MICA Polymorphism Is Associated With Type 1 Diabetes in the Korean Population

YONGSOO PARK, MD
HONGKYU LEE, MD
CARANI B. SANJEEVI, MD
GEORGE S. EISENBARTH, MD, PHD

OBJECTIVE — Recent studies have demonstrated that MICA (major histocompatibility complex class I chain-related genes) on the short arm of the chromosome 6 are associated with susceptibility to various autoimmune diseases in Caucasians. The aim of our study was to investigate the role of MICA in type 1 diabetes susceptibility independent of the HLA DR-DQ polymorphism in genetically distinct Koreans.

RESEARCH DESIGN AND METHODS — A total of 119 patients selected from Korean Seoul type 1 diabetes registry and 134 nondiabetic unrelated control subjects were typed for exon 5 polymorphism of MICA in addition to HLA DR-DQ typing. A total of 52 simplex families of type 1 diabetes were also studied.

RESULTS — The MICA microsatellite allele consisting of six repetitions of GCT/AGC (A6) was present at a significantly lower frequency in the diabetic patient group ($P_c < 0.01$; $P_c = P$ value after Bonferroni correction) than in the control population. The MICA microsatellite allele consisting of four repetitions (A4) was present at a higher frequency in diabetic patients ($P < 0.05$). This deviated distribution was not changed even after controlling for the HLA DRB1-DQB1 haplotype. Transmission/disequilibrium test revealed significant deviation of transmission for alleles at the A6 polymorphism within the MICA gene ($P < 0.05$).

CONCLUSIONS — We could assess that the MICA gene might be associated with type 1 diabetes transracially independent of the HLA gene.

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Although there were major discrepancies among studies on the genes associated with type 1 diabetes transracially, highly significant linkage for HLA was revealed in all reports (1,2). It is known that more than one genetic locus, even within HLA, is important for disease risk. A primary role of some antigen-presenting HLA DR and DQ molecules has been established (1–4); however, the relative importance of HLA class II genes remains to be determined in each population by experimental data. The contribution of HLA class II genes to type 1 diabetes in some Asian populations is less important because a significant portion of the diabetic patients do not have the high-risk HLA genotype (5,6). The HLA DR3/4 (DQB1*0302) heterozygous genotype occurs in ~0.9% of children born in Seoul, Korea, and is present in ~9.3% of children developing type 1 diabetes. There is a larger probability for the role of genes other than HLA class II genes in Asians.

Nevertheless, the population frequency of DR3/4 genotype is still 10–20 times higher than the prevalence of type 1 diabetes associated with this genotype (1,7). DRB1 subtyping might change the risk of type 1 diabetes in the DQ high-risk population, even though it is assumed to be accounted for only 10% (7,8). This implies that additional protective genes and/or environmental factors influence susceptibility to the disease. Studies both in animals and in humans have also indicated that other major histocompatibility complex (MHC)-linked genes are participating in the susceptibility of HLA complex (9,10). These other MHC-linked genes have not been mapped. The HLA complex encompasses 3.5 Mb of DNA from the centromeric HLA-DPB2 locus to the telomeric HLA-F locus on chromosome 6p21, and the strong linkage disequilibrium (LD) between genes in the complex makes this a difficult task.

One Japanese group reported that HLA class I molecule A and B were associated with early-onset type 1 diabetes (10). Furthermore, a novel family of the human MHC class I genes termed MICA (MHC class I chain-related genes) has been recently identified near the HLA-B gene on the short arm of human chromosome 6 (11,12). This gene has been known to carry numerous nonsynonymous polymorphisms, and when these are overlaid onto a three-dimensional structure, most lie along the edge of the peptide-binding groove in the α2 extracellular domain (13,14). The polymorphism of the MICA gene (11,12) and its location in the HLA region warrant studies aimed at identifying an association with the risk for autoimmune diseases. However, the contribution of this HLA locus to type 1 diabetes susceptibility might be unclear because of the strong LD around this area. In this study, we investigated whether the MICA gene as well as HLA class II polymorphism influenced the genetic predisposition to type 1 diabetes in Korea.

