differences in immunosenescence which could account for the sex ratio of GBS.

NK cell function in GBS could be further studied using functional assays of NK cell activity, including the role of cytokines (i.e. IL-2, IL-15, IL-18, IFNγ) as well as cytotoxicity. Parallel to this, polymorphisms in other genes related to NK cell function could be studied, including CD94:NKG2 receptors.

Much work remains to be done following the GWAS of GBS. One would expect that with expansion of the sample size further genetic loci of interest could be identified, similar to the experience in the large GWASs in MS. The genetic loci identified will need to be reproduced in an independent cohort of subjects with GBS. Finally, functional studies of the proteins coded for by the identified genes could lead to a better understanding of GBS, and possibly could direct investigations into new therapies for this illness.

Appendix A – Guillain Barré Syndrome: Modern theories on Etiology

This review was written in collaboration with Dr. Todd Hardy and Dr. Stephen Reddel from Concord Hospital, Sydney. It summarizes some of the theoretical groundwork for this thesis.

Curr Allergy Asthma Rep (2011) 11:197–204
DOI 10.1007/s11882-011-0190-y

Guillain-Barré Syndrome: Modern Theories of Etiology

Todd A. Hardy · Stefan Blum · Pamela A. McCombe · Stephen W. Reddel

Published online: 31 March 2011
© Her Majesty in Right of Australia 2011

Abstract Guillain-Barré syndrome (GBS) is a classic failure of the immune system with a life-threatening attack upon a critical self-component. The active phase of the disease is short, concordant with the latency of a primary adaptive immune response. Triggers for GBS include infection and (rarely) vaccination; cross-reactivity between infectious and neural epitopes has been well demonstrated, particularly for *Campylobacter jejuni* and motor axonal forms of GBS in which non-protein gangliosides are antigenic. Most people are probably exposed to a GBS trigger, but only rarely does the disease develop. We propose that GBS illustrates competing determinants of the immune system's decision about whether to mount a response, and that in unlucky affected individuals, co-presentation of cross-reactive antigens with danger signals activating pattern-recognition receptors overcomes normal self-recognition such that a primary response is initiated that attacks the nerve. Then, in most cases of GBS, the response rapidly turns off, and second attacks rarely occur. This suggests active restoration of tolerance, and specific privileged site attributes of nerve and declining danger signals as the trigger wanes may contribute to this restoration. Standard immunosuppression has not been effective in GBS. We suggest this is because immune tolerance is already being restored by the time such therapies are initiated. This in turn suggests that improvements in GBS outcomes are likely to come from better protection of the nerve cells under attack while normal resumption of tolerance is permitted to proceed rather than exploring more aggressive immunosuppressive approaches.

Keywords Guillain-Barré syndrome · Acute inflammatory demyelinating polyradiculoneuropathy · Acute motor axonal neuropathy · Immune pathogenesis · Autoimmune disease

T. A. Hardy
Department of Neurology,
Concord Hospital,
Sydney, Australia
e-mail: thardy@med.usyd.edu.au

S. Blum · P. A. McCombe
Department of Neurology, Royal Brisbane and Women's Hospital,
Brisbane, Australia

S. Blum
e-mail: stefan_blum@health.qld.gov.au

P. A. McCombe
e-mail: pamela.mccombe@uq.edu.au

S. W. Reddel (✉)
Departments of Neurology and Molecular Medicine,
University of Sydney, Concord Hospital,
Clinical Sciences Building,
Sydney, New South Wales 2139, Australia
e-mail: stephen.reddel@sydney.edu.au

Introduction

"The immune system has co-evolved with microbes that cause acute infectious disease. Immune responses must be appropriate to allow survival of both the individual and the species" [1]. Guillain-Barré syndrome (GBS) is a disorder in which the immune response is inappropriate; the affected individual is often profoundly damaged and might die without modern hospital care. The immune attack on nerves appears to occur because targets in the nerve are cross-reactive with targets in infectious organisms, and is considered to be autoimmune. However, for reasons that we discuss, this is a most atypical autoimmune disease.

GBS is an acute, acquired peripheral neuropathy with a course that is usually monophasic. In the United States, it has an estimated incidence of 2 per 100,000 people [2]. It is very serious compared with most other neuropathies. Within a few days of onset, many sufferers become so weak that they cannot walk, and a significant minority require periods of intensive care ventilation. In contrast, most other neuropathies progress slowly over a period of years, and many cause no significant motor deficit. GBS usually manifests following a diarrheal or respiratory illness and is characterized by ascending paralysis and hyporeflexia or areflexia with variable amounts of sensory and autonomic involvement. Generally, most patients recover reasonably well but may take months to years to do so. Those with respiratory muscle weakness are at higher risk of complications, and the modern in-hospital mortality rate is 2.7%. Significant permanent impairment is seen in 15% to 20% of patients, but many more patients have minor residual symptoms, and the mean cost per patient with GBS was estimated at a tremendous $318,966 (US dollars). Treatment with either plasma exchange or intravenous immunoglobulins improves functional outcomes [3]. The presentation, diagnosis, investigation, and therapeutic options are well-reviewed elsewhere [3, 4].

The chronology of GBS is important. GBS progresses to peak severity often over only 2 weeks and by definition no more than 4 weeks. If progression lasts longer than this, the patient is defined as having chronic inflammatory demyelinating polyneuropathy (CIDP) [5]. The key point is that having attacked and often devastated the peripheral nerves of the host, the immune system rapidly "realizes" the error of its ways and does not continue to attack, leaving phagocytic processes to clean up and nerves to repair. If the damage is confined to myelin, then Schwann cell precursors proliferate and repair damage, with clinical recovery within weeks. If the damage has spilled over to axons or the axons are the primary target, then axonal regrowth is required, which occurs at a rate of 0.5 to 1 cm/wk; therefore, clinical recovery takes months to years and is often incomplete. However, if the axonal attack causes electrical block of conduction but not axonal lysis or, alternatively, only the short terminal motor axon is affected, then recovery again can be swift. Thus, the duration of illness is a function of the type of cell damage, the location of the damage, and the ability to repair damage but is not related to an ongoing effect of the immune system.

GBS is not a single disease state but rather represents a spectrum of entities affecting peripheral nerves, the most common of which in Western countries is acute inflammatory demyelinating polyradiculopathy (AIDP). Classification of GBS into subtypes depends on an understanding of the nerve fiber type involved (sensory, motor, and autonomic) and the predominant nature of peripheral nerve or nerve root injury (eg, axonal versus demyelinating). Important subtypes include the following:

- Acute motor axonal neuropathy (AMAN)
- Acute motor and sensory axonal neuropathy (AMSAN)
- Miller Fisher syndrome (MFS)
- Acute pandysautonomia

Related Conditions and Animal Models

GBS has an excellent animal model, experimental allergic neuritis (EAN), which can be triggered by vaccination with whole nerve extract and peripheral myelin proteins. A related experimental neuropathy also has been induced with various gangliosides. CIDP is a chronic but less severe disease. Animal models of CIDP are limited, and some require suppression of immunoregulation. Acute disseminated/demyelinating encephalomyelitis and (chronic) multiple sclerosis affect the central nervous system, with acute disseminated/demyelinating encephalomyelitis sharing many features with GBS.

Triggers of Guillain-Barré Syndrome

Antecedent Infections in Guillain-Barré Syndrome

It is not understood what triggers the immune-mediated destruction of nerve tissue in GBS. Antecedent infections are common in GBS, with about two thirds of patients reporting symptoms of gastrointestinal or respiratory infection in the weeks preceding onset. This strongly suggests that GBS is a postinfectious, immune-mediated disease. In most cases, a causative organism is not identified, but several viral and bacterial pathogens have been found in the serum of GBS patients in case–control studies. *Campylobacter jejuni* is the most commonly recognized organism. Only a minority of patients infected with *C. jejuni* will acquire GBS, suggesting that host factors confer susceptibility. Other common pathogens that precede GBS include cytomegalovirus, Epstein-Barr virus, and *Mycoplasma pneumoniae*.

Vaccinations

It is controversial whether GBS can be triggered by vaccinations. Concerns were first raised following an apparent increase in the incidence of GBS in those who received the "swine flu" vaccination in the United States in 1976 [6]. Most subsequent studies have failed to show an association. Combined data from the 1992–1993 and 1993–1994 influenza vaccinations in the United States demon-

strated an increase in GBS of 1 extra case per 1 million people in the 6 weeks following vaccination [7]. Currently, data from two large US studies monitoring the latest H1N1 "swine flu" influenza vaccinations do not support an increased risk of GBS above that seen with other influenza vaccines [8]. Multiple other vaccines have been implicated as potential triggers for GBS, but evidence is lacking or unconvincing [9]. The data are much clearer for older rabies vaccines. For example, in 1980, the Semple rabies vaccine, which contains brain protein, was discontinued in many countries due to the production of a GBS-like illness in 7% of those vaccinated.

Guillain-Barré Syndrome Subtypes

Despite significant advances over the past two decades, the pathogenesis of the various GBS subtypes is not understood. Why some develop AIDP while others develop AMAN despite *C. jejuni* infection being commonly antecedent to both is far from clear. Racial predisposition may be a factor.

Acute Inflammatory Demyelinating Polyradiculopathy

AIDP is by far the most common form of GBS among Caucasians, accounting for 90% to 95% of all cases in Europe, North America, and Australia. Nerve conduction studies may show evidence of demyelination, with prolonged distal motor latencies, conduction slowing or block, temporal dispersion of compound action potentials, and prolonged F-wave latencies [4]. Sensory nerve studies also may be abnormal. Pathologically, a multifocal mononuclear cell infiltration throughout the peripheral nervous system is observed along with evidence of active demyelination. The distribution of inflammation corresponds to the clinical deficit [10]. The immune mechanisms leading to demyelination in AIDP are incompletely understood, but cell-mediated and antibody-mediated effects have been implicated. The target antigen in AIDP is less clear than the target antigens in AMAN. It is proposed that activated CD4 T-helper cells bind to specific antigens on myelin-producing Schwann cells or on the myelin sheath itself [11]. This is supported by the observation that T cells reactive to myelin proteins and gangliosides have been found in patients with AIDP [12]. These cells also produce interferon-γ, an important proinflammatory cytokine, in response to stimulation with ganglioside GM1 [13]. The number, but not the function, of $CD4^{+}CD25^{+}$ regulatory T cells is reduced in the acute phase of GBS [14, 15].

Antibody-mediated damage also appears to be important, with plasmapheresis and intravenous immunoglobulin being the only partially effective therapies. Antibodies putatively bind to Schwann cell epitopes, leading to complement activation and demyelination in a mechanism that may precede macrophage invasion [16]. In the past, because myelin proteins cause EAN, they have been studied as a possible target in AIDP and have been found in a minority of GBS patients at the peak of their disease [17]. However, they are thought to be unlikely to be important targets in most GBS patients. The binding of antibodies to myelin-specific gangliosides or steric mixtures of gangliosides is being actively studied. Activated macrophages attracted to the area of immune activity contribute to further immune destruction of nerve tissue and stimulate local inflammation via cytokine release. However, experimental autoimmune encephalomyelitis murine models suggest that macrophage release of growth factors may also be important for axonal regrowth and remyelination.

Acute Motor Axonal Neuropathy

AMAN is a form of GBS distinguished from AIDP by its axonal rather than demyelinating pathology and by a lack of sensory nerve involvement [18, 19]. Although it is less common than AIDP in Western countries, AMAN is the predominant form of GBS in Northern China, occurring in 65% of patients. In this population, AMAN occurs more often in summer and is frequently associated with antecedent *C. jejuni* infection. Similar reports have revealed that AMAN is more common than AIDP in other parts of Asia and in Mexico.

In AMAN, rather than macrophage invasion of myelin sheaths leading to denudation of peripheral nerve axons (as in AIDP), macrophages instead attack the nodes of Ranvier beneath the intact Schwann cell and invade periaxonally early in the disease [4, 20]. The macrophages appear to be targeting antibodies deposited at the site of ganglioside antigens on the axolemma. The term *ganglioside* refers to a large group of glycosphingolipids that are frequently found on the outer surface of cell membranes. In AMAN, particular gangliosides are targeted, including GM1, GD1a, GalNac-GD1a, and GM1b [21, 22]. As patients with AMAN usually recover, the resulting damage to the axon appears to be short-lived and may reflect a temporary blockade of conduction, although axonal degeneration distally is possible. Infrequently, patients are severely affected, in keeping with axonal degeneration at the level of the ventral nerve root [4].

Evidence to support a juxtanodal location for the ganglioside antigens GM1 and GalNac-GD1a comes from studies in a rabbit model of AMAN [23]. GalNacT knockout mice suggest that the GM1 antigen is important for supporting paranodal voltage-gated sodium channel clusters and other elements of paranodal ultrastructure. There may also be a differential expression of epitopes such

as GD1a between motor and sensory nerves that could account for the relatively selective involvement of motor nerves in AMAN [24].

Neural complement activation in AMAN is well-defined [25]. Presumably, the presence of bound anti-ganglioside antibodies activates complement deposition, and the classical pathway appears to be principally involved [26]. Postmortem studies show that complement accumulates at the site of nerve damage at the axolemma [27]. In mice, the anti-complement monoclonal antibody eculizumab prevents anti-ganglioside-mediated neuropathy, further supporting a role for complement in antibody-mediated axonal damage [28•].

Acute Motor and Sensory Axonal Neuropathy

AMSAN is the name given to the motor axonal form of the disease when there is coexistent sensory involvement; this is often very severe. The pathology is similar to that seen in AMAN, with involvement of ventral nerve roots and peripheral motor axons targeted as a site of macrophage invasion periaxonally. In AMSAN, dorsal nerve roots and sensory fibers also appear to be involved. Once again, there appears to be a limited role for demyelination or lymphocytic inflammation [20]. Immunologic studies have shown that patients with AMAN and AMSAN share a common profile of anti-GM1, anti-GD1a, and anti-GM1b antibodies, which supports an antibody-mediated pathogenesis in both conditions [29].

Acute Inflammatory Demyelinating Polyradiculopathy/Acute Motor Axonal Neuropathy/Acute Motor and Sensory Axonal Neuropathy Overlap

The diagnosis of AIDP versus other GBS subtypes, such as AMAN or AMSAN, is largely reliant on neurophysiologic studies, which have limitations. For example, conduction block, a neurophysiologic finding typical of demyelination, can be present in axonal disease if blocking of the nodes of Ranvier occurs [30]. This could lead to the misdiagnosis of AMAN as AIDP and may have some impact on the reported prevalence of the different forms of GBS in epidemiologic studies.

A further level of complexity relates to the finding that axonal changes are frequently seen in more severe AIDP. This picture is referred to as *bystander damage*. According to this hypothesis, axons are susceptible to changes in their milieu relating to inflammation and may become compromised due to endoneurial edema, dysimmune attack on axon epitopes situated beneath myelin targets, ischemia, or intra-axonal accumulation of sodium and calcium resulting from voltage-gated ion channel dysfunction [31, 32].

Anti-GQ1b Antibody Syndromes

MFS (Fisher's syndrome) is a GBS variant with ophthalmoplegia, ataxia, and areflexia associated with anti-GQ1b antibodies [33]. Anti-GQ1b antibodies also appear to underlie the related condition Bickerstaff's brainstem encephalitis, in which an altered level of consciousness, extensor plantar responses, and other central signs are also present. The pathophysiology of these conditions involves the paranode where clusters of GQ1b antigen have been localized using immunohistochemical techniques. Not surprisingly, given the clinical features, cranial nerves innervating the extraocular muscles appear to be more densely replete with GQ1b antigen than other cranial nerves. Ataxia is thought to reflect GQ1b antibody acting on antigens in the dorsal root ganglia and nerve terminal of the muscle spindle.

The Immunopathology of Guillain-Barré Syndrome

Molecular Mimicry and Evidence That Antibodies Are Pathogenic

Increasing evidence indicates that the immunopathogenesis, particularly of AMAN and MFS, is a result of molecular mimicry. The relationship is less clear for AIDP. The term describes the dual recognition of host and microbe antigens by T-cell receptors or antibodies that leads to an erroneous recognition of autoantigens and the triggering of an immune response against host tissues [34]. The evidence for molecular mimicry includes the epidemiologic association between the infectious agent and GBS and the identification of T cells and antibodies against host target antigens.

Support for molecular mimicry also comes from the identification of microbial mimics of the target antigen. The *C. jejuni* lipo-oligosaccharide (LOS) has structural similarities to human gangliosides GM1 and GD1a in peripheral nerve axons [35]. Specific gene variants have been identified in *C. jejuni* from patients with GBS that produce LOS with greater structural homology to gangliosides [36]. *C. jejuni* LOS seems to correlate with the specificity of anti-ganglioside antibodies and the associated variant of GBS [37]. Patients with AMAN seem to have *C. jejuni* that express GM1- and GD1a-like LOS. Alternatively, patients with MFS have isolates expressing GD3-, GT1a-, or GD1c-like LOS. Anti-disialoside (GD) antibodies were demonstrated to kill perisynaptic Schwann cells and damage motor nerve terminals in a murine model [38].

Further evidence for molecular mimicry arises from the reproduction of the disease in an animal model [23, 35]. Immunizing rabbits with GM1-like LOS from *C. jejuni* of a

patient with AMAN yielded an axonal neuropathy with similar clinical characteristics to that of the patient. Similar types of cross-reactivity have been identified in patients with preceding infections from other organisms, including *Haemophilus influenzae*. In humans, widespread parenteral administration of gangliosides for pain and other symptoms has in sporadic cases led to the development of AMAN in combinations of serum anti-GM1 and anti-GD1a IgG antibodies [39].

Adaptive Immunity to Gangliosides and the Role of CD1

Cross-reactive IgG antibodies to LOS and gangliosides are of the type IgG_1 and IgG_3, suggesting that T lymphocytes help in their production. In contrast to peptides, lipid antigens cannot be expressed by *HLA* molecules, as they are not soluble in water and are associated with membranes or lipid-binding proteins in tissues and biological fluids. To be recognized by T cells, lipid antigens must be presented by CD1 antigen-presenting molecules, which can bind lipids [40, 41]. This implies that only lipids that can bind to CD1 are immunogenic for T cells. In humans, five closely related genes located on chromosome 1 (q22–23), *CD1a*, *CD1b*, *CD1c*, *CD1d*, and *CD1e*, are all expressed by professional antigen-presenting cells (APCs). T cells specific for *CD1d* share many of the properties of innate immune cells and carry natural killer cell markers and a semi-invariant T-cell receptor. Because of these properties, they have been labeled invariant natural killer T cells [42]. Polymorphisms in *CD1a* and *CD1e* genes as a susceptibility factor in human GBS patients have been studied, with conflicting results.

The Blood–Nerve Barrier

The blood–nerve barrier (BNB) refers to the tight junctions formed between vascular endothelial cells that restrict the passage of solutes and immune molecules to nerves, where changes may occur in GBS and EAN. In one study, T cells reactive to the P2 component of myelin were injected into rat sciatic nerve along with intraperitoneal demyelinating sera or control [43]. Increased vascular permeability of the BNB occurred only in the presence of circulating anti-myelin antibodies, suggesting that T cells alone are not sufficient to cause BNB disruption but also require antibodies. Likewise, T cells migrating to an intraneurally injected non-neural antigen (ovalbumin) only produced significant changes in the presence of demyelinating sera, suggesting that T-cell presence in the nerve is more important than T-cell specificity [44]. Similar results were obtained using intraneural tumor necrosis factor (TNF)-α, implying a role in pathogenesis.

Host Factors

It has been estimated that fewer than 1 in 1,000 patients who contract a *C. jejuni* infection will develop GBS. Even when several family members are infected with the same *C. jejuni* variant, not all will develop GBS. Epidemics of *C. jejuni* have not been associated with outbreaks of GBS [45]. Also, the wide variety of triggering events and associated organisms makes molecular mimicry as the sole explanation for GBS unlikely. This suggests that host factors, including genetic and interacting environmental issues, must also play a role in determining who will manifest the disease, but no clear factors influencing susceptibility have been identified.

Studies investigating possible associations between *HLA* class I and II alleles in several different populations have yielded conflicting results. At least in the older studies, this may be partially due to the lack of differentiation between AIDP and AMAN. Single nucleotide polymorphisms of several genes linked to the host immune response have been investigated as possible susceptibility factors. Although no consistent association with development of GBS has been found, several of these genes (mannose-binding lection, *FcγRIII*, *MMP-9*, and *TNF-α*) may be associated with more severe disease.

Guillain-Barré Syndrome: Not a Typical Autoimmune Disease

GBS is not like other autoimmune diseases, as it is typically monophasic and does not respond to immunosuppressive therapies such as corticosteroids. It also does not tend to cluster with other autoimmune diseases. The exceptions are the few relapsing cases that tend to be milder and do have an association with other autoimmunity, and may be thought of as relapsing CIDP [46•]. Epidemiologic observations atypical for autoimmunity include a slight male preponderance of GBS and increasing incidence with advancing age. A very curious feature of GBS is that the adaptive immune response turns off. Is this simply a passive process—that the response to the triggering alloantigen has been successful and the response can be downregulated—or is there an active correction and deletion or suppression of autoreactive lymphocytes?

Recently, Steiner et al. [47•] also questioned whether GBS truly should be viewed as an autoimmune disease and noted the occasional association with HIV infection and more commonly with infections such as Epstein-Barr virus that are well-known to affect the immune system. They suggested it be viewed as a transient or sometimes chronic breakdown in immune tolerance in association with immunosuppression, and related to an exogenous insult

such as infection [47•]. According to their hypothesis, a pathogen itself could cause a transient breakdown in tolerance, with an associated short-lived, immune-mediated attack on peripheral nerves. Clearance of the precipitating infection stops the nerve damage and results in resumption of normal immune response and tolerance, enabling recovery, although HIV seems a curious example given that this is a rare cause of GBS, and other cases are not obviously immunosuppressed. These authors argue that such a mechanism provides an explanation for the monophasic nature of the disease and obviates a role for host susceptibility.

A Hypothesis for Guillain-Barré Syndrome Involving Competing Determinants of Immune Regulation

GBS may be well considered as bridging several competing determinants of whether the adaptive immune system should mount a response to an antigen: the Burnet–Janeway non-self or self-determinant; the Matzinger pattern-recognition receptor/danger signal determinant; and, additionally, the role of the particular tissue involved influencing the nature of the response [48]. The chronology and intensity of this response are illustrated in Fig. 1. The innate and adaptive immune systems attack the trigger in the relevant tissue (eg, *C. jejuni* in the gut). The initial infection or vaccination results in presentation of allo- as well as cross-reactive epitopes in combination with danger signals. The adaptive system should not respond to those with self-homology but should respond to other infectious epitopes that are not shared with self. However, new or previously silenced lymphocytes under the influence of potent danger signal activation of APCs with local chance effects and permissive host factors do, unfortunately, result in the subsequent initiation of an adaptive response to the shared antigens.

