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Contributions of vitamin D response elements and HLA promoters to multiple sclerosis risk

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ABSTRACT

Objective: The identification of a vitamin D–responsive (VDRE) motif within the HLA-DRB1*15:01 promoter region provides an attractive explanation for the combined effects of HLA-DR inheritance and vitamin D exposure on multiple sclerosis (MS) risk. We therefore sought to incorporate HLA-DRB1 promoter variation, including the VDRE motif, in an assessment of HLA-DRB1–associated MS risk.

Methods: We utilized 32 homozygous HLA cell lines (covering 17 DRB1 alleles) and 53 heterozygote MS samples (20 DRB1 alleles) for HLA-DRB1 promoter sequencing. The influence of HLA-DRB1 variation on MS risk was then assessed among 466 MS cases and 498 controls.

Results: The majority of HLA*DRB1 alleles (including HLA-DRB1*15:01) express the functional VDRE motif, apart from HLA-DRB1*04, *07, and *09 alleles that comprise the HLA-DR53 serologic group. Allele-specific variation within functional X-box and Y-box motifs was also associated with serologically defined HLA-DR haplotypes. Incorporating these results in an analysis of MS risk, we identified a strong protective effect of HLA-DRB1*04, *07, and *09 (DR53) alleles ($p = 10^{-12}$) and elevated risk associated with DRB1*15 and *16 (DR51) and *08 (DR8) alleles ($p < 10^{-18}$).

Conclusions: HLA-DRB1 groups corresponding to serologic HLA-DR profiles as well as promoter polymorphism haplotypes effectively stratified MS risk over an 11-fold range, suggesting functional relationships between risk-modifying HLA-DRB1 alleles. An independent contribution of VDRE motif variation to increase MS risk was not discernible, although vitamin D–dependent regulation of HLA-DR expression may still play an important role given that HLA-DRB1*04/*07/*09 (DR53) alleles that express the "nonresponsive" VDRE motif were associated with significantly reduced risk of MS. ***Neurology*® 2012;79:538–546**

GLOSSARY

ABMDR = Australian Bone Marrow Donor Registry; **AH** = ancestral haplotype; **HARP** = heterozygous ambiguity resolving primer; **MS** = multiple sclerosis; **OR** = odds ratio; **PDDD** = Perth Demyelinating Disease Database; **VDR** = vitamin D receptor; **VDRE** = vitamin D–responsive element.

The strong effect of geographic location on multiple sclerosis (MS) risk, described in 1921[1] and refined in studies incorporating meteorologic data,[2,3] reflects a protective influence of sunlight exposure that is most evident in early life,[4] and may even be relevant to in utero development.[5,6] These benefits are mediated at least in part via vitamin D, derived naturally from sunlight exposure or through supplementation.[7] Irrespective of the source, activated vitamin D exerts a range of immune-modulating functions[8,9] via vitamin D receptors (VDR) and their target nuclear vitamin D response elements (VDREs).[10]

In this context, recent data[11] have suggested a direct link between vitamin D and the dominant genetic risk factor HLA-DRB1*15:01, present in more than 50% of MS cases.[12] This

Supplemental data at www.neurology.org

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study identified a VDRE motif within the HLA-DRB1*15:01 promoter region that enhances gene expression in response to vitamin D stimulus[11] (also observed for HLA-DRB1*03:01 in an unrelated study[13]), while other HLA-DRB1 alleles studied were found to carry an alternative promoter sequence that is nonresponsive to vitamin D. These authors[11] provided a cogent argument that for HLA-DRB1*15 bearing individuals, a lack of vitamin D during early life could allow autoreactive T cells to escape thymic deletion,[11] providing a plausible common pathway for the effects of sunlight exposure and HLA-DRB1*15:01 carriage on MS susceptibility. We have sought to further delineate this effect through a comprehensive analysis of HLA-DRB1 promoter sequence variation and allelic diversity in relation to MS disease risk.

METHODS

Research participants. A total of 466 Caucasian participants in the Perth Demyelinating Disease Database (PDDD) with a diagnosis of clinically definite or probable MS according to the Poser criteria[14] or of MS using the McDonald criteria[15] were included in the study. Of these, 425 (91.2%) were classified as relapsing-remitting MS. The study population included 354 female cases (76%), of whom 94% had relapsing-remitting MS, and 112 male cases (81% with relapsing-remitting MS). Patients were assessed by the same 2 neurologists (A.G.K. and W.M.C.) at the time of collecting blood samples. The control group consisted of 498 healthy Caucasian individuals (288 [58%] female) from the Australian Bone Marrow Donor Registry (ABMDR). With regard to participation in other studies of genetic susceptibility to MS, 1 case only from these 466 cases was included in the International MS Genetic Consortium, and 90 cases were included in the ANZGene study cohort. No control subjects were involved in these studies.