RESEARCH DESIGN AND METHODS

Subjects

The samples used in this study included three groups. First, for the case-control association study, 119 type 1 diabetic patients were selected randomly from the Korean Seoul Registry (5,6,15). All patients

From the Department of Internal Medicine (Y.P., H.L.), Hanyang and Seoul National University, School of Medicine, Seoul, Korea; the Department of Molecular Medicine (C.B.S.), Karolinska Hospital, Stockholm, Sweden; and the Barbara Davis Center for Childhood Diabetes (G.S.E.), University of Colorado Health Sciences Center, Denver, Colorado.

Address correspondence and reprint requests to Yongsoo Park, MD, Department of Internal Medicine, Hanyang University Hospital, 249-1 Kyomun-dong, Kuri, Kyunggi-do, 471-020, Korea. E-mail: parkys@email.hanyang.ac.kr.

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Abbreviations: LD, linkage disequilibrium; MHC, major histocompatibility complex; MICA, MHC class I chain-related genes; PCR, polymerase chain reaction; RR, relative risk; TDT, transmission/disequilibrium test.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

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Table 1—*MICA alleles and genotypes in Korean type 1 diabetic and healthy subjects*

MICA alleles and genotypes	Patients (n = 119)	Healthy subjects (n = 134)	RR	P	P_c
A4	56 (23.5)	41 (15.3)	1.70	0.023	0.11 (NS)
A5	58 (24.4)	78 (29.1)	0.79	NS	NS
A5.1	53 (22.3)	43 (16.0)	1.50	NS	NS
A6	30 (12.6)	66 (24.6)	0.44	0.00062	0.0031
A9	41 (17.2)	40 (14.9)	1.19	NS	NS
A6/A6	0 (0)	6 (4.5)	0.08	0.002	0.030
A6/X (X = other than A6)	30 (25.2)	54 (40.3)	0.50	0.016	0.24 (NS)
X/X (X = other than A6)	89 (74.8)	74 (55.2)	2.41	0.0015	0.0225

Data are n (%) unless otherwise indicated. P_c, corrected P value (correction factor was 5 for MICA alleles and 15 for MICA genotypes).

were on insulin therapy upon hospital discharge, <15 years of age, and residents of Seoul at the time of disease onset. Their mean age was 13 years (range 3–22). The second group consisted of 134 nondiabetic control subjects with no family history of diabetes who were selected from the same geographical area. Their mean age was 34 years (14–46). In addition, a third group of 52 simplex families with type 1 diabetes was recruited from the Seoul Registry in Korea. All individuals or their parents gave appropriate informed consent to participate in the study.

HLA genotyping

Peripheral blood lymphocytes from all donors were used for the molecular typing of the low-resolution typing of HLA-DRB1 and most common HLA-DQA and -B chain gene allelic forms (5,6,16). HLA DRB1/DQA1/DQB1 was genotyped using polymerase chain reaction (PCR)–sequence-specific oligonucleotide techniques according to previous reports (5,16). The nomenclature used to define the HLA-DR and -DQ alleles was that of the official nomenclature for factors of the HLA System (17).

MICA genotyping

For analysis of microsatellite repeat polymorphism in the transmembrane (TM) region of the MICA gene, PCR primers flanking the TM region (MICA5F: 5′-CCTTTTTTTCAGGGAAAGTGC-3′; MICA5R: 5′-CCTTACCATCTCCAGAAACTGC-3′) were designed (18,19). Genotypes were determined using a fluorescent-based method as reported previously (18,19). Briefly, reverse PCR primer was labeled with 6-FAM, and the PCR products were electrophoresed in 6% denaturing polyacrylamide gel using a Model 373 DNA sequencer (Applied Biosystems, Foster City, CA) with Genescan 500 TAMRA as an internal lane size standard. PCR fragments were sized with Genescan 672 software (Applied Biosystems), genotyped with Genotyper software (Applied Biosystems), and alleles were called using a histogram.

Data analysis

Data from family-based samples allowed unambiguous assignment of haplotypes in nearly all families. However, the haplotypic data in type 1 diabetes families did not allow us to define all of the extended haplotypes, including HLA and MICA in both isolated type 1 diabetes cases and the control group. Therefore, associations of HLA with MICA alleles were assessed using the Δ value for nonrandom assortment of alleles (20). Moreover, to minimize the influence of the strong LD that exists between HLA and MICA, we calculated the relative risk (RR) conferred by the MICA*A6 or *A4 allele by using HLA haplo-identical control subjects and diabetic patients (21).

