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Diversity of MICA and Linkage Disequilibrium with HLA-B in Two North American Populations

Xiaojiang Gao, Richard M. Single, Peter Karacki, Darlene Marti, Stephen J. O'Brien, and Mary Carrington

ABSTRACT: The MICA gene has a high degree of polymorphism. Allelic variation of MICA may influence binding of these ligands to the NK cell receptor NKG2D and may affect organ transplantation and/or disease pathogenesis. Knowledge of the population distribution of MICA alleles and their linkage disequilibrium (LD) with class I human leukocyte antigen (HLA) will enhance our understanding of the potential functional significance of the MICA polymorphism. In the present study, we characterized the MICA and HLA-B polymorphisms in two North American populations: European and African. The individual racial groups showed rather limited variation at the MICA locus, where the same set of three most common alleles, MICA*00201, *004, and *00801, account for 64 and 71% of the allele frequency in European-Americans and African-Americans, respectively. Other common alleles (allele frequency >5% in a population) include MICA*00901 and *010. MICA alleles showed strong linkage disequilibrium with HLA-B. Typically, a common MICA allele has strong LD with several HLA-B alleles, whereas most HLA-B alleles and their related serological groups are associated with a single MICA allele. The lack of evidence for an active diversification of the MICA gene after racial separation indicates an evolutionary history distinct from that of the classical HLA genes. *Human Immunology 67, 152–158 (2006).* © American Society for Histocompatibility and Immunogenetics, 2006. Published by Elsevier Inc.

KEYWORDS: MICA polymorphism; HLA-B; Linkage disequilibrium

ABBREVIATIONS

HLA	human leukocyte antigen
MHC	major histocompatibility complex
MIC	major histocompatibility complex class I chain-related
TCR	T-cell receptor
LD	linkage disequilibrium
PCR–SSOP	polymerase chain reaction–sequence-specific oligonucleotide probing
HWE	Hardy–Weinberg equilibrium

INTRODUCTION

Major histocompatibility complex (MHC) class I chain-related (MIC) molecules are stress-inducible proteins encoded by genes on chromosome 6 mapping close to HLA-B and -C [1]. The molecular structure of MIC proteins bears similarity to classical class I HLA but the lack of the β_2-microglobulin association, inability to bind peptides, and highly restricted tissue distribution of MIC proteins indicate a clear distinction in their specialized functions relative to those of the classical HLA molecules. The MICA protein is known to serve as a ligand for the activating natural killer cell receptor NKG2D, which is also expressed on the surface of all CD8 $\alpha\beta$ T cells [2–4]. *In vitro* binding experiments have shown that the allelic variation of MICA influences the affinity and efficiency of ligand binding between MICA and NKG2D [5] and may therefore influence TCR-

From the Laboratory of Genomic Diversity, NCI-Frederick, Basic Research Program, SAIC Frederick, Frederick, MD, USA (X.G., D.M., M.C.); Department of Mathematics and Statistics, University of Vermont, Burlington, VT, USA (R.M.S.); Georgetown University School of Medicine, Georgetown, DC, USA (P.K.); and Laboratory of Genomic Diversity, National Cancer Institute, Frederick, MD, USA (S.J.O'B.).

Address reprint requests to: Mary Carrington, P.O. Box B, Laboratory of Genomic Diversity, NCI-Frederick, Frederick, MD 21702, USA; Tel: (301)846-1390; Fax: (301)846-6771; E-mail: carringt@ncifcrf.gov.

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mediated antigen recognition on target cells. MICA polymorphism may also affect graft survival in organ transplantation since allo-antibodies against specific MICA alleles have been found in organ transplant recipients [6].

Like HLA, the MICA gene exhibits a high degree of allelic variation with at least 58 MICA alleles recognized so far. The functional relevance of the allelic diversity and its role in disease development are yet to be established. Due to the proximity of the MICA gene to HLA-B (46.4 kb centromeric to HLA-B), the alleles of these two loci are in strong linkage disequilibrium, which complicates identification of independent MICA effects in disease association studies. Knowledge of population distribution of MICA alleles and their LD relationships with HLA may provide clues with regard to the role of MICA in disease and insights into the evolutionary history of the MHC. Using PCR–SSOP-based typing technologies, we examined 1850 individuals from two North American populations for the distribution of MICA alleles and MICA-HLA-B haplotypes.