The next step is the response to the cross-reactive neural tissue—often building 1 or 2 weeks after the primary event is clinically reported to be improving, which we suggest corresponds to the latency of a second primary adaptive response. This is the acute GBS phase. Note that the duration of that response is short. There is, however, plenty of neural target newly determined to be worthy of a response, but instead of finishing off every nerve in the body (which is what the host might hope for if the target was an infection and not its own nerves), the response appears to be actively switched off. This occurs almost as fast as one could imagine the adaptive immunity switching off at all when one considers T-cell survival times and circulating antibody half-lives. Why? We propose the combination of a lack of danger signal in this native tissue along with regulatory factors in neural tissue that favor nonresponse (because of the extreme harm done by immune activation in this milieu) and continued self-signals co-presented to trafficking APCs phagocytosing the debris.

This active re-establishment of tolerance potentially addresses the question of why GBS stops so rapidly and relapses so rarely despite further lifetime exposure to triggers in many, and why immunotherapies such as corticosteroids are ineffective. At the time of clinical GBS, the immune system is already back on the path to tolerance, and therapy may just as easily hinder the regulatory pathways as help. It is noted that both the oral and intravenous corticosteroid trials showed a trend toward an increased relapse rate in the treated arm. Antibody-directed therapies such as intravenous immunoglobulin and plasmapheresis will with this concept have their demonstrated modest benefit. Implications arising include using antibody-mediating therapies as intensively but as early as possible, minimizing death of axons and Schwann cells even with bound antibody via use of complement inhibition, and eliminating any ongoing trigger that may be present. Additionally, perhaps neuroprotective agents should be tried in treating GBS despite their relative failure in stroke, given that neuroprotective drugs could be administered concurrently with available therapies and in this disease have good tissue penetration and time to act.

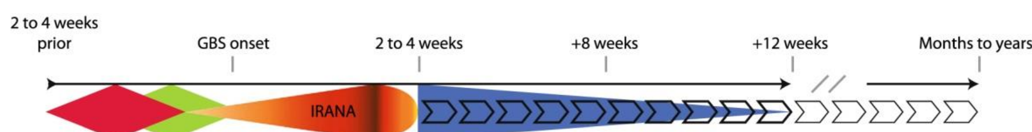


Fig. 1 The chronology and intensity of immune responses in Guillain-Barré syndrome (GBS). *Red* indicates a primary trigger (eg, infection or vaccination) usually resolved before GBS symptoms are present; if not, primary vascular or rare neural infection should be suspected. *Green* indicates a specific immune response against the trigger. During this period, presentation of cross-reactive natural epitopes with activation of danger pathways in a susceptible host results in IRANA (immunologic response against nerve antigens), a second immunologic response against neural targets with myelin, and/or axonal injury, with continued presentation of self-identifiers and diminishing danger pathway signals resulting in rapid re-establishment of tolerance. *Blue* indicates regeneration of myelin from Schwann cell precursors. *Chevrons* indicate slow regrowth of axons with time to and likelihood of functional recovery of motor units dependent upon regrowth distance required

Conclusions

GBS is a classic failure of the immune system with a life-threatening attack upon a critical self-component, yet unlike typical autoimmune diseases, the response promptly ceases and rarely recurs. The active phase of the disease is short, concordant with the latency of a primary adaptive immune response. GBS triggers include infection and (rarely) vaccination; cross-reactivity between infectious and neural epitopes has been well-demonstrated, particularly for *C. jejuni* and motor axonal forms of GBS. Standard immunosuppression has not been effective in GBS, and we suggest this is because immune tolerance is already being restored by the time such therapies are given. This suggests that improvements in GBS outcomes are likely to come from better protection of the nerve cells under attack while normal resumption of tolerance is permitted to proceed, rather than exploring more aggressive immunosuppressive approaches.

Acknowledgments Dr. Blum has been funded by the Royal Brisbane and Women's Hospital Research Scholarship.

Dr. Reddel has received honoraria, sponsorship, therapeutic trial payments, and/or grant funding from the National Health and Medical Research Council of Australia, the Muscular Dystrophy Association of the United States, and the Muscular Dystrophy Association of New South Wales. He also wishes to acknowledge helpful discussions with John Pollard, Judy Spies, and Hugh Willison.

Disclosure Dr. Reddel has received honoraria, sponsorship, therapeutic trial payments, and/or grant funding from Bayer Schering Pharma and Genzyme Corp. Drs. Hardy, Blum, and McCombe reported no potential conflicts of interest relevant to this article.

References

Papers of particular interest, published recently, have been highlighted as:
• Of importance

1. Zinkernagel R. On observing and analyzing disease versus signals. Nat Immunol. 2007;8:8–10.
2. Alshekhlee A, Hussain Z, Sultan B, Katirji B. Guillain-Barre syndrome: incidence and mortality rates in US hospitals. Neurology. 2008;70:1608–13.
3. van Doorn PA, Ruts L, Jacobs BC. Clinical features, pathogenesis, and treatment of Guillain-Barré syndrome. Lancet Neurol. 2008;7:939–50.
4. Hughes RAC, Cornblath DR. Guillain-Barre syndrome. Lancet. 2005;366:1653–66.
5. Ruts L, Drenthen J, Jacobs BC, Van Doorn PA. Distinguishing acute-onset CIDP from fluctuating Guillain-Barre syndrome: a prospective study. Neurology. 2010;74:1680–6.
6. Schonberger LB, Bregman DJ, Sullivan-Bolyai JZ, et al. Guillain-Barre syndrome following vaccination in the National Influenza Immunization Program, United States, 1976–1977. Am J Epidemiol. 1979;110:105–23.
7. Lasky T, Terracciano GJ, Magder L, et al. The Guillain-Barre syndrome and the 1992–1993 and 1993–1994 influenza vaccines. N Engl J Med. 1998;339:1797–802.
8. 2009 H1N1 flu. Available at: http://www.cdc.gov/h1n1flu.
9. Haber P, Sejvar J, Mikaeloff Y, DeStefano F. Vaccines and Guillain-Barre syndrome. Drug Saf. 2009;32:309–23.
10. Asbury AK, Arnason BG, Adams RD. The inflammatory lesion in idiopathic polyneuritis. Medicine. 1969;48:173–215.
11. Kieseier BC, Kiefer R, Gold R, et al. Advances in understanding and treatment of immune-mediated disorders of the peripheral nervous system. Muscle Nerve. 2004;30:131–56.
12. Csurhes PA, Sullivan AA, Green K, et al. T cell reactivity to P0, P2, PMP-22, and myelin basic protein in patients with Guillain-Barre syndrome and chronic inflammatory demyelinating polyradiculoneuropathy. J Neurol Neurosurg Psychiatry. 2005;76:1431–9.
13. McCombe PA, Csurhes PA. T cells from patients with Guillain-Barre syndrome produce interferon-gamma in response to stimulation with the ganglioside GM1. J Clin Neurosci. 2010;17:537–8.
14. Chi L-J, Wang H-B, Zhang Y, Wang W-Z. Abnormality of circulating CD4(+)CD25(+) regulatory T cell in patients with Guillain-Barre syndrome. J Neuroimmunol. 2007;192:206–14.
15. Harness J, McCombe PA. Increased levels of activated T-cells and reduced levels of CD4/CD25+ cells in peripheral blood of Guillain-Barré syndrome patients compared to controls. J Clin Neurosci. 2008;15:1031–5.
16. Hafer-Macko CE, Sheikh KA, Li CY, et al. Immune attack on the Schwann cell surface in acute inflammatory demyelinating polyneuropathy. Ann Neurol. 1996;39:625–35.
17. Inglis HR, Csurhes PA, McCombe PA. Antibody responses to peptides of peripheral nerve myelin proteins P0 and P2 in patients with inflammatory demyelinating neuropathy. J Neurol Neurosurg Psychiatry. 2007;78:419–22.
18. Feasby TE, Gilbert JJ, Brown WF, et al. An acute axonal form of Guillain-Barre polyneuropathy. Brain. 1986;109:1115–26.
19. McKhann GM, Cornblath DR, Griffin JW, et al. Acute motor axonal neuropathy: a frequent cause of acute flaccid paralysis in China. Ann Neurol. 1993;33:333–42.
20. Griffin JW, Li CY, Ho TW, et al. Pathology of the motor-sensory axonal Guillain-Barre syndrome. Ann Neurol. 1996;39:17–28.
21. Ang CW, Yuki N, Jacobs BC, et al. Rapidly progressive, predominantly motor Guillain-Barre syndrome with anti-GalNAc-GD1a antibodies. Neurology. 1999;53:2122–7.
22. Ho TW, Willison HJ, Nachamkin I, et al. Anti-GD1a antibody is associated with axonal but not demyelinating forms of Guillain-Barre syndrome. Ann Neurol. 1999;45:168–73.
23. Yuki N, Yamada M, Koga M, et al. Animal model of axonal Guillain-Barre syndrome induced by sensitization with GM1 ganglioside. Ann Neurol. 2001;49:712–20.
24. Lopez PHH, Zhang G, Bianchet MA, et al. Structural requirements of anti-GD1a antibodies determine their target specificity. Brain. 2008;131:1926–39.
25. Kaida K, Kusunoki S. Antibodies to gangliosides and ganglioside complexes in Guillain-Barre syndrome and Fisher syndrome: Mini-review. J Neuroimmunol. 2010;223:5–12.
26. Rinaldi S, Willison HJ. Ganglioside antibodies and neuropathies. Curr Opin Neurol. 2008;21:540–6.
27. Hafer-Macko C, Hsieh ST, Li CY, et al. Acute motor axonal neuropathy: an antibody-mediated attack on axolemma. Ann Neurol. 1996;40:635–44.
28. • Halstead SK, Zitman FMP, Humphreys PD, et al: Eculizumab prevents anti-ganglioside antibody-mediated neuropathy in a murine model. Brain 2008, 131:1197–1208. *This study demonstrated a realistic therapy for possible trials in human GBS.*

29. Yuki N, Kuwabara S, Koga M, Hirata K. Acute motor axonal neuropathy and acute motor-sensory axonal neuropathy share a common immunological profile. J Neurol Sci. 1999;168:121–6.
30. Uncini A, Manzoli C, Notturno F, Capasso M. Pitfalls in electrodiagnosis of Guillain-Barré syndrome subtypes. J Neurol Neurosurg Psychiatry. 2010;81:1157–63.
31. Madrid RE, Wiśniewski HM. Axonal degeneration in demyelinating disorders. J Neurocytol. 1977;6:103–17.
32. Sobottka B, Harrer MD, Ziegler U, et al. Collateral bystander damage by myelin-directed CD8+ T cells causes axonal loss. Am J Pathol. 2009;175:1160–6.
33. Chiba A, Kusunoki S, Shimizu T, Kanazawa I. Serum IgG antibody to ganglioside GQ1b is a possible marker of Miller Fisher syndrome. Ann Neurol. 1992;31:677–9.
34. Damian RT. Molecular mimicry: antigen sharing by parasite and host and its consequences. Am Nat. 1964;98:129–49.
35. Yuki N, Kuwabara S. Axonal Guillain-Barre syndrome: carbohydrate mimicry and pathophysiology. J Peripher Nerv Syst. 2007;12:238–49.
36. van Belkum A, van den Braak N, Godschalk P, et al. A Campylobacter jejuni gene associated with immune-mediated neuropathy. Nat Med. 2001;7:752–3.
37. Koga M, Takahashi M, Masuda M, et al. Campylobacter gene polymorphism as a determinant of clinical features of Guillain-Barre syndrome. Neurology. 2005;65:1376–81.
38. Halstead SK, O'Hanlon GM, Humphreys PD, et al. Anti-disialoside antibodies kill perisynaptic Schwann cells and damage motor nerve terminals via membrane attack complex in a murine model of neuropathy. Brain. 2004;127:2109–23.
39. Illa I, Ortiz N, Gallard E, et al. Acute axonal Guillain-Barre syndrome with IgG antibodies against motor axons following parenteral gangliosides. Ann Neurol. 1995;38:218–24.
40. Shamshiev A, Donda A, Prigozy TI, et al. The alphabeta T cell response to self-glycolipids shows a novel mechanism of CD1b loading and a requirement for complex oligosaccharides. Immunity. 2000;13:255–64.
41. Shamshiev A, Donda A, Carena I, et al. Self glycolipids as T-cell autoantigens. Eur J Immunol. 1999;29:1667–75.
42. Godfrey DI, Kronenberg M. Going both ways: immune regulation via CD1d-dependent NKT cells. J Clin Investig. 2004;114:1379–88.
43. Spies JM, Westland KW, Bonner JG, Pollard JD. Intraneural activated T cells cause focal breakdown of the blood–nerve barrier. Brain. 1995;118:857–68.
44. Pollard JD, Westland KW, Harvey GK, et al. Activated T cells of nonneural specificity open the blood–nerve barrier to circulating antibody. Ann Neurol. 1995;37:467–75.
45. Ho TW, Mishu B, Li CY, et al. Guillain-Barre syndrome in northern China. Relationship to Campylobacter jejuni infection and anti-glycolipid antibodies. Brain. 1995;118:597–605.
46. • Kuitwaard K, van Koningsveld R, Ruts L, et al: Recurrent Guillain Barre syndrome. Journal of Neurology, Neurosurgery & Psychiatry 2009, 80:56–59. *This article confirmed the low rate of recurrent GBS and demonstrated that the few patients who do have recurrences have other features of autoimmunity, and that the standard on-off case does not.*
47. • Steiner I, Rosenberg G, Wirguin I: Transient immunosuppression: a bridge between infection and the atypical autoimmunity of Guillain-Barre syndrome? Clin Exp Immunol 2010. *This article presents an interesting related hypothesis that suggests more of a role for transient immunosuppression as an initiating feature but also deals well with the reasons why GBS is not a typical autoimmune disease.*
48. Matzinger P. Friendly and dangerous signals: is the tissue in control? Nat Immunol. 2007;8:11–3.

Appendix B - Genetics of Guillain Barré Syndrome (GBS) and Chronic Inflammatory Demyelinating Polyradiculoneuropathy (CIDP): current knowledge and future directions

Blum S and McCombe PA

This review outlines the current knowledge of genetic factors in GBS pathogenesis. The manuscript has been accepted for publication in the Journal of the Peripheral Nervous System in April 2014. It was written in collaboration with Prof. Pamela McCombe.

Abstract

Guillain Barre Syndrome (GBS) and chronic inflammatory demyelinating polyradicloneuropathy (CIDP) are thought to be autoimmune diseases, which are thought to arise after exposure to environmental triggers in genetically susceptible individuals. The evidence that GBS has a genetic basis includes the finding of occasional families with GBS. In CIDP there is an overlap with hereditary neuropathy. There have been many attempts to find an HLA association with GBS and CIDP with little success. These studies are reviewed. There have been studies of other plausible genes in GBS and CIDP and the role of these genes in GBS and CIDP and the data from these genetic studies is reviewed. Some of the genes that have been studied are immune related and some others have nervous system effects. The studies are limited by small numbers. Some of the genes show association with disease severity rather than disease susceptibility. The need for more detailed molecular studies of the role of HLA molecules and the need for modern genetic approaches to GBS and CIDP is explained.

(1) Introduction

Guillain Barré syndrome (GBS) is a severe acute polyradiculoneuropathy. Several sub-groups of GBS have been described, with corresponding diagnostic criteria (Asbury and Cornblath, 1990; Hadden et al., 1998). Acute inflammatory demyelinating polyradiculoneuropathy (AIDP) is characterized pathologically by inflammation and demyelination (Asbury et al., 1969). More recently, acute motor axonal neuropathy (AMAN), where there is primary axonal dysfunction has been described (McKhann et al., 1993). Another variant is acute motor and sensory axonal neuropathy (AMSAN) which may be a severe form of AIDP. There are other variants of GBS, such as Miller Fisher Syndrome (MFS) (Winer, 2011), where the pathology is more focal. Chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) is a chronic or relapsing motor and sensory neuropathy, with inflammation and demyelination of the peripheral nerve (McCombe et al., 1987). GBS and CIDP are thought to be immune mediated (Hardy et al., 2011) and abnormalities of T cells, antibodies and gene expression have been reported in the peripheral blood of subjects with GBS and CIDP (Chang et al., 2012).

GBS and CIDP fulfil most of the criteria for autoimmune diseases (Rose and Bona, 1993), except for the lack of a clear HLA association, lack of a clear target antigen and male predominance. Autoimmune diseases are considered to arise in genetically susceptible people after exposure to environmental triggers. The environmental triggers for GBS and CIDP are commonly infections, but the genetic basis of GBS and CIDP is unknown. Genetic variation in immune related genes or in the peripheral nerves could contribute to the risk of acquiring disease and to severity of disease. In diseases, such as multiple sclerosis MS, where extensive genetic studies have been performed, there are associations with many genes that have a small risk (Sawcer et al., 2011; Beecham et al., 2013) . In this review, GBS and CIDP are considered separately, although there is considerable overlap in the pathology and there are patients who develop CIDP after a first episode that is typical of GBS (Ruts et al., 2010). We review the evidence that there is a genetic basis to GBS and CIDP, the data obtained from studying candidate genes and discuss future options for investigation. The numbers of subjects in each study are listed in the tables.

(2) Evidence for genetic basis to susceptibility to GBS and CIDP:

If there were a genetic basis to GBS and CIDP, it might be expected that disease would occur in families. There have been a number of reports of familial GBS (Saunders and Rake, 1965; MacGregor, 1965; Wilmshurst et al., 1999; Naik et al., 2012; Senanayake et al., 2010; Barzegar et al., 2012) including one of siblings born to a consanguineous marriage (Aquil et al., 2011). There has also been a report of MFS occurring simultaneously in two siblings (Peeples, 2011). The largest report from The Netherlands described 12 families with GBS (Geleijns et al., 2004a) and found that among siblings of subjects with GBS, the observed incidence was 2.6-fold increased compared to the expected incidence (Geleijns et al., 2004a). These studies support a possible role for genetic factors in GBS. Studies investigating disease prevalence in twins can be used to evaluate the genetic predisposition, but there are no such studies in GBS or CIDP.

There is one report of familial CIDP (Gabreels-Festen et al., 1986). However, there is considerable difficulty in making such a diagnosis because familial demyelinating neuropathy would very likely be diagnosed as a hereditary demyelinating neuropathy. There have been clinical studies that indicate an overlap between hereditary and inflammatory demyelinating neuropathy; some patients with inherited neuropathy are responsive to treatment with corticosteroids, suggesting a super-imposed inflammatory component (Dyck et al., 1982), some subjects with Charcot Marie Tooth disease (CMT) are found to have inflammatory changes on biopsy (Vital et al., 2003), including seven subjects with CMT1A and one with CMTX (Ginsberg et al., 2004), there is a report of CIDP in a subject with CMT1A (Rajabally et al., 2000) and children with CMT1A have been reported with superimposed inflammatory neuropathy (Desurkar et al., 2009). In GBS, there are reports of GBS in a subject with CMT1A, caused by PMP22 duplication (Munch et al., 2008), in subjects with hereditary neuropathy with pressure palsy, due to PMP22 gene deletion (Korn-Lubetzki et al., 2002; Remiche et al., 2013), and axonal GBS in a patient with axonal Charcot Marie Tooth (CMT) disease (Odaka et al., 2003). Another degenerative neurological disease that has been associated with GBS is hereditary spastic paraparesis (Alhashel et al., 2004). Hereditary amyloid neuropathy due to mutations of transthyretin has been found in subjects thought to have CIDP (Mathis et al., 2012). The finding of GBS in families supports a role for genetics in GBS. The finding of inflammatory

neuropathy in people with inherited neuropathies also could suggest that abnormalities in the peripheral nerve could make people more vulnerable to superimposed inflammation. Twin studies and studies of the prevalence of disease in relatives can be used to evaluate the genetic predisposition. There are no twin studies in GBS and CIDP. The study of familial GBS found that among siblings of subjects with GBS, the observed incidence was 2.6-fold increased compared to the expected incidence (Geleijns et al., 2004a).

Another genetic factor that is associated with GBS is gender. Being male, which requires carriage of at least one Y chromosome, is a substantial risk factor for GBS (Blum et al., 2013b). Male predominance is an unusual feature for an autoimmune disease, which generally are more prevalent in females (McCombe et al., 2009). The Y chromosome encodes at least 27 proteins, some of which are confined to the testes whilst others are more widely expressed (Skaletsky et al., 2003). The genetic risk of being male can be due to the effects of male gonadal hormones but also could be related to the *Yaa* gene cluster, which predisposes to autoimmunity in mice, and is a translocation of a cluster of X chromosome genes, including the *Tlr7* gene (Subramanian et al., 2006), which could also be linked to the increased incidence in males.

(3) Specific genes associated with GBS and CIDP

In GBS and CIDP there have been studies of a number of candidate genes that could plausibly be involved in the pathogenesis of GBS and CIDP. Below we describe studies that have examined the effects of genotype in the development of GBS and CIDP, on the severity of disease and on response to treatment, particularly IVIG. The HLA genes are listed in Tables 1 and 2, and the other genes are summarized in Table 3.

The MHC is a highly polymorphic region that contains many genes that are of importance to immunity (Trowsdale and Knight, 2013). HLA associations are common in autoimmune disease and the presence of an HLA association is one of the criteria for diagnosis of an autoimmune disease (Rose and Bona, 1993). Disease associations are reported with single genes, most commonly with the HLADRB1 gene, but often with extended haplotypes of linked genes. As shown in Tables 1 and 2 there was an expectation that there would be a HLA association with

GBS and CIDP and many studies have been undertaken. Early studies (using serological methods) found no HLA associations with GBS (Stewart et al., 1978) (Latovitzki et al., 1979) (Hafez et al., 1985), except for one from the USA of subjects with GBS found a slight reduction in the frequency of HLA A11 (Kaslow et al., 1984). Studies from the UK found no association of HLA Class I or class II genes with GBS (Winer et al., 1988) and from Sweden found no association with MHC class II molecules (Hillert et al., 1991). One study in Mexico found that GBS was associated with carriage of HLA-DR3 (Gorodezky et al., 1983). A study from The Netherlands found no association of HLA DRB1 or HLA DQB1 with GBS (Geleijns et al., 2005b). A study from North India reported no association of GBS with HLA DRB1, but increased HLA DQ1*06 in GBS and that HLA DRB1*0701 was increased in those with preceding infection (Sinha et al., 2010). A study from Japan showed no association with MHC class I or MHC class II genes (Ma et al., 1998b) and one from China showed no association with MHC class II molecules (Li et al., 2000).