The allele and haplotype frequencies were obtained by the method of gene counting. Case-control association studies were carried out for the allele frequencies using a two-by-two contingency χ^2 test (two-tailed) or the Fisher's exact test. Estimates of the RR in the various individuals were calculated using Woolf's method, as modified by Haldane for small sample sizes when appropriate (22,23). The Bonferroni correction for multiple comparisons was applied. Both the P values before the correction (P) and after the correction (P_c) are shown.

Intrafamilial association studies were carried out using the transmission/disequilibrium test (TDT) as described by Spielman et al. (24).

RESULTS — MICA genotypes were successfully determined for 119 diabetic patients and 134 control subjects by using exon 5 microsatellite polymorphism. Among the MICA alleles, the frequency of the MICA*A6 allele was decreased in our cohort of Korean type 1 diabetic patients compared with the healthy control subjects (RR = 0.44, $P_c < 0.01$). (Table 1). The frequencies of MICA*A4 were higher in diabetic patients than in control subjects (RR = 1.70, $P < 0.05$), albeit the differences were not statistically significant after Bonferroni correction. None of the other MICA alleles was found to be associated, either positively or negatively, with risk for the disease. Among the MICA genotypes, both A6/A6 and X/X (X = genotypes other than A6) were significantly associated with type 1 diabetes in Korea. Although not statistically lower after correction for multiple comparison, the A6/X (X = other than A6) genotype was also lower in diabetic patients. Type 1 diabetic patients had more A4/A4 or A4/X genotype (X = other than A4) (RR = 1.7, $P < 0.05$) and less X/X (X = other than A4) than control subjects (RR = 0.59, $P < 0.05$). In our Korean simplex families, there was a significant decrease in transmission of the A6 allele (32.3%, $P < 0.05$). There was a similar deviation of transmission (but in the opposite direction from that for transmission of A6) of the A4 allele, although statistical significance was not reached (Table 2). These results suggested that MICA was significantly associated with type 1 diabetes.

Table 2—*TDT of the MICA alleles in Korean type 1 diabetic patients*

Allele	Transmitted (%)	Not transmitted (%)	P
A4	22 (64.7)	12 (35.3)	NS
A5	22 (51.2)	21 (48.8)	NS
A5.1	15 (55.6)	12 (44.4)	NS
A6	11 (32.3)	23 (67.7)	<0.05
A9	10 (45.4)	12 (54.6)	NS

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To determine whether the apparent MICA association with type 1 diabetes was a result of LD with HLA class II molecule, we evaluated linkage disequilibria, using Δ values for nonrandom assortment in the random control subjects. Table 3 presents the Δ and χ^2 values between HLA and the MICA*A6 (or MICA*A4). The frequencies of the MICA*A6 allele were significantly above random expectations on two HLA haplotypes (DR4-DQB1*0401 and DR1-DQB1*0301) ($P < 0.05$). In addition, the frequencies of MICA*A6 were different from random expectations on several other haplotypes, although statistical significance was not reached. In contrast, the frequencies of the MICA*A4 allele were significantly above random expectations on two HLA haplotypes (DR15-DQB1*0602 and DR13-DQB1*0603) and significantly below expectations on the DR8-DQB1*0601 haplotype ($P < 0.05$).

To minimize the influence of the strong LD that exists between HLA class II and MICA, we estimated the RR conferred by the MICA*A6 and *A4 allele by using HLA haplo-identical control subjects and diabetic patients (Table 4). The frequencies of the MICA*A6 allele in diabetic patients were decreased in all significant comparisons (DR4-DQB1*0401 and DR4-DQB1*0302), whereas those of the MICA*A4 allele were increased in all significant comparisons (DR4-DQB1*0401 and DR8-DQB1*0601).

Table 3—Δ *Values for nonrandom assortment of HLA with the protective MICA*A6 allele or the susceptible MICA*A4 allele in the random control group*

	+/+	+/−	−/+	−/−	Δ† ($\times 10^{-3}$)	χ^2
HLA/A6 allele						
DRB1-DQ haplotypes						
DR4-DQB1*0401	25	47	107	357	+8.4	4.6‡
DR1-DQB1*0301	2	0	130	404	+1.6	6.1‡
DR7-DQB1*0201	3	23	129	381	−3.8	2.5
DR15-DQB1*0602	5	31	127	373	−4.3	2.4
DR14-DQB1*0503	4	24	128	380	−3.2	1.7
DR12-DQB1*0301	14	26	118	378	+4.6	2.5
DR8-DQB1*0301	2	2	130	402	+1.1	1.4
DR4-DQB1*0302	8	30	124	374	−1.5	
DR9-DQB1*0303	14	46	118	358	−0.9	
DR13-DQB1*0604	7	29	125	375	−2.1	
DR3-DQB1*0201	2	8	130	396	−0.5	
DR1-DQB1*0501	8	20	124	384	+1.2	
DR8-DQB1*0302	3	7	129	397	+0.6	
DR8-DQB1*0601	8	24	124	380	+0.1	
DR15-DQB1*0601	8	24	124	380	+0.1	
DR4-DQB1*0301	2	8	130	396	−0.5	
DR10-DQB1*0501	5	11	127	393	+1.2	
DR11-DQB1*0301	5	13	127	391	+0.6	
DR8-DQB1*0401	0	4	132	400	−1.1	
DR13-DQB1*0603	2	4	130	400	+0.6	
DR7-DQB1*0303	0	4	132	400	−1.1	
Others§	5	19	1447	4425	−0.1	
HLA/A4 allele						
DRB1-DQ haplotypes						
DR8-DQB1*0601	0	32	82	422	−5.1	6.1‡
DR15-DQB1*0602	10	26	72	428	+4.7	4.6‡
DR13-DQB1*0603	3	3	79	451	+2.1	5.6‡
DR16-DQB1*0301	1	1	81	453	+0.7	1.9
DR10-DQB1*0501	4	12	78	442	+1.6	
DR1-DQB1*0501	6	22	76	432	+1.8	
DR9-DQB1*0303	7	53	75	401	−2.3	
DR8-DQB1*0301	0	4	82	450	−0.6	
DR8-DQB1*0401	0	4	82	450	−0.6	
DR4-DQB1*0401	9	63	73	391	−2.2	
DR13-DQB1*0604	7	29	75	425	+1.6	
DR1-DQB1*0301	0	2	82	452	−0.3	
DR7-DQB1*0201	5	21	77	433	+1.1	
DR14-DQB1*0503	5	23	77	431	+0.7	
DR12-DQB1*0301	6	34	76	420	−0.1	
DR4-DQB1*0302	5	33	77	421	−0.9	
DR3-DQB1*0201	2	8	80	446	+0.5	
DR8-DQB1*0302	1	9	81	445	−0.5	
DR15-DQB1*0601	5	27	77	427	+0.1	
DR4-DQB1*0301	1	9	81	445	−0.5	
DR11-DQB1*0301	3	15	79	439	+0.3	
DR7-DQB1*0303	1	3	81	451	+0.4	
Others¶	1	19	737	4067	−0.2	

Data are number of individuals unless otherwise indicated. †Positive Δ values indicate positive association, and negative Δ values indicate negative association between the assessed HLA and MICA allele; ‡$P < 0.05$; §denotes DR16-DQB1*0301, DR12-DQB1*0302, DR15-DQB1*0502, DR16-DQB1*0502, DR13-DQB1*0502, DR13-DQB1*0302, DR15-DQB1*0301, DR11-DQB1*0303, DR1-DQB1*0301, and DR14-DQB1*0301; ¶denotes DR12-DQB1*0302, DR15-DQB1*0502, DR16-DQB1*0502, DR13-DQB1*0502, DR13-DQB1*0302, DR15-DQB1*0301, DR11-DQB1*0303, DR1-DQB1*0301, and DR14-DQB1*0301.