MATERIALS AND METHODS

Study Populations

A total of 1850 unrelated individuals from our collection of AIDS-related cohorts, including 1395 HIV-positive and 456 HIV-negative individuals, were typed for MICA and HLA-B polymorphisms. These individuals belong to two racial groups: European-Americans ($N = 1245$) and African-Americans ($N = 605$).

DNA Preparation

Genomic DNA was prepared from B-cell lines previously transformed from peripheral blood lymphocytes. DNA extraction was performed using the Qiagen DNA Purification Kit (Qiagen Inc., Valencia, CA, USA) following the manufacturer's instructions.

MICA and HLA-B Typing

MICA typing was carried out using a PCR–SSOP-based method modified from previously published protocols [7]. Briefly, the segment of the MICA gene containing exon 2, intron 2, exon 3, intron 3, and exon 4 was selectively amplified using a pair of locus-specific primers, P6823 (5′-CGTTCTTGTCCCTTTGCCYGTGTGC) and P8999 5′-(GATGCTGCCCCCATTCCCTTCCCAA) [8]. For some samples a second-round PCR using the PCR product from the first PCR as the template and a pair of nested primers MAE2-15 (5′-AGCCCCACAGTCTTC) and MAE4-275 (5′-CAGAGGGCACAG) was necessary to enhance the yield of the PCR product. PCR products were blotted on Hybond-N^+ membrane (Amersham Biosciences UK Limited, UK) and hybridized with a panel of 62 oligonucleotide probes designed to match all known sequence variations in exons 2, 3, and 4. Alleles were assigned on the basis of SSOP hybridization patterns predicted from known MICA alleles. HLA-B typing was performed following the PCR–SSOP typing protocols recommended by the 13th International Histocompatibility Workshop (http://www.ihwg.org/components/ssopr.htm). Ambiguity was resolved by sequencing the entire sequence from exon 2 through exon 3.

Data Analyses

Allele frequencies of MICA were calculated by direct gene counting. Hardy–Weinberg equilibrium (HWE) was tested using the Monte Carlo version of the exact test of Guo and Thomson [9]. p values were corrected for the six tests performed using Hochberg's method for multiple comparisons [10]. Two-locus haplotype frequencies and linkage disequilibrium between pairs of MICA and HLA-B alleles were estimated by the maximum likelihood expectation using the HAPL-E software package developed in the Laboratory of Genomic Diversity, National Cancer Institute (G Nelson et al., unpublished). The relative strength and significance of LD between individual alleles were assessed using Lewontin's [11] D'ij statistic (written as D' in what follows) and the single degree of freedom χ^2 statistic. The strength of overall LD between the two loci was measured using a multiallelic statistic [12], a weighted average of the individual D' values with weights given by the product of allele frequencies. Values for this statistic range from zero (linkage equilibrium) to one (complete LD). The significance of overall LD was tested using a permutation test based on the likelihood ratio statistic [13]. The null hypothesis that the distribution of allele frequencies is compatible with neutral expectations, based on the sample homozygosity statistic, was tested using the exact test developed by Slatkin [14, 15].

RESULTS

In both European-Americans and African-Americans HIV-positive and HIV-negative groups manifested no significant differences in the frequency distribution of MICA alleles as shown in Table 1. We also did not detect significant associations of HLA-B alleles with HIV infection in HIV-positive and HIV-negative groups nor among different clinical groups (data not shown) even though previous survival analyses have identified particular HLA-B alleles associated with altered rates of disease progression [16, 17]. Thus, this sample is appropriate for population allele/haplotype frequency analyses. For the purpose of anthropological analyses, study subjects were pooled by their racial origins into European-Americans

TABLE 1 Allele frequencies of MICA in European-American and African-American populations