When GBS patients from China were classified as having AIDP or AMAN, it was found that certain DQB1 molecules are associated with AIDP but not axonal neuropathy (Magira et al., 2003). Another study from China showed that HLA DRB1*13 was increased in subjects with AIDP compared to AMAN (Monos et al., 1997) . A study from Japan found a tendency for increased HLA DRB1*0803 in subjects with GBS associated with Campylobacter jejuni infection (Ma et al., 1998b) and a study from Greece reported GBS associated with Campylobacter jejuni infection in a subject who was positive for HLA-B35 and HLA-DR8 (Chatzipanagiotou et al., 2003). A study from the United Kingdom found no association of MHC class II alleles with susceptibility to GBS or MFS, but increased HLA DQB1*03 in subjects with GBS or MFS with preceding Campylobacter jejuni infection (Rees et al., 1995).

HLA C molecules can be assigned to two groups (C1 and C2) based on the presence of Asparagine or Lysine at position 80. Patients with GBS are significantly more likely to carry HLA-C2 than controls (Blum et al., 2013a). This is important because this polymorphism determines interaction with Killer immunoglobulin-like receptor (KIR) molecules, which are crucial for NK cell activation.. HLA Bw4 can be classified according to the presence of Threonine or Isoleucine at position 80. Patients with GBS are significantly more likely to carry

Isoleucine at this position than controls (Blum et al., 2013a) and again this is important in KIR binding.

In CIDP, one early study found increased frequency of carriage of the HLA-Aw30, HLA B8 and HLA Dw3 (Stewart et al., 1978) and a similar finding was reported from another two small studies (Adams et al., 1979;Vaughan et al., 1990). A later study found increased HLA DR2 (Feeney et al., 1990). A study from The Netherlands found no HLA associations with CIDP (van Doorn et al., 1991). We found a significant association of CIDP with HLA DR2, which is stronger in women than men (Greer et al., 2004) . In Tunisia there is a report of an association of CIDP with HLA DRB1*13 allele (Mrad et al., 2013).

The T cell receptor recognizes antigen presented by HLA molecules, and genetic variation in the T cell receptor can influence immune reactivity. There is evidence of T cell involvement in the pathogenesis of GBS and CIDP, with T cell infiltration into the peripheral nerves CIDP (Schmidt et al., 1996; Zweiman et al., 1983) and reports of T cell reactivity to peripheral nerve antigens (Csurhes et al., 2005b; Csurhes et al., 2005a). In CIDP, there are studies that indicate that the T cell repertoire is altered in CIDP, mostly in the CD8 population (Schneider-Hohendorf et al., 2012;Mausberg et al., 2013). Another study found no association of T cell receptor genes with GBS, although there was a trend to an influence on the severity of disease (Ma et al., 1998b). There are no studies of T cell receptor genes in CIDP.

Glycolipid antigens, such as gangliosides, are the target of antibodies found in some forms of GBS and CIDP. Production of antibodies usually requires T cell help. In addition, in GBS there are reports of T cells which are reactive with GM1 (McCombe and Csurhes, 2010). Gangliosides are presented to T cells in association with CD1 (Porcelli et al., 1998;Ulrichs and Porcelli, 2000) and so it would seem likely that CD1-restricted pathways would be important in disease pathogenesis. There are five closely related CD1 genes (CD1A-CD1E), and there is allelic variation in CD1A, CD1D and CD1E (Porcelli et al., 1998;Han et al., 1999). In GBS, one small

study found an association of susceptibility of GBS with CD1 polymorphisms (Caporale et al., 2006), but another study of did not confirm this (Kuijf et al., 2008). A meta-analysis of data from 1590 cases of GBS found no significant association with CD1A or CD1E polymorphisms (Wu et al., 2012). In CIDP, one small study found no association with CD1 polymorphisms (De Angelis et al., 2007).

Killer immunoglobulin-like receptors (KIRs) constitute the largest family of human NK receptors, with multiple inhibitory and activating members showing extensive polymorphisms(Middleton and Gonzelez, 2010). Generally, KIR with a long cytoplasmic tail (i.e. KIR 2DL1) are inhibitory, whereas KIR with a short tail are activating (i.e. KIR 2DS1). KIRs are found on NK cells, but also on subgroups of CD4+ and CD8+ T lymphocytes (van et al., 2004). For a functional interaction both KIR and its specific Class 1 HLA-ligand need to be present. The importance of these interactions has been shown in a number of diseases, e.g. in multiple sclerosis, where the KIR 2DS1 / HLA-C2 pair appears to be a risk factor (Fusco et al., 2010). KIR and their HLA ligands have also been found to have a role in susceptibility to a range of viruses, but have not been studied for the infective agents more commonly associated with GBS (Kulkarni et al., 2008). The intricacies of the KIR/HLA interaction have been reviewed elsewhere (Rajalingam, 2012;Boyton and Altmann, 2007). We recently reported that the frequency of KIR genes did not differ between GBS patients and controls. However, KIR molecules need HLA ligands for signaling, and we found that GBS patients lacked inhibitory HLA ligands for their KIR ligands (Blum et al., 2013a). We have found similar results in CIDP (unpublished data). The importance of an alteration in KIR/HLA combinations in GBS and CIDP is that it suggests a role for innate immunity.

Interleukin 10 (IL10) is a cytokine that has a role in the regulation of innate immunity (Ng et al., 2013) and is thought to be anti-inflammatory. IL10 has numerous polymorphisms that have been implicated in disease susceptibility (Wang et al., 2013). One experimental study found that transgenic mice with over-expression of IL10 develop a macrophage mediated neuropathy that resembles CIDP (Dace et al., 2009). Polymorphisms in the promoter regions of IL10 were

associated with susceptibility to GBS, but not with disease severity (Myhr et al., 2003). There are no studies of IL10 polymorphisms in CIDP. Thus although IL10 is an attractive candidate gene for autoimmune disease, there is little evidence as yet to support a role in GBS and CIDP.

Tumor necrosis factor (TNF) is a cytokine produced mainly by macrophages and monocytes. It interacts with the TNF receptor to influence immunity and inflammation (Chen and Goeddel, 2002). Circulating levels of TNF are increased in GBS (Radhakrishnan et al., 2003). Episodes of GBS have occurred after the use of anti-TNF therapy in rheumatology (Shin et al., 2006). One study found that polymorphisms of TNF were not associated with the development of GBS but were associated with disease severity (Geleijns et al., 2007). A study from China showed that TNF polymorphisms were associated with the AMAN and AMSAN forms of GBS (Jiao et al., 2012;Zhang et al., 2007). One study from Japan found that TNF polymorphisms were linked to GBS (Ma et al., 1998a), while another showed an association of TNF polymorphisms with axonal forms of GBS and higher levels of circulating TNF (Prasad et al., 2010) . A meta-analysis of 1590 cases of GBS found that polymorphisms of TNFA 308G/A were significantly associated with risk of GBS (Wu et al., 2012). There are no studies of TNF in CIDP. Thus there is considerable evidence that polymorphisms of TNF can influence susceptibility to GBS.

Matrix metalloproteinases (MMP) can play a role in inflammation, and possibly can be involved in opening the blood nerve barrier. MMP9 is expressed in the peripheral nerve in GBS (Kieseier et al., 1998) and in CIDP (Leppert et al., 1999). MMP9 gene expression is upregulated in peripheral blood leukocytes in GBS (Chang et al., 2012) and elevated levels of MMP9 are found in GBS (Nyati et al., 2010a). A study of polymorphisms of MMP9 found that these were not associated with the development of GBS but were associated with disease severity (Geleijns et al., 2007). There are no studies of MMP9 polymorphisms in CIDP.

Fc receptors bind immunoglobulin molecules in immune complexesThere are polymorphisms of these receptors and these have been correlated with autoimmune and infectious disease such as systemic lupus erythematosus (SLE) (Zidan et al., 2013) and Graves disease (Yesmin et al., 2010). In CIDP there is impaired expression of the inhibitory receptor

FcIIB (Tackenberg et al., 2009a) and this is restored by treatment with IVIG (Creange et al., 2003). A study from North India found that FcgammaR IIA and Fcgamma IIIA, but not FCgamma RIIIB were associated with GBS (Sinha et al., 2010). In a study from Norway found no association of FcgammaR IIA, Fcgamma IIIA or FCgamma RIIIB with GBS, but polymorphisms of the FCgamma RIIIB were found to influence the severity of GBS (Vedeler et al., 2000). The FcgammaRIIIa gene was associated with severity of GBS in studies from United Kingdom and The Netherlands (van Sorge et al., 2005). However, a meta-analysis of data from 1590 cases of GBS found no significant association with FcgammaR IIA, Fcgamma IIIA or FCgamma RIIIB (Wu et al., 2012). The rare -386C/-120A FcgammaRIIB promoter polymorphism resulting in reduced promoter activity previously associated with autoimmune phenotypes was overrepresented in CIDP (Tackenberg et al., 2009a).

FcR-like molecules (also known as FcR homologues) are structurally similar to Fc receptors but do not bind immunoglobulin. These molecules are expressed on B cells and appear to have an immunoregulatory function (Davis, 2007). FcR like 3 is expressed on marginal zone B cells (Won et al., 2006). Mutations of FcR-like 3 have been associated with autoimmune diseases (Yang et al., 2013). Polymorphisms of the FcRL3 gene gave been linked with incidence of GBS in study of Han Chinese subjects (Sang et al., 2012). There are no studies of FcRL3 genes in CIDP.

Fas cell surface death receptor (CD95) is involved in apoptosis (Wajant, 2002) and plays a role in killing of target cells and lymphocyte homeostasis (Strasser et al., 2009). Fas mediated apoptosis is impaired in subjects with CIDP compared to controls (Comi et al., 2006) and is impaired in CIDP compared to GBS subjects (Comi et al., 2009). In a study from the Netherlands, Fas polymorphisms were not associated with the development of GBS but were associated with the development of antiganglioside antibodies (Geleijns et al., 2005a). There are no studies in CIDP.

Toll-like receptors (TLR) detect molecules released by pathogens. Activation of these receptors leads to intracellular signaling of cells of the innate immune system (Lester and Li, 2013). TLR4 is a toll-like receptor that responds to lipopolysaccharide. Polymorphisms of TLR4 have been implicated in susceptibility to various diseases including cancer (Zhang et al., 2013). Since GBS and CIDP frequently occur after infections, it is expected that signaling through the innate immune system would be involved in the pathogenesis. A Dutch found that polymorphisms of TLR 4 were not associated with GBS (Geleijns et al., 2004b). However, a study from North India found that TLR4 polymorphisms were associated with risk of GBS (Nyati et al., 2010b). There are no studies in CIDP.

CD14 is a glycosylphosphatidylinositol (GPI)-anchored receptor known to serve as a co-receptor for several Toll-like Receptors (TLRs) both at the cell surface and in the endosomal compartment (Zanoni and Granucci, 2013). CD14 is involved in the response to LPS (Ziegler-Heitbrock and Ulevitch, 1993). Given the frequent occurrence of GBS and relapses of CIDP after infection, a role molecules involved in the innate immune response seems possible. A Dutch study found that polymorphisms of CD14 were not associated with GBS (Geleijns et al., 2004b). There are no studies of CD14 in CIDP.

Glucocorticoid receptors (GR) act directly in the nucleus to regulate gene transcription, or interact with other transcription factors (Ratman et al., 2013). GR are involved in the immune response (Pandolfi et al., 2013). There are polymorphisms of the GR genes that influence the sensitivity of the GR and susceptibility to rheumatoid arthritis (van Oosten et al., 2010). In GBS, one study found that polymorphisms of the GR are not linked to susceptibility but are related to the clinical course (Dekker et al., 2009). There are no studies in CIDP.

T cell specific adaptor proteins function in signal transduction in T cells (Lapinski et al., 2009) and play a role in the regulation of autoimmune disease (Marti et al., 2005). This gene has been associated with MS (Dai et al., 2001;Lorentzen et al., 2008) . One study showed that a

polymorphism of the SH2D2A T cell adaptor protein was associated with CIDP in a study of 48 patients and healthy controls (Notturno et al., 2008). There are no studies of this gene in GBS.

Alpha-1 antitrypsin (SERPIN1A) is a serine protease inhibitor. It also has anti-inflammatory properties (Bergin et al., 2012). In an old study, using protein electrophoresis distinguish alpha1 antitrypsin alleles, one study found an association of with GBS and CIDP (McCombe et al., 1985) . Since alpha-1 antitrypsin plays a role in protease inhibition and in immunology, it would be worth further investigation with modern genetic approaches.

Transient axonal glycoprotein-1 (TAG-1) (contactin 2) is a membrane-linked glycoprotein that functions as a cell adhesion molecule. It is found in the juxtanodal region, which is a key region of pathology in inflammatory neuropathy (Pollard and Armati, 2011). It also has a role in endocytosis (Dang et al., 2012). There are no studies of TAG-1 in GBS. In CIDP, polymorphisms of TAG1 are correlated with responsiveness to IVIG in Japanese subjects with CIDP (Iijima et al., 2011). There was no association with CIDP in a small series of Chinese subjects with CIDP (Pang et al., 2012).

IgG heavy chain allotype (Gm markers) are markers of variability of the heavy chains of IgG. Molecular studies have subsequently shown these to be due to polymorphisms of the heavy chain of immunoglobulin genes (Grubb, 1995; Lefranc and Lefranc, 2012). Allelic variation of these genes leads to differences in immune function and disease associations (Oxelius and Pandey, 2013). In GBS and CIDP, there has been one study showing an association of Gm allotypes with CIDP but not with GBS (Feeney et al., 1989) while and another showed no association (Pandey et al., 2005). Immunoglobulin kappa light chain allotypes (KM markers) are similar to Gm markers and are due to polymorphisms of the Kappa light chain of immunoglobulin molecules. One study showed that KM allotypes are associated with autoantibodies to GD1a in GBS, but not with susceptibility to disease (Pandey et al., 2005).

Apoliprotein E (Apo E) is involved in cholesterol transport and is important in neuronal biology (Holtzman et al., 2012). Apo E has three major alleles e2, e3, and e4 (Seripa et al., 2011). It is well known to be a risk factor for Alzheimer's disease (Kline, 2012). Recently ApoE has been found to have an immunomodulatory role in GBS and EAE (Zhang et al., 2010). One study found that ApoE genotype did not influence the outcome after GBS (Pritchard et al., 2003). There are no studies in CIDP.

CD59 (MAC-inhibitory protein) is a molecule that prevents the activation of complement and the formation of the membrane attack complex (Tandon et al., 1992). CD59 has a number of polymorphisms. There is evidence of complement deposition in nerves in GBS (Hays et al., 1988) and of activation of the membrane attack complex in GBS (Koski et al., 1987). CD59 is upregulated in peripheral nerves in GBS, possibly as a compensatory response to complement activation (Wanschitz et al., 2003). Eculizumab, a monoclonal antibody that block formation of the membrane attack complex, is effective in treating experimental autoimmune neuritis, the animal model of GBS (Halstead et al., 2008). There are no disease association studies of GBS or CIDP with CD59. However, there is a report that a patient with CD59 deficiency had associated CIDP (Nevo et al., 2013). This is intriguing and suggests that a genetic study would be of interest.

In summary, a number of plausible candidate genes have been investigated in GBS and CIDP. Some of these have been replicated but in general these are only single studies of small numbers of subjects. The genes that have shown an association with GBS and CIDP have been implicated in the pathology of the disease. Many of the candidate genes have been implicated in other autoimmune diseases. In autoimmunity there is considerable evidence of genetic susceptibility to specific autoimmune disease. These are also reports of a familial susceptibility to autoimmunity in general (McCombe et al., 1990a). However, GBS and CIDP have some features that are atypical for autoimmune disease and it is possible that GBS and CIDP will have a different genetic background. The strong relationship of GBS and CIDP to infection suggests that genes of the innate immune response could be important.

(4) Future directions

GBS and CIDP are important diseases that cause significant morbidity and use of resources, so there is a need to understand more about the cause of these diseases and especially the genes that predispose to disease, because this could suggest possible therapies. The numbers of subjects that have been studied are small, so there would be benefit in repeating the studies mentioned above. There appear to be genes that make subjects susceptible to the development of disease and genes that regulate the severity of disease. Such genes are promising candidates for further study because knowledge of the biological pathways that modify the severity of disease could suggest treatments for disease.

There could also be further studies of the differences between GBS and CIDP. GBS and CIDP have similar pathological features but differ in the course of disease. Of the candidate genes, many show similar results in GBS and CIDP. However, it would seem very likely that subjects with GBS would differ from subjects with CIDP in their ability to regulate immune attack on peripheral nerve, with GBS being an acute self-limited disease and CIDP an ongoing disease. In experimental autoimmune neuritis, acute disease can be converted to a chronic relapsing form of disease with low dose immunosuppression (McCombe et al., 1990b), suggesting that relapses are prevented by immune mechanisms. It is possible that GBS and CIDP will differ in genes that regulate the immune response, while showing similarity in genes that are directly involved in the pathogenesis of disease. Therefore more studies comparing GBS and CIDP would be of interest.

There is no clear HLA association in GBS or CIDP. The lack of strong HLA class II association with inflammatory neuropathy could suggest that MHC restricted antigen presentation is not of prime importance in susceptibility to GBS and CIDP. However, there are some instances where the fine specificity of HLA molecules has been shown to be important. The first example is the finding that alleles that encode HLA proteins that have an RLD motif at residues 55-57 ($RLD^{55\text{-}57}$) and an $ED^{70\text{-}71}$ motif in the DQβ chain and a $E^{9}V^{11}H^{13}$ motif in the DRβ chain(Magira et al., 2003) were associated with the development of AIDP, but not AMAN,

in patients from Northern China. These motifs occur primarily in DRB1*04 and DQB1*06 and *04 alleles, with these DR and DQ alleles not being in linkage-disequilibrium. The other examples are our findings that patients with GBS are significantly more likely to carry Lysine than Arginine at position 80 of HLA C molecules and that patients with GBS are significantly more likely than controls to carry Isoleucine at position 80 of Bw4 than controls (Blum et al., 2013a). Therefore, the very fine specificity of HLA molecules could be worth further exploration, looking at the detailed molecular interactions that occur with different polymorphisms of genes in the HLA region, looking for causative genes rather than for disease associations.

Genotyping with Single nucleotide polymorphisms (SNPs) or whole exome sequencing (WES) has now become standard. Full sequencing of the entire genome has also become more common. As yet there are no published GWAS studies in GBS and CIDP. It will be important that GWAS studies indicate whether subjects have AIDP or AMAN, and whether the subjects have any family history of neuropathy. GWAS studies are able to look at the effects of relatively common polymorphisms that are selected for inclusion, but will not distinguish less common alleles. Future studies with whole exome sequencing (WES) will be able to look for rare alleles that predispose to disease (Casals et al., 2012). More detailed sequencing of the non-transcribed regions of the chromosome is likely to be needed to account for all the genetic basis of disease. Analysis of SNP studies requires making allowance for the many comparisons by requiring a very low probability that differences between disease and control groups occurred by chance. This is done in an attempt to avoid false positive results (Type I error) but has the risk of leading to false negative results (Type II error). To look for evidence that disease is hereditable, a new approach to this is to consider all SNPs at the same time, to see if there are overall differences between groups when using all the data (Yang et al., 2010; Lee et al., 2011) .

Taken together it seems likely that there is a genetic element in predisposition to GBS and CIDP, that at least some of the genes will be involved in immunity and that there may well be genes that modify the severity of disease. In the future large cohorts of subjects and modern techniques will be required to test this.