CONCLUSIONS — Significant associations between type 1 diabetes and MICA gene polymorphisms were observed in our Korean population. Our data showed a protective effect for the A6 allele of MICA, which is still statistically significant after correction for multiple comparisons. Although the statistical significance is lost after correction of the *P* value, the uncorrected *P* value is suggestive of a positive association of the A4 allele with disease, especially when combined with the transmission data. The TDT analyses of our type 1 diabetes families done independently of case-control comparison showed that the transmission of A4 was similar (but in the opposite direction) to that of A6, suggesting a trend for predisposition to disease of the A4 allele. These results are novel and of interest for understanding the genetic basis of type 1 diabetes. The question is whether this observed association is independent of other type 1 diabetes genes in the MHC complex. Any analysis of association of alleles of genetic

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Table 4—*MICA associations with type 1 diabetes in HLA haplo-identical subjects*

	Diabetic patients A6/X or A6/A6	X/X†	Control subjects A6/X or A6/A6	X/X	RR	*P*
DRB1-DQ haplotypes						
DR4-DQB1*0401	10	31	22	19	0.3	0.012
DR4-DQB1*0302	4	23	8	11	0.2	0.048
DR8-DQB1*0302	0	6	3	2	0.06	NS (0.060)
DR11-DQB1*0301	0	6	4	5	0.09	NS
DR1-DQB1*0301	0	0	1	1	1.0	NS
DR7-DQB1*0201	6	5	3	10	4.0	NS
DR13-DQB1*0604	11	4	7	11	4.3	NS
DR15-DQB1*0602	0	3	5	13	0.4	NS
DR14-DQB1*0503	1	2	4	10	1.3	NS
DR12-DQB1*0301	1	1	12	8	0.7	NS
DR8-DQB1*0301	0	0	2	0	0.2	NS
DR9-DQB1*0303	13	29	14	16	0.5	NS
DR3-DQB1*0201	5	25	2	3	0.3	NS
DR1-DQB1*0501	4	14	7	7	0.3	NS
DR8-DQB1*0601	1	4	7	9	0.3	NS
DR15-DQB1*0601	1	0	7	9	3.8	NS
DR4-DQB1*0301	0	0	2	3	1.4	NS
DR10-DQB1*0501	0	1	3	5	0.5	NS
DR8-DQB1*0401	1	2	0	2	3.0	NS
DR13-DQB1*0603	0	3	2	1	0.09	NS
DR15/16-DQB1*0301	0	1	0	2	1.7	NS
DR15/16-DQB1*0502	0	3	1	2	0.2	NS
DR7-DQB1*0303	0	1	0	2	1.7	NS
Others‡	0	0	4	2	0.6	NS
	Diabetic patients A4/A4 or A4/X	X/X†	Control subjects A4/A4 or A4/X	X/X		
DRB1-DQ haplotypes						
DR4-DQB1*0401	27	14	9	32	6.9	0.0001
DR8-DQB1*0601	2	3	0	16	23.6	0.047
DR11-DQB1*0301	2	4	2	7	1.8	NS
DR15/16-DQB1*0502	3	0	0	3	49.0	NS
DR1-DQB1*0301	0	0	1	1	1.0	NS
DR7-DQB1*0201	6	5	5	8	1.9	NS
DR15-DQB1*0602	2	1	8	10	2.5	NS
DR14-DQB1*0503	1	2	5	9	0.9	NS
DR12-DQB1*0301	1	1	6	14	2.3	NS
DR8-DQB1*0301	0	0	0	2	5.0	NS
DR4-DQB1*0302	8	19	5	14	1.2	NS
DR9-DQB1*0303	13	29	7	23	1.5	NS
DR13-DQB1*0604	7	8	7	11	1.4	NS
DR3-DQB1*0201	9	21	2	3	0.3	NS
DR1-DQB1*0501	9	9	6	8	0.3	NS
DR8-DQB1*0302	2	4	1	4	2.0	NS
DR15-DQB1*0601	0	1	4	12	0.9	NS
DR4-DQB1*0301	0	0	1	4	3.0	NS
DR10-DQB1*0501	0	1	4	4	0.3	NS
DR8-DQB1*0401	1	2	0	2	3.0	NS
DR13-DQB1*0603	3	0	3	0	1.0	NS
DR15/16-DQB1*0301	0	1	1	1	0.3	NS
DR7-DQB1*0303	0	1	1	1	0.3	NS
Others‡	0	0	0	6	13.0	NS

Data are *n* unless otherwise indicated. †X = other than A6 or A4; ‡denotes DR12-DQB1*0302, DR13-DQB1*0502, DR13-DQB1*0302, DR13-DQB1*0301, DR11-DQB1*0303, and DR14-DQB1*0301.

loci located on chromosome 6p with type 1 diabetes susceptibility must necessarily take into account the strong effects of the DR and DQ loci and the strong LD that exists across the entire HLA region.

