Journal of Neuroimmunology 261 (2013) 92–97

Contents lists available at SciVerse ScienceDirect

Journal of Neuroimmunology

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

The influence of non-HLA gene polymorphisms and interactions on disease risk in a Western Australian multiple sclerosis cohort

Wei Qiu [a,b,c,d], Kym Pham [b,c,d], Ian James [e,f], David Nolan [g], Allison Castley [g], Frank T. Christiansen [g,h], Petra Czarniak [i], Yuebei Luo [b,c,d], Jingshan Wu [b,c,d], Michael Garlepp [i], Steve Wilton [b,c,d], William M. Carroll [b,c,d], Frank L. Mastaglia [b,c,d], Allan G. Kermode [b,c,d,j,*]

[a] Department of Neurology, The Third Affiliated Hospital of Sun Yat-sen University, Guangzhou, China
[b] Centre for Neuromuscular and Neurological Disorders, University of Western Australia, Australia
[c] Department of Neurology, Sir Charles Gairdner Hospital, Queen Elizabeth II Medical Centre, Perth, Western Australia, Australia
[d] Demyelinating Diseases Centre, Australian Neuro-Muscular Research Institute, Queen Elizabeth II Medical Centre, Perth, Western Australia, Australia
[e] Centre for Clinical Immunology & Biomedical Statistics, Murdoch University, Australia
[f] Royal Perth Hospital, Perth, Western Australia, Australia
[g] Department of Clinical Immunology and Immunogenetics, Royal Perth Hospital, Perth, Western Australia, Australia
[h] School of Pathology and Laboratory Medicine, University of Western Australia, Perth, Western Australia, Australia
[i] School of Pharmacy, Curtin University, Perth, Western Australia, Australia
[j] Institute of Immunology and Infectious Diseases, Murdoch University, Western Australia, Australia

ARTICLE INFO

Article history:
Received 19 August 2012
Received in revised form 17 March 2013
Accepted 30 April 2013

Keywords:
Multiple sclerosis
Non-HLA genes
Single nucleotide polymorphisms
HLA

ABSTRACT

Non-Human Leukocyte Antigen (HLA) genes have concomitant, although modest, effects on multiple sclerosis (MS) susceptibility; however findings have varied in different populations. Here we present the results of an association study of 16 single nucleotide polymorphisms (SNPs) in 10 non-HLA genes (*IL7R*, *IL2RA*, *CLEC-16A*, *TYK2*, *CD58*, *IRF5*, *STAT3*, *CTLA-4*, *APOE*, *ICAM-1*) in a Western Australian cohort of 350 MS patients and 498 population control subjects. Our results indicate that in this population, SNPs in *IL7R*, *TYK2*, *IRF5* and *APOE* have modifying effects on MS susceptibility. We also found evidence of interactive protective effects between polymorphisms in the *IL7R*/*CD58*, *CLEC-16A*/*CTLA-4*, and *TYK2*/*IRF5* genes, which in some instances are restricted within HLA- or gender-defined groups.

1. Introduction

Multiple sclerosis (MS) is a common immune-related neurological disease characterized by myelin loss, axonal damage, and progressive neurological dysfunction. The causes of MS are largely unknown; however it is clear that multiple genetic and environmental components play an important role in this complex disease.

Within the past six years several independent genome wide association studies (GWAS) in MS have identified association with single-nucleotide polymorphisms (SNPs) in at least 23 different non-Human Leukocyte Antigen (HLA) genes with modest effect size and odds ratio (OR) ranging from 1.0 to 1.3 (Hafler et al., 2007; ANZgene, 2009; Baranzini et al., 2009; WTCCC, 2011). The few identified non-HLA alleles are common in the general population. It is well-established that as supplements to HLA genes, the multiple non-HLA loci make concomitant contributions to MS susceptibility, and are part of new pathways with biological effect involved in the pathogenesis of this multifactorial disease. However, investigation of non-HLA genes has progressed relatively slowly due to their lower level effects as well as complicated underlying allelic interactions among them or with epigenetic factors. Meanwhile, the function of most of the non-HLA candidate genes in the pathogenesis of MS still remains unclear.