Table 1 Studies of HLA associations in GBS

Gene	No of subjects	Country/Date	Method	Result	Reference
MHC Class I HLA A					
HLA-A	59 GBS/561 controls	United Kingdom/1977	Serological	No association with GBS	(Adams et al., 1977)
HLA-A	22 GBS, 322 controls	Australia/1978	Serological	No association with GBS	(Stewart et al., 1978)
HLA-A	18 GBS, 103 controls	USA/1979	Serological	No association with GBS	(Latovitzki et al., 1979)
HLA-A	92 GBS, 100 controls	USA/1984	Serological	Slight reduction in HLA A11 in GBS	(Kaslow et al., 1984)
HLA-A	32 GBS, 234 controls	Egypt/1985	Serological	No association with GBS	(Hafez et al., 1985)
HLA-A	89 GBS, unknown controls	United Kingdom/1988	Serological	No association with GBS	(Winer et al., 1988)
HLA-A	81 GBS, 87 controls	Japan/1998	Serological	No association with GBS	(Ma et al., 1998b)
HLA-A	195 GBS, 225 controls	Australia/2013	PCR-SSP	No increase in HLA A Bw4 alleles	(Blum et al., 2013a)
MHC Class I HLA B					
HLA-B	22 GBS, 322 controls	Australia/1978	Serological	No association with GBS	(Stewart et al., 1978)
HLA-B	18 GBS, 103 controls	USA/1979	Serological	No association with GBS	(Latovitzki et al., 1979)
HLA-B	92 GBS, 100 controls	USA/1984	Serological	No association with GBS	(Kaslow et al., 1984)
HLA-B	32 GBS, 234 controls	Egypt/1985	Serological	No association with GBS	(Hafez et al., 1985)
HLA-B	89 GBS, unknown	United	Serological	No association with GBS	(Winer et al.,

	controls	Kingdom/1988			1988)
HLA-B	81 GBS, 87 controls	Japan/1998	Serological	No association with GBS	(Ma et al., 1998b)
HLA-B	195 GBS, 225 controls	Australia/2013	PCR-SSP	Increased carriage of isoleucine at position 80 of Bw4 alleles	(Blum et al., 2013a)
MHC Class I HLA C					
HLA-C	92 GBS, 100 controls	USA/1984	Serological	No association with GBS	(Kaslow et al., 1984)
HLA-C	81GBS, 87 controls	Japan/1998	Serological	No association with GBS	(Ma et al., 1998b)
HLA-C	195 GBS, 225 controls	Australia/2013	PCR-SSP	Increased carriage of alleles with lysine at position 80.	(Blum et al., 2013a)
MHC Class II HLA-DPB1					
HLA-DPB1	49 GBS, 100 controls	Sweden/1991	RFLP	No association with GBS	(Hillert et al., 1991)
HLA- DPB1	47 AMAN, 25 AIDP, 97 controls	China/2003	PCR/sequencing	No association with AIDP or AMAN	(Magira et al., 2003)
MHC Class II HLA-DQA1					
HLA DQA1	47 GBS, 50 controls	China/2000	PCR-SSP	No association with GBS, increased DQA1*03 with GM1 antibodies.	(Li et al., 2000)
HLA DQA1	74 GBS, 158 controls	Australia/2006	Serological/PCR-SSP	No association with GBS	(McCombe et al., 2006)
MHC Class I I HLA-DQB1					
HLA-DQB1	90 GBS, 7MFS, 100 controls	United Kingdom/1995	PCR	No association with susceptibility to GBS, increased HLA DQB1*03 with C jejuni infection	(Rees et al., 1995)
HLA-DQB1	49 GBS, 100 controls	Sweden/1991	RFLP	No association with GBS	(Hillert et al., 1991)

HLA-DQB1	31 AMAN, 12 AIDP, 34 controls	China/1997	PCR	No association with GBS	(Monos et al., 1997)
HLA-DQB1	81 GBS, 87 controls	Japan/1998	PCR 2 digit typing	No association with GBS	(Ma et al., 1998b)
HLA DQB1	47 GBS, 50 controls	China/2000	PCR-SSP	No association with GBS	(Li et al., 2000)
HLA-DQB1	47 AMAN, 25 AIDP, 97 controls	China/2003	PCR/sequencing	DQ epitopes associated with AIDP but not AMAN	(Magira et al., 2003)
HLA-DQB1	167 GBS, 207 controls	The Netherlands/2005	PCR with SSPs	No association with GBS	(Geleijns et al., 2005b)
HLA-DQB1	74 GBS, 158 controls	Australia/2006	Serological/PCR-SSP	No association with GBS	(McCombe et al., 2006)
HLA-DQB1	54 GBS, 202 controls	North India/2010	PCR with SSPs	Increased DQ*06 in GBS	(Sinha et al., 2010)
MHC Class I	**HLA- DRB1**				
HLA-DRB1	18 GBS, 103 controls	USA/1979	Serological	No association with GBS	(Latovitzki et al., 1979)
HLA-DRB1	38 GBS, 100 healthy controls	Mexico/1983	Serotyping	Increased DR3 in GBS	(Gorodezky et al., 1983)
HLA-DRB1	79 GBS patients	United Kingdom/1988	Serological	No association with GBS	(Winer et al., 1988)
HLA-DRB1	49 GBS, 100 controls	Sweden/1991	RFLP	No association with GBS	(Hillert et al., 1991)
HLA-DRB1	90 GBS, 7MFS, 100 controls	United Kingdom/1995	PCR	No association with susceptibility to GBS	(Rees et al., 1995)
HLA- DRB1	31 AMAN, 12 AIDP, 34 controls	China/1997	PCR	Increased DRB1*13 in AIDP	(Monos et al., 1997)
HLA-DRB1	81 GBS, 87 controls	Japan/1998	PCR 2 digit typing	No association with GBS	(Ma et al., 1998b)

HLA DRB1	47 GBS, 50 controls	China/2000	PCR-SSP	No association with GBS	(Li et al., 2000)
HLA-DRB1	47 AMAN, 25 AIDP, 97 controls	China/2003	PCR/sequencing	DRB1 epitopes associated with AIDP, no association with AMAN	(Magira et al., 2003)
HLA-DRB1	167 GBS, 207 controls	The Netherlands/2005	PCR-SSP	No association with GBS	(Geleijns et al., 2005b)
HLA-DRB1	74 GBS, 158 controls	Australia/2006	Serological/PCR-SSP	No association with GBS	(McCombe et al., 2006)
HLA-DRB1	54 GBS, 202 controls	North India/2010	PCR with SSPs	No association with GBS, but increased DRB1*0701 in patients with preceding infection	**(Sinha et al., 2010)**

Table 2 Studies of HLA associations in CIDP

Gene	No of subjects	Country	Method	Result	Reference
MHC Class I HLA- A					
HLA-A	16 CIDP, 322 controls	Australia/1978	Serological	Increased HLA A Aw30 in CIDP	(Stewart et al., 1978)
HLA-A	14 CIDP, 351 controls	United Kingdom/1979	Serological	No association with CIDP	(Adams et al., 1979)
HLA-A	31 CIDP, unknown controls	United Kingdom/1990	RFLP	No association with CIDP	(Vaughan et al., 1990)
HLA-A	52 CIDP, 504 controls	The Netherlands/1991	Serological	No association with CIDP	(van Doorn et al., 1991)
HLA-A	71 CIDP, 2516 controls	Australia/1990	Serological	Increased HLA A3	(Feeney et al., 1990)
MHC Class 1 HLA-B					
HLA-B	16 CIDP, 322 controls	Australia/1978	Serological	Increased HLA B8 in CIDP	(Stewart et al., 1978)
HLA-B	14 CIDP, 351 controls	United Kingdom/1979	Serological	Increased HLA B8	(Adams et al., 1979)

HLA-B	31 CIDP, unknown controls	United Kingdom/1990	RFLP	Increased HLA-B7	(Vaughan et al., 1990)
HLA-B	52 CIDP, 504 controls	The Netherlands/1991	Serological	No association with CIDP	(van Doorn et al., 1991)
MHC Class I HLA-C					
HLA-C	31 CIDP, unknown controls	United Kingdom/1990	RFLP	Increased HLA-Cw7	(Vaughan et al., 1990)
HLA-C	52 CIDP, 504 controls	The Netherlands/1991	Serological	No association with CIDP	(van Doorn et al., 1991)
MHC Class II HLA- DPB1					
HLA-DPB1	31 CIDP, unknown controls	United Kingdom/1990	RFLP	No association with CIDP	(Vaughan et al., 1990)
MHC Class II HLA-DQA1					
HLA-DQA1	104 CIDP, 296 controls	Australia/2006	Serological	No association with CIDP	**(McCombe et al., 2006)**
MHC Class II HLA-DQB1					
HLA-DQB1	31 CIDP, unknown controls	United Kingdom/1990	RFLP	No association with CIDP	(Vaughan et al., 1990)
HLA-DQB1	52 CIDP, 504 controls	The Netherlands/1991	Serological	No association with CIDP	(van Doorn et al., 1991)

HLA-DQB1	102 CIDP, 300 control	Australia/2006	Serological	No association with CIDP	(McCombe et al., 2006)
HLA-DQB1	36 CIDP, 100 controls	Tunisia/2013	PCR-SSP	No association with CIDP	(Mrad et al., 2013)
MHC Class II HLA-DRB1					
HLA-DRB1	16 CIDP, 322 controls	Australia/1978	Serological	Increased HLA Dw3 in CIDP	(Stewart et al., 1978)
HLA-DRB1	14 CIDP, 351 controls	United Kingdom/1979	Serological	Increased HLA Dw3	(Adams et al., 1979)
HLA-DRB1	31 CIDP, unknown controls	United Kingdom/1990	RFLP	No association with CIDP	(Vaughan et al., 1990)
HLA-DRB1	52 CIDP, 504 controls	The Netherlands/1991	Serological	No association with CIDP	(van Doorn et al., 1991)
HLA-DRB1	102 CIDP, 300 control	Australia/2006	Serological	No association with CIDP in total group, but increased HLA DR2 in females	(McCombe et al., 2006)
HLA-DRB1	36 CIDP, 100 controls	Tunisia/2013	PCR-SSP	Increased HLA DRB1*13	(Mrad et al., 2013)

Table 3 Non-HLA genes investigated in GBS and CIDP

Protein	Gene	Chromosome location	Link to GBS	Link to CIDP
Alpha1 antitrypsin	SERPINA1	14q32.1	Yes (McCombe et al., 1985)	Yes (McCombe et al., 1985)
Apolipoprotein E	APOE	19q13.2	No influence on outcome (Pritchard et al., 2003).	Not studied in CIDP
CD1a	CD1A	1q22-23	Yes (Caporale et al., 2006) No(Kuijf et al., 2008) (Wu et al., 2012)	No(De Angelis et al., 2007)
CD1d	CD1d	1q22-23	No (Caporale et al., 2006)	Not studied in CIDP
CD1e	CD1E	1q22-23	Yes(Caporale et al., 2006) No(Kuijf et al., 2008) (Wu et al., 2012)	No (De Angelis et al., 2007)
CD14	CD14	5q31.1	No (Geleijns et al., 2004b).	Not studied in CIDP
CD59	CD59	11p13	Not studied	One subject with CD59 deficiency had CIDP (Nevo et al., 2013)
Contactin 2 (Transient axonal glycoprotein-1)	CNTN2	1q32.1	Not studied	Yes – response to IVIG (Iijima et al., 2011) No (Pang et al., 2012)
Fas (CD95)	FAS	10q24.1	Linked to production of ganglioside antibodies (Geleijns et al., 2005a)	Not studied in CIDP
Fcgamma receptor IIa	FCGR2A	1q23	Yes(Sinha et al., 2010)	Not studied in CIDP

			No (Vedeler et al., 2000)	
Fcgamma receptor IIb	FCGR2B		Not studied	Associated with CIDP (Tackenberg et al., 2009b)
Fcgamma receptor IIIa	FCGR3A	1q23	Yes(Sinha et al., 2010) No(Vedeler et al., 2000) No effect in incidence but effect on severity (van Sorge et al., 2005)	Not studied in CIDP
Fcgammma receptor IIIb	FCGR3B	1q23	No(Sinha et al., 2010) No effect in incidence but effect on severity(Vedeler et al., 2000) (van Sorge et al., 2005)	Not studied in CIDP
FcR- like 3	FCRL3	1q21	Yes (Sang et al., 2012)	Not studied in CIDP
Glucocorticoid receptor	NRC31	5q31.3	Related to clinical course (Dekker et al., 2009)	Not studied in CIDP
G1M marker	IGHG1	14q32.33	Yes(Feeney et al., 1989) No(Pandey et al., 2005)	No(Feeney et al., 1989)
G2M marker	IGHG2	14q32.33	Yes(Feeney et al., 1989) No(Pandey et al., 2005)	No(Feeney et al., 1989)
G3M marker	IGHG3	14q32.33	Yes(Feeney et al., 1989) No(Pandey et al., 2005)	No(Feeney et al., 1989)
Interleukin 10	IL 10	1q31	Linked to disease susceptibility (Myhr et al., 2003)	Not studied in CIDP
Killer	KIR2DL1,	19q13.4	No(Blum et al.,	No studied in

immunoglobulin-like receptors (KIR)	KIR2DL2,KIR2DL3, KIR2DL4, KIR2DL5A, KIR2DL5B, KIR2DS1, KIR2DS2, KIR2DS3, KIR2DS4, KIR3DL1, KIR3DL2, KIR3DL3, KIR3DS1		2013a)	CIDP
KM marker	IGKC	2p12	Linked to antibody production(Pandey et al., 2005)	Not studied in CIDP
MMP9	MMP9	20q11.2	No effect on susceptibility, but link with severity (Geleijns et al., 2007)	Not studied in CIDP
T cell specific adaptor protein	SH2D2a	1q21	No studies	Linked to CIDP (Notturno et al., 2008).
TNF alpha	TNF	6p21.3	Yes (Ma et al., 1998a;Wu et al., 2012) Linked to severity (Geleijns et al., 2007) Associated with AMAN (Jiao et al., 2012;Zhang et al., 2007;Prasad et al., 2010)	Not studied in CIDP
Toll-like receptor 4	TLR4	9q33.1	No (Geleijns et al., 2004b) Yes (Nyati et al., 2010b)	Not studied in CIDP
Peripheral myelin protein 22* **	PMP22	17p12	Reports of subject with GBS and CMT1A (Munch et al., 2008)	Reports of subjects with CIDP and CMT1A (Rajabally et al., 2000;Desurkar et al., 2009)

****** This gene is included because of the reports of GBS and CIDP in subjects with CMT.**

Reference List

Abecasis, G. R., D. Altshuler, A. Auton, L. D. Brooks, R. M. Durbin, R. A. Gibbs, M. E. Hurles and G. A. McVean (2010). "A map of human genome variation from population-scale sequencing." Nature **467**(7319): 1061-1073.

Abecasis, G. R., E. Noguchi, A. Heinzmann, J. A. Traherne, S. Bhattacharyya, N. I. Leaves, G. G. Anderson, Y. Zhang, N. J. Lench, A. Carey, L. R. Cardon, M. F. Moffatt and W. O. Cookson (2001). "Extent and distribution of linkage disequilibrium in three genomic regions." Am J Hum Genet **68**(1): 191-197.

Adams, D., H. Festenstein, J. D. Gibson, R. A. Hughes, J. Jaraquemada, C. Papasteriadis, J. Sachs and P. K. Thomas (1979). "HLA antigens in chronic relapsing idiopathic inflammatory polyneuropathy." Journal of neurology, neurosurgery, and psychiatry **42**(2): 184-186.

Afshar, G. and J. P. Murnane (1999). "Characterization of a human gene with sequence homology to Saccharomyces cerevisiae SIR2." Gene **234**(1): 161-168.

Albers, J. W., P. D. Donofrio and T. K. McGonagle (1985). "Sequential electrodiagnostic abnormalities in acute inflammatory demyelinating polyradiculoneuropathy." Muscle & Nerve **8**(6): 528-539.

Alhashel, J. Y., A. F. Alshubaili, A. Montasser and T. Anim (2004). "Guillain-Barre syndrome in a patient with hereditary spastic paraparesis. A case report." Medical principles and practice : international journal of the Kuwait University, Health Science Centre **13**(6): 369-371.

Alshekhlee, A., Z. Hussain, B. Sultan and B. Katirji (2008). "Guillain-Barre syndrome: incidence and mortality rates in US hospitals." Neurology **70**(18): 1608-1613.

Ambasudhan, R., M. Talantova, R. Coleman, X. Yuan, S. Zhu, S. A. Lipton and S. Ding (2011). "Direct reprogramming of adult human fibroblasts to functional neurons under defined conditions." Cell Stem Cell **9**(2): 113-118.

Amirzargar, A., F. Khosravi, S. Dianat, F. Hushmand, P. Maryousef, A. R. Foroushani, J. Lotfi and B. Nikbin (2007). "Profile of cytokine gene polymorphisms in Iranian multiple sclerosis patients." Mult Scler **13**(2): 253-255.

Andorfer, B., B. C. Kieseier, E. Mathey, P. Armati, J. Pollard, N. Oka and H. P. Hartung (2001). "Expression and distribution of transcription factor NF-kappaB and inhibitor IkappaB in the inflamed peripheral nervous system." Journal of Neuroimmunology **116**(2): 226-232.

Ang, C. W., M. A. De Klerk, H. P. Endtz, B. C. Jacobs, J. D. Laman, F. G. van der Meche and P. A. van Doorn (2001). "Guillain-Barre syndrome- and Miller Fisher syndrome-associated Campylobacter jejuni lipopolysaccharides induce anti-GM1 and anti-GQ1b Antibodies in rabbits." Infection & Immunity **69**(4): 2462-2469.

Ang, C. W., B. C. Jacobs and J. D. Laman (2004). "The Guillain-Barre; syndrome: a true case of molecular mimicry." Trends in Immunology **25**(2): 61-66.

Ang, C. W., N. Yuki, B. C. Jacobs, M. Koga, P. A. Van Doorn, P. I. Schmitz, M. Van Der, x00E and F. G. (1999). "Rapidly progressive, predominantly motor Guillain-Barre syndrome with anti-GalNAc-GD1a antibodies." Neurology **53**(9): 2122-2127.

Ang, S. F., Z. S. Zhao, L. Lim and E. Manser (2010). "DAAM1 is a formin required for centrosome re-orientation during cell migration." PLoS One **5**(9).

Anonymous (1978). "Criteria for diagnosis of Guillain-Barre syndrome." Annals of Neurology **3**(6): 565-566.

Anonymous (1991). "Research criteria for diagnosis of chronic inflammatory demyelinating polyneuropathy (CIDP). Report from an Ad Hoc Subcommittee of the American Academy of Neurology AIDS Task Force." Neurology **41**(5): 617-618.
Aquil, N., I. A. Khan and B. Soomro (2011). "Guillain Barre syndrome in a family: a case report of four siblings." J Coll Physicians Surg Pak **21**(3): 179-181.
Arnett, D. K., R. B. Devereux, D. C. Rao, N. Li, W. Tang, R. Kraemer, S. A. Claas, J. M. Leon and U. Broeckel (2009). "Novel genetic variants contributing to left ventricular hypertrophy: the HyperGEN study." Journal of Hypertension **27**(8): 1585-1593.
Asbury, A., B. Arnason and R. Adams (1969). "The inflammatory lesion in idiopathic polyneuritis." Medicine **48**: 173-215.
Asbury, A. K. and D. R. Cornblath (1990). "Assessment of current diagnostic criteria for Guillain-Barre syndrome." Annals of Neurology **27 Suppl**: S21-24.
Baker, M. G., A. Kvalsvig, J. Zhang, R. Lake, A. Sears and N. Wilson (2013). "Declining Guillain-Barre syndrome after campylobacteriosis control, New Zealand, 1988-2010." Emerging Infectious Diseases **18**(2): 226-233.
Baldwin, R. T., R. R. Pierce and O. H. Frazier (1992). "Guillain-Barre syndrome after heart transplantation." Journal of Heart & Lung Transplantation **11**(4 Pt 1): 817-819.
Bamford, R. N., A. P. DeFilippis, N. Azimi, G. Kurys and T. A. Waldmann (1998). "The 5' untranslated region, signal peptide, and the coding sequence of the carboxyl terminus of IL-15 participate in its multifaceted translational control." J Immunol **160**(9): 4418-4426.
Barondes, S. H., D. N. Cooper, M. A. Gitt and H. Leffler (1994). "Galectins. Structure and function of a large family of animal lectins." J Biol Chem **269**(33): 20807-20810.
Barrett, J. C., B. Fry, J. Maller and M. J. Daly (2005). "Haploview: analysis and visualization of LD and haplotype maps." Bioinformatics **21**(2): 263-265.
Baxter, R., N. Lewis, N. Bakshi, C. Vellozzi, N. P. Klein and C. Network (2012). "Recurrent Guillain-Barre syndrome following vaccination." Clinical Infectious Diseases **54**(6): 800-804.
Beecham, A. H., N. A. Patsopoulos, D. K. Xifara, M. F. Davis, A. Kemppinen, C. Cotsapas, T. S. Shah, C. Spencer, D. Booth, A. Goris, A. Oturai, J. Saarela, B. Fontaine, B. Hemmer, C. Martin, F. Zipp, S. D'Alfonso, F. Martinelli-Boneschi, B. Taylor, H. F. Harbo, I. Kockum, J. Hillert, T. Olsson, M. Ban, J. R. Oksenberg, R. Hintzen, L. F. Barcellos, C. Agliardi, L. Alfredsson, M. Alizadeh, C. Anderson, R. Andrews, H. B. Sondergaard, A. Baker, G. Band, S. E. Baranzini, N. Barizzone, J. Barrett, C. Bellenguez, L. Bergamaschi, L. Bernardinelli, A. Berthele, V. Biberacher, T. M. Binder, H. Blackburn, I. L. Bomfim, P. Brambilla, S. Broadley, B. Brochet, L. Brundin, D. Buck, H. Butzkueven, S. J. Caillier, W. Camu, W. Carpentier, P. Cavalla, E. G. Celius, I. Coman, G. Comi, L. Corrado, L. Cosemans, I. Cournu-Rebeix, B. A. Cree, D. Cusi, V. Damotte, G. Defer, S. R. Delgado, P. Deloukas, S. A. di, A. T. Dilthey, P. Donnelly, B. Dubois, M. Duddy, S. Edkins, I. Elovaara, F. Esposito, N. Evangelou, B. Fiddes, J. Field, A. Franke, C. Freeman, I. Y. Frohlich, D. Galimberti, C. Gieger, P. A. Gourraud, C. Graetz, A. Graham, V. Grummel, C. Guaschino, A. Hadjixenofontos, H. Hakonarson, C. Halfpenny, G. Hall, P. Hall, A. Hamsten, J. Harley, T. Harrower, C. Hawkins, G. Hellenthal, C. Hillier, J. Hobart, M. Hoshi, S. E. Hunt, M. Jagodic, I. Jelcic, A. Jochim, B. Kendall, A. Kermode, T. Kilpatrick, K. Koivisto, I. Konidari, T. Korn, H. Kronsbein, C. Langford, M. Larsson, M. Lathrop, C. Lebrun-Frenay, J. Lechner-Scott, M. H. Lee, M. A. Leone, V. Leppa, G. Liberatore, B. A. Lie, C. M. Lill, M. Linden, J. Link, F. Luessi, J. Lycke, F. Macciardi, S. Mannisto, C. P. Manrique, R. Martin, V. Martinelli, D. Mason, G. Mazibrada, C. McCabe, I. L. Mero, J. Mescheriakova, L. Moutsianas, K. M. Myhr, G. Nagels, R. Nicholas, P. Nilsson, F. Piehl, M. Pirinen, S. E. Price, H. Quach, M. Reunanen, W. Robberecht, N. P. Robertson, M. Rodegher, D. Rog, M. Salvetti, N. C. Schnetz-Boutaud, F. Sellebjerg, R. C. Selter, C. Schaefer, S. Shaunak, L.

Shen, S. Shields, V. Siffrin, M. Slee, P. S. Sorensen, M. Sorosina, M. Sospedra, A. Spurkland, A. Strange, E. Sundqvist, V. Thijs, J. Thorpe, A. Ticca, P. Tienari, D. C. van, E. M. Visser, S. Vucic, H. Westerlind, J. S. Wiley, A. Wilkins, J. F. Wilson, J. Winkelmann, J. Zajicek, E. Zindler, J. L. Haines, M. A. Pericak-Vance, A. J. Ivinson, G. Stewart, D. Hafler, S. L. Hauser, A. Compston, G. McVean, J. P. De, S. J. Sawcer and J. L. McCauley (2013). "Analysis of immune-related loci identifies 48 new susceptibility variants for multiple sclerosis." Nature genetics **45**(11): 1353-1360.

Ben-Smith, A., J. S. Gaston, P. C. Barber and J. B. Winer (1996). "Isolation and characterisation of T lymphocytes from sural nerve biopsies in patients with Guillain-Barre syndrome and chronic inflammatory demyelinating polyneuropathy." J Neurol Neurosurg Psychiatry **61**(4): 362-368.

Bierbaum, S., C. Sengler, K. Gerhold, R. Berner and A. Heinzmann (2006). "Polymorphisms within interleukin 15 are associated with juvenile idiopathic arthritis." Clinical & Experimental Rheumatology **24**(2): 219.

Blaser, M. J., A. Olivares, D. N. Taylor, D. R. Cornblath and G. M. McKhann (1991). "Campylobacter serology in patients with Chinese paralytic syndrome." Lancet **338**(8762): 308.

Blum, S., S. Reddel, J. Spies and P. McCombe (2013). "Clinical features of subjects with Guillain Barre Syndrome at seven hospitals on the East Coast of Australia." Journal of the Peripheral Nervous System **18**: 316-320.

