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The killer immunoglobulin-like receptor KIR3DL1 combination with HLA-Bw4 is protective against multiple sclerosis in African Americans

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Abstract

We investigated the role of the *KIR* loci and their *HLA* class I ligands in a large cohort of African American MS patients (N=907) and controls (N=1456). No significant differences in carrier frequencies for any *KIR* locus or haplotype were observed between cases and controls. However, examination of *KIR* in the context of their cognate HLA ligands revealed a strong protective effect for *KIR3DL1* in combination with *HLA-A* and *–B* alleles bearing the Bw4 motif ($p=10^{-8}$; OR=0.60, CI=0.50–0.71) and the Bw4 ligand alone (Table 3; $p<10^{-6}$; OR=0.63, CI=0.53–0.75). The observed effect cannot be explained by either a specific *HLA-B* allele or by linkage disequilibrium with *HLA-DRB1* or *HLA-A*. The protective effect was observed only in individuals who were not positive for the MS risk allele *HLA-DRB1*15:01* ($p<10^{-6}$; OR=0.61, CI=0.51–0.74). Our study, the first investigation of *KIR* and MS in African Americans, confirms and refines previous findings in a European cohort.

Introduction

The important role of variation in genes of the human leukocyte antigen (HLA) complex in genetic predisposition to multiple sclerosis (MS) has long been recognized. However, in addition to their role in antigen presentation to T cells, HLA class I molecules also serve as ligands for KIR molecules. An ever-increasing pace of investigation over the last decade has demonstrated definitively that killer immunoglobulin-like (KIR) receptors play critical roles in transplantation success and disease pathogenesis (1–4).

Expressed on the surface of natural killer (NK) cells, *KIR* serve to mediate cytolytic killing and cytokine secretion (5). The *KIR* gene complex on human chromosome 19q13.4 encodes

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both inhibitory and activating receptors, with significant variation in gene content between individuals and populations. Any given *KIR* haplotype may contain from 4–14 genes, and are generally categorized into two groups, termed A and B (6). *KIR* molecules recognize specific epitopes on *HLA* class I: *KIR3DL2* and *KIR2DS4* recognize *HLA-A* molecules with the *A3/A11* epitope, while *KIR2DL1, KIR2DL2/3*, *KIR2DS1*, *KIR2DS2* and *KIR2DS4* interact with either the C1 or C2 epitopes on *HLA-C*; a small subset of *HLA-B* molecules also carry the C1 epitope, and are capable of interacting with these KIR. Finally, *KIR3DL1* binds *HLA-A* and *-B* molecules that carry the *Bw4* specificity (2, 7–9).

Only a handful of studies have been conducted examining *KIR* variation in MS, but overall, the analysis of *KIR* gene-content variation has pointed to a role for the *KIR* in disease susceptibility. In patients of European ancestry, predisposition to MS has been variably associated with the absence of the inhibitory *KIR2DL3* (10) or the presence of activating *KIR2DL5* and *KIR3DS1*. In other studies, *KIR2DS1* was found to be protective in MS (11, 12). In the largest previous study to-date, the *HLA* class I *Bw4* motif was found to be protective in a Norwegian cohort, but no association was observed between *KIR* carrier frequencies and MS (13).

All prior examination of the role of *KIR* in MS has been conducted in cohorts of European origin. While conventional wisdom has held that MS is much less common in African Americans relative to European Americans, more recent data suggests that risk for MS in African Americans is higher than expected and its incidence in this group is increasing (14, 15). Importantly, African Americans are more likely to have a more acute disease course and it appears that this increased severity is partially associated with African ancestry (16, 17). Examination of *HLA* associations with MS in African Americans has served to clarify *HLA* class II haplotypic associations (18) and revealed a role for *HLA-DRB5* (19). Here, we investigate the role of the *KIR* loci and their *HLA* class I ligands in a large cohort of African American MS patients and controls. In addition to being the first investigation of *KIR* and autoimmune disease in African Americans, this study represents the largest case-control analysis conducted to-date in any population examining *KIR* and *HLA* variation in MS.

Results and Discussion

No significant difference in carrier frequencies for any *KIR* locus (or allele in the case of *KIR2DL2/3* and *KIR3DL1/S1*), were observed between cases and controls (Table 1). We also examined whether specific haplotypic structures of the *KIR* were associated, rather than individual loci, both across the entire *KIR* cluster and within each of the centromeric and telomeric regions. No significant associations were observed for any specific *KIR* gene-content haplotype or particular combinations of *KIR* A and B haplotypes (data not shown).

However, examination of *KIR* in the context of their cognate *HLA* ligands (Table 2) revealed a strong protective effect for *KIR3DL1* in combination with *HLA-A* and *–B* alleles bearing the Bw4 motif ($p=10^{-8}$; OR=0.60, CI=0.50–0.71). Because nearly every individual in this cohort is positive for *KIR3DL1* (Table 1), a protective effect is also expected and observed for the Bw4 ligand alone (Table 3; $p<10^{-6}$; OR=0.63, CI=0.53–0.75). These results are in keeping with those observed in the Norwegian cohort (13), where, as in most populations

(20) the carrier frequency of *KIR3DL1* is also very high. No other *HLA* ligand was independently associated with disease.

We tested all *HLA-B* alleles for association with disease in order to determine whether the Bw4-mediated effect could be attributed to one or a few specific *HLA-B* alleles. Only one *HLA-B* allele, *HLA-B*53* would be considered significantly protective in this cohort after Bonferroni correction for multiple tests (<0.002). While this allele does have the Bw4 motif, it is not present at sufficient frequency in this cohort (<4% in cases and controls; Supplemental table 1) to account for the observed Bw4 association. We therefore considered whether the observed association with Bw4 could be attributed to linkage disequilibrium of Bw4-bearing *HLA-B* alleles with specific MS-associated *HLA* alleles at other loci.

When two locus haplotypes (*HLA-B~HLA-DRB1*and *HLA-A~HLA-B*) are considered with HLA-B alleles coded for either the Bw4 or Bw6 motif, in each case the most significantly protective haplotype bears a Bw4 HLA-B allele, while the *HLA-A* and *HLA-DRB1* alleles vary (Table 4). For example, when *HLA-DRB1* is considered alone, protection is conferred by the common allele *HLA-DRB1*11* (p=0.0001; OR=0.69, CI=0.57–0.84). Likewise, the most highly significant protective *HLA-DRB1~HLA-Bw4/6* haplotype is *HLA-DRB1*11~Bw4* (p<10–6; OR=0.50, CI=0.34–0.72). In contrast, the *HLA-DRB1*11~Bw6* haplotype is not significantly associated with disease. Similarly, the two other haplotypes observed to be protective in this cohort are *HLA-DRB1*13~Bw4* (p<0.05; OR=0.67, CI=0.49–0.93) and *HLA-DRB1*07~Bw4* (p<0.05; OR=0.58, CI=0.40–0.85), but their Bw6-bearing counterparts are not. In each of these cases, the Bw4 and Bw6 bearing HLA-DRB1 haplotypes are present at roughly equal frequencies, thus ruling out linkage disequilibrium as a source of the disparate effects.

When we consider common (frequency >0.03%) haplotypes with *HLA-A*, only *HLA-A*02~Bw4* is significantly protective in this cohort (p=0.003; OR=0.70,CI=0.55–0.88). When HLA-A is considered alone (Supplemental table 1), *HLA-A*02* is not protective, nor is it protective in the context of Bw6 rather than Bw4 bearing *HLA-B* alleles (Table 4). Taken together, the haplotype data indicate that the protective effect is being mediated by the presence of the Bw4 epitope that confers *KIR3DL1* ligand status to *HLA-B* alleles. The observed effect cannot be explained by either a specific *HLA-B* allele defined with respect to the antigen-binding domain (at single-field resolution) or by any protective *HLA-DRB1* or *HLA-A* alleles in linkage disequilibrium with Bw-bearing *HLA-B* alleles, supporting the relevance of the *KIR-HLA* framework in disease risk.

The strong predisposing effect of *HLA-DRB1*15:01* in MS has long been recognized, and in this cohort *HLA-DRB1*15:01*, like nearly most others examined to-date (21), is the primary predisposing *HLA* variant with OR>2 (Supplemental table 1). Given the strong and significant role for *HLA-DRB1*15:01*, we explored whether protection mediated via *KIR3DL1/HLA-Bw4* is differentially associated with regard to *HLA-DRB1*15:01* status. Cases and controls were stratified according to having 0, 1 or 2 copies of *HLA-DRB1*15:01*, and association of KIR3DL1/Bw4 with disease was examined within each stratum (Table 5). The protective effect was observed only in individuals who were not positive for *HLA-DRB1*15:01* ($p<10^{-6}$; OR=0.61, CI=0.51–0.74). These data suggest that

while the KIR-ligand combination mediates a robust protective effect, that effect is overridden in the presence of the strong predisposing effect of HLA-DRB1*15:01. Analysis of *HLA-B~HLA-DRB1* haplotypes in *HLA-DRB1*15:01*-negative individuals yields results similar to those described above for the entire cohort (data not shown), implying that differential association of *KIR3DL1/Bw4* is not mediated by linkage disequilibrium between *HLA-DRB1* and *HLA-B* alleles.

Given the extensive allelic polymorphism of KIR3DL1, future studies examining KIR in MS will benefit from high-resolution genotyping, particularly in populations with African ancestry. For example, a non-expressed variant, KIR3DL1*004 (22) is observed at relatively high frequencies in a West African population (23). It is also interesting to note that KIR3DL1 and HLA-Bw4 were found to be subject to co-evolution in this same population, suggesting a selective advantage for this KIR-HLA combination.

In conclusion, our data in a large African American cohort confirm and refine a previous finding in a large Norwegian cohort (13) of protection from MS mediated by the *KIR3DL1* ligand *HLA-Bw4*, either alone or in combination with *KIR3DL1*. The fact that our results in a large African American cohort also implicate Bw4, despite the fact that individuals with European and African ancestry have vastly different *HLA-B* allelic variation and frequency distributions (24), supports the notion that the functional properties of the Bw4 motif with respect to *KIR3DL1* and NK cell inhibition and/or licensing are the key determinants in protection from disease.

Materials and Methods

The study cohort consisted of 907 African American MS cases and 1456 African American controls. All multiple sclerosis subjects met established diagnostic criteria (25, 26). Ascertainment protocols and clinical and demographic characteristics have been summarized elsewhere (16, 27). Principal component analysis and pruned genome-wide autosomal non-MHC SNPs with minor allele frequency (MAF) > 1% were used to assess ancestry and control for the effects of population stratification (28).

KIR genotyping

DNA samples were collected from patients and KIR typing was performed for presence or absence of *KIR* genes by the LABType® SSO KIR typing (OneLambda, Inc. Canoga Park, CA). The typing system can distinguished 8 inhibitory genes (*KIR2DL1-2DL5, KIR3DL1-3DL3*), 6 activating genes (*KIR2DS1-2DS5, KIR3DS1*) and 2 pseudogenes (*KIR2DP1* and *KIR3DP1*).

HLA genotyping

Genotypes for *HLA-DRB1*, *HLA-A*, *-B* and *–C* were obtained by SSOP using the LABType® SSO *HLA* typing (OneLambda, Inc. Canoga Park, CA).

Statistical analysis

All statistical analysis was performed using the R language for statistical computing (29). All single-locus tests for association were performed using standard chi-squared analysis with the 'chisq.test' function in the R base package. Odds ratios and confidence intervals were calculated using the 'epitools' package for R (30). In the case of low-frequency cells, as is common in *HLA* data, alleles with expected counts <5 were combined into a single "binned" category prior to analysis (31). Haplotype estimation and association tests were performed using the 'haplo.stats' package (32, 33).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

KIR carrier frequencies in MS cases and controls.

KIR	Case	Control
2DL1	0.98	0.97
2DL2	0.54	0.53
2DL3	0.89	0.88
2DL4	1	1
2DL5	0.53	0.54
2DP1	0.99	0.98
2DS1	0.21	0.23
2DS2	0.47	0.46
2DS3	0.30	0.26
2DS4	0.98	0.98
2DS5	0.31	0.34
3DL1	0.98	0.98
3DS1	0.17	0.18
3DL2	1	1
3DL3	1	1
3DP1	1	1

Table 2

Frequency of KIR~HLA ligand combinations in MS cases and controls

KIR~HLA combination	Case	Control	p-value
KIR2DL1~HLA-C2	0.72	0.74	ns
KIR2DL2~HLA-C1	0.38	0.38	ns
KIR2DL3~HLA-C1	0.62	0.61	ns
KIR3DL1~HLA-Bw4	0.62	0.73	10^{-8}
KIR3DL2~HLA-A3/11	0.17	0.13	ns

Table 3

Carrier frequency of HLA ligands in MS cases and controls.

Ligand	Case	Control	p-value
HLA-C1	0.69	0.70	ns
HLA-C2	0.74	0.76	ns
HLA-Bw4	0.64	0.74	$<10^{-6}$
HLA-A3/11	0.17	0.13	ns

Table 4

HLA-DRB1~HLA-B and HLA-A~HLA-B haplotype frequencies in MS cases and controls.

HLA-DRB1	HLA-B	Case	Control	p-value
HLA-HLA-DRB1*03	Bw6	0.136	0.112	0.004
HLA-HLA-DRB1*13	Bw6	0.085	0.083	NS
HLA-HLA-DRB1*13	Bw4	0.064	0.081	0.035
HLA-HLA-DRB1*15:03	Bw6	0.085	0.060	0.004
HLA-HLA-DRB1*11	Bw6	0.059	0.070	NS
HLA-HLA-DRB1*11	Bw4	0.038	0.065	$<10^{-4}$
HLA-HLA-DRB1*15:03	Bw4	0.047	0.058	NS
HLA-HLA-DRB1*07	Bw6	0.050	0.050	NS
HLA-HLA-DRB1*07	Bw4	0.039	0.054	0.015
HLA-HLA-DRB1*15:01	Bw6	0.057	0.029	$<10^{-6}$
HLA-HLA-DRB1*01	Bw6	0.042	0.038	NS
HLA-HLA-DRB1*08	Bw6	0.039	0.031	NS
HLA-HLA-DRB1*04	Bw6	0.030	0.035	NS
HLA-HLA-DRB1*08	Bw4	0.033	0.031	NS
HLA-HLA-DRB1*03	Bw4	0.032	0.027	NS

HLA-A	HLA-B	Case	Control	p-value
HLA-A*02	Bw6	0.172	0.155	NS
HLA-A*02	Bw4	0.105	0.140	0.003
HLA-A*03	Bw6	0.096	0.070	0.01
HLA-A*30	Bw6	0.077	0.082	NS
HLA-A*01	Bw6	0.086	0.063	0.02
HLA-A*23	Bw6	0.069	0.069	NS
HLA-A*30	Bw4	0.041	0.054	NS
HLA-A*03	Bw4	0.057	0.040	0.02
HLA-A*23	Bw4	0.042	0.045	NS
HLA-A*01	Bw4	0.034	0.031	NS

The most fifteen most common HLA-DRB1~HLA-B and ten most common HLA-A~HLA-B (frequency >3%) haplotypes are given.

Table 5

Frequency of KIR3DL1~Bw4 combination in HLA-DRB1*15:01 negative MS cases (n=784) and controls (n=1357).

	Case	Control
KIR3DL1~Bw4 positive	0.639	0.744
KIR3DL1~Bw4 negative	0.361	0.256