MICA*	Position 129	European-American Total $N = 1245$	European-American HIV+ $N = 964$	European-American HIV− $N = 282$	African-American Total $N = 605$	African-American HIV+ $N = 431$	African-American HIV− $N = 174$
001	M	0.0108	0.0114	0.0089	0.0140	0.0128	0.0172
00201	M	0.1410	0.1447	0.1281	0.2537	0.2494	0.2644
00202	M	0.0016	0.0016	0.0018	0.0000	0.0000	0.0000
004	V	0.0747	0.0674	0.0996	0.1909	0.1995	0.1695
006	V	0.0032	0.0031	0.0036	0.0008	0.0012	0.0000
00701	M	0.0458	0.0472	0.0409	0.0066	0.0081	0.0029
00702	M	0.0048	0.0052	0.0036	0.0058	0.0046	0.0086
00801	V	0.4305	0.4196	0.4680	0.2661	0.2622	0.2759
00802	V	0.0028	0.0021	0.0053	0.0512	0.0499	0.0546
00901	V	0.0711	0.0731	0.0641	0.0174	0.0174	0.0172
00902	V	0.0137	0.0119	0.0196	0.0215	0.0197	0.0259
010	V	0.0578	0.0607	0.0480	0.0116	0.0116	0.0115
011	M	0.0289	0.0311	0.0214	0.0256	0.0255	0.0259
01201	M	0.0193	0.0213	0.0125	0.0124	0.0139	0.0086
015	M	0.0020	0.0021	0.0018	0.0562	0.0568	0.0546
016	V	0.0229	0.0228	0.0231	0.0017	0.0023	0.0000
017	M	0.0112	0.0135	0.0036	0.0058	0.0046	0.0086
018	M	0.0390	0.0420	0.0285	0.0298	0.0302	0.0287
019	M	0.0120	0.0130	0.0089	0.0083	0.0058	0.0144
021	M	0.0028	0.0026	0.0036	0.0017	0.0023	0.0000
024	V	0.0012	0.0005	0.0036	0.0008	0.0012	0.0000
030	M	0.0016	0.0016	0.0018	0.0033	0.0035	0.0029
035	M	0.0004	0.0005	0.0000	0.0000	0.0000	0.0000
041	M	0.0000	0.0000	0.0000	0.0050	0.0070	0.0000
043	M	0.0000	0.0000	0.0000	0.0033	0.0046	0.0000
044	V	0.0000	0.0000	0.0000	0.0008	0.0000	0.0029
045	M	0.0004	0.0005	0.0000	0.0033	0.0035	0.0029
046	M	0.0000	0.0000	0.0000	0.0025	0.0023	0.0029
047	M	0.0004	0.0005	0.0000	0.0000	0.0000	0.0000

χ^2 test detected no significant differences in the frequency distributions of MICA alleles in HIV+ and HIV− groups in both populations.

($N = 1245$) and African-Americans ($N = 605$). A total of 29 MICA alleles were detected in the present experiments (Table 1), of which 25 and 26 were detected in European-Americans and African-Americans, respectively. In both populations MICA*00201, *004, and *00801 were the three most common alleles and the only alleles with frequency greater than 10%. Among European-Americans, MICA*00801 had the highest allele frequency (43.05%), followed by *00201 (14.1%). Twelve other alleles showed frequencies between 1 and 7.47%, and the remaining 11 alleles had frequencies of less than 1%. Among African-Americans, the same three alleles, *00201, *004, and *00801, account for 25.37, 19.09, and 26.61% of the gene frequency, respectively. Nine other alleles had frequencies higher than 1% and 14 alleles were observed at frequencies of <1%. All the alleles with a frequency greater than 1% in either population were also found in the other population. Population-specific alleles were rare and only sporadically detected in one of the two racial groups. The distribution of MICA alleles and genotypes in the two study populations did not show significant deviation from HWE. Also, in both populations, the distribution of MICA allele frequencies did not show a significant deviation from neutral expectations.

A total of 88 HLA-B alleles at the four-digit resolution level were detected in the present study, including 70 in European-Americans and 61 in African-Americans (Table 2). When only the alleles with a frequency higher than 1% were counted, however, the numbers dropped to 22 and 25 in the two populations, respectively.

Table 3 shows inferred haplotype frequencies and LD values between pairs of MICA and HLA-B alleles. Haplotype distribution in the two racial groups showed a high degree of variation that was largely dependent on the distribution of HLA-B alleles. For example, the MICA*00801-B*0702 haplotype is the most common haplotype in European-Americans (12.2%), and it is the second most common haplotype in African-Americans (6.67%). Both alleles on this haplotype are commonly detected in both study populations. The MICA*00201-B*5301 haplotype, on the other hand, is the most com-

TABLE 2 Frequencies of HLA-B alleles in European-American and African-American populations

