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Short communication

Analysis of HLA-DRB3 alleles and supertypical genotypes in the MHC Class II region in sporadic inclusion body myositis

Arada Rojana-udomsart [a,b], Chalermchai Mitrpant [a,c], Ian James [d], Campbell Witt [e], Merrilee Needham [f,g], Timothy Day [h,i], Lynette Kiers [h,i], Alastair Corbett [j], Patricia Martinez [e], Steve D. Wilton [a], Frank L. Mastaglia [a,*]

[a] Australian Neuro-muscular Research Institute and Centre for Neuromuscular and Neurological disorders, The University of Western Australia, Queen Elizabeth II Medical Centre, Perth, Western Australia, Australia
[b] Department of Medicine, Yala Hospital, Yala, Thailand
[c] Department of Biochemistry, Faculty of Medicine, Siriraj Hospital, Mahidol University, Bangkok, Thailand
[d] Centre for Clinical Immunology & Biomedical Statistics, Institute for Immunology & Infectious Diseases, Murdoch University & Royal Perth Hospital, Western Australia, Australia
[e] Department of Clinical Immunology, PathWest, Laboratory Medicine, Royal Perth Hospital, Perth, Western Australia, Australia
[f] Department of Neurology, Royal North Shore Hospital, St Leonards, NSW, Australia
[g] Department of Medicine, Northern Clinical School, University of Sydney, Sydney, NSW, Australia
[h] Department of Neurology & Neurophysiology, Royal Melbourne Hospital, Parkville, Victoria, Australia
[i] Department of Medicine, University of Melbourne, Parkville, Victoria, Australia
[j] Department of Neurology, Concord Hospital, Concord, NSW, Australia

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ABSTRACT

We compared the carriage frequencies of HLA-DRB3 and its major alleles and of HLA-DRB4 and HLA-DRB5 in an Australian sIBM cohort and a population control group who had previously been genotyped for the HLA-DRB1*03:01 risk allele. There was a strong disease association with the carriage of the DRB3*01:01 allele which was accounted for by its linkage disequilibrium with DRB1*03:01. The carriage of HLA-DRB4 was found to be strongly protective and abrogated the risk effect of HLA-DRB1*03:01. The findings indicate that haplotypic combinations of alleles at the HLA-DRB1 and secondary HLA-DRB loci have important risk modifying effects in sIBM.

1. Introduction

Sporadic inclusion body myositis (sIBM) is a late-onset myopathy with a well-defined clinical phenotype and a combination of inflammatory and myodegenerative features (Dalakas, 2006; Needham and Mastaglia, 2007). Its etiopathogenesis remains elusive (Needham and Mastaglia, 2008) and has been the subject of ongoing investigation (Askanas and Engel, 2008; Dalakas, 2008; Schmidt et al., 2008; Greenberg, 2011; Hohlfeld, 2011).

Genetic factors contribute to susceptibility (Needham et al., 2007). In Caucasians, sIBM is strongly associated with HLA-DRB1*03:01 and the 8.1 ancestral haplotype (AH) characterised by HLA-A*01:01, HLA-B*08:01, HLA-DRB1*03:01, HLA-DRB3*01:01 and HLA-DQA1*05:01 (Garlepp et al., 1994; Lampe et al., 2003; Badrising et al., 2004; O'Hanlon et al., 2005; Needham et al., 2008; Mastaglia et al., 2009; Rojana-udomsart et al., 2012). Price et al. (2004) found that the carriage of HLA-DR3 (DRB1*03:01) without other 8.1 AH alleles was less frequent in sIBM than in controls, and suggested that the risk allele may be in linkage disequilibrium with DRB1*03:01. A recombinant mapping study by Scott et al. (2011) localised the susceptibility region to a 172 kb segment in the Class II MHC region adjacent to the HLA-DRB1 locus and encompassing the HLA-DRA and HLA-DRB3 genes which encode the α and β subunits of the HLA-DR molecules. Whereas HLA-DRA is highly conserved and has not been directly associated with any disease, HLA-DRB3 is polymorphic (Marsh, 2005) and its DRB3*01:01 allele is carried on the 8.1 AH (Price et al., 1999).

In this study we investigated the role of HLA-DRB3 in sIBM susceptibility by comparing its carriage frequency and that of its major alleles DRB3*01:01, DRB3*02:02 and DRB3*03:01 in an Australian sIBM cohort and an ethnically-matched population control group. We also analysed the carriage frequency of HLA-DRB4 and HLA-DRB5 which, like DRB3, may be expressed in individuals with a second DRB locus and encode the Class II supertypes HLA-DR53, HLA-DR51 and HLA-DR52 (Dorak et al., 2002).

* Corresponding author. Tel.: +61 893461611; fax: +61 893463487.
E-mail address: francis.mastaglia@anri.uwa.edu.au (F.L. Mastaglia).

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2. Subjects and methods

2.1. Subjects

Seventy four Caucasian sIBM patients (45 males), who fulfilled the clinical and biopsy criteria for definite or probable sIBM (Griggs et al., 1995; Mastaglia and Phillips, 2002), were included. All had been included in our previous HLA-DRB1 genotyping study (Rojana-udomsart et al., 2012). The age-at-onset was 37–63 years (mean 60.3 ± 9.4 years); disease duration was 8.7 ± 5.1 years. The mean modified Rankin Score at presentation was 1.4 ± 0.5. Only two patients were confined to a wheelchair after intervals of 21 and 26 years. The controls comprised 189 randomly selected community dwelling Caucasians from the Busselton Community Health Study (Welborn, 1998). The study was approved by the Sir Charles Gairdner Hospital Human Ethics Committee (Approval Number 2006–073).