Standard protocol approvals, registrations, and patient consents. The study protocol was approved by the Sir Charles Gairdner Hospital Human Research Ethics Committee, and informed consent was obtained from all participants in the PDDD cohort. Approval for use of control samples was provided by the ABMDR ethics committee.

B-cell lines. To ascertain DRB1 promoter sequences we utilized DNA extracted from cell lines with known ancestral haplotypes (AH). Thirty-two cell lines were selected to obtain sequence data for 17 DRB1 alleles; some represented more than once in these cell lines (AH 8.1, 7.1, 52.1, 58.1, 37.1, 46.1, 44.2, and 57.1). In addition, 53 samples from the PDDD cohort were selected based on HLA-DRB1 alleles present—including 8 homozygous and 42 heterozygous HLA-DRB1*15:01-positive samples—in order to obtain sequence data for 20 DRB1 alleles.

HLA-DRB1 typing. High-resolution 4-digit HLA-DRB1 genotyping was performed in the Department of Clinical Immunology, PathWest, Royal Perth Hospital by DNA sequencing for both MS cases and control samples, using a previously reported method.[16] Automated sequencing was carried out on the ABI Prism 3730 and 3730xl Genetic Analyzers and HLA-DRB1 analysis was carried out using ASSIGN V4.0.1.36 (Conexio Genomics). All HLA typing results for MS samples were resolved to the 4-digit level using heterozygous ambiguity resolving primers (HARPs) where applicable. For control samples, sequence-based typing methods provided 2-digit resolution and NMDP codes of possible heterozygous HLA-DRB1 combinations (see reference 17 for further information).

HLA-DRB1 promoter genotyping. HLA-DRB1 promoter genotyping was also performed in the Department of Clinical Immunology PathWest, Royal Perth Hospital, using the following methods. DNA was extracted from whole blood using the QIAGEN BioRobot M48 machine using QIAGEN Magattract DNA Blood Mini M48 kit. The PCR was performed in a 30 μL volume, consisting of 2 μL genomic DNA (at approximately 50 ng/μL), 10 × PCR buffer (15 mM $MgCl_2$, 100 mM Tris-HCl pH 8.4, 500 mM KCl, 1 mg/mL of gelatin, 0.2% of NP-40), 1 mM of each dNTP (Life Technologies, Rockville, MD), and 2.5 units of Taq Platinum polymerase (Life Technologies). The forward primer sequences 5′CAATTAAAGTTTTACATG and 5′CAATTAAAGTGTTTTACACG were tagged with the universal M13F sequence, and the reverse primer 5′TGTCCCCAGMCAAAGCCAGT was tagged with the universal M13R sequence. Concentrations of all primers were initially tested at 20 pmol and adjustments to individual primer concentrations were made if an allele was suboptimally amplified.

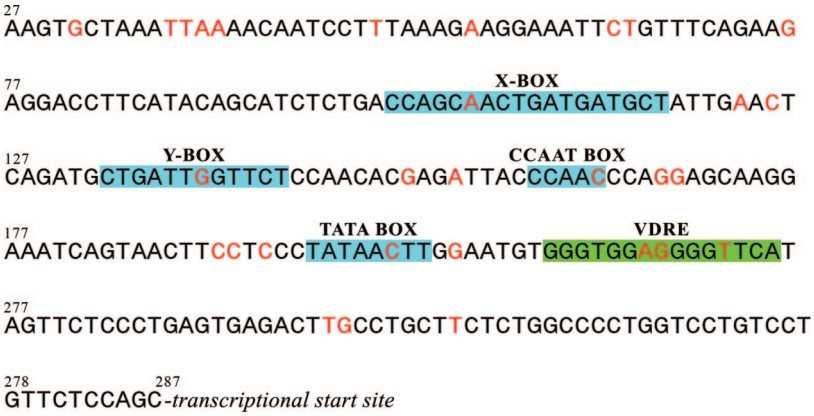
The DNA were amplified following initial denaturation at 96°C for 6 minutes followed by 35 cycles of 96°C for 30 seconds, 70°C for 30 seconds, and 72°C for 2 minutes. A final extension step was performed at 72°C for 10 minutes. PCR amplification was confirmed following electrophoresis using 1% SE Agarose gel and the PCR amplicons were purified using MIOBIO® PCR purification system. Automated sequencing was performed using ABI Big Dye Terminator chemistry, as per manufacturer's instructions, using universal M13F and M13R sequencing primers as well as group-specific sequencing primers (5′TCTGACCAGCGACTGAT and 5′TGTCAGAACTGCCATGCA) when required. Samples were purified using the Agencourt® CleanSEQ® PCR purification system prior to sequencing on ABI Prism 3730 and 3730xl Genetic Analyzers. Finally, the HLA-DRB1 promoter analysis was carried out using ASSIGN ATF 1.0.2.45 (Conexio Genomics).