To distinguish associations resulting from LD from associations resulting from HLA's effects on type 1 diabetes susceptibility, we performed an LD analysis followed by an association analysis using HLA haplo-identical subjects. The frequencies of the MICA*A6 allele were increased on two HLA haplotypes (DR4-DQB1*0401 and DR1-DQB1*0301). Because DR4-DQB1*0401 is not decreased among type 1 diabetic patients, the pattern of LD between HLA class II and MICA may not explain the associations between MICA and type 1 diabetes. The DR1-DQB1*0301 haplotype is rare both in cases and control groups, and Δ is very small ($+1.6 \times 10^{-3}$). In contrast, the frequencies of the MICA*A4 allele were significantly increased on two HLA haplotypes (DR15-DQB1*0602 and DR13-DQB1*0603) and significantly decreased on the DR8-DQB1*0601 haplotype. Because the DR15-DQB1*0602 and the DR13-DQB1*0603 haplotypes are significantly decreased in patients with type 1 diabetes and are positively associated with the susceptible MICA*A4 allele, the pattern of LD between HLA class II and MICA might not explain the associations between MICA and type 1 diabetes. However, because the peculiar Asian haplotype DR8-DQB1*0601 is significantly decreased among type 1 diabetes patients, the negative association of MICA*A4 with HLA class II could explain the associations between MICA and type 1 diabetes.

To minimize the influence of the strong LD that exists between HLA class II and MICA, we estimated the RR conferred by the MICA*A6 and *A4 allele by using HLA haplo-identical control subjects and diabetic patients. The frequencies of the MICA*A6 allele in diabetic patients were decreased in all significant comparisons (the DR4-DQB1*0401 and the DR4-DQB1*0302 haplotypes), whereas those of the MICA*A4 allele in diabetic patients were increased in all significant comparisons (the DR4-DQB1*0401 haplotype and especially the DR8-DQB1*0601 haplotype, which was shown to be in negative association with MICA*A4). Because the HLA haplo-identical analysis used diabetic patients and control subjects who have one identical HLA haplotype, the nonmatched haplotype could still cause differences in the MICA gene fre-

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quencies between diabetic patients and the control group. However, the consistent negative associations of allele A6 and the consistent positive associations of allele A4 controlling for the different HLA haplotypes might suggest an independent effect of MICA gene on type 1 diabetes susceptibility in Koreans. Although these analyses might be useful, they did not completely eliminate the influence of HLA class II genes. The extended HLA haplotype comparisons will entirely eliminate any bias resulting from LD (20,21). However, the haplotypic data in type 1 diabetes families did not allow us to define all of the extended haplotypes, including HLA and MICA in both isolated type 1 diabetes cases and control subjects. Moreover, another possibility may be that the observed association could be because of the other genes in the complex.

The frequency of the MICA*A6 allele was decreased in our Korean type 1 diabetic patients. Both A6/A6 and X/X (X = other than A6) were significantly decreased in type 1 diabetic patients in Korea. The A6/X genotype also was lower in the diabetic patients. This might result from the influence of the accompanying protective HLA DR-DQ haplotypes. However, we could not find a significant LD between the MICA*A6 allele and the DR15-DQB1*0602 or the DR11/12-DQB1*0301 haplotype, which are dominantly protective in Koreans (5,6). We confirmed the protective influence of allele A6 in our small Korean simplex families. There was a significant decrease in transmission of the A6 allele. The specific molecular mechanism underlying this influence of MICA*A6 is unknown.