Compared to HLA genes, findings on non-HLA genes have varied across different populations and studies. Even the findings in GWAS are not consistent in all the studies, possibly due to the minimal contribution by some alleles, or rare variants other than common variants, as well as epigenetic modifications that cannot be detected. For example, the rs6498169 SNP in *CLEC-16A* was suggested to be disease-associated by the International Multiple Sclerosis Genetics Consortium (IMSGC) (Hafler et al., 2007), but was not verified in the Australia and New Zealand Multiple Sclerosis Genetics Consortium (ANZgene) study (ANZgene, 2009). Despite the success of GWAS in identifying modest-effect genes, a few SNP-based candidate gene studies have also been

Abbreviations: *APOE*, Apolipoprotein E; *CLEC-16A*, C-type lectin domain family 16; *CTLA-4*, Cytotoxic T-lymphocyte antigen 4; *ICAM-1*, Intercellular adhesion molecule 1; *IL2RA*, Interleukin 2 receptor a; *IL7R*, Interleukin 7 receptor; *IRF5*, Interferon regulatory factor 5; *STAT3*, Signal transducer and activator of transcription 3; *TYK2*, Tyrosine kinase 2.

* Corresponding author at: Australian Neuromuscular Research Institute, Sir Charles Gairdner Hospital, 6009 Perth, WA, Australia. Tel.: +61 8 93881865; fax: +61 8 93882149.
E-mail address: kermode@me.com (A.G. Kermode).

0165-5728/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jneuroim.2013.04.022

Table 1
Demographic data of MS patients and controls.

	MS cases (n = 350)	Controls (n = 498)
Gender (F/M)	268/82	288/210
Age (years)	48.5 ± 12.4	52.0 ± 4.1
Disease type (bout-onset/chronic-onset)	332/18	–
Age at onset (years)	36.1 ± 11.3	–
Disease duration (years)	12.4 ± 9.7	–
EDSS	3.2 ± 2.3	–

conducted to validate the data from GWAS (Rubio et al., 2008; Hoppenbrouwers et al., 2009; Perera et al., 2009). For example, two SNPs (rs2104286 and rs12722489) in the *IL2RA* gene were first reported to be associated with MS by IMSGC (Hafler et al., 2007; IMSGC, 2008), and subsequently the novel rs791589 SNP in *IL2RA* was also shown to be associated in a SNP-based candidate gene study by the Australian Tasmanian group (Perera et al., 2009). Therefore, additional studies in different populations may be helpful in elucidating the contribution of non-HLA loci.

Recently, our group found that in a Western Australian MS patient cohort polymorphism at the HLA-DRB1 locus has modifying effects both in disease susceptibility and the clinical phenotype, and confirmed the contribution of diplotype interactions at the HLA-DRB1 locus on disease risk (Wu et al., 2010). To more fully characterize the genetic contribution to susceptibility in this population we have now investigated the influence of the following non-HLA genes: *IL7R*, *IL2RA*, *CLEC-16A*, *TYK2*, *CD58*, *IRF5*, *STAT3*, *CTLA-4*, *APOE*, and *ICAM-1* in this patient cohort. The rationale for selecting these genes for analysis is based on the following evidence: (i) Six of these genes have previously been found to be associated with MS in GWAS or case–control studies: *IL7R* (Gregory et al., 2007; Lundmark et al., 2007; ANZgene, 2009), *IL2RA* (Hafler et al., 2007; ANZgene, 2009), *CLEC-16A* (IMSGC, 2008), *TYK2* (ANZgene, 2009; Ban et al., 2009), *CD58* (Hafler et al., 2007; Rubio et al., 2008; ANZgene, 2009), *STAT3* (Jakkula et al., 2010); *IRF5* from a case–control study with a large sample size (Kristjansdottir et al., 2008); (ii) Genes which have previously been suggested to be linked with MS pathogenesis: ICAM-1 which mediates a key function in T lymphocyte migration across the blood–brain barrier (Steiner et al., 2010), and CTLA-4 which is biologically important in T lymphocyte activation (McCoy and Le Gros, 1999), both of which are early events in the pathogenesis of MS. *APOE* has a key role in lipid transport in the nervous system and is known to be involved in neuronal maintenance and repair and neuroplasticity, and has also been implicated in neuroinflammation and neurodegeneration (Huang et al., 2004; Verghese et al., 2011). The selection of the individual SNPs used in this study was based on previously reported associations with MS or other diseases. We aimed to address the genetic impact of polymorphisms in these non-HLA genes in our MS cohort using an SNP-based candidate gene approach, and to investigate epistatic interactions between non-HLA and HLA loci.