Blumenthal, D., D. Prais, E. Bron-Harlev and J. Amir (2004). "Possible association of Guillain-Barre syndrome and hepatitis A vaccination." Pediatr Infect Dis J **23**(6): 586-588.

Bobbala, D., X. L. Chen, C. Leblanc, M. Mayhue, J. Stankova, T. Tanaka, Y. G. Chen, S. Ilangumaran and S. Ramanathan (2012). "Interleukin-15 plays an essential role in the pathogenesis of autoimmune diabetes in the NOD mouse." Diabetologia **55**(11): 3010-3020.

Bock, J., L. H. Mochmann, C. Schlee, N. Farhadi-Sartangi, S. Gollner, C. Muller-Tidow and C. D. Baldus (2013). "ERG transcriptional networks in primary acute leukemia cells implicate a role for ERG in deregulated kinase signaling." PLoS One **8**(1): e52872.

Bogliun, G., E. Beghi and G. B. S. R. S. G. Italian (2004). "Incidence and clinical features of acute inflammatory polyradiculoneuropathy in Lombardy, Italy, 1996." Acta Neurologica Scandinavica **110**(2): 100-106.

Bohn, A. B., L. Nederby, T. Harbo, A. Skovbo, T. Vorup-Jensen, J. Krog, J. Jakobsen and M. E. Hokland (2011). "The effect of IgG levels on the number of natural killer cells and their Fc receptors in chronic inflammatory demyelinating polyradiculoneuropathy." European Journal of Neurology **18**(6): 919-924.

Bondarenko, S., A. L. Catapano and G. D. Norata (2013). "The CD1d-Natural Killer T Cell Axis in Atherosclerosis." J Innate Immun.

Bouchard, C., C. Lacroix, V. Plante, D. Adams, F. Chedru, J. M. Guglielmi and G. Said (1999). "Clinicopathologic findings and prognosis of chronic inflammatory demyelinating polyneuropathy." Neurology **52**(3): 498-503.

Caporale, C. M., F. Notturno, M. Pace, A. Aureli, V. Di Tommaso, G. De Luca, D. Farina, A. Giovannini and A. Uncini (2011). "CD1A and CD1E gene polymorphisms are associated with susceptibility to multiple sclerosis." Int J Immunopathol Pharmacol **24**(1): 175-183.

Caporale, C. M., F. Papola, M. A. Fioroni, A. Aureli, A. Giovannini, F. Notturno, D. Adorno, V. Caporale and A. Uncini (2006). "Susceptibility to Guillain-Barre syndrome is associated to polymorphisms of CD1 genes." Journal of Neuroimmunology **177**(1-2): 112-118.

Caporale, C. M., A. Uncini and A. Giovannini (2008). ""Susceptibility to Guillain-Barre syndrome is associated to polymorphisms of CD1 genes" by Bang et al. in the J of Neuroimmunology (2007) 186, 201-202." Journal of Neuroimmunology **194**(1-2): 193-194.

Carninci, P. and Y. Hayashizaki (2007). "Noncoding RNA transcription beyond annotated genes." Current Opinion in Genetics & Development **17**(2): 139-144.
Carr, W. H., M. J. Pando and P. Parham (2005). "KIR3DL1 polymorphisms that affect NK cell inhibition by HLA-Bw4 ligand." Journal of Immunology **175**(8): 5222-5229.
Carson, W. E., M. E. Ross, R. A. Baiocchi, M. J. Marien, N. Boiani, K. Grabstein and M. A. Caligiuri (1995). "Endogenous production of interleukin 15 by activated human monocytes is critical for optimal production of interferon-gamma by natural killer cells in vitro." J Clin Invest **96**(6): 2578-2582.
Cavdar Koc, E., W. Burkhart, K. Blackburn, A. Moseley and L. L. Spremulli (2001). "The small subunit of the mammalian mitochondrial ribosome. Identification of the full complement of ribosomal proteins present." J Biol Chem **276**(22): 19363-19374.
Cencioni, M. T., F. Notturno, C. M. Caporale, B. Creati, V. Prencipe, L. Battistini and A. Uncini (2009). "T cell response in acute motor axonal neuropathy." International Journal of Immunopathology & Pharmacology **22**(4): 1043-1050.
Centers for Disease, C. and Prevention (2010). "Preliminary results: surveillance for Guillain-Barre syndrome after receipt of influenza A (H1N1) 2009 monovalent vaccine - United States, 2009-2010." MMWR - Morbidity & Mortality Weekly Report **59**(21): 657-661.
Chaari, A., M. Bahloul, H. Dammak, G. Nourhene, N. Rekik, C. Hedi, B. H. Chokri and M. Bouaziz (2010). "Guillain-Barre syndrome related to pandemic influenza A (H1N1) infection." Intensive Care Medicine **36**(7): 1275.
Chang, K. H., T. J. Chuang, R. K. Lyu, L. S. Ro, Y. R. Wu, H. S. Chang, C. C. Huang, H. C. Kuo, W. C. Hsu, C. C. Chu and C. M. Chen (2012). "Identification of gene networks and pathways associated with Guillain-Barre syndrome." PLoS.ONE. **7**(1): e29506.
Chi, L.-J., H.-B. Wang, Y. Zhang and W.-Z. Wang (2007). "Abnormality of circulating CD4(+)CD25(+) regulatory T cell in patients with Guillain-Barre syndrome." Journal of Neuroimmunology **192**(1-2): 206-214.
Chi, L. J., H. B. Wang and W. Z. Wang (2008). "Impairment of circulating CD4+CD25+ regulatory T cells in patients with chronic inflammatory demyelinating polyradiculoneuropathy." Journal of the peripheral nervous system : JPNS **13**(1): 54-63.
Chiba, A., S. Kusunoki, S. Kuwata, T. Juji, Y. Shibata and I. Kanazawa (1995). "HLA and anti-GQ1b IgG antibody in Miller Fisher syndrome and Guillain-Barre syndrome." Journal of Neuroimmunology **61**(1): 85-88.
Chio, A., D. Cocito, M. Leone, M. T. Giordana, G. Mora, R. Mutani and S. Piemonte and Valle d'Aosta Register for Guillain-Barre (2003). "Guillain-Barre syndrome: a prospective, population-based incidence and outcome survey." Neurology **60**(7): 1146-1150.
Cho, J. H. and P. K. Gregersen (2011). "Genomics and the multifactorial nature of human autoimmune disease." New England Journal of Medicine **365**(17): 1612-1623.
Christensen, U., A. Haagerup, H. G. Binderup, J. Vestbo, T. A. Kruse and A. D. Borglum (2006). "Family based association analysis of the IL2 and IL15 genes in allergic disorders." European Journal of Human Genetics **14**(2): 227-235.
Claverie, J.-M. (2005). "Fewer Genes, More Noncoding RNA." Science **309**(5740): 1529-1530.
Collins, F. S., E. D. Green, A. E. Guttmacher and M. S. Guyer (2003). "A vision for the future of genomics research." Nature **422**(6934): 835-847.
Consortium, E. P., B. E. Bernstein, E. Birney, I. Dunham, E. D. Green, C. Gunter and M. Snyder (2012). "An integrated encyclopedia of DNA elements in the human genome." Nature **489**(7414): 57-74.
Corbo, M., A. Quattrini, N. Latov and A. P. Hays (1993). "Localization of GM1 and Gal(beta 1-3)GalNAc antigenic determinants in peripheral nerve." Neurology **43**(4): 809-814.

Cornblath, D. R., E. D. Mellits, J. W. Griffin, G. M. McKhann, J. W. Albers, R. G. Miller, T. E. Feasby and S. A. Quaskey (1988). "Motor conduction studies in Guillain-Barre syndrome: description and prognostic value." Annals of Neurology **23**(4): 354-359.

Creange, A., T. Sharshar, T. Planchenault, C. Christov, F. Poron, J. C. Raphael and R. K. Gherardi (1999). "Matrix metalloproteinase-9 is increased and correlates with severity in Guillain-Barre syndrome." Neurology **53**(8): 1683-1691.

Csurhes, P. A., A.-A. Sullivan, K. Green, J. M. Greer, M. P. Pender and P. A. McCombe (2005). "Increased circulating T cell reactivity to GM1 ganglioside in patients with Guillain-Barré syndrome." Journal of Clinical Neuroscience **12**(4): 409-415.

Csurhes, P. A., A. A. Sullivan, K. Green, M. P. Pender and P. A. McCombe (2005). "T cell reactivity to P0, P2, PMP-22, and myelin basic protein in patients with Guillain-Barre syndrome and chronic inflammatory demyelinating polyradiculoneuropathy." Journal of Neurology, Neurosurgery & Psychiatry **76**(10): 1431-1439.

Dalakas, M. C. (2011). "Advances in the diagnosis, pathogenesis and treatment of CIDP." Nature reviews. Neurology **7**(9): 507-517.

Damian (1964). "Molecular mimicry: antigen sharing by parasite and host and its consequences." Amercan Naturalist **98**.

Davidson, D. L., A. F. O'Sullivan and K. D. Morley (1992). "HLA antigens in familial Guillain-Barre syndrome." Journal of Neurology, Neurosurgery & Psychiatry **55**(6): 508-509.

dbSNP, N. (2013). "NCBI dbSNP Build 138." Retrieved November 9, 2013, from http://www.ncbi.nlm.nih.gov/SNP/snp_summary.cgi?view+summary=view+summary&build_id=138.

De Libero, G. and L. Mori (2005). "Recognition of lipid antigens by T cells." Nature Reviews Immunology **5**(6): 485-496.

de Rham, C., S. Ferrari-Lacraz, S. Jendly, G. Schneiter, J. M. Dayer and J. Villard (2007). "The proinflammatory cytokines IL-2, IL-15 and IL-21 modulate the repertoire of mature human natural killer cell receptors." Arthritis Res Ther **9**(6): R125.

Dekker, M. J., E. L. van den Akker, J. W. Koper, L. Manenschijn, K. Geleijns, L. Ruts, W. van Rijs, A. P. Tio-Gillen, P. A. van Doorn, S. W. Lamberts and B. C. Jacobs (2009). "Effect of glucocorticoid receptor gene polymorphisms in Guillain-Barre syndrome." Journal of the Peripheral Nervous System **14**(2): 75-83.

Deng, H., X. Yang, T. Jin, J. Wu, L. S. Hu, M. Chang, X. J. Sun, A. Adem, B. Winblad and J. Zhu (2008). "The role of IL-12 and TNF-alpha in AIDP and AMAN." European Journal of Neurology **15**(10): 1100-1105.

DePaolo, R. W., V. Abadie, F. Tang, H. Fehlner-Peach, J. A. Hall, W. Wang, E. V. Marietta, D. D. Kasarda, T. A. Waldmann, J. A. Murray, C. Semrad, S. S. Kupfer, Y. Belkaid, S. Guandalini and B. Jabri (2011). "Co-adjuvant effects of retinoic acid and IL-15 induce inflammatory immunity to dietary antigens." Nature **471**(7337): 220-224.

DeVivo, D. C. and W. K. Engel (1970). "Remarkable recovery of a steroid-responsive recurrent polyneuropathy." J Neurol Neurosurg Psychiatry **33**(1): 62-69.

Di, S. A., S. A. Calarota, F. Vidali, T. T. Macdonald and G. R. Corazza (2011). "Role of IL-15 in immune-mediated and infectious diseases." Cytokine & growth factor reviews **22**(1): 19-33.

Dick, D. M., F. Aliev, R. F. Krueger, A. Edwards, A. Agrawal, M. Lynskey, P. Lin, M. Schuckit, V. Hesselbrock, J. Nurnberger, Jr., L. Almasy, B. Porjesz, H. J. Edenberg, K. Bucholz, J. Kramer, S. Kuperman and L. Bierut (2011). "Genome-wide association study of conduct disorder symptomatology." Mol Psychiatry **16**(8): 800-808.

Dionne, A., M. W. Nicolle and A. F. Hahn (2010). "Clinical and electrophysiological parameters distinguishing acute-onset chronic inflammatory demyelinating polyneuropathy from acute inflammatory demyelinating polyneuropathy." Muscle & Nerve **41**(2): 202-207.

Dorak, M. (2007). "Role of Natural Killer Cells and Killer Immunoglobulin-Like Receptor Polymorphisms." METHODS IN MOLECULAR MEDICINE **134**: 123.
Drenthen, J., N. Yuki, J. Meulstee, E. M. Maathuis, P. A. van Doorn, G. H. Visser, J. H. Blok and B. C. Jacobs (2011). "Guillain–Barré syndrome subtypes related to Campylobacter infection." Journal of Neurology, Neurosurgery & Psychiatry **82**(3): 300-305.
Dryden, S. C., F. A. Nahhas, J. E. Nowak, A. S. Goustin and M. A. Tainsky (2003). "Role for human SIRT2 NAD-dependent deacetylase activity in control of mitotic exit in the cell cycle." Mol Cell Biol **23**(9): 3173-3185.
Dudbridge, F. and A. Gusnanto (2008). "Estimation of significance thresholds for genomewide association scans." Genet Epidemiol **32**(3): 227-234.
Duncan, R. and P. G. Kennedy (1987). "Guillain-Barre syndrome following acute head trauma." Postgraduate Medical Journal **63**(740): 479-480.
Dyck, P. J., A. C. Lais, M. Ohta, J. A. Bastron, H. Okazaki and R. V. Groover (1975). "Chronic inflammatory polyradiculoneuropathy." Mayo Clinic Proceedings **50**(11): 621-637.
Dyck, P. J., P. O'Brien, C. Swanson, P. Low and J. Daube (1985). "Combined azathioprine and prednisone in chronic inflammatory-demyelinating polyneuropathy." Neurology **35**(8): 1173-1176.
Dyck, P. J., P. C. O'Brien, K. F. Oviatt, R. P. Dinapoli, J. R. Daube, J. D. Bartleson, B. Mokri, T. Swift, P. A. Low and A. J. Windebank (1982). "Prednisone improves chronic inflammatory demyelinating polyradiculoneuropathy more than no treatment." Annals of Neurology **11**(2): 136-141.
Dyck, P. J., C. J. Swanson, P. A. Low, J. D. Bartleson and E. H. Lambert (1982). "Prednisone-responsive hereditary motor and sensory neuropathy." Mayo Clinic Proceedings **57**(4): 239-246.
Efthimiou, P., R. A. Flavell, A. Furlan, G. Gasbarrini, A. Gava, I. Kone-Paut, R. Manna, L. Punzi, F. S. Sutterwala, I. Touitou and A. Doria (2008). "Autoinflammatory syndromes and infections: pathogenetic and clinical implications." Clin Exp Rheumatol **26**(1 Suppl 48): S53-61.
Eftimov, F., J. B. Winer, M. Vermeulen, R. de Haan and I. N. van Schaik (2009). "Intravenous immunoglobulin for chronic inflammatory demyelinating polyradiculoneuropathy." Cochrane Database Syst Rev(1): CD001797.
Fauriat, C., M. A. Ivarsson, H.-G. Ljunggren, K.-J. Malmberg and J. Michaelsson (2010). "Education of human natural killer cells by activating killer cell immunoglobulin-like receptors." Blood **115**(6): 1166-1174.
Feeney, D. J., J. D. Pollard, J. G. McLeod, G. J. Stewart and T. J. Doran (1990). "HLA antigens in chronic inflammatory demyelinating polyneuropathy." Journal of neurology, neurosurgery, and psychiatry **53**(2): 170-172.
Fehniger, T. A. and M. A. Caligiuri (2001). "Interleukin 15: biology and relevance to human disease." Blood **97**(1): 14-32.
Fisher, M. (1956). "Syndrome of ophthalmoplegia, ataxia and areflexia." New England Journal of Medicine **255**: 57-65.
FitzPatrick, D. R., E. Germain-Lee and D. Valle (1995). "Isolation and characterization of rat and human cDNAs encoding a novel putative peroxisomal enoyl-CoA hydratase." Genomics **27**(3): 457-466.
Frenzen, P. D. (2008). "Economic cost of Guillain-Barré syndrome in the United States." Neurology **71**(1): 21-27.
Fujisaki, H., H. Kakuda, C. Imai, C. G. Mullighan and D. Campana (2009). "Replicative potential of human natural killer cells." British Journal of Haematology **145**(5): 606-613.

Fusco, C., F. R. Guerini, G. Nocera, G. Ventrella, D. Caputo, M. A. Valentino, C. Agliardi, J. Gallotti, V. B. Morra, C. Florio, M. Clerici and M. L. Lombardi (2010). "KIRs and their HLA ligands in Remitting-Relapsing Multiple Sclerosis." J Neuroimmunol.
Gabriel, S. B., S. F. Schaffner, H. Nguyen, J. M. Moore, J. Roy, B. Blumenstiel, J. Higgins, M. DeFelice, A. Lochner, M. Faggart, S. N. Liu-Cordero, C. Rotimi, A. Adeyemo, R. Cooper, R. Ward, E. S. Lander, M. J. Daly and D. Altshuler (2002). "The structure of haplotype blocks in the human genome." Science **296**(5576): 2225-2229.
Galeazzi, M., G. Gasbarrini, A. Ghirardello, S. Grandemange, H. M. Hoffman, R. Manna, M. Podswiadek, L. Punzi, G. D. Sebastiani, I. Touitou and A. Doria (2006). "Autoinflammatory syndromes." Clin Exp Rheumatol **24**(1 Suppl 40): S79-85.
Gauderman, W. J. (2002). "Sample size requirements for matched case-control studies of gene-environment interaction." Stat Med **21**(1): 35-50.
Geleijns, K., B. A. Brouwer, B. C. Jacobs, J. J. Houwing-Duistermaat, C. M. van Duijn and P. A. van Doorn (2004). "The occurrence of Guillain-Barre syndrome within families." Neurology **63**(9): 1747-1750.
Geleijns, K., M. Emonts, J. D. Laman, W. van Rijs, P. A. van Doorn, P. W. Hermans and B. C. Jacobs (2007). "Genetic polymorphisms of macrophage-mediators in Guillain-Barre syndrome." Journal of Neuroimmunology **190**(1-2): 127-130.
Geleijns, K., B. C. Jacobs, W. Van Rijs, A. P. Tio-Gillen, J. D. Laman and P. A. van Doorn (2004). "Functional polymorphisms in LPS receptors CD14 and TLR4 are not associated with disease susceptibility or Campylobacter jejuni infection in Guillain-Barre patients." Journal of Neuroimmunology **150**(1-2): 132-138.
Geleijns, K., J. D. Laman, W. van Rijs, A. P. Tio-Gillen, R. Q. Hintzen, P. A. van Doorn and B. C. Jacobs (2005). "Fas polymorphisms are associated with the presence of anti-ganglioside antibodies in Guillain-Barre syndrome." Journal of Neuroimmunology **161**(1-2): 183-189.
Geleijns, K., G. M. Schreuder, B. C. Jacobs, K. Sintnicolaas, R. van Koningsveld, J. Meulstee, J. D. Laman and P. A. van Doorn (2005). "HLA class II alleles are not a general susceptibility factor in Guillain-Barre syndrome." Neurology **64**(1): 44-49.
Genome.gov. (2012). "A Catalog of Published Genome-wide Association Studies." 2014, from http://www.genome.gov/page.cfm?pageid=26525384&clearquery=1#result_table.
Gilmore, T. D. (2006). "Introduction to NF-kappaB: players, pathways, perspectives." Oncogene **25**(51): 6680-6684.
Ginsberg, L., O. Malik, A. R. Kenton, D. Sharp, J. R. Muddle, M. B. Davis, J. B. Winer, R. W. Orrell and R. H. M. King (2004). "Coexistent hereditary and inflammatory neuropathy." Brain **127**(1): 193-202.
Giron-Michel, J., A. Caignard, M. Fogli, D. Brouty-Boye, D. Briard, M. van Dijk, R. Meazza, S. Ferrini, C. Lebousse-Kerdiles, D. Clay, H. Bompais, S. Chouaib, B. Peault and B. Azzarone (2003). "Differential STAT3, STAT5, and NF-kappaB activation in human hematopoietic progenitors by endogenous interleukin-15: implications in the expression of functional molecules." Blood **102**(1): 109-117.
Gladstone, D. E., A. A. Prestrud and T. H. Brannagan, 3rd (2005). "High-dose cyclophosphamide results in long-term disease remission with restoration of a normal quality of life in patients with severe refractory chronic inflammatory demyelinating polyneuropathy." J Peripher Nerv Syst **10**(1): 11-16.
Godfrey, D. I. and M. Kronenberg (2004). "Going both ways: immune regulation via CD1d-dependent NKT cells." Journal of Clinical Investigation **114**(10): 1379-1388.
Godschalk, P. C. R., M. L. Kuijf, J. Li, F. St Michael, C. W. Ang, B. C. Jacobs, M.-F. Karwaski, D. Brochu, A. Moterassed, H. P. Endtz, A. van Belkum and M. Gilbert (2007). "Structural characterization of Campylobacter jejuni lipooligosaccharide outer cores

associated with Guillain-Barre and Miller Fisher syndromes." Infection & Immunity **75**(3): 1245-1254.
Goldstein, D. B. (2001). "Islands of linkage disequilibrium." Nature genetics **29**(2): 109-111.
Gordon, P. H. and A. J. Wilbourn (2001). "Early electrodiagnostic findings in Guillain-Barre syndrome." Archives of Neurology **58**(6): 913-917.
Gorodezky, C., B. Varela, L. E. Castro-Escobar, A. Chavez-Negrete, A. Escobar-Gutierrez and J. Martinez-Mata (1983). "HLA-DR antigens in Mexican patients with Guillain-Barre syndrome." Journal of Neuroimmunology **4**(1): 1-7.
Grabstein, K., J. Eisenman, K. Shanebeck, C. Rauch, S. Srinivasan, V. Fung, C. Beers, J. Richardson, M. Schoenborn, M. Ahdieh and a. et (1994). "Cloning of a T cell growth factor that interacts with the beta chain of the interleukin-2 receptor." Science **264**(5161): 965-968.
Griffin, J. W., C. Y. Li, T. W. Ho, M. Tian, C. Y. Gao, P. Xue, B. Mishu, D. R. Cornblath, C. Macko, G. M. McKhann and A. K. Asbury (1996). "Pathology of the motor-sensory axonal Guillain-Barre syndrome." Annals of Neurology **39**(1): 17-28.
Guillain, G. e. a. (1916). "Sur un syndrome de radiculoneurite avec hyperalbuminose du liquide cephalo-rachidien sans reaction cellulaire." Bull. Mem. Soc. Med. Hop. Paris **40**: 1462-1470.
Hadden, R. D., H. Karch, H. P. Hartung, J. Zielasek, B. Weissbrich, J. Schubert, A. Weishaupt, D. R. Cornblath, A. V. Swan, R. A. Hughes, K. V. Toyka and G. Plasma Exchange/Sandoglobulin Guillain-Barre Syndrome Trial (2001). "Preceding infections, immune factors, and outcome in Guillain-Barre syndrome." Neurology **56**(6): 758-765.
Haeseleer, F. and K. Palczewski (2000). "Short-chain dehydrogenases/reductases in retina." Methods Enzymol **316**: 372-383.
Hafez, M., M. Nagaty, Y. Al-Tonbary, F. A. el-Shennawy, A. el-Mongui, S. el-Sallab and S. Attia (1985). "HLA-antigens in Guillain-Barre syndrome." J Neurogenet **2**(4): 285-290.
Hahn, A. F., C. F. Bolton, N. Pillay, C. Chalk, T. Benstead, V. Bril, K. Shumak, M. K. Vandervoort and T. E. Feasby (1996). "Plasma-exchange therapy in chronic inflammatory demyelinating polyneuropathy. A double-blind, sham-controlled, cross-over study." Brain **119 (Pt 4)**: 1055-1066.
Halstead, S. K., F. M. Zitman, P. D. Humphreys, K. Greenshields, J. J. Verschuuren, B. C. Jacobs, R. P. Rother, J. J. Plomp and H. J. Willison (2008). "Eculizumab prevents anti-ganglioside antibody-mediated neuropathy in a murine model." Brain : a journal of neurology **131**(Pt 5): 1197-1208.
Handunnetthi, L., S. V. Ramagopalan, G. C. Ebers and J. C. Knight (2010). "Regulation of major histocompatibility complex class II gene expression, genetic variation and disease." Genes Immun **11**(2): 99-112.
Hankey, G. J. (1987). "Guillain-Barre syndrome in Western Australia, 1980-1985." Medical Journal of Australia **146**(3): 130-133.
Harness, J. and P. A. McCombe (2008). "Increased levels of activated T-cells and reduced levels of CD4/CD25+ cells in peripheral blood of Guillain-Barré syndrome patients compared to controls." Journal of Clinical Neuroscience **15**(9): 1031-1035.
Hemachudha, T., D. E. Griffin, W. W. Chen and R. T. Johnson (1988). "Immunologic studies of rabies vaccination-induced Guillain-Barre syndrome." Neurology **38**(3): 375-378.
Hirschhorn, J. N. and M. J. Daly (2005). "Genome-wide association studies for common diseases and complex traits." Nature reviews. Genetics **6**(2): 95-108.
Ho, T. W., H. J. Willison, I. Nachamkin, C. Y. Li, J. Veitch, H. Ung, G. R. Wang, R. C. Liu, D. R. Cornblath, A. K. Asbury, J. W. Griffin and G. M. McKhann (1999). "Anti-GD1a antibody is associated with axonal but not demyelinating forms of Guillain-Barre syndrome." Annals of Neurology **45**(2): 168-173.