Type 1 diabetes is a polygenic disorder with an autoimmune basis for disease development (1). In addition to the HLA class II region, a second susceptibility locus for type 1 diabetes has been suggested to lie near the class I region (7,9,10). MICA is located in the MHC class III region near class I and is expressed by monocytes, keratinocytes, and endothelial cells (13,14). Sequence determination of MICA gene identified trinucleotide (GCT) repeat microsatellite polymorphism in exon 5. Five alleles with four, five, six, and nine repetitions of GCT or five repetitions of GCT with one additional nucleotide insertion (GGCT) were identified. The alleles are A4, A5, A6, A9, and A5.1 (11,12). The aim of our study was to find an association of MICA alleles with type 1 diabetes. A negative association was observed between type 1 diabetes and the A6 allele, and a similar, but slightly weak, positive association of type 1 diabetes with the A4 allele was observed in our Korean population. One recent Taiwanese report indicated that only the allele frequency of A9 was significantly higher in Taiwanese children with type 1 diabetes (25). We could not confirm what they found. However, the genotype and allele frequencies in Korean diabetic patients and control subjects are similar to the Japanese population (26), in which the frequency of the A4 allele was significantly higher and that of the A6 allele was significantly lower among type 1 diabetic patients. By contrast, they suggested that the A4 allele was associated with DR4-DQB1*0401 and the A6 allele was associated with the DR2-DQB1*0601 haplotype. However, they did not perform the LD analysis; they correlated various alleles. Nor did they present the family data in deducing the HLA DR-DQ haplotype. In our Korean data using the LD analyses, MICA*A4 is associated with HLA DR15-DQB1*0602, DR13-DQB1*0603, and DR8-DQB1*0601 rather than DR2-DQB1*0601, the sequences of which we confirmed, whereas MICA*A6 is associated with HLA DR4-DQB1*0401 and DR1-DQB1*0301. Contrary to their report, we found an influence of the MICA gene on type 1 diabetes susceptibility independent of the HLA DR-DQ haplotype.

One of us (C.B.S.) reported that allele A5 was associated with type 1 diabetes in the European Caucasoid population (19), and allele A5.1 was thought to be associated 100% in Latvian type 1 diabetic patients carrying DQB1*0201 compared with DQB1*0201-negative patients (A. Shtauvere, M. Ghaderi, I. Rumba, C.B.S., unpublished data). Our observations from the Caucasian Addison's disease patients suggested that allele A5.1 is an important risk marker for the autoimmune Addison's disease (18). It might be possible for different alleles to be associated with a disease or similar autoimmune diseases in different populations. If the two alleles at the locus in question were both in LD with the real susceptibility allele, this could easily be true. But it is also quite possible with polymorphic loci, such as HLA, that different alleles have similar effects. It has been known in a complex autoimmune disease like type 1 diabetes that more than one autoantigen can trigger the autoimmune response (27). There have been some reports that DR3 uses GAD, whereas DR4 uses insulin and ICA512/IA-2 (27,28). In Basque patients, because DR3 is at a very high frequency (and it seems to be a high-risk DR3 haplotype), the strongest type 1 diabetes association is seen with the DR3 haplotype (29). Because there are many DR4 and few or no DR3 in Sardinian patients, the strongest association of the DR4 haplotype with type 1 diabetes is seen (3). In our recent study of the susceptibility influence of MICA on Addison's disease, we also found a significant protective effect of the A6 allele independent of the HLA polymorphism in Caucasians in addition to the susceptibility effect of A5.1 (Y.P., C.B.S., L. Yu, M. Rewers, P.A. Gottlieb, P. Fain, G.S.E., unpublished data).

Our type 1 diabetic subjects were selected from a population-based incidence registry (15), and control subjects were selected randomly according to a standardized international protocol (30) and have been described previously (5,6), permitting precise estimates of population gene frequency. The genotype distribution for the control population was tested for Hardy-Weinberg equilibrium and was not different from that expected. Because our patients were all <15 years of age at onset, we could not assess the heterogeneity of age of onset for the susceptibility influence of MICA on type 1 diabetes. From this and the TDT, we can suggest that the MICA gene might be associated with type 1 diabetes transracially independent of the HLA class II gene. Additional investigation of adjacent markers in a large number of type 1 diabetic patients including those of different age of onset is required to clarify the contribution of the MICA gene to type 1 diabetes susceptibility.

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