Sequencing-Based Genotyping and Association Analysis of the MICA and MICB Genes in Type 1 Diabetes

Sarah F. Field, Sergey Nejentsev, Neil M. Walker, Joanna M.M. Howson, Lisa M. Godfrey, Jennifer D. Jolley, Matthew P.A. Hardy, and John A. Todd

OBJECTIVE—The nonclassical major histocompatibility complex (MHC) class I chain-related molecules (MICs), encoded within the MHC, function in immunity. The transmembrane polymorphism in *MICA* (*MICA-STR*) has been reported to be associated with type 1 diabetes. In this study, we directly sequenced both of the highly polymorphic MIC genes (*MICA* and *MICB*) in order to establish whether they are associated with type 1 diabetes independently of the known type 1 diabetes MHC class II genes *HLA-DRB1* and *HLA-DQB1*.

RESEARCH DESIGN AND METHODS—We developed a sequencing-based typing method and genotyped *MICA* and *MICB* in 818 families (2,944 individuals) with type 1 diabetes from the U.K. and U.S. (constructing the genotype from single nucleotide polymorphisms in exons 2–4 of *MICA* and 2–5 of *MICB*) and additionally genotyped the *MICA-STR* in 2,023 type 1 diabetic case subjects and 1,748 control subjects from the U.K. We analyzed the association of the *MICA* and *MICB* alleles and genotypes with type 1 diabetes using regression methods.

RESULTS—We identified known *MICA* and *MICB* alleles and discovered four new *MICB* alleles. Based on this large-scale and detailed genotype data, we found no evidence for association of *MICA* and *MICB* with type 1 diabetes independently of the MHC class II genes (*MICA* $P = 0.08$, *MICA-STR* $P = 0.76$, *MICB* $P = 0.03$, after conditioning on *HLA-DRB1* and *HLA-DQB1*).

CONCLUSIONS—Common *MICA* and *MICB* genetic variations including the *MICA-STR* are not associated, in a primary way, with susceptibility to type 1 diabetes. ***Diabetes* 57:1753–1756, 2008**

Approximately 50% of the familial clustering of type 1 diabetes is attributable to the major histocompatibility complex (MHC) region on human chromosome 6p21, and the MHC class II genes *HLA-DRB1* and *HLA-DQB1* account for a large proportion of this clustering (1). However, association studies in the MHC region indicate that other genes have effects in type 1 diabetes independently of the class II genes (2–4). Identification of these genes is complicated because genes in the MHC have multiple alleles and exhibit extensive linkage disequilibrium (LD), and the class II genes *HLA-DQB1* and *HLA-DRB1* show complicated dominance and epistatic effects. Importantly, any putative new effect must be distinguished from the effect of these class II genes (2,3).

The nonclassical MHC class I chain-related molecule (MIC) genes *MICA* and *MICB* are located 46.4 and 141.2 kb, respectively, centromeric of *HLA-B* and ~2 Mb telomeric of *HLA-DRB1* (5) and have been associated both with immunity and with autoimmune diseases (5). In common with most MHC HLA genes, alleles of *MICA* and *MICB* are named based on haplotypes of nonsynonymous single nucleotide polymorphisms (nsSNPs). However, the most studied polymorphism in either of these genes is a short tandem repeat (STR) in the transmembrane domain of *MICA* (*MICA-STR*). Whether these genes are associated with type 1 diabetes is uncertain, with multiple conflicting reports in the literature (3,6–14). Most of these studies failed to demonstrate convincingly that the association is independent of the MHC class II genes, and all were restricted to analyses of the *MICA-STR*. The *MICA-STR* is a GCT repeat microsatellite in *MICA* exon 5. (Alleles of the *MICA-STR* are named based on the number of repeat units.) In European populations, the most common allele of the *MICA-STR* is *MICA-STR**A5.1 (frequency 0.50), which contains five GCT repeats and a G insertion that causes a frame shift, leading to a premature stop codon and the truncation of the cytoplasmic tail. (*MICA-STR**A5 contains five GCT repeats but not the G insertion and is in frame.) Here, we genotyped the *MICA-STR* in a large case/control collection. However, different alleles of the *MICA-STR* associate with multiple alleles of other polymorphisms in *MICA* (5). Therefore, to capture the allelic variation of *MICA* more comprehensively, we designed a sequencing-based typing (SBT) method that yields the genotype information for both *MICA* and *MICB* for exons 2–5 and used it to genotype two collections of type 1 diabetic families. Various approaches have been described to obtain a complete genotype of the *MICA* and *MICB* genes (15,16). Our method is similar to other SBT methods but incorporates a novel scoring method that increases throughput.

RESEARCH DESIGN AND METHODS

MICA and *MICB* share 83% DNA sequence homology. Each gene consists of six exons: exon 1, which codes for a signal peptide, exons 2–4 for three extracellular domains (α1, α2, and α3), exon 5 for a transmembrane domain, and exon 6 for a carboxy-terminal cytoplasmic tail (5).

Genotyping of *MICA-STR*. Genotyping of *MICA-STR* was done using PCR (Note 1 of the online appendix [available at http://dx.doi.org/10.2337/db07-1402]) followed by capillary electrophoresis of the amplified product (17). We

From the Juvenile Diabetes Research Foundation/Wellcome Trust Diabetes and Inflammation Laboratory, Department of Medical Genetics, Cambridge Institute for Medical Research, University of Cambridge, Addenbrooke's Hospital, Cambridge, U.K.

Corresponding author: John A. Todd, JDRF/WT Diabetes and Inflammation Laboratory, Cambridge Institute for Medical Research, Wellcome Trust/MRC Building, Addenbrooke's Hospital, Hills Road, Cambridge, CB2 0XY, U.K. E-mail: john.todd@cimr.cam.ac.uk.

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LD, linkage disequilibrium; MHC, major histocompatibility complex; MIC, MHC class I chain-related molecule, nsSNP, nonsynