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 killer immunoglobulin-like receptor (KIR) molecules. As the ever-increasing pace of investigation over the last decade has demonstrated definitively that 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 both inhibitory and activating receptors, with significant variation in gene content between individuals and populations. Any given KIR haplotype may contain from 4 to 14 genes, and are generally categorized between individuals and populations. Any given KIR haplotype may contain from 4 to 14 genes, and are generally categorized into two groups, termed A and B.5 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 (ref. 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 haplotype associations18 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).

1Department of Neurology, University of California, San Francisco, CA, USA; 2Department of Laboratory Medicine and Pathology, Mayo Clinic in Arizona, AZ, USA and 3ATIP-Avenir Inserm Unit 1064, Hospital and University of Nantes, Nantes, France. Correspondence: Dr JA Hollenbach, Department of Neurology, University of California, San Francisco School of Medicine, 675 Nelson Rising Lane, Mission Bay Campus, San Francisco, CA 94158, USA.
E-mail: jill.hollenbach@neurology.ucsf.edu
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SHORT COMMUNICATION
The killer immunoglobulin-like receptor KIR3DL1 in combination with HLA-Bw4 is protective against multiple sclerosis in African Americans

JA Hollenbach1, MJ Pando2, SJ Caillier1, P-A Gourraud1,3 and JR Oksenberg1

We investigated the role of the KIR loci and their HLA class I ligands in a large cohort of African American multiple sclerosis (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; odds ratio (OR) = 0.60, confidence interval (CI) = 0.50–0.71) and the Bw4 ligand alone (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.

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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 ($P = 10^{-8}$; odds ratio (OR) = 0.60, confidence interval (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; $P < 10^{-5}$; OR = 0.63, CI = 0.53–0.75). These results are in keeping with those observed in the Norwegian cohort, where, as in most populations, the carrier frequency of KIR3DL1 in the context of their cognate ligands (Table 2) revealed a strong protective effect for HLA-Bw4. The Bw4 motif (Table 2) is protective in this cohort (OR = 0.60, CI = 0.49–0.84), but the 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 (OR = 0.003; OR = 0.70, CI = 0.55–0.88). When HLA-A is considered alone (Supplementary 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

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      |

Abbreviations: HLA, human leukocyte antigen; NS, nonsignificant.

Table 4. HLA-DRB1 ~ HLA-B and HLA-A ~ HLA-B haplotype frequencies in MS cases and controls

| 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 | Bw4 | 0.059 | 0.070 | NS |
| HLA-HLA-DRB1*11 | Bw4 | 0.038 | 0.065 | < 10^{-4} |
| HLA-HLA-DRB1*13 | Bw4 | 0.047 | 0.058 | NS |
| HLA-HLA-DRB1*07 | Bw4 | 0.050 | 0.050 | NS |
| HLA-HLA-DRB1*07 | Bw4 | 0.039 | 0.054 | 0.015 |
| HLA-HLA-DRB1*07:01 | 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     | 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*23  | 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 |

Abbreviations: HLA, human leukocyte antigen; NS, nonsignificant. The 15 most common HLA-DRB1 ~ HLA-B and 10 most common HLA-A ~ HLA-B (frequency > 3%) haplotypes are given.
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-baring 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 mostly others examined to-date,21 is the primary predisposing HLA variant with OR > 2 (Supplementary Table 1). 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 < 10−5; 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 (ref. 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 cohort19 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.16,18 Principal component analysis and pruned genome-wide autosomal non-major histocompatibility complex (MHC) single nucleotide polymorphisms (SNPs) with minor allele frequency >1% were used to assess ancestry and control for the effects of population stratification.27

### 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    |

### HLA genotyping

Genotypes for HLA-DRB1, HLA-A, -B and -C were obtained by sequence specific oligonucleotide probes using the LABType SSO HLA typing (OneLambda, Inc.).

### Statistical analysis

All statistical analysis was performed using the R language for statistical computing.28 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.29 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.30 Haplotype estimation and association tests were performed using the ‘haplo.stats’ package.31,32

### CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Supplementary Information accompanies this paper on Genes and Immunity website (http://www.nature.com/gene)