Statistical analysis. Comparisons of carriage frequencies of alleles between cases and controls were carried out by Fisher exact tests for individual alleles or case-control logistic regressions for multivariable analyses. p Values of < 0.05 were considered statistically significant. Analyses were carried out using the TIBCO Spotfire S+ statistical package (TIBCO Software Inc., Palo Alto, CA). For analyses of HLA-DR associations with MS risk, 2-digit resolution HLA results were utilized in order to maximize data availability among controls.

RESULTS

HLA-DRB1 allele-specific VDRE sequence variation. We first sought to examine sequence variation in the HLA-DRB1 promoter region, including the previously identified VDRE motif. As shown in figure 1 and table 1, we confirmed the association between HLA-DRB1*15:01 and the VDRE motif ^{211}GGGTGGAGGGGTTCA^{225} associated with vitamin D–responsive HLA-DRB1 gene expression. This VDRE

Figure 1 Sequence variation in the HLA-DRB1 promoter region



HLA-DRB1 5′UTR reference sequence (HLA-DRB1*15:01) showing variation sites (red) identified by sequence-based typing (see table 1), relative to known regulatory motifs (in blue, as defined in reference 17) and vitamin D–responsive element motif (in green, reference 11).

sequence was also found in all HLA-DRB1 alleles examined other than HLA-DRB1*04, *07, and *09 alleles where an alternative GGGTGGA*GA*GGGGT-CA sequence was identified (differences italicized) that introduced an internal GA insertion between positions 216 and 217 of the DRB1*15:01 reference sequence shown in figure 1, which is likely to disrupt VDR binding by shifting the adenine from position 7 where it is invariably located within functional VDRE motifs.[10] The resulting loss of VDRE function is consistent with previous findings that the "alternative" (HLA-DRB1*04/07/09-associated) VDRE is not responsive to vitamin D stimulation.[11] Among the 85 samples in which promoter sequencing was undertaken, all conformed to the expected patterns of allele-specific promoter polymorphism identified in table 1, including 53 DRB1*15:01-positive samples (3 homozygous cell lines and 50 MS cohort samples: 8 homozygous and 42 heterozygous at the DRB1 locus).

HLA-DRB1 promoter sequence variation. Investigating the promoter region more generally (table 1), we identified sequence identity throughout the entire promoter region between HLA-DRB1*15:01 and the closely related DRB1*15:02 and DRB1*16:01 alleles that make up the DR51 haplotype group,[18] as well as HLA-DRB1*01 alleles (4 DRB1*01:01, 1 DRB1*01:02, and 3 DRB1*01:03-positive samples) that share a distant ancestral history with HLA-DRB1*15:01[19] but which display only limited sequence similarity and epitope binding preferences.[20,21] It is also notable that the HLA-DRB1*15:01, DRB1*15:02, and DRB1*16:01 alleles within the HLA-DR51 haplotype group share an association with HLA-DRB5, while loss of this additional HLA-DRB locus has been identified as a feature of the evolutionary divergence of HLA-DRB1*01 from the ancestral HLA-DR51 group.[19] All 53 HLA-DRB1*15:01-positive samples showed sequence identity across the promoter region (figure 1).

Looking beyond the VDRE motif region, we identified HLA allele-associated polymorphism within regulatory X-box and Y-box motifs (see figure 1) that have been previously shown to influence constitutive and cytokine-stimulated HLA-DR expression, respectively.[18,22] With regard to the MS-associated HLA-DRB1*15:01 allele, its X-box sequence has been associated with high constitutive gene expression, 4- to 5-fold higher than observed with DR53-associated HLA-DRB1*04, *07, and *09 alleles, in which the canonical motif is disrupted by the A-to-G transition at position 106 (figure 1 and table 1).[22] Similarly, polymorphic residues at positions 139 (G) within the Y-box domain and 164 (C) within the CCAAT domain of HLA-DRB1*15:01 have been associated with ~2-fold increase in DRB1 expression in response to cytokine stimuli (e.g., interferon-γ and tumor necrosis factor-α[18]), while the HLA-DR53-associated sequence (139C, 164T) was again associated with nonresponsiveness.[18]

Taken together, these findings provide evidence for shared promoter sequence variation within the HLA-DR51 group (DRB1*15:01, 15:02, 16:01) and HLA-DRB*01:01, characterized functionally by high constitutive as well as inducible gene expression in response to vitamin D/VDR as well as cytokine-mediated stimuli. This functional VDRE motif is also shared by HLA-DRB1 alleles within the HLA-DR52 group (table 1) as well as DRB1*10:01 and DRB1*08, although these alleles lack the cytokine responsiveness conferred by the DR51-specific Y-box and CCAAT motifs. In contrast, the HLA-DR53 group of alleles (DRB1*04, *07, and *09) share promoter polymorphisms that confer both lower constitutive gene expression as well as nonresponsiveness to both vitamin D– and cytokine-mediated stimuli.