HLA-B*	Eur. A. $N = 1238$	Afr. A. $N = 587$	HLA-B*	Eur. A. $N = 1238$	Afr. A. $N = 587$
0702	0.1244	0.0792	3904	0.0000	0.0010
0705	0.0024	0.0076	3905	0.0004	0.0000
0801	0.1001	0.0382	3906	0.0041	0.0010
1302	0.0332	0.0115	4001	0.0506	0.0191
1401	0.0134	0.0067	4002	0.0142	0.0019
1402	0.0231	0.0181	4006	0.0004	0.0010
1403	0.0000	0.0019	4008	0.0004	0.0000
1501	0.0563	0.0095	4011	0.0008	0.0000
1503	0.0020	0.0706	4012	0.0000	0.0010
1504	0.0000	0.0000	4101	0.0041	0.0048
1507	0.0004	0.0000	4102	0.0045	0.0029
1508	0.0004	0.0010	4103	0.0000	0.0010
1509	0.0004	0.0010	4201	0.0004	0.0515
1513	0.0000	0.0029	4202	0.0000	0.0048
1516	0.0016	0.0200	4402	0.0823	0.0219
1517	0.0049	0.0029	4403	0.0482	0.0468
1518	0.0024	0.0029	4404	0.0004	0.0000
1524	0.0016	0.0000	4405	0.0012	0.0000
1537	0.0000	0.0057	4501	0.0057	0.0525
1538	0.0004	0.0000	4701	0.0028	0.0000
1545	0.0004	0.0000	4801	0.0008	0.0000
1801	0.0429	0.0277	4901	0.0178	0.0219
1803	0.0012	0.0000	5001	0.0085	0.0162
2702	0.0049	0.0000	5101	0.0571	0.0210
2703	0.0000	0.0029	5102	0.0000	0.0010
2705	0.0409	0.0057	5107	0.0004	0.0010
2707	0.0004	0.0000	5109	0.0008	0.0000
2708	0.0004	0.0000	5201	0.0089	0.0210
2709	0.0004	0.0000	5301	0.0081	0.1174
3501	0.0462	0.0725	5302	0.0000	0.0010
3502	0.0126	0.0010	5501	0.0190	0.0048
3503	0.0174	0.0038	5502	0.0004	0.0000
3504	0.0008	0.0000	5601	0.0041	0.0019
3505	0.0000	0.0010	5701	0.0454	0.0115
3508	0.0041	0.0000	5702	0.0004	0.0086
3512	0.0004	0.0000	5703	0.0012	0.0439
3517	0.0004	0.0000	5704	0.0000	0.0029
3524	0.0004	0.0000	5705	0.0000	0.0010
3701	0.0138	0.0057	5801	0.0093	0.0363
3801	0.0251	0.0019	5802	0.0004	0.0420
3802	0.0004	0.0000	7301	0.0004	0.0000
3901	0.0146	0.0029	7801	0.0000	0.0057
3902	0.0000	0.0010	8101	0.0000	0.0229
3903	0.0012	0.0000	8201	0.0000	0.0019

mon haplotype in African-Americans (9.49%) but was observed much less frequently in European-Americans (0.65%). This difference is based on the frequency of B*5301, which is found predominantly in populations of African origin, whereas MICA*00201 is a universally common allele. Overall, population-specific haplotypes are confined to those involving a population-specific HLA-B allele.

Overall LD between MICA and HLA-B was significant in both populations. The strength of overall LD, measured by Hedrick's multiallelic statistic, was greater in European-Americans (0.95) than in African-Americans (0.87).

Relative strength of LD between individual alleles was measured by D', where D' = 1 indicates complete association between the two alleles on the haplotype. Significant LD between MICA and HLA-B was observed in both racial groups, based on the χ^2 analysis (Table 3). In European-Americans, all the inferred haplotypes with a frequency greater than 0.5% were in significant LD ($p < 0.05$) and 81% (25/31) of these haplotypes have a D' greater than 0.7. Similarly, in the African-American group, only 1 of the 35 inferred haplotypes did not reach statistical significance and 65% (23/35) of the haplotypes showed a D' greater than 0.7. Upon restricting the analysis to haplotypes with frequencies of 0.5% or greater, common MICA alleles were found to have multiple associations with HLA-B. For instance, the two dominant MICA alleles *00201 and *00801 are both associated with several B alleles. MICA*00201 is associated with HLA-B*35, *53, and *58 related subtypes while MICA*00801 is associated with HLA-B*07, *08, *13, *37, *40, and *4402 alleles. In contrast, very few HLA-B alleles showed multiple associations with MICA, including some of the most common B alleles, after removing the low-frequency haplotypes from consideration.

Most LD relationships between MICA and HLA-B are shared by both populations. A smaller proportion of the haplotypes is apparently population specific and these haplotypes normally involve a MICA or HLA-B allele that has a highly restricted population distribution. One exception was the MICA*004 association with HLA-B*5101 and B*5201, which was evident only in African-Americans even though all of these alleles were common in both populations. In European-Americans B*5101 and B*5201 are associated with MICA*00901 (an allele closely related to MICA*004) and, to a lesser extent, with MICA*010.