2. Patients and methods

2.1. Patients

Three hundred and fifty consecutive Caucasian MS patients from the Perth Demyelinating Diseases Database who had undergone high-resolution HLA-DRB1 genotyping were enrolled in the study. All were diagnosed with clinically definite MS according to the Poser criteria (Poser et al., 1983) or McDonald criteria (McDonald et al., 2001). The patients were classified as bout-onset MS (relapsing remitting/secondary progressive) or chronic-onset MS (primary progressive/progressive relapsing). Healthy Caucasoid subjects from the Western Australian branch of the Australian Bone Marrow Donor Registry (ABMDR) were used as controls. The demographic data are shown in Table 1. This study was approved by the Sir Charles Gairdner Hospital Human Research Ethics Committee and Board of the ABMDR and informed consent was obtained from all the participants.

2.2. Genotyping

Sixteen SNPs in ten genes (Table 2) were genotyped. DNA extractions were performed from whole blood on the Biorobot M48 instrument using the MagAttract DNA blood M48 Mini kit (Qiagen). The HLA-DRB1 typing of the patients and controls was performed using a previously reported sequencing based method (Sayer et al., 2001). SNPs were genotyped using TaqMan allelic discrimination assay performed in accordance to the manufacturer's instructions on the ABI7900HT (Applied Biosystems Life Technologies) using 384 well plates at the Australian Neurological Research Institute. Allelic discrimination analysis was performed using SDS 2.3 software.

Table 2
Case–control comparison of allelic frequencies in the Western Australia MS cohort.

Genes	SNPs	Major[a]/minor allele	Allele carriage frequency		*P* value[b]	OR (95% CI)
			MS cases (n = 350)	Controls (n = 498)		
IL7R	rs6897932	C[#]/T	329/349	458/497	0.27	1.4 (0.78–2.6)
	rs3194051	A[#]/G	332/347	448/486	0.04*	1.9 (0.99–3.7)
IL2RA	rs12722489	C/T[#]	91/341	137/494	0.75	0.95 (0.69–1.3)
	rs2104286	T[#]/C	326/347	465/498	0.78	1.1 (0.61–2.0)
CLEC-16A	rs6498146	C/T[#]	116/348	185/495	0.24	0.84 (0.62–1.1)
TYK2	rs34536443	G/C[#]	24/349	52/498	0.09	0.63 (0.37–1.1)
CD58	rs2300747	A[#]/G	344/349	487/496	0.79	1.3 (0.38–4.9)
	rs12044852	C/A[#]	54/350	86/496	0.51	0.87 (0.59–1.3)
IRF5	rs4728142	G[#]/A	267/348	413/497	0.02*	0.67 (0.47–0.96)
	rs3807306	G[#]/T	233/325	382/496	0.10	0.76 (0.54–1.1)
STAT3	rs744166	A/G[#]	239/341	337/494	0.59	1.1 (0.80–1.5)
APOE	rs429358	T[#]/C	338/349	486/497	0.51	0.70 (0.27–1.8)
	rs7412	C/T[#]	69/332	67/498	0.007*	1.7 (1.1–2.5)
ICAM-1	rs5498	A/G[#]	240/348	318/495	0.16	1.2 (0.91–1.7)
CTLA-4	rs231775	A/G[#]	209/348	316/498	0.35	0.87 (0.65–1.2)
	rs5742909	C/T[#]	61/339	59/492	0.02*	1.6 (1.1–2.4)

[#] Denotes the more significant allele (risk or protective) which is calculated for *P* value.
[a] Major allele is the more frequent one of the two alleles in the combined cases and controls.
[b] *P* value is calculated without any adjustment.
* denotes $P < 0.05$