HLA-DRB1 alleles and VDRE variants as MS risk factors. Having established associations between promoter sequence variation and HLA-DRB1 alleles, we sought to investigate the potential relevance of these observations to genetic risk of MS among Caucasian MS cases (n = 466) and controls (n = 498). Table 2 describes the rates of HLA DRB1 allele carriage and homozygosity frequencies among cases and controls, with odds ratios (ORs) for HLA allele carriage among MS cases and controls represented in figure 2. As expected, HLA-DRB1*15 was overrepresented among cases (n = 256, 54.9%) compared with controls (n = 135, 27.1%), providing an OR of 3.27 ($p < 0.001$). The closely related HLA-DRB1*16 allele, although low in prevalence (17 cases, 3.6%; 8

Table 1 HLA-DRB1 promoter sequence variation identified in this study[a]

HLA haplotype	DRB1 allele	DRw serology	DRB3, 4, 5 locus	Changes in SNP sites when compared to reference sequence																				VDRE					
				31	37–40	50	56	65–66	76	106	123	125	139	152	155	164	168	169	190	191	193	201	205	216–217	224	247	248	255	Variant
	15:01	DRw	DRB3, 4, 5	G	TTAA	T	A	CT	G	A	A	C	G	G	A	C	G	G	C	C	C	C	G	(GA)	T	T	G	T	
7.1	*15:01	51	5																										1
7.1	*15:01	51	5																										1
18.1	*15:01	51	5																										1
52.1	*15:02	51	5																										1
52.1	15:02	51	5																										1
37	*16:01	51	5																										1
7.2	*01:01	1																											1
37	*10:01	52		A	DEL	G							C	T		T			A	DEL								G	2
	14:04	52	3	A	DEL	G							C	T		T			A	DEL								G	2
	12:01	52	3	A	DEL	G							C	T		T			A									G	3
18.2	*03:01	52	3	A	DEL	G							C	T		T			A									G	3
8.1	*03:01	52	3	A	DEL	G							C	T		T			A									G	3
8.1	*03:01	52	3	A	DEL	G							C	T		T			A									G	3
58.1	*03:01	52	3	A	DEL	G							C	T		T			A									G	3
58.1	*03:01	52	3	A	DEL	G							C	T		T			A									G	3
42.1	*03:02	52	3	A	DEL	G							C	T		T			A									G	3
35.1	11	52	3	A	DEL	G							C	T		T			A									G	3
62.3	*13:01	52	3	A	DEL	G							C	T		T			A									G	3
44.4	*13:02	52	3	A	DEL	G							C	T		T			A									G	3
60.2	*08:01	8		A	DEL	G							C	T	T	T			A									G	4
46.2	*08:03	8		A	DEL	G							C	T	T	T			A									G	4
44.1	*04:01	53	4	A				DEL	A	G	T		C	T		T	C			T		T		INS	DEL	C	A	C	5
62.1	*04:01	53	4	A				DEL	A	G	T		C	T		T	C			T		T		INS	DEL	C	A	C	5
38.1	*04:02	53	4	A				DEL	A	G	T		C	T		T	C			T		T		INS	DEL	C	A	C	5
54.1	*04:05	53	4	A				DEL	A	G	T		C	T		T	C			T		T		INS	DEL	C	A	C	5
13.1	*07:01	53	4	A			G	DEL		G	T	A	C	T		T	C	A		T		T		INS	DEL	C	A	C	6
57.1	*07	53	4	A			G	DEL		G	T	A	C	T		T	C	A		T		T		INS	DEL	C	A	C	6
57.1	*07	53	4	A			G	DEL		G	T	A	C	T		T	C	A		T		T		INS	DEL	C	A	C	6
44.2	*07	53	4	A			G	DEL		G	T	A	C	T		T	C	A		T		T		INS	DEL	C	A	C	6
44.2	*07	53	4	A			G	DEL		G	T	A	C	T		T	C	A		T		T		INS	DEL	C	A	C	6
46.1	*09:01	53	4	A			G	DEL		G	T	A	C	T		T	C	A		T		T	A	INS	DEL		A	C	7
46.1	*09:01	53	4	A			G	DEL		G	T	A	C	T		T	C	A		T		T	A	INS	DEL		A	C	7

[a] Unless otherwise specified, all results are derived from homozygous cell lines. DRB1*12:01 and *14:04 results were obtained from DRB1*15:01 heterozygous samples. Other results from heterozygote samples: DRB1*01:02 and *01:03 share sequence identity with DRB1*01:01 (variant 1); DRB1*011:04 shares sequence identity with DRB1*11:01 (variant 3); DRB1*04:03, *04:04, and *04:06 share sequence identity with DRB1*04:01 (variant 5).

Table 2 HLA allele carriage (phenotype) and homozygosity frequency in cases and controls

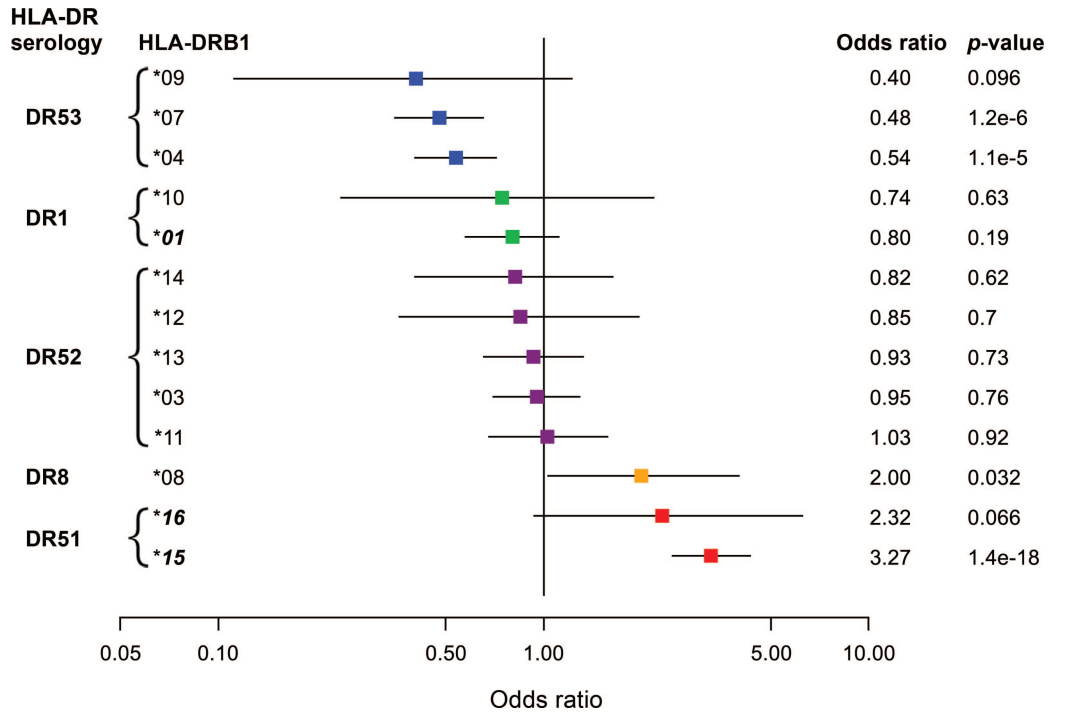
	Allele carriage				Homozygosity			
	Cases (466)		Controls (498)		Cases (466)		Controls (498)	
	No.	%	No.	%	No.	%	No.	%
DRB1*01	83	17.8	106	21.3	1	0.2	5	1.0
DRB1*03	110	23.6	122	24.5	15	3.2	10	2.0
DRB1*04	113	24.2	186	37.3	5	1.1	22	4.4
DRB1*07	84	18.0	157	31.5	5	1.1	6	1.2
DRB1*08	29	6.2	16	3.2	0	0.0	1	0.2
DRB1*09	5	1.1	13	2.6	0	0.0	0	0.0
DRB1*10	7	1.5	10	2.0	0	0.0	0	0.0
DRB1*11	53	11.4	55	11	1	0.2	1	0.2
DRB1*12	12	2.6	15	3.0	1	0.2	1	0.2
DRB1*13	75	16.1	85	17.1	4	0.9	6	1.2
DRB1*14	17	3.6	22	4.4	0	0.0	0	0.0
DRB1*15	256	54.9	135	27.1	39	8.4	14	2.8
DRB1*16	17	3.6	8	1.6	0	0.0	0	0.0

controls, 1.6%), was also associated with increased MS risk (OR 2.32, $p = 0.066$), particularly among HLA-DRB1*15-negative individuals (OR 3.62, $p = 0.005$). Similarly, carriage of HLA-DRB1*08 was associated with MS risk in the overall dataset (29 cases, 6.2%; 16 controls, 3.2%: OR 2.00, $p = 0.032$) with a more pronounced effect after exclusion of HLA-DRB1*15 (OR 2.90, $p = 0.006$). No other HLA-DRB1 alleles were associated with increased MS risk, either within a restricted dataset of relapsing-remitting disease (table e-1 and figure e-1 on the *Neurology*® Web site at www.neurology.org) or in the presence or absence of HLA-DRB1*15 (figure e-2A and e-2B).

Reduced MS risk was associated with carriage of the prevalent alleles HLA-DRB1*04 (113 cases, 24.2%; 186 controls, 37.3%: OR 0.54, $p < 0.0001$) and HLA-DRB1*07 (84 cases, 18.0%; 157 controls, 31.5%: OR 0.48, $p < 0.0001$). A trend toward reduced risk was also found for the low-prevalence HLA-DRB1*09 allele (5 cases, 1.1%; 13 controls, 2.6%: OR 0.40, $p = 0.096$). Notably, HLA-DRB1*04 remained protective among HLA-DRB1*15 heterozygous individuals (OR 0.55, $p = 0.023$), while the protective effect of HLA-DRB1*07 was observed in the HLA-DRB1*15-negative group (OR 0.42, $p < 0.0001$), but not among HLA-DRB1*15 carriers (OR 1.48, $p = 0.29$).

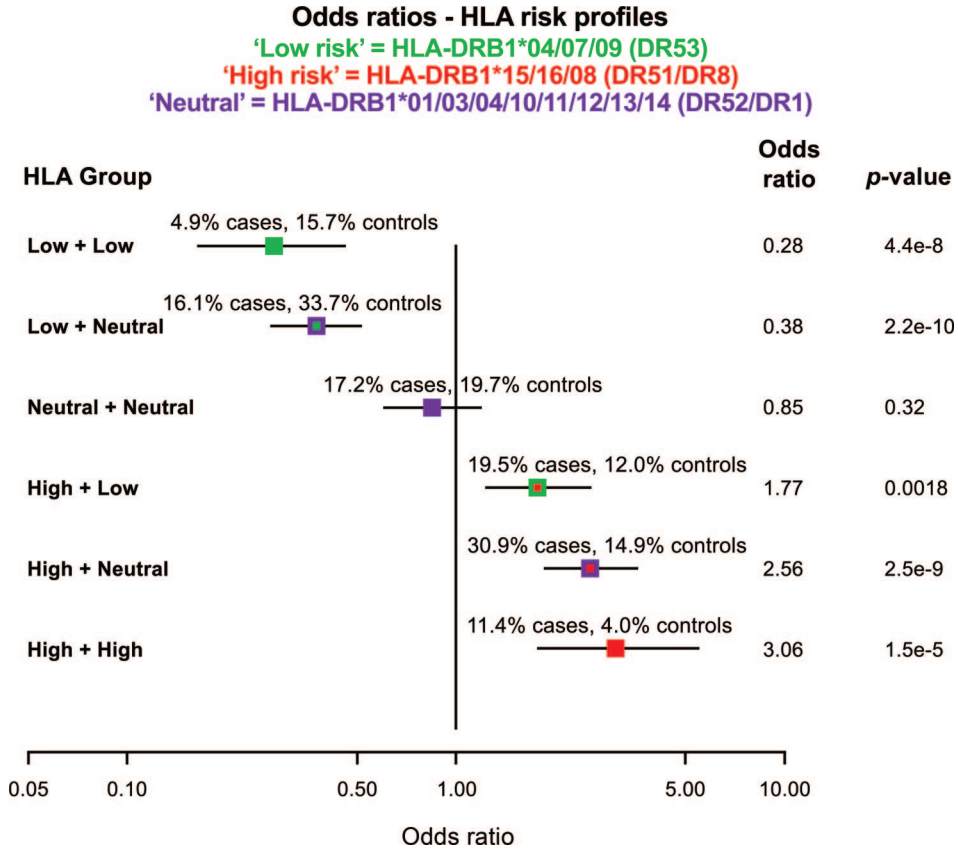
Overall, the effects of HLA-DRB1 allele carriage on MS risk show consistent correlations with the original HLA-DR serologic groups defined prior to the availability of sequence-based HLA typing tech-

Figure 2 Relative risk of multiple sclerosis according to HLA carriage: Odds ratios and 95% confidence intervals



HLA serologic groups are indicated by color and defined in the left-hand column. Alleles that share promoter sequence identity with HLA-DRB1*15:01 are indicated with bold italics.