Our cohorts included six individuals who have one copy of HLA-B*4801, an allele known to be associated with a MICA null allele caused by a deletion involving the entire MICA gene [18–20]. In all six samples, MICA typing showed a "homozygous" pattern with only one MICA allele detected, and the detected MICA alleles are all in LD with the respective non-B*4801 HLA-B allele of each individual. Though the typing methods used in the present study could not directly detect MICA null alleles, the typing results support the previous observation with regard to the HLA-B*4801 association with a MICA null allele.

TABLE 3 Two-locus haplotype frequencies and linkage disequilibrium of MICA with HLA-B in European-American and African-American populations

European-Americans (N = 1238)				African-Americans (N = 587)			
MICA*-HLA-B*	Frequency	D′	χ^2	MICA*-HLA-B*	Frequency	D′	χ^2
00801----0702	0.1220	0.9770	171.87	00201----5301	0.0949	0.7842	114.50
00801----0801	0.0990	0.9928	140.00	00801----0702	0.0667	0.8870	84.88
00801----4402	0.0771	0.8958	95.18	00801----1503	0.0590	0.8264	69.89
010----1501	0.0529	0.9389	493.53	00201----3501	0.0581	0.7961	69.82
00801----4001	0.0493	0.9575	64.73	004----4201	0.0469	0.9525	92.24
00901----5101	0.0493	0.8548	390.91	00802----1510	0.0428	0.9400	196.78
004----4403	0.0459	0.9522	375.73	004----4403	0.0393	0.9004	73.78
00701----2705	0.0388	0.9379	401.52	00201----5802	0.0377	0.9142	51.06
00201----3501	0.0371	0.7750	171.86	00801----0801	0.0351	0.9068	44.88
00201----5701	0.0327	0.6779	136.60	015----4501	0.0349	0.6718	131.36
00801----1302	0.0323	0.9570	41.98	00201----5801	0.0316	0.8676	40.74
018----1801	0.0295	0.6762	285.64	004----4901	0.0205	0.9507	39.91
00201----3801	0.0230	0.9062	118.48	00801----4402	0.0205	0.9402	26.88
011----1402	0.0222	0.9639	279.70	00801----8101	0.0197	0.9431	25.93
004----4901	0.0170	0.9509	137.59	004----5703	0.0181	0.2981	11.25
01201----5501	0.0170	0.8915	240.29	00801----4001	0.0163	0.9319	21.15
00801----4002	0.0141	1.0000	19.14	011----1402	0.0163	0.9020	98.68
00801----3701	0.0133	0.9480	16.92	001----1801	0.0137	0.5451	85.38
00201----3901	0.0129	0.8707	64.45	004----5201	0.0128	0.6080	16.98
016----3502	0.0121	0.9670	166.09	00902----5001	0.0128	0.7847	78.09
017----5701	0.0113	0.2414	120.19	004----5101	0.0120	0.4866	12.77
001----1801	0.0105	0.2373	114.33	018----5703	0.0094	0.1996	25.14
00901----3503	0.0093	0.4986	53.00	010----1501	0.0086	0.9080	66.92
00201----5801	0.0089	0.9494	46.99	00801----1516	0.0077	0.1704	1.19
00901----5201	0.0081	0.9021	65.21	00902----4501	0.0071	0.1203	16.50
00902----5001	0.0077	0.9034	121.85	018----5301	0.0070	0.0328	3.42
00201----5301	0.0065	0.7672	29.34	00801----1302	0.0068	0.5457	5.09
0011----1401	0.0065	0.4694	60.05	015----5702	0.0068	0.8806	29.30
0019----1401	0.0065	0.4785	86.94	04----7801	0.0060	1.0000	12.04
00201----3503	0.0064	0.2658	11.02	017----5701	0.0060	0.5357	49.17
0016----3501	0.0056	0.1004	23.55	018----1801	0.0060	0.2179	16.98
				00901----5101	0.0060	0.2787	25.91
				00801----3701	0.0051	1.0000	7.09
				041----5301	0.0051	0.0405	15.54
				00801----0705	0.0051	0.6478	4.56

Only the haplotypes with a frequency greater than 0.5% are included.