2.2. HLA genotyping

High-resolution HLA-DRB3 and HLA-DRB1 genotyping was performed in the PathWest Clinical Immunology Laboratories at Royal Perth Hospital by DNA sequencing as described previously (Rojana-udomsart et al., 2012). To confirm the presence or absence of DRB3, DRB4, DRB5 in the sIBM cohort a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique was used (Ishihara et al., 1995). The carriage of HLA-DRB4 and HLA-DRB5 was determined by the presence or absence of amplified HLA-DRB4 or HLA-DRB5 specific amplicons. Homozygosity for the alleles of HLA-DRB3, DRB4 and DRB5 was considered to be present if there were no other DRB3, 4 or 5 alleles and the DRB1 alleles did not include DRB1*01, DRB1*08 or DRB1*10, in which case the second expressed DRB allele would only be present in single copy (Dorak et al., 2002).

2.3. Statistical analysis

Comparisons of carriage frequencies for alleles, haplotypes and diplotypes between cases and controls were analysed by Pearson's chi-square and Fisher exact tests. Case–control logistic regression was used for multivariable comparisons. Two-sided p values <0.05 were considered significant. Analyses were carried out using the IBM SPSS statistics 20 package (IBM, New York, USA) and TIBCO Spotfire S+ ver. 8.2 (TIBCO Software, Inc., Palo Alto, California).

3. Results

3.1. HLA-DRB3, HLA-DRB4 and HLA-DRB5

The carriage frequencies for HLA-DRB3, DRB4 and DRB5 are given in Table 1. The majority of sIBM patients and controls carried a second expressed HLA-DRB gene (95.9% vs 98.4%). In sIBM this was usually HLA-DRB3, which was higher in frequency than in controls. In contrast, the frequency of HLA-DRB4 was significantly lower in sIBM. Homozygosity for HLA-DRB3 was more frequent in sIBM than in controls (32.4% vs 12.7%, $p<0.001$), while HLA-DRB3 + DRB4 heterozygotes were less frequent (6.8% vs 29.6%, $p<0.001$). Joint case–control logistic regression analysis including HLA-DRB3, DRB4, DRB5 and the alleles of HLA-DRB3 confirmed that HLA-DRB4 was strongly protective ($p=0.0000005$), while HLA-DRB5 was also marginally protective ($p=0.02$). There were no differences in disease severity or clinical course between HLA-DRB4 carriers and non-carriers.

3.2. Frequency of HLA-DRB3 alleles and diplotypes

As shown in Table 1, HLA-DRB3*01:01 was the most frequent allele in the sIBM group and was a significant risk factor in joint analyses ($p=0.013$). The most common allele in the control group was HLA-DRB3*02:02, and the least common allele in both groups was HLA-DRB3*03:01. When considering allele combinations, those involving HLA-DRB3*01:01 were more common in the patient group. Homozygosity for HLA-DRB3*01:01 was more frequent in the sIBM group than in the controls and the HLA-DRB3*01:01 + 02:02 diplotype was also significantly associated with disease.

3.3. Joint analyses with HLA-DRB1*0301

The carriage frequency of HLA-DRB1*03:01 was higher in the sIBM group than in the controls (73% vs 23.3%, $p<0.001$) and was not significantly higher than the carriage of HLA-DRB3*01:01 (68.9%, $p=0.37$, McNemar test). After adjusting for the carriage of HLA-DRB1*03:01 in joint analyses, the association with HLA-DRB3*01:01 in the sIBM group was no longer significant ($p=0.62$), suggesting that the effect of HLA-DRB3*01:01 is due to its linkage disequilibrium with HLA-DRB1*03:01. HLA-DRB4 retained its protective significance in the joint model ($p=0.00015$). HLA-DRB1*03:01 ($p=0.0000004$, OR 5.47) and HLA-DRB4 ($p=0.0001$, OR 0.29) were jointly independently significant with no significant interaction between the two ($p=0.23$). The effects of HLA-DRB1*03:01 and HLA-DRB4 were estimated to be of almost equal but opposite size, the carriage of both HLA-DRB4 and HLA-DRB1*03:01

Table 1
Carriage frequencies of secondary HLA-DRB loci and HLA-DRB3 alleles and diplotypes.

HLA-	Case carriage		Control carriage		Odds ratio (95%CI)		p-Value
	Frequency	% (n)	Frequency	% (n)			
DRB3	82.4	(61)	60.3	(114)	3.09	(1.54, 6.54)	<0.001[a]
DRB3*01:01	68.9	(51)	24.9	(47)	6.70	(3.56, 12.70)	<0.001[a]
DRB3*02:01	27.0	(20)	33.3	(63)	0.74	(0.39, 1.39)	0.38
DRB3*03:01	5.4	(4)	10.1	(19)	0.51	(0.12, 1.62)	0.33
DRB4	17.6	(13)	61.9	(117)	0.13	(0.06, 0.26)	<0.001[a]
DRB5	16.2	(12)	23.3	(44)	0.64	(0.29, 1.34)	0.24
HLA-DRB3 diplotypes							
DRB3*01:01 + 01:01	12.2	9	3.2	6	4.22	(1.28, 14.92)	0.014[a]
DRB3*01:01 + 02:02	14.9	11	5.8	11	2.83	(1.05, 7.55)	0.025[a]
DRB3*01:01 + 03:01	4.1	3	1.1	2	3.95	(0.44, 47.92)	0.14
DRB3*02:02 + 02:02	1.4	1	1.6	3	0.85	(0.02, 10.78)	1
DRB3*02:02 + 03:01	0	0	1.1	2	0	(0.00, 13.64)	1
DRB3*03:01 + 03:01	0	0	0	0			

[a] Indicates statistical significance.