Figure 3 Combined effects of HLA-DR profiles on multiple sclerosis risk



Stratification of multiple sclerosis risk according to combined HLA allele profiles, including prevalence rates in cases (n = 466) and controls (n = 498): odds ratios and 95% confidence intervals.

niques (figure 2), which reflect both the peptide-binding preferences of these HLA-DRB1 allele groups[19–21] as well as their haplotypic associations with additional HLA-DRB loci (i.e., HLA-DRB5 [DR51], DRB3 [DR52], and DRB4 [DR53]). Thus, HLA-DRB1 alleles from the DR53 group appear to be protective, while those from the DR51 and DR8 group are associated with excess risk. The remaining DR52 and DR1-associated HLA-DRB1 alleles did not significantly influence MS risk in this population, and it is notable in particular that carriage of HLA-DRB1*01 was not associated with increased MS risk in the overall group (OR 0.80, $p = 0.19$) or in the absence of HLA-DRB1*15 (OR 0.95, $p = 0.84$) despite sharing complete promoter sequence identity with HLA-DRB1*15 and DRB1*16 alleles. Thus, there was no evidence that promoter sequence variation—either within the VDRE motif or across the promoter region more generally—acted as a dominant independent risk factor for MS in this study.

Considering these findings in terms of the inheritance of serologic HLA-DR profiles, there was a clear linear effect of HLA-DR53 allele copy number (p value for trend $= 1.9 \times 10^{-12}$) as well as HLA-DR51/DR8 allele copy number ($p < 10^{-18}$). Utilizing these data to stratify genetic risk (figure 3), the overall effect of these compound genotypes appears to be consistent with a dominant influence of "high risk" HLA-DR51 and HLA-DR8 alleles (present in 61.8% of cases and 30.9% of controls), and a recessive protective effect of HLA-DR53 alleles (21.0% of cases and 49.4% of controls), although independent allele dosage models cannot be excluded.

DISCUSSION The identification of a vitamin D response element in the promoter region of the HLA-DRB1 gene,[11] and the role of allele-specific sequence variation within this VDRE motif in determining responsiveness to vitamin D stimulus,[11,13] has provided an attractive mechanism by which the 2 dominant risk factors for MS—HLA-DRB1 allele carriage and low environmental vitamin D exposure—may be directly linked. In this study we have further explored the role of promoter polymorphism in relation to HLA-DRB1 alleles represented in Caucasian populations, demonstrating that the HLA-DRB1*15:01-associated VDRE motif is widely represented among Caucasian HLA-DRB1 alleles with the exception of HLA-DRB1*04, DRB1*07, and DRB1*09 alleles.

Furthermore, we identified promoter sequence variation at other regulatory sites within the DRB1 promoter region that have been previously shown to influence both constitutive and cytokine-stimulated expression levels.[18,22] In this regard, the MS risk-associated HLA-DRB1*15:01 allele is characterized by relatively high constitutive expression as well as responsiveness to both vitamin D/VDR as well as cytokine stimulation, combined traits that are shared by several DRB1 alleles that show sequence identity throughout the promoter region (DRB1*15:02, 16:01, and 01:01). Conversely, those DRB1 alleles that lack a vitamin D–responsive VDRE motif (DRB1*04, DRB1*07, and DRB1*09) are also characterized by promoter sequence variation within X-box and Y-box domains that confer low constitutive gene expression and nonresponsiveness to cytokine stimuli.[18,22]

In light of these observations, it is interesting that risk of MS in a large case-control study involving Caucasian participants from Western Australia could be effectively stratified according to groups of HLA-DRB1 alleles that reflect these promoter haplotypes in part, but which are also defined by their evolutionary ancestry,[19,21] antigen-binding preferences,[20] and linkage disequilibrium with additional HLA-DRB loci (i.e., HLA-DRB3/4/5).[23] In this respect it is also notable that the serologic HLA-DR groups originally defined before the advent of high-resolution sequence-based typing methods appear to provide a very useful classification system for assessing MS risk in this study. Thus, the HLA-DR53 group of alleles (DRB1*04, DRB1*07, and DRB1*09, each in linkage disequilibrium with HLA-DRB4) demonstrated a consistent association with reduced MS risk, while increased MS risk was associated with the HLA-DR51 group (DRB1*15:01, DRB1*16:01, associated with HLA-DRB5 carriage) as well as DR8 (DRB1*08, which has no additional HLA-DRB loci within the extended HLA-DR haplotype). Both protective and risk effects were dependent on allele dose and were highly significant, contributing to a genetic profile for this study population that could effectively stratify low- and high-risk groups, providing an 11-fold range in disease susceptibility across the study population (ORs 0.28–3.06, figure 3). These results need to be interpreted in light of the limitations of the study, both in terms of the influence of sample size on statistical power as well as the use of low-resolution (2-digit) DRB1 typing results in this analysis, so that we were unable to assess any differential effects of closely related alleles (e.g., DRB1*15:01 vs 15:02) on MS risk. Nevertheless, it is interesting that similar results were obtained from an analysis of HLA-DR serologic profiles in a Finnish study.[24]