DISCUSSION

The PCR–SSOP-based typing method used in this study provides accurate, reliable, and high-resolution typing of the MICA gene and is particularly suitable for large sample sizes. The recognition of the MICA null allele in this study, however, relied on its known association with HLA-B*4801. For populations with higher frequencies of MICA null alleles, especially null alleles without a clear-cut HLA association, an alternative typing method for direct detection of null alleles will be necessary.

It has been suggested that the allelic variation of MIC proteins may have been maintained by overdominant selection [21], similar to that proposed for HLA genes [22]. However, allele frequency distributions for MICA in our data were not significantly different from neutral expectations in both racial groups. In fact, the observed homozygosity was slightly higher than expected under neutrality for both groups. For HLA-B, both populations had observed homozygosity values lower than neutral expectations, even though there did not reach statistical significance. Our results showed moderately high MICA diversity. The observed MICA polymorphism, however, has limited variation across racial groups with little evidence of active microevolution occurring after the separation of racial groups, which has been clearly observed at the HLA loci. Though 29 MICA alleles were detected in our study sample, a large number of the alleles were found only sporadically (13 alleles have a frequency $<1\%$). The same group of three alleles (*00201, *00801, and *004) accounted for more than 64 and 71% of the allele frequencies in the two populations, respectively, and these alleles have been commonly found

in other populations also [23]. Nevertheless, some exceptions have been reported, such as MICA*027, which is common in Asians [24] but not in other ethnic groups. This may indicate a relatively newer origin of *027 generated after the separation of Asian populations from other racial groups.

Individual MICA alleles that have high frequencies showed multiple LD relationships with HLA-B, whereas individual HLA-B alleles, including the common B*07 and B*08 alleles, were generally observed with only a single MICA association. This "one-way" LD relationship suggests that the common MICA alleles are very old, predating major branches of HLA-B alleles. One exception to this generalization is the association of B*51 with both MICA*004 and *00901 in African-Americans, perhaps reflecting a common origin of the two MICA alleles. In fact, MICA*004 and *00901 are identical in nucleotide sequence except for a nonsynonymous single-base mutation at the end of exon 3. Thus, the less frequent MICA*00901 may have been generated from MICA*004 on a B*5101-bearing haplotype. Similarly, MICA*001 and *018 are identical except for a nonsynonymous mutation in exon 3, codon 125, with *018 retaining the MICA consensus codon 125. The two alleles are both linked to B*1801, indicating that MICA*001 might have been generated from MICA*018 through a point mutation.

LD relationships between MICA and HLA-B also reflect the evolutionary history of HLA-B alleles. For example, B*35, *53, and *58 share a high degree of sequence homology and are considered to have been generated from the same progenitor allele. The fact that they are all linked with the same MICA allele (*00201), consistently detected both in the present and in previously reported populations around the world [24, 25, 26], further supports the notion of a common ancestry. Similarly, the MICA*004 association with B*41 and *42 and the MICA*00201 LD with B*38 and *39 also reflect the shared common ancestry of the B alleles. Individual MICA alleles tend to associate with serological HLA-B groups. One of the few exceptions is B*44, where the two B*44 subtypes *4402 and *4403 were found to be associated with different MICA alleles (MICA*00801 and *004, respectively) in both racial groups. Interestingly, in our cohorts the two B*44 subtypes were also found to have distinct LD relationships with HLA-Cw alleles (data not shown). These observations may reflect the long and distinctive evolutionary histories of the two B*44 alleles and their extended MHC haplotypes, even though they still share the same serological determinants.

It has been demonstrated *in vitro* that the allelic diversity at the MICA locus affects ligand binding between the MICA and the NK-cell receptor NKG2D, potentially affecting NK-cell activation and the modulation of T-cell responses [5]. Alleles at the MICA locus can be defined as strong or weak binders on the basis of their capacity to bind NKG2D, which has been suggested to be attributed to the methionine vs valine substitution in position 129 of the MICA protein. The strong NKG2D binding alleles share methionine in position 129, while weak binding alleles have valine in this position. In the two study populations, the combined frequencies of strong (methionine 129) vs weak (valine 129) NKG2D binders are 32/68% in European-Americans and 44/56% in African-Americans. European-Americans showed a lower ratio of strong to weak NKG2D binding alleles than African-Americans resulting from their extremely high frequency of the valine 129 (weak binding) allele MICA*00801 (43%). In our cohorts we did not detect any association of individual MICA alleles with HIV infection, nor did we find a correlation of NKG2D binding with HIV status. Examination of the overall capacity of MIC proteins to interact with NK receptors across populations, however, may provide useful information for other population-based disease studies.

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