Hoffman, H. M., J. L. Mueller, D. H. Broide, A. A. Wanderer and R. D. Kolodner (2001). "Mutation of a new gene encoding a putative pyrin-like protein causes familial cold autoinflammatory syndrome and Muckle-Wells syndrome." Nat Genet **29**(3): 301-305.
Hogan, J. C., T. P. Briggs and P. J. Oldershaw (1992). "Guillain-Barre syndrome following cardiopulmonary bypass." International Journal of Cardiology **35**(3): 427-428.
Hollenbach, J. A., M. B. Ladner, K. Saeteurn, K. D. Taylor, L. Mei, T. Haritunians, D. P. B. McGovern, H. A. Erlich, J. I. Rotter and E. A. Trachtenberg (2009). "Susceptibility to Crohn's disease is mediated by KIR2DL2/KIR2DL3 heterozygosity and the HLA-C ligand." Immunogenetics **61**(10): 663-671.
Hou, Y. F., Y. C. Zhang, Y. L. Jiao, L. C. Wang, J. F. Li, Z. L. Pan, Q. R. Yang, H. S. Sun and Y. R. Zhao (2010). "Disparate distribution of activating and inhibitory killer cell immunoglobulin-like receptor genes in patients with systemic lupus erythematosus." Lupus **19**(1): 20-26.
Huflejt, M. E., E. T. Jordan, M. A. Gitt, S. H. Barondes and H. Leffler (1997). "Strikingly different localization of galectin-3 and galectin-4 in human colon adenocarcinoma T84 cells. Galectin-4 is localized at sites of cell adhesion." J Biol Chem **272**(22): 14294-14303.
Hughes, R. A., J. M. Newsom-Davis, G. D. Perkin and J. M. Pierce (1978). "Steroids in acute polyneuropathy." Lancet **2**(8104-5): 1383.
Hughes, R. A., A. V. Swan and P. A. van Doorn (2010). "Corticosteroids for Guillain-Barre syndrome." Cochrane database of systematic reviews (Online) **2**: CD001446.
Hughes, R. A. C., D. Allen, A. Makowska and N. A. Gregson (2006). "Pathogenesis of chronic inflammatory demyelinating polyradiculoneuropathy." Journal of the Peripheral Nervous System **11**(1): 30-46.
Hughes, R. A. C. and J. H. Rees (1997). "Clinical and Epidemiologic Features of Guillain Barre Syndrome." The Journal of Infectious Diseases **176**(s2): S92-S98.
Hughes, R. A. C., A. V. Swan, J.-C. Raphaël, D. Annane, R. van Koningsveld and P. A. van Doorn (2007). "Immunotherapy for Guillain-Barré syndrome: a systematic review." Brain **130**(9): 2245-2257.
Hughes, R. A. C., E. F. M. Wijdicks, E. Benson, D. R. Cornblath, A. F. Hahn, J. M. Meythaler, J. T. Sladky, R. J. Barohn, J. C. Stevens and G. Multidisciplinary Consensus (2005). "Supportive care for patients with Guillain-Barre syndrome." Archives of Neurology **62**(8): 1194-1198.
Hugot, J. P., M. Chamaillard, H. Zouali, S. Lesage, J. P. Cezard, J. Belaiche, S. Almer, C. Tysk, C. A. O'Morain, M. Gassull, V. Binder, Y. Finkel, A. Cortot, R. Modigliani, P. Laurent-Puig, C. Gower-Rousseau, J. Macry, J. F. Colombel, M. Sahbatou and G. Thomas (2001). "Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease." Nature **411**(6837): 599-603.
Iijima, M., H. Koike, M. Katsuno and G. Sobue (2011). "Polymorphism of transient axonal glycoprotein-1 in chronic inflammatory demyelinating polyneuropathy." Journal of the peripheral nervous system : JPNS **16 Suppl 1**: 52-55.
Illes, Z., T. Kondo, J. Newcombe, N. Oka, T. Tabira and T. Yamamura (2000). "Differential expression of NK T cell V alpha 24J alpha Q invariant TCR chain in the lesions of multiple sclerosis and chronic inflammatory demyelinating polyneuropathy." Journal of Immunology **164**(8): 4375-4381.
Ilyin, G. P., A. L. Serandour, C. Pigeon, M. Rialland, D. Glaise and C. Guguen-Guillouzo (2002). "A new subfamily of structurally related human F-box proteins." Gene **296**(1-2): 11-20.
Inglis, H. R., P. A. Csurhes and P. A. McCombe (2007). "Antibody responses to peptides of peripheral nerve myelin proteins P0 and P2 in patients with inflammatory demyelinating neuropathy." Journal of Neurology, Neurosurgery & Psychiatry **78**(4): 419-422.

Jamil, K. M. and S. I. Khakoo (2011). "KIR/HLA Interactions and Pathogen Immunity." Journal of Biomedicine and Biotechnology **2011**.

Jamshidian, A., A.-R. Nikseresht, M. Vessal and E. Kamali-Sarvestani (2010). "Association of CD1A +622 T/C, +737 G/C and CD1E +6129 A/G Genes Polymorphisms with Multiple Sclerosis." Immunological Investigations **39**(8): 874-889.

Jiao, H., W. Wang, H. Wang, Y. Wu and L. Wang (2012). "Tumor necrosis factor alpha 308 G/A polymorphism and Guillain-Barre syndrome risk." Molecular Biology Reports **39**(2): 1537-1540.

Johnson, A. D. and C. J. O'Donnell (2009). "An open access database of genome-wide association results." BMC medical genetics **10**: 6.

Kalani, M., M. Rasouli, A. Moravej, S. Kiany and H. R. Rahimi (2011). "Association of interleukin-15 single nucleotide polymorphisms with resistance to brucellosis among Iranian patients." Tissue Antigens **78**(5): 352-358.

Kanda, T. (2013). "Biology of the blood-nerve barrier and its alteration in immune mediated neuropathies." Journal of neurology, neurosurgery, and psychiatry **84**(2): 208-212.

Kaslow, R. A., J. Z. Sullivan-Bolyai, B. Hafkin, L. B. Schonberger, L. Kraus, M. J. Moore, E. Yunis and R. M. Williams (1984). "HLA antigens in Guillain-Barre syndrome." Neurology **34**(2): 240-242.

Kennedy, M. K., M. Glaccum, S. N. Brown, E. A. Butz, J. L. Viney, M. Embers, N. Matsuki, K. Charrier, L. Sedger, C. R. Willis, K. Brasel, P. J. Morrissey, K. Stocking, J. C. Schuh, S. Joyce and J. J. Peschon (2000). "Reversible defects in natural killer and memory CD8 T cell lineages in interleukin 15-deficient mice." The Journal of experimental medicine **191**(5): 771-780.

Khalili-Shirazi, A., N. A. Gregson, M. Londei, L. Summers and R. A. Hughes (1998). "The distribution of CD1 molecules in inflammatory neuropathy." Journal of the Neurological Sciences **158**(2): 154-163.

Kieseier, B. C., J. M. Clements, H. B. Pischel, G. M. Wells, K. Miller, A. J. Gearing and H. P. Hartung (1998). "Matrix metalloproteinases MMP-9 and MMP-7 are expressed in experimental autoimmune neuritis and the Guillain-Barre syndrome." Ann Neurol **43**(4): 427-434.

Kieseier, B. C., R. Kiefer, R. Gold, B. Hemmer, H. J. Willison and H.-P. Hartung (2004). "Advances in understanding and treatment of immune-mediated disorders of the peripheral nervous system." Muscle & Nerve **30**(2): 131-156.

Kim, J. G., R. C. Armstrong, D. v Agoston, A. Robinsky, C. Wiese, J. Nagle and L. D. Hudson (1997). "Myelin transcription factor 1 (Myt1) of the oligodendrocyte lineage, along with a closely related CCHC zinc finger, is expressed in developing neurons in the mammalian central nervous system." J Neurosci Res **50**(2): 272-290.

Klein, R. J., C. Zeiss, E. Y. Chew, J.-Y. Tsai, R. S. Sackler, C. Haynes, A. K. Henning, J. P. SanGiovanni, S. M. Mane, S. T. Mayne, M. B. Bracken, F. L. Ferris, J. Ott, C. Barnstable and J. Hoh (2005). "Complement Factor H Polymorphism in Age-Related Macular Degeneration." Science **308**(5720): 385-389.

Koga, M., M. Gilbert, M. Takahashi, J. Li, S. Koike, K. Hirata and N. Yuki (2006). "Comprehensive analysis of bacterial risk factors for the development of Guillain-Barre syndrome after Campylobacter jejuni enteritis." Journal of Infectious Diseases **193**(4): 547-555.

Koga, M., N. Yuki and K. Hirata (2001). "Antecedent symptoms in Guillain-Barre syndrome: an important indicator for clinical and serological subgroups." Acta Neurologica Scandinavica **103**(5): 278-287.

Koga, M., N. Yuki, M. Takahashi, K. Saito and K. Hirata (1998). "Close association of IgA anti-ganglioside antibodies with antecedent Campylobacter jejuni infection in Guillain-Barre and Fisher's syndromes." Journal of Neuroimmunology **81**(1-2): 138-143.

Koller, H., M. Schroeter, B. C. Kieseier and H.-P. Hartung (2005). "Chronic inflammatory demyelinating polyneuropathy--update on pathogenesis, diagnostic criteria and therapy." Current Opinion in Neurology **18**(3): 273-278.

Kools, P., G. Van Imschoot and F. van Roy (2000). "Characterization of three novel human cadherin genes (CDH7, CDH19, and CDH20) clustered on chromosome 18q22-q23 and with high homology to chicken cadherin-7." Genomics **68**(3): 283-295.

Korn-Lubetzki, I., Z. Argov, A. Raas-Rothschild, I. Wirguin and I. Steiner (2002). "Family with inflammatory demyelinating polyneuropathy and the HNPP 17p12 deletion." Am J Med Genet **113**(3): 275-278.

Koski, C. L., M. Baumgarten, L. S. Magder, R. J. Barohn, J. Goldstein, M. Graves, K. Gorson, A. F. Hahn, R. A. C. Hughes, J. Katz, R. A. Lewis, G. J. Parry, P. van Doorn and D. R. Cornblath (2009). "Derivation and validation of diagnostic criteria for chronic inflammatory demyelinating polyneuropathy." Journal of the Neurological Sciences **277**(1-2): 1-8.

Kuhr, T., K. Hala, H. Dietrich, M. Herold and G. Wick (1994). "Genetically determined target organ susceptibility in the pathogenesis of spontaneous autoimmune thyroiditis: aberrant expression of MHC-class II antigens and the possible role of virus." J Autoimmun **7**(1): 13-25.

Kuijf, M. L., K. Geleijns, N. Ennaji, W. van Rijs, P. A. van Doorn and B. C. Jacobs (2008). "Susceptibility to Guillain-Barre syndrome is not associated with CD1A and CD1E gene polymorphisms." Journal of Neuroimmunology **205**(1-2): 110-112.

Kuijf, M. L., P. C. R. Godschalk, M. Gilbert, H. P. Endtz, A. P. Tio-Gillen, C. W. Ang, P. A. van Doorn and B. C. Jacobs (2007). "Origin of ganglioside complex antibodies in Guillain-Barre syndrome." Journal of Neuroimmunology **188**(1-2): 69-73.

Kuitwaard, K., R. van Koningsveld, L. Ruts, B. C. Jacobs and P. A. van Doorn (2009). "Recurrent Guillain Barre syndrome." Journal of Neurology, Neurosurgery & Psychiatry **80**(1): 56-59.

Kulkarni, S., M. P. Martin and M. Carrington (2008). "The Yin and Yang of HLA and KIR in human disease." Seminars in Immunology **20**(6): 343-352.

Kurzawski, M., A. Pawlik, B. Czerny, L. Domanski, J. Rozanski and M. Drozdzik (2005). "Frequencies of the common promoter polymorphisms in cytokine genes in a Polish population." Int J Immunogenet **32**(5): 285-291.

Kuwabara, S., S. Misawa, M. Mori, N. Tamura, M. Kubota and T. Hattori (2006). "Long term prognosis of chronic inflammatory demyelinating polyneuropathy: a five year follow up of 38 cases." Journal of Neurology, Neurosurgery & Psychiatry **77**(1): 66-70.

Lander, E. S., L. M. Linton, B. Birren, C. Nusbaum, M. C. Zody, J. Baldwin, K. Devon, K. Dewar, M. Doyle, W. FitzHugh, R. Funke, D. Gage, K. Harris, A. Heaford, J. Howland, L. Kann, J. Lehoczky, R. LeVine, P. McEwan, K. McKernan, J. Meldrim, J. P. Mesirov, C. Miranda, W. Morris, J. Naylor, C. Raymond, M. Rosetti, R. Santos, A. Sheridan, C. Sougnez, N. Stange-Thomann, N. Stojanovic, A. Subramanian, D. Wyman, J. Rogers, J. Sulston, R. Ainscough, S. Beck, D. Bentley, J. Burton, C. Clee, N. Carter, A. Coulson, R. Deadman, P. Deloukas, A. Dunham, I. Dunham, R. Durbin, L. French, D. Grafham, S. Gregory, T. Hubbard, S. Humphray, A. Hunt, M. Jones, C. Lloyd, A. McMurray, L. Matthews, S. Mercer, S. Milne, J. C. Mullikin, A. Mungall, R. Plumb, M. Ross, R. Shownkeen, S. Sims, R. H. Waterston, R. K. Wilson, L. W. Hillier, J. D. McPherson, M. A. Marra, E. R. Mardis, L. A. Fulton, A. T. Chinwalla, K. H. Pepin, W. R. Gish, S. L. Chissoe, M. C. Wendl, K. D. Delehaunty, T. L. Miner, A. Delehaunty, J. B. Kramer, L. L. Cook, R. S. Fulton, D. L.

Johnson, P. J. Minx, S. W. Clifton, T. Hawkins, E. Branscomb, P. Predki, P. Richardson, S. Wenning, T. Slezak, N. Doggett, J. F. Cheng, A. Olsen, S. Lucas, C. Elkin, E. Uberbacher, M. Frazier, R. A. Gibbs, D. M. Muzny, S. E. Scherer, J. B. Bouck, E. J. Sodergren, K. C. Worley, C. M. Rives, J. H. Gorrell, M. L. Metzker, S. L. Naylor, R. S. Kucherlapati, D. L. Nelson, G. M. Weinstock, Y. Sakaki, A. Fujiyama, M. Hattori, T. Yada, A. Toyoda, T. Itoh, C. Kawagoe, H. Watanabe, Y. Totoki, T. Taylor, J. Weissenbach, R. Heilig, W. Saurin, F. Artiguenave, P. Brottier, T. Bruls, E. Pelletier, C. Robert, P. Wincker, D. R. Smith, L. Doucette-Stamm, M. Rubenfield, K. Weinstock, H. M. Lee, J. Dubois, A. Rosenthal, M. Platzer, G. Nyakatura, S. Taudien, A. Rump, H. Yang, J. Yu, J. Wang, G. Huang, J. Gu, L. Hood, L. Rowen, A. Madan, S. Qin, R. W. Davis, N. A. Federspiel, A. P. Abola, M. J. Proctor, R. M. Myers, J. Schmutz, M. Dickson, J. Grimwood, D. R. Cox, M. V. Olson, R. Kaul, C. Raymond, N. Shimizu, K. Kawasaki, S. Minoshima, G. A. Evans, M. Athanasiou, R. Schultz, B. A. Roe, F. Chen, H. Pan, J. Ramser, H. Lehrach, R. Reinhardt, W. R. McCombie, l. B. M. de, N. Dedhia, H. Blocker, K. Hornischer, G. Nordsiek, R. Agarwala, L. Aravind, J. A. Bailey, A. Bateman, S. Batzoglou, E. Birney, P. Bork, D. G. Brown, C. B. Burge, L. Cerutti, H. C. Chen, D. Church, M. Clamp, R. R. Copley, T. Doerks, S. R. Eddy, E. E. Eichler, T. S. Furey, J. Galagan, J. G. Gilbert, C. Harmon, Y. Hayashizaki, D. Haussler, H. Hermjakob, K. Hokamp, W. Jang, L. S. Johnson, T. A. Jones, S. Kasif, A. Kaspryzk, S. Kennedy, W. J. Kent, P. Kitts, E. V. Koonin, I. Korf, D. Kulp, D. Lancet, T. M. Lowe, A. McLysaght, T. Mikkelsen, J. V. Moran, N. Mulder, V. J. Pollara, C. P. Ponting, G. Schuler, J. Schultz, G. Slater, A. F. Smit, E. Stupka, J. Szustakowski, D. Thierry-Mieg, J. Thierry-Mieg, L. Wagner, J. Wallis, R. Wheeler, A. Williams, Y. I. Wolf, K. H. Wolfe, S. P. Yang, R. F. Yeh, F. Collins, M. S. Guyer, J. Peterson, A. Felsenfeld, K. A. Wetterstrand, A. Patrinos, M. J. Morgan, J. P. de, J. J. Catanese, K. Osoegawa, H. Shizuya, S. Choi and Y. J. Chen (2001). "Initial sequencing and analysis of the human genome." Nature **409**(6822): 860-921.

Latovitzki, N., N. Suciu-Foca, A. S. Penn, M. R. Olarte and A. M. Chutorian (1979). "HLA typing and Guillain-Barre syndrome." Neurology **29**(5): 743-745.

Lee, T., M. A. Mosing, J. D. Henry, J. N. Trollor, A. Lammel, D. Ames, N. G. Martin, M. J. Wright and P. S. Sachdev (2012). "Genetic influences on five measures of processing speed and their covariation with general cognitive ability in the elderly: the older Australian twins study." Behav Genet **42**(1): 96-106.

Lee, Y., A. Mattai, R. Long, J. L. Rapoport, N. Gogtay and A. M. Addington (2012). "Microduplications disrupting the MYT1L gene (2p25.3) are associated with schizophrenia." Psychiatr Genet **22**(4): 206-209.

Lee, Y. and T. Shin (2002). "Expression of constitutive endothelial and inducible nitric oxide synthase in the sciatic nerve of Lewis rats with experimental autoimmune neuritis." Journal of Neuroimmunology **126**(1-2): 78-85.

Li, W., X. Wang, J. Zhao, J. Lin, X. Q. Song, Y. Yang, C. Jiang, B. Xiao, G. Yang, H. X. Zhang and L. X. Lv (2012). "Association study of myelin transcription factor 1-like polymorphisms with schizophrenia in Han Chinese population." Genes Brain Behav **11**(1): 87-93.

Liu, J.-X., H. J. Willison, D. Pedrosa, x00F and F. f (2009). "Immunolocalization of GQ1b and related gangliosides in human extraocular neuromuscular junctions and muscle spindles." Investigative Ophthalmology & Visual Science **50**(7): 3226-3232.

Liu, Y. Z., Y. F. Pei, Y. F. Guo, L. Wang, X. G. Liu, H. Yan, D. H. Xiong, Y. P. Zhang, S. Levy, J. Li, C. K. Haddock, C. J. Papasian, Q. Xu, J. Z. Ma, T. J. Payne, R. R. Recker, M. D. Li and H. W. Deng (2009). "Genome-wide association analyses suggested a novel mechanism for smoking behavior regulated by IL15." Molecular Psychiatry **14**(7): 668-680.

Lo, Y. L. (2007). "Clinical and immunological spectrum of the Miller Fisher syndrome." Muscle & Nerve **36**(5): 615-627.