With regard to the potential influence of VDRE polymorphism on MS risk, we can conclude that the trait of vitamin D–responsive HLA-DRB1 gene expression is not specific to the high-risk DRB1*15:01 allele, and indeed that the functional VDRE motif is common among a broad range of Caucasian HLA-DRB1 genotypes, including DRB1 alleles that have no discernible influence on MS risk. This finding, however, does not exclude the possibility that early-life vitamin D exposure may interact with HLA-DRB1 inheritance as a risk factor for the disease, or indeed that interactions between the HLA-DRB1 promoter region and environmental- or infection-derived stimuli such as vitamin D and inflammatory cytokines may play an important role in modulating disease risk through epigenetic mechanisms.[25] We did not assess sunlight exposure as a potential variable in the analysis of MS risk in this cohort, but anticipate that such a study would be more usefully

Comment: The HLA region in multiple sclerosis

Nolan et al.[1] investigate human leukocyte antigen (HLA)-DRB1 promoter sequence variation, including a previously characterized vitamin D response element (VDRE),[2] addressing the challenge of a mechanistic explanation for the association of HLA with multiple sclerosis (MS) and the suggested link with vitamin D.[2]

The HLA-DRB1 gene is highly polymorphic, with many different forms or haplotypes
that are evolutionarily related. The latest MS genome-wide association study identified DRB1*1501, DRB1*03:01, and the group of DRB1*13:03 and DRB1*08:01 haplotypes as increasing risk of MS.[3] Nolan et al. reveal differences in the sequence of functional promoter elements among haplotypes, ranging from haplotypes with high constitutive expression levels that can be further induced by vitamin D and cytokines (DRB1*15, *16 and *01 haplotypes), to those with low constitutive gene expression and nonresponsiveness to vitamin D and cytokines (DRB1*04, *07, and *09 haplotypes).[1] Combining promoter sequence differences with high-resolution association studies, not all cytokine and vitamin D responsive haplotypes are associated with MS. For example, the high-risk MS haplotype HLA-DRB1*15:01 (in the Nolan et al. study) is identical in promoter sequence, including the VDRE, to related haplotypes that are not associated with MS, such as DRB1*15:02.[1,3] This study hence suggests that none of the HLA-DRB1 promoter characteristics can on its own account for the association of HLA with MS. Whereas the current knowledge of MS genetics provides further evidence for both the HLA and the vitamin D metabolism pathways,[3] the study by Nolan et al.[1] does not support response to vitamin D as a major explanation for the association of HLA with MS. Further studies coupling detailed knowledge of sequence variation to imputation and conditional analyses in large datasets are needed to gain additional insights.

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undertaken in a more geographically diverse population given that study participants were largely derived from metropolitan Perth, which is located at a latitude (32°) where early-life UV exposure is likely to be sufficient to protect against excess MS risk.[2,3]

These findings suggest that the functional relationships between HLA-DR alleles, both in terms of their promoter sequence characteristics[18,22] as well as their peptide-binding characteristics,[19–21,26] can be usefully explored to identify HLA-DRB1 allele-specific and shared genetic traits that contribute to MS risk. We therefore submit that the results of this study provide a platform for further analyses in larger study populations with sufficient statistical power to comprehensively examine the influence of HLA-DRB1 promoter sequence variation, allele distribution, and serologic profiles on MS risk. In this regard, the collaborative efforts of the International Multiple Sclerosis Genetic Consortium have now provided an unprecedented opportunity to conduct such analyses based on existing data,[27] with the additional benefit of a broad geographic distribution that also allows for the incorporation of environmental sunlight exposure as an additional variable. It is our hope that further exploration of these important genetic risk factors will contribute toward an improved understanding of the role of HLA-restricted adaptive immunity in MS susceptibility.

AUTHOR CONTRIBUTIONS

David Nolan: study design, manuscript preparation and analysis. Allison Castley: HLA-DRB1 promoter sequencing and high-resolution sequence-based HLA typing, manuscript preparation and analysis. Monika Tschochner: manuscript preparation and analysis. Ian James: statistical analysis and manuscript preparation. Wei Qiu: patient recruitment, Perth demyelinating disease cohort data entry and curation, manuscript preparation. David Sayer: HLA-DRB1 promoter sequence analysis and allele assignment. Frank Christiansen: HLA-DRB1 analysis and immunogenetics expertise, manuscript preparation. Campbell Witt: HLA-DRB1 analysis and immunogenetics expertise, manuscript preparation. Frank Mastaglia: patient assessment, clinical management and recruitment, manuscript preparation. William Carroll: patient assessment, clinical management and recruitment, manuscript preparation. Allan Kermode: patient assessment, clinical management and recruitment, manuscript preparation.

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