Lodolce, J. P., P. R. Burkett, R. M. Koka, D. L. Boone and A. Ma (2002). "Regulation of lymphoid homeostasis by interleukin-15." Cytokine & Growth Factor Reviews **13**(6): 429-439.
Lyu, R. K., L. M. Tang, S. Y. Cheng, W. C. Hsu and S. T. Chen (1997). "Guillain-Barre syndrome in Taiwan: a clinical study of 167 patients." Journal of Neurology, Neurosurgery & Psychiatry **63**(4): 494-500.
Ma, J. J., M. Nishimura, H. Mine, S. Kuroki, M. Nukina, M. Ohta, H. Saji, H. Obayashi, H. Kawakami, T. Saida and T. Uchiyama (1998). "Genetic contribution of the tumor necrosis factor region in Guillain-Barre syndrome." Ann Neurol **44**(5): 815-818.
Ma, J. J., M. Nishimura, H. Mine, S. Kuroki, M. Nukina, M. Ohta, H. Saji, H. Obayashi, T. Saida, H. Kawakami and T. Uchiyama (1998). "HLA and T-cell receptor gene polymorphisms in Guillain-Barre syndrome." Neurology **51**(2): 379-384.
Ma, Y. and M. Fang (2013). "Immunosenescence and age-related viral diseases." Science China. Life sciences **56**(5): 399-405.
Madrid, R. E., Wi, x015B and H. M. niewski (1977). "Axonal degeneration in demyelinating disorders." Journal of Neurocytology **6**(1): 103-117.
Magira, E. E., M. Papaioakim, I. Nachamkin, A. K. Asbury, C. Y. Li, T. W. Ho, J. W. Griffin, G. M. McKhann and D. S. Monos (2003). "Differential distribution of HLA-DQ beta/DR beta epitopes in the two forms of Guillain-Barre syndrome, acute motor axonal neuropathy and acute inflammatory demyelinating polyneuropathy (AIDP): identification of DQ beta epitopes associated with susceptibility to and protection from AIDP." Journal of Immunology **170**(6): 3074-3080.
Martin, A. M., J. K. Kulski, S. Gaudieri, C. S. Witt, E. M. Freitas, J. Trowsdale and F. T. Christiansen (2004). "Comparative genomic analysis, diversity and evolution of two KIR haplotypes A and B." Gene **335**: 121-131.
Martin, M. P., A. Bashirova, J. Traherne, J. Trowsdale and M. Carrington (2003). "Cutting edge: expansion of the KIR locus by unequal crossing over." Journal of Immunology **171**(5): 2192-2195.
Matzinger, P. (2002). "The Danger Model: A Renewed Sense of Self." Science **296**(5566): 301-305.
Mazzeo, A., M. Aguennouz, C. Messina and G. Vita (2004). "Immunolocalization and activation of transcription factor nuclear factor kappa B in dysimmune neuropathies and familial amyloidotic polyneuropathy." Archives of Neurology **61**(7): 1097-1102.
McCombe, P. A., P. Clark, J. A. Frith, S. R. Hammond, G. J. Stewart, J. D. Pollard and J. G. McLeod (1985). "Alpha-1 antitrypsin phenotypes in demyelinating disease: an association between demyelinating disease and the allele PiM3." Annals of neurology **18**(4): 514-516.
McCombe, P. A., P. A. Csurhes and J. M. Greer (2006). "Studies of HLA associations in male and female patients with Guillain-Barre syndrome (GBS) and chronic inflammatory demyelinating polyradiculoneuropathy (CIDP)." Journal of Neuroimmunology **180**(1-2): 172-177.
McCombe, P. A., J. M. Greer and I. R. Mackay (2009). "Sexual dimorphism in autoimmune disease." Current Molecular Medicine **9**(9): 1058-1079.
McCombe, P. A., J. D. Pollard and J. G. McLeod (1987). "Chronic inflammatory demyelinating polyradiculoneuropathy. A clinical and electrophysiological study of 92 cases." Brain **110**(Pt 6): 1617-1630.
McKhann, G. M., D. R. Cornblath, J. W. Griffin, T. W. Ho, C. Y. Li, Z. Jiang, H. S. Wu, G. Zhaori, Y. Liu, L. P. Jou and et al. (1993). "Acute motor axonal neuropathy: a frequent cause of acute flaccid paralysis in China." Annals of Neurology **33**(4): 333-342.
McKhann, G. M., D. R. Cornblath, T. Ho, C. Y. Li, A. Y. Bai, H. S. Wu, Q. F. Yei, W. C. Zhang, Z. Zhaori, Z. Jiang and et al. (1991). "Clinical and electrophysiological aspects of

acute paralytic disease of children and young adults in northern China." Lancet **338**(8767): 593-597.
Mehndiratta, M. M. and R. A. Hughes (2012). "Plasma exchange for chronic inflammatory demyelinating polyradiculoneuropathy." Cochrane Database Syst Rev **9**: CD003906.
Mercurio, F., H. Zhu, B. W. Murray, A. Shevchenko, B. L. Bennett, J. w. Li, D. B. Young, M. Barbosa, M. Mann, A. Manning and A. Rao (1997). "IKK-1 and IKK-2: Cytokine-Activated IκB Kinases Essential for NF-κB Activation." Science **278**(5339): 860-866.
Meyer, K. J., M. S. Axelsen, V. C. Sheffield, S. R. Patil and T. H. Wassink (2012). "Germline mosaic transmission of a novel duplication of PXDN and MYT1L to two male half-siblings with autism." Psychiatr Genet **22**(3): 137-140.
Middleton, D. and F. Gonzelez (2010). "The extensive polymorphism of KIR genes." Immunology **129**(1): 8-19.
Mori, M., S. Kuwabara, T. Fukutake, N. Yuki and T. Hattori (2001). "Clinical features and prognosis of Miller Fisher syndrome." Neurology **56**(8): 1104-1106.
Mrad, M., N. Fekih-Mrissa, M. Mansour, A. Seyah, A. Riahi, N. Gritli and R. Mrissa (2013). "Association of HLA-DR/DQ polymorphism with chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) in Tunisian patients." Transfusion and apheresis science : official journal of the World Apheresis Association : official journal of the European Society for Haemapheresis **49**(3): 623-626.
Mrozek, E., P. Anderson and M. A. Caligiuri (1996). "Role of interleukin-15 in the development of human CD56+ natural killer cells from CD34+ hematopoietic progenitor cells." Blood **87**(7): 2632-2640.
Münch, C., J. T. Epplen, M. Meins, R. Meyer, J. R. Weber and T. Meyer (2008). "Severe Guillain–Barré syndrome associated with chromosome 17p11.2-12 duplication." Muscle & Nerve **37**(2): 256-258.
Murray, N. M. and D. T. Wade (1980). "The sural sensory action potential in Guillain-Barre syndrome." Muscle & Nerve **3**(5): 444.
Myhr, K. M., K. S. Vagnes, T. H. Maroy, J. H. Aarseth, H. I. Nyland and C. A. Vedeler (2003). "Interleukin-10 promoter polymorphisms in patients with Guillain-Barre syndrome." Journal of Neuroimmunology **139**(1-2): 81-83.
Naik, K. R., A. O. Saroja and B. P. Patil (2012). "Familial Guillain-Barre syndrome: First Indian report." Ann Indian Acad Neurol **15**(1): 44-47.
Nevo, Y., B. Ben-Zeev, A. Tabib, R. Straussberg, Y. Anikster, Z. Shorer, A. Fattal-Valevski, A. Ta-Shma, S. Aharoni, M. Rabie, S. Zenvirt, H. Goldshmidt, Y. Fellig, A. Shaag, D. Mevorach and O. Elpeleg (2013). "CD59 deficiency is associated with chronic hemolysis and childhood relapsing immune-mediated polyneuropathy." Blood **121**(1): 129-135.
Nielsen, N. M., T. Westergaard, K. Rostgaard, M. Frisch, H. Hjalgrim, J. Wohlfahrt, N. Koch-Henriksen and M. Melbye (2005). "Familial Risk of Multiple Sclerosis: A Nationwide Cohort Study." American Journal of Epidemiology **162**(8): 774-778.
Nishimura, T., H. Honda and M. Takeichi (2012). "Planar cell polarity links axes of spatial dynamics in neural-tube closure." Cell **149**(5): 1084-1097.
Nobile-Orazio, E., D. Cocito, S. Jann, A. Uncini, E. Beghi, P. Messina, G. Antonini, R. Fazio, F. Gallia, A. Schenone, A. Francia, D. Pareyson, L. Santoro, S. Tamburin, R. Macchia, G. Cavaletti, F. Giannini and M. Sabatelli (2012). "Intravenous immunoglobulin versus intravenous methylprednisolone for chronic inflammatory demyelinating polyradiculoneuropathy: a randomised controlled trial." Lancet neurology **11**(6): 493-502.
Notturno, F., M. Pace, M. V. De Angelis, C. M. Caporale, A. Giovannini and A. Uncini (2008). "Susceptibility to chronic inflammatory demyelinating polyradiculoneuropathy is associated to polymorphic GA repeat in the SH2D2A gene." Journal of Neuroimmunology **197**(2): 124-127.

Nyati, K. K., K. N. Prasad, A. Verma and V. K. Paliwal (2010). "Correlation of matrix metalloproteinases-2 and -9 with proinflammatory cytokines in Guillain-Barre syndrome." Journal of Neuroscience Research **88**(16): 3540-3546.

Odaka, M., N. Yuki, N. Kokubun, K. Hirata and S. Kuwabara (2003). "Axonal Guillain-Barre syndrome associated with axonal Charcot-Marie-Tooth disease." Journal of the Neurological Sciences **211**(1-2): 93-97.

Olivier, N., H. Laribi, M. Pages, W. Camu and R. Juntas Morales (2010). "Recurrent Guillain-Barre syndrome after surgery." Revue Neurologique **166**(6-7): 644-647.

Ollier, W. E. (2004). "Cytokine genes and disease susceptibility." Cytokine **28**(4-5): 174-178.

Pang, S. Y., K. H. Chan, W. W. Mak, M. H. Kung, C. N. Lee, T. H. Tsoi, E. K. Yip and S. L. Ho (2012). "Single-nucleotide polymorphism of transient axonal glycoprotein-1 and its correlation with clinical features and prognosis in chronic inflammatory demyelinating polyneuropathy." Journal of the peripheral nervous system : JPNS **17**(1): 72-75.

Pang, Z. P., N. Yang, T. Vierbuchen, A. Ostermeier, D. R. Fuentes, T. Q. Yang, A. Citri, V. Sebastiano, S. Marro, T. C. Sudhof and M. Wernig (2011). "Induction of human neuronal cells by defined transcription factors." Nature **476**(7359): 220-223.

Patil, N., A. J. Berno, D. A. Hinds, W. A. Barrett, J. M. Doshi, C. R. Hacker, C. R. Kautzer, D. H. Lee, C. Marjoribanks, D. P. McDonough, B. T. Nguyen, M. C. Norris, J. B. Sheehan, N. Shen, D. Stern, R. P. Stokowski, D. J. Thomas, M. O. Trulson, K. R. Vyas, K. A. Frazer, S. P. Fodor and D. R. Cox (2001). "Blocks of limited haplotype diversity revealed by high-resolution scanning of human chromosome 21." Science **294**(5547): 1719-1723.

Patterson, N., A. L. Price and D. Reich (2006). "Population structure and eigenanalysis." PLoS genetics **2**(12): e190.

Pe'er, I., R. Yelensky, D. Altshuler and M. J. Daly (2008). "Estimation of the multiple testing burden for genomewide association studies of nearly all common variants." Genet Epidemiol **32**(4): 381-385.

Pearson, T. A. and T. A. Manolio (2008). "How to interpret a genome-wide association study." JAMA **299**(11): 1335-1344.

Pellett, F., F. Siannis, I. Vukin, P. Lee, M. B. Urowitz and D. D. Gladman (2007). "KIRs and autoimmune disease: studies in systemic lupus erythematosus and scleroderma." Tissue Antigens **69 Suppl 1**: 106-108.

Pinol-Roma, S., M. S. Swanson, J. G. Gall and G. Dreyfuss (1989). "A novel heterogeneous nuclear RNP protein with a unique distribution on nascent transcripts." J Cell Biol **109**(6 Pt 1): 2575-2587.

Piradov, M. A., A. G. Dolbin, R. Voylokova and V. V. Yazdovsky (1995). "HLA antigens in severe forms of Guillain-Barre syndrome." Neurology **45**(7): 1419-1420.

Porcelli, S. A. (1995). "The CD1 family: a third lineage of antigen-presenting molecules." Advances in Immunology **59**: 1-98.

Prasad, K. N., K. K. Nyati, A. Verma, A. Rizwan and V. K. Paliwal (2010). "Tumor necrosis factor-alpha polymorphisms and expression in Guillain-Barre syndrome." Human Immunology **71**(9): 905-910.

Price, A. L., N. J. Patterson, R. M. Plenge, M. E. Weinblatt, N. A. Shadick and D. Reich (2006). "Principal components analysis corrects for stratification in genome-wide association studies." Nature genetics **38**(8): 904-909.

Prineas, J. W. (1971). "Demyelination and remyelination in recurrent idiopathic polyneuropathy. An electron microscope study." Acta Neuropathologica **18**(1): 34-57.

Prineas, J. W. (1981). "Pathology of the Guillain-Barre syndrome." Annals of Neurology **9 Suppl**: 6-19.

Pritchard, J., R. A. C. Hughes, J. H. Rees, H. J. Willison and J. A. R. Nicoll (2003). "Apolipoprotein E genotypes and clinical outcome in Guillain-Barre syndrome." J Neurol Neurosurg Psychiatry **74**(7): 971-973.

Radhakrishnan, V. V., M. G. Sumi, S. Reuben, A. Mathai and M. D. Nair (2003). "Circulating tumour necrosis factor alpha & soluble TNF receptors in patients with Guillain-Barre syndrome." Indian J Med Res **117**: 216-220.

Rai, E. and E. K. Wakeland (2011). "Genetic predisposition to autoimmunity--what have we learned?" Semin Immunol **23**(2): 67-83.

Rajabally, Y. A., G. Nicolas, F. Pieret, P. Bouche and P. Y. K. Van den Bergh (2009). "Validity of diagnostic criteria for chronic inflammatory demyelinating polyneuropathy: a multicentre European study." Journal of Neurology, Neurosurgery & Psychiatry **80**(12): 1364-1368.

Rajalingam, R. (2012). "Overview of the killer cell immunoglobulin-like receptor system." Methods in Molecular Biology **882**: 391-414.

Randall, D. P. (2010). "Guillain-Barre Syndrome and Immunizations." Disease-a-Month **56**(5): 293-298.

Raschetti, R., M. Maggini, P. Popoli, B. Caffari, R. Da Cas, F. Menniti-Ippolito, S. Spila-Alegiani and G. Traversa (1995). "Gangliosides and Guillain-Barré syndrome." Journal of Clinical Epidemiology **48**(11): 1399-1405.

Rechreche, H., G. V. Mallo, G. Montalto, J. C. Dagorn and J. L. Iovanna (1997). "Cloning and expression of the mRNA of human galectin-4, an S-type lectin down-regulated in colorectal cancer." Eur J Biochem **248**(1): 225-230.

Remiche, G., M. Abramowicz and N. Mavroudakis (2013). "Chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) associated to hereditary neuropathy with liability to pressure palsies (HNPP) and revealed after influenza AH1N1 vaccination." Acta neurologica Belgica **113**(4): 519-522.

Renaud, S., A. P. Hays, T. H. Brannagan Iii, H. W. Sander, M. Edgar, L. H. Weimer, M. R. Olarte, M. C. Dalakas, Z. Xiang, M. J. Danon and N. Latov (2005). "Gene expression profiling in chronic inflammatory demyelinating polyneuropathy." Journal of Neuroimmunology **159**(1–2): 203-214.

Rose, N. R. and C. Bona (1993). "Defining criteria for autoimmune diseases (Witebsky's postulates revisited)." Immunology Today **14**(9): 426-430.

Ruckert, R., K. Brandt, M. Ernst, K. Marienfeld, E. Csernok, C. Metzler, V. Budagian, E. Bulanova, R. Paus and S. Bulfone-Paus (2009). "Interleukin-15 stimulates macrophages to activate CD4+ T cells: a role in the pathogenesis of rheumatoid arthritis?" Immunology **126**(1): 63-73.

Rusconi, F., E. Mancinelli, G. Colombo, R. Cardani, L. Da Riva, I. Bongarzone, G. Meola and R. Zippel (2010). "Proteome profile in Myotonic Dystrophy type 2 myotubes reveals dysfunction in protein processing and mitochondrial pathways." Neurobiol Dis **38**(2): 273-280.

Ruts, L., J. Drenthen, B. C. Jacobs and P. A. Van Doorn (2010). "Distinguishing acute-onset CIDP from fluctuating guillain-barre syndrome: A prospective study." Neurology **74**(21): 1680-1686.

Ruts, L., R. van Koningsveld and P. A. van Doorn (2005). "Distinguishing acute-onset CIDP from Guillain–Barré syndrome with treatment related fluctuations." Neurology **65**(1): 138-140.

Salemi, S. and R. D'Amelio (2010). "Vaccinations and autoimmune diseases." International Reviews of Immunology **29**(3): 239-246.

Sang, D., Q. Chen, X. Liu, H. Qu, D. Wei, L. Yin and L. Zhang (2012). "Fc receptor like 3 in Chinese patients of Han nationality with Guillain-Barre syndrome." Journal of Neuroimmunology **246**(1-2): 65-68.

Sanvito, L., A. Makowska, N. Gregson, R. Nemni and R. A. Hughes (2009). "Circulating subsets and CD4(+)CD25(+) regulatory T cell function in chronic inflammatory demyelinating polyradiculoneuropathy." Autoimmunity **42**(8): 667-677.

Sanvito, L., A. Makowska, M. Mahdi-Rogers, R. D. M. Hadden, M. Peakman, N. Gregson, R. Nemni and R. A. C. Hughes (2009). "Humoral and cellular immune responses to myelin protein peptides in chronic inflammatory demyelinating polyradiculoneuropathy." Journal of Neurology, Neurosurgery & Psychiatry **80**(3): 333-338.

Saunders, M. and M. Rake (1965). "Familial Guillain-Barre syndrome." Lancet **2**(7422): 1106-1107.

Scelsa, S. N., V. Ghali, S. Herskovitz, P. Bieri, D. L. Shank, D. D. J. MacGowan and S. Liau (2004). "Blood gammadelta T cells, Campylobacter jejuni, and GM1 titers in Guillain-Barré syndrome." Muscle & Nerve **30**(4): 423-432.

Schmidt, B., K. V. Toyka, R. Kiefer, J. Full, H. P. Hartung and J. Pollard (1996). "Inflammatory infiltrates in sural nerve biopsies in Guillain-Barre syndrome and chronic inflammatory demyelinating neuropathy." Muscle Nerve **19**(4): 474-487.

Schonberger, L. B., D. J. Bregman, J. Z. Sullivan-Bolyai, R. A. Keenlyside, D. W. Ziegler, H. F. Retailliau, D. L. Eddins and J. A. Bryan (1979). "Guillain-Barre syndrome following vaccination in the National Influenza Immunization Program, United States, 1976--1977." American Journal of Epidemiology **110**(2): 105-123.

Sekiguchi, Y., A. Uncini, N. Yuki, S. Misawa, F. Notturno, S. Nasu, K. Kanai, Y. Noto, Y. Fujimaki, K. Shibuya, S. Ohmori, Y. Sato and S. Kuwabara (2012). "Antiganglioside antibodies are associated with axonal Guillain-Barre syndrome: a Japanese-Italian collaborative study." Journal of neurology, neurosurgery, and psychiatry **83**(1): 23-28.

Shamshiev, A., A. Donda, I. Carena, L. Mori, L. Kappos and G. De Libero (1999). "Self glycolipids as T-cell autoantigens." European Journal of Immunology **29**(5): 1667-1675.

Shamshiev, A., A. Donda, T. I. Prigozy, L. Mori, V. Chigorno, C. A. Benedict, L. Kappos, S. Sonnino, M. Kronenberg and G. De Libero (2000). "The alphabeta T cell response to self-glycolipids shows a novel mechanism of CD1b loading and a requirement for complex oligosaccharides." Immunity **13**(2): 255-264.

Sharma, K. R., D. Saadia, A. G. Facca, R. Bhatia, D. R. Ayyar and W. Sheremata (2008). "Chronic inflammatory demyelinating polyradiculoneuropathy associated with multiple sclerosis." Journal of clinical neuromuscular disease **9**(4): 385-396.

Shi, Y., Y. Ding, Y. P. Lei, X. Y. Yang, G. M. Xie, J. Wen, C. Q. Cai, H. Li, Y. Chen, T. Zhang, B. L. Wu, L. Jin, Y. G. Chen and H. Y. Wang (2012). "Identification of novel rare mutations of DACT1 in human neural tube defects." Hum Mutat **33**(10): 1450-1455.

Shilling, H. G., L. A. Guethlein, N. W. Cheng, C. M. Gardiner, R. Rodriguez, D. Tyan and P. Parham (2002). "Allelic polymorphism synergizes with variable gene content to individualize human KIR genotype." Journal of Immunology **168**(5): 2307-2315.

Shin, I. S., A. N. Baer, H. J. Kwon, E. J. Papadopoulos and J. N. Siegel (2006). "Guillain-Barre and Miller Fisher syndromes occurring with tumor necrosis factor alpha antagonist therapy." Arthritis Rheum **54**(5): 1429-1434.

Shuert, G. T. and J. W. Gamble (1972). "Guillain-Barre syndrome after mandibular surgery: report of case." Journal of Oral Surgery **30**(12): 913-915.

Sinha, S., K. N. Prasad, D. Jain, K. K. Nyati, S. Pradhan and S. Agrawal (2010). "Immunoglobulin IgG Fc-receptor polymorphisms and HLA class II molecules in Guillain-Barre syndrome." Acta Neurologica Scandinavica **122**(1): 21-26.

Skarka, A., L. Skarydova, H. Stambergova and V. Wsol (2013). "Purification and reconstitution of human membrane-bound DHRS7 (SDR34C1) from Sf9 cells." Protein Expr Purif **95C**: 44-49.
Skarydova, L. and V. Wsol (2012). "Human microsomal carbonyl reducing enzymes in the metabolism of xenobiotics: well-known and promising members of the SDR superfamily." Drug Metab Rev **44**(2): 173-191.
Snyder, M. R., C. M. Weyand and J. J. Goronzy (2004). "The double life of NK receptors: stimulation or co-stimulation?" Trends in Immunology **25**(1): 25-32.
Sobottka, B., M. D. Harrer, U. Ziegler, K. Fischer, H. Wiendl, x00Fc, T. nig, B. Becher and N. Goebels (2009). "Collateral bystander damage by myelin-directed CD8+ T cells causes axonal loss." American Journal of Pathology **175**(3): 1160-1166.
Solana, R., R. Tarazona, I. Gayoso, O. Lesur, G. Dupuis and T. Fulop (2012). "Innate immunosenescence: effect of aging on cells and receptors of the innate immune system in humans." Seminars in immunology **24**(5): 331-341.
Stambergova, H., L. Skarydova, J. E. Dunford and V. Wsol (2014). "Biochemical properties of human dehydrogenase/reductase (SDR family) member 7." Chem Biol Interact **207**: 52-57.
Steel, J. C., T. A. Waldmann and J. C. Morris (2012). "Interleukin-15 biology and its therapeutic implications in cancer." Trends in Pharmacological Sciences **33**(1): 35-41.
Steiner, I., G. Rosenberg and I. Wirguin (2010). "Transient immunosuppression: a bridge between infection and the atypical autoimmunity of Guillain-Barre syndrome?" Clinical & Experimental Immunology **162**(1): 32-40.
Stewart, C. A., F. Laugier-Anfossi, F. Vely, X. Saulquin, J. Riedmuller, A. Tisserant, L. Gauthier, F. Romagne, G. Ferracci, F. A. Arosa, A. Moretta, P. D. Sun, S. Ugolini and E. Vivier (2005). "Recognition of peptide-MHC class I complexes by activating killer immunoglobulin-like receptors." Proceedings of the National Academy of Sciences of the United States of America **102**(37): 13224-13229.
Storey, E., M. Cook, R. Peppard, H. Newton-John and E. Byrne (1989). "Guillain Barre Syndrome and related conditions in Victorian teaching hospital 1980-1984." Australian and New Zealand Journal of Medicine **19**(6): 687-693.
Subramanian, S., K. Tus, Q. Z. Li, A. Wang, X. H. Tian, J. Zhou, C. Liang, G. Bartov, L. D. McDaniel, X. J. Zhou, R. A. Schultz and E. K. Wakeland (2006). "A Tlr7 translocation accelerates systemic autoimmunity in murine lupus." Proc Natl Acad Sci U S A **103**(26): 9970-9975.
Tang, F., B. Sally, C. Ciszewski, V. Abadie, S. A. Curran, V. Groh, O. Fitzgerald, R. J. Winchester and B. Jabri (2013). "Interleukin 15 Primes Natural Killer Cells to Kill via NKG2D and cPLA2 and This Pathway Is Active in Psoriatic Arthritis." PLoS One **8**(9): e76292.
Theodorou, P., V. Limmroth, W. Perbix, M. Gerbershagen, F. Knam and G. Spilker (2008). "Guillain-Barre syndrome after lightning strike." Burns **34**(5): 722-726.
Theofilopoulos, A. N., R. Gonzalez-Quintial, B. R. Lawson, Y. T. Koh, M. E. Stern, D. H. Kono, B. Beutler and R. Baccala (2010). "Sensors of the innate immune system: their link to rheumatic diseases." Nat Rev Rheumatol **6**(3): 146-156.
Thorsby, E. (2011). "On the future of HLA." Tissue Antigens **78**(4): 229-240.
Tokuhara, D., H. Hirokawa, T. Yamano and Y. Okano (2010). "Protein-losing gastropathy in a child and Guillain-Barre syndrome in a father caused by intrafamilial infection." Pediatrics International **52**(3): e167-170.
Touitou, I. and I. Kone-Paut (2008). "Autoinflammatory diseases." Best Pract Res Clin Rheumatol **22**(5): 811-829.
Trowsdale, J. (2001). "Genetic and functional relationships between MHC and NK receptor genes." Immunity **15**(3): 363-374.

Turner, S., L. L. Armstrong, Y. Bradford, C. S. Carlson, D. C. Crawford, A. T. Crenshaw, M. de Andrade, K. F. Doheny, J. L. Haines, G. Hayes, G. Jarvik, L. Jiang, I. J. Kullo, R. Li, H. Ling, T. A. Manolio, M. Matsumoto, C. A. McCarty, A. N. McDavid, D. B. Mirel, J. E. Paschall, E. W. Pugh, L. V. Rasmussen, R. A. Wilke, R. L. Zuvich and M. D. Ritchie (2011). Quality Control Procedures for Genome-Wide Association Studies. Current Protocols in Human Genetics, John Wiley & Sons, Inc.

Uncini, A. and S. Kuwabara (2012). "Electrodiagnostic criteria for Guillain-Barre syndrome: a critical revision and the need for an update." Clinical neurophysiology : official journal of the International Federation of Clinical Neurophysiology **123**(8): 1487-1495.

Uncini, A., C. Manzoli, F. Notturno and M. Capasso (2010). "Pitfalls in electrodiagnosis of Guillain–Barré syndrome subtypes." Journal of Neurology, Neurosurgery & Psychiatry **81**(10): 1157-1163.

Uncini, A., F. Notturno, M. Pace and C. M. Caporale (2011). "Polymorphism of CD1 and SH2D2A genes in inflammatory neuropathies." Journal of the Peripheral Nervous System **16 Suppl 1**: 48-51.

Van den Bergh, P. Y. K. and F. Pieret (2004). "Electrodiagnostic criteria for acute and chronic inflammatory demyelinating polyradiculoneuropathy." Muscle & Nerve **29**(4): 565-574.

van Doorn, P. A., L. Ruts and B. C. Jacobs (2008). "Clinical features, pathogenesis, and treatment of Guillain-Barré syndrome." Lancet Neurology **7**(10): 939-950.

van, D. P. A., G. M. Schreuder, M. Vermeulen, J. d'Amaro and A. Brand (1991). "HLA antigens in patients with chronic inflammatory demyelinating polyneuropathy." Journal of neuroimmunology **32**(2): 133-139.

Van Rhijn, I., L. H. Van den Berg, W. M. Bosboom, H. G. Otten and T. Logtenberg (2000). "Expression of accessory molecules for T-cell activation in peripheral nerve of patients with CIDP and vasculitic neuropathy." Brain **123**(Pt 10): 2020-2029.

Van, R. I., T. Logtenberg, C. W. Ang and d. B. L. H. Van (2003). "Gammadelta T cell non-responsiveness in Campylobacter jejuni-associated Guillain-Barre syndrome patients." Neurology **61**(7): 994-996.

van Sorge, N. M., W. L. van der Pol, M. D. Jansen, K. P. Geleijns, S. Kalmijn, R. A. Hughes, J. H. Rees, J. Pritchard, C. A. Vedeler, K. M. Myhr, C. Shaw, I. N. van Schaik, J. H. Wokke, P. A. van Doorn, B. C. Jacobs, J. G. van de Winkel and L. H. van den Berg (2005). "Severity of Guillain-Barre syndrome is associated with Fc gamma Receptor III polymorphisms." Journal of Neuroimmunology **162**(1-2): 157-164.

Vandenbroeck, K. (2012). "Cytokine gene polymorphisms and human autoimmune disease in the era of genome-wide association studies." J Interferon Cytokine Res **32**(4): 139-151.

Vedeler, C. A., G. Raknes, K. M. Myhr and H. Nyland (2000). "IgG Fc-receptor polymorphisms in Guillain-Barre syndrome." Neurology **55**(5): 705-707.

Velentgas, P., A. A. Amato, R. L. Bohn, K. A. Chan, T. Cochrane, D. P. Funch, I. Dashevsky, A. L. Duddy, P. Gladowski, S. A. Greenberg, J. M. Kramer, C. McMahill-Walraven, C. Nakasato, C. M. Spettell, B. L. Syat, P. M. Wahl, A. M. Walker, F. Zhang, J. S. Brown and R. Platt (2012). "Risk of Guillain-Barre syndrome after meningococcal conjugate vaccination." Pharmacoepidemiol Drug Saf **21**(12): 1350-1358.

Venstrom, J. M., T. A. Gooley, S. Spellman, J. Pring, M. Malkki, B. Dupont, E. Petersdorf and K. C. Hsu (2010). "Donor activating KIR3DS1 is associated with decreased acute GVHD in unrelated allogeneic hematopoietic stem cell transplantation." Blood **115**(15): 3162-3165.

Venter, J. C., M. D. Adams, E. W. Myers, P. W. Li, R. J. Mural, G. G. Sutton, H. O. Smith, M. Yandell, C. A. Evans, R. A. Holt, J. D. Gocayne, P. Amanatides, R. M. Ballew, D. H. Huson, J. R. Wortman, Q. Zhang, C. D. Kodira, X. H. Zheng, L. Chen, M. Skupski, G. Subramanian, P. D. Thomas, J. Zhang, M. G. L. Gabor, C. Nelson, S. Broder, A. G. Clark, J.

Nadeau, V. A. McKusick, N. Zinder, A. J. Levine, R. J. Roberts, M. Simon, C. Slayman, M. Hunkapiller, R. Bolanos, A. Delcher, I. Dew, D. Fasulo, M. Flanigan, L. Florea, A. Halpern, S. Hannenhalli, S. Kravitz, S. Levy, C. Mobarry, K. Reinert, K. Remington, J. Abu-Threideh, E. Beasley, K. Biddick, V. Bonazzi, R. Brandon, M. Cargill, I. Chandramouliswaran, R. Charlab, K. Chaturvedi, Z. Deng, F. V. Di, P. Dunn, K. Eilbeck, C. Evangelista, A. E. Gabrielian, W. Gan, W. Ge, F. Gong, Z. Gu, P. Guan, T. J. Heiman, M. E. Higgins, R. R. Ji, Z. Ke, K. A. Ketchum, Z. Lai, Y. Lei, Z. Li, J. Li, Y. Liang, X. Lin, F. Lu, G. V. Merkulov, N. Milshina, H. M. Moore, A. K. Naik, V. A. Narayan, B. Neelam, D. Nusskern, D. B. Rusch, S. Salzberg, W. Shao, B. Shue, J. Sun, Z. Wang, A. Wang, X. Wang, J. Wang, M. Wei, R. Wides, C. Xiao, C. Yan, A. Yao, J. Ye, M. Zhan, W. Zhang, H. Zhang, Q. Zhao, L. Zheng, F. Zhong, W. Zhong, S. Zhu, S. Zhao, D. Gilbert, S. Baumhueter, G. Spier, C. Carter, A. Cravchik, T. Woodage, F. Ali, H. An, A. Awe, D. Baldwin, H. Baden, M. Barnstead, I. Barrow, K. Beeson, D. Busam, A. Carver, A. Center, M. L. Cheng, L. Curry, S. Danaher, L. Davenport, R. Desilets, S. Dietz, K. Dodson, L. Doup, S. Ferriera, N. Garg, A. Gluecksmann, B. Hart, J. Haynes, C. Haynes, C. Heiner, S. Hladun, D. Hostin, J. Houck, T. Howland, C. Ibegwam, J. Johnson, F. Kalush, L. Kline, S. Koduru, A. Love, F. Mann, D. May, S. McCawley, T. McIntosh, I. McMullen, M. Moy, L. Moy, B. Murphy, K. Nelson, C. Pfannkoch, E. Pratts, V. Puri, H. Qureshi, M. Reardon, R. Rodriguez, Y. H. Rogers, D. Romblad, B. Ruhfel, R. Scott, C. Sitter, M. Smallwood, E. Stewart, R. Strong, E. Suh, R. Thomas, N. N. Tint, S. Tse, C. Vech, G. Wang, J. Wetter, S. Williams, M. Williams, S. Windsor, E. Winn-Deen, K. Wolfe, J. Zaveri, K. Zaveri, J. F. Abril, R. Guigo, M. J. Campbell, K. V. Sjolander, B. Karlak, A. Kejariwal, H. Mi, B. Lazareva, T. Hatton, A. Narechania, K. Diemer, A. Muruganujan, N. Guo, S. Sato, V. Bafna, S. Istrail, R. Lippert, R. Schwartz, B. Walenz, S. Yooseph, D. Allen, A. Basu, J. Baxendale, L. Blick, M. Caminha, J. Carnes-Stine, P. Caulk, Y. H. Chiang, M. Coyne, C. Dahlke, A. Mays, M. Dombroski, M. Donnelly, D. Ely, S. Esparham, C. Fosler, H. Gire, S. Glanowski, K. Glasser, A. Glodek, M. Gorokhov, K. Graham, B. Gropman, M. Harris, J. Heil, S. Henderson, J. Hoover, D. Jennings, C. Jordan, J. Jordan, J. Kasha, L. Kagan, C. Kraft, A. Levitsky, M. Lewis, X. Liu, J. Lopez, D. Ma, W. Majoros, J. McDaniel, S. Murphy, M. Newman, T. Nguyen, N. Nguyen, M. Nodell, S. Pan, J. Peck, M. Peterson, W. Rowe, R. Sanders, J. Scott, M. Simpson, T. Smith, A. Sprague, T. Stockwell, R. Turner, E. Venter, M. Wang, M. Wen, D. Wu, M. Wu, A. Xia, A. Zandieh and X. Zhu (2001). "The sequence of the human genome." Science **291**(5507): 1304-1351.

Vierbuchen, T., A. Ostermeier, Z. P. Pang, Y. Kokubu, T. C. Sudhof and M. Wernig (2010). "Direct conversion of fibroblasts to functional neurons by defined factors." Nature **463**(7284): 1035-1041.

Vital, A., C. Vital, A. Lagueny, X. Ferrer, Ribi, x00E, C. re-Bachelier, P. Latour and K. G. Petry (2003). "Inflammatory demyelination in a patient with CMT1A." Muscle & Nerve **28**(3): 373-376.

Vivier, E., D. H. Raulet, A. Moretta, M. A. Caligiuri, L. Zitvogel, L. L. Lanier, W. M. Yokoyama and S. Ugolini (2011). "Innate or adaptive immunity? The example of natural killer cells." Science **331**(6013): 44-49.

Voelter-Mahlknecht, S., A. D. Ho and U. Mahlknecht (2005). "FISH-mapping and genomic organization of the NAD-dependent histone deacetylase gene, Sirtuin 2 (Sirt2)." Int J Oncol **27**(5): 1187-1196.

Vucic, S., M. C. Kiernan and D. R. Cornblath (2009). "Guillain-Barre syndrome: an update." Journal of Clinical Neuroscience **16**(6): 733-741.

Wall, J. D. and J. K. Pritchard (2003). "Haplotype blocks and linkage disequilibrium in the human genome." Nat Rev Genet **4**(8): 587-597.

Wang, S., W. Kang, M. Y. Go, J. H. Tong, L. Li, N. Zhang, Q. Tao, X. Li, K. F. To, J. J. Sung and J. Yu (2012). "Dapper homolog 1 is a novel tumor suppressor in gastric cancer through inhibiting the nuclear factor-kappaB signaling pathway." Mol Med **18**: 1402-1411.
Wang, T., Z. Zeng, T. Li, J. Liu, J. Li, Y. Li, Q. Zhao, Z. Wei, Y. Wang, B. Li, G. Feng, L. He and Y. Shi (2010). "Common SNPs in myelin transcription factor 1-like (MYT1L): association with major depressive disorder in the Chinese Han population." PLoS One **5**(10): e13662.
Weber, F., R. Rudel, P. Aulkemeyer and H. Brinkmeier (2000). "Anti-GM1 antibodies can block neuronal voltage-gated sodium channels." Muscle & Nerve **23**(9): 1414-1420.
Willison, H. J. and N. Yuki (2002). "Peripheral neuropathies and anti-glycolipid antibodies." Brain **125**(Pt 12): 2591-2625.
Wilmshurst, J. M., K. R. Pohl, R. W. Vaughan and R. A. Hughes (1999). "Familial Guillain-Barre syndrome." European Journal of Neurology **6**(4): 499-503.
Winer, J. B. (2001). "Guillain Barre syndrome." Mol Pathol **54**(6): 381-385.
Winer, J. B., D. Briggs, K. Welsh and R. A. Hughes (1988). "HLA antigens in the Guillain-Barre syndrome." J Neuroimmunol **18**(1): 13-16.
Winklmeier, A., V. Contreras-Shannon, S. Arndt, C. Melle and A. K. Bosserhoff (2009). "Cadherin-7 interacts with melanoma inhibitory activity protein and negatively modulates melanoma cell migration." Cancer Sci **100**(2): 261-268.
Woronicz, J. D., X. Gao, Z. Cao, M. Rothe and D. V. Goeddel (1997). "IκB Kinase-β: NF-κB Activation and Complex Formation with IκB Kinase-α and NIK." Science **278**(5339): 866-870.
Wu, L. Y., Y. Zhou, C. Qin and B. L. Hu (2012). "The effect of TNF-alpha, FcgammaR and CD1 polymorphisms on Guillain-Barre syndrome risk: evidences from a meta-analysis." J Neuroimmunol **243**(1-2): 18-24.
Wu, L. Y., Y. Zhou, C. Qin and B. L. Hu (2012). "The effect of TNF-alpha, FcR and CD1 polymorphisms on Guillain-Barre syndrome risk: evidences from a meta-analysis." Journal of Neuroimmunology **243**(1-2): 18-24.
Yamakawa, T., K. Yamada, T. Sasamura, N. Nakazawa, M. Kanai, E. Suzuki, M. E. Fortini and K. Matsuno (2012). "Deficient Notch signaling associated with neurogenic pecanex is compensated for by the unfolded protein response in Drosophila." Development **139**(3): 558-567.
Yan, W. X., J. J. Archelos, H. P. Hartung and J. D. Pollard (2001). "P0 protein is a target antigen in chronic inflammatory demyelinating polyradiculoneuropathy." Annals of Neurology **50**(3): 286-292.
Yawata, M., N. Yawata, L. Abi-Rached and P. Parham (2002). "Variation within the human killer cell immunoglobulin-like receptor (KIR) gene family." Critical Reviews in Immunology **22**(5-6): 463-482.
Ye, Y., K. Wang, F. Deng and Y. Xing (2013). "Electrophysiological subtypes and prognosis of Guillain-Barre syndrome in Northeastern China." Muscle & Nerve **47**(1): 68-71.
Ye, Y., D. Zhu, K. Wang, J. Wu, J. Feng, D. Ma, Y. Xing and X. Jiang (2010). "Clinical and electrophysiological features of the 2007 Guillain-Barre syndrome epidemic in northeast China." Muscle Nerve **42**(3): 311-314.
Yokogawa, T., N. Shimada, N. Takeuchi, L. Benkowski, T. Suzuki, A. Omori, T. Ueda, K. Nishikawa, L. L. Spremulli and K. Watanabe (2000). "Characterization and tRNA recognition of mammalian mitochondrial seryl-tRNA synthetase." J Biol Chem **275**(26): 19913-19920.
Yokoyama, S., N. Watanabe, N. Sato, P.-Y. Perera, L. Filkoski, T. Tanaka, M. Miyasaka, T. A. Waldmann, T. Hiroi and L. P. Perera (2009). "Antibody-mediated blockade of IL-15

reverses the autoimmune intestinal damage in transgenic mice that overexpress IL-15 in enterocytes." Proceedings of the National Academy of Sciences **106**(37): 15849-15854.
Yoo, A. S., A. X. Sun, L. Li, A. Shcheglovitov, T. Portmann, Y. Li, C. Lee-Messer, R. E. Dolmetsch, R. W. Tsien and G. R. Crabtree (2011). "MicroRNA-mediated conversion of human fibroblasts to neurons." Nature **476**(7359): 228-231.
Yoshii, F. and Y. Shinohara (1998). "Natural killer cells in patients with Guillain-Barre syndrome." Journal of the Neurological Sciences **157**(2): 175-178.
Yuki, N., S. Handa, T. Tai, M. Takahashi, K. Saito, Y. Tsujino and T. Taki (1995). "Ganglioside-like epitopes of lipopolysaccharides from Campylobacter jejuni (PEN 19) in three isolates from patients with Guillain-Barre syndrome." J Neurol Sci **130**(1): 112-116.
Yuki, N., Y. Ichihashi and T. Taki (1995). "Subclass of IgG antibody to GM1 epitope-bearing lipopolysaccharide of Campylobacter jejuni in patients with Guillain-Barre syndrome." J Neuroimmunol **60**(1-2): 161-164.
Yuki, N. and S. Kuwabara (2007). "Axonal Guillain-Barre syndrome: carbohydrate mimicry and pathophysiology." Journal of the Peripheral Nervous System **12**(4): 238-249.
Yuki, N., S. Kuwabara, M. Koga and K. Hirata (1999). "Acute motor axonal neuropathy and acute motor-sensory axonal neuropathy share a common immunological profile." J Neurol Sci **168**(2): 121-126.
Yuki, N. and Y. Tsujino (1995). "Familial Guillain-Barre syndrome subsequent to Campylobacter jejuni enteritis." J Pediatr **126**(1): 162.
Yuki, N., M. Yamada, M. Koga, M. Odaka, K. Susuki, Y. Tagawa, S. Ueda, T. Kasama, A. Ohnishi, S. Hayashi, H. Takahashi, M. Kamijo and K. Hirata (2001). "Animal model of axonal Guillain-Barre syndrome induced by sensitization with GM1 ganglioside." Annals of Neurology **49**(6): 712-720.
Zanoni, I. and F. Granucci (2013). "Role of CD14 in host protection against infections and in metabolism regulation." Front Cell Infect Microbiol **3**: 32.
Zhang, J., H. Dong, B. Li, C. Y. Li and L. Guo (2007). "Association of tumor necrosis factor polymorphisms with Guillain-Barre syndrome." Eur.Neurol. **58**(1): 21-25.
Zhang, L., X. Gao, J. Wen, Y. Ning and Y. G. Chen (2006). "Dapper 1 antagonizes Wnt signaling by promoting dishevelled degradation." The Journal of biological chemistry **281**(13): 8607-8612.
Zhu, W., E. Mix, I. Nennesmo, A. Adem and J. Zhu (2004). "Anti-cytokine autoantibodies in experimental autoimmune neuritis in Lewis rats." Experimental Neurology **190**(2): 486-494.
Zhu, W., E. Mix and J. Zhu (2003). "Inflammation and proinflammatory cytokine production, but no demyelination of facial nerves, in experimental autoimmune neuritis in Lewis rats." Journal of Neuroimmunology **140**(1-2): 97-101.
Zidan, H. E., N. A. Sabbah, H. A. Hagrass, E. A. Tantawy, E. E. El-Shahawy, G. S. Nageeb and A. B. Abdul-Sattar (2013). "Association of FcgammaRIIB and FcgammaRIIA R131H gene polymorphisms with renal involvement in Egyptian systemic lupus erythematosus patients." Mol Biol Rep.
Zingoni, A., M. Ardolino, A. Santoni and C. Cerboni (2012). "NKG2D and DNAM-1 activating receptors and their ligands in NK-T cell interactions: role in the NK cell-mediated negative regulation of T cell responses." Frontiers in immunology **3**: 408.
Zingoni, A., T. Sornasse, B. G. Cocks, Y. Tanaka, A. Santoni and L. L. Lanier (2005). "NK cell regulation of T cell-mediated responses." Molecular immunology **42**(4): 451-454.
Zondervan, K. T. and L. R. Cardon (2004). "The complex interplay among factors that influence allelic association." Nat Rev Genet **5**(2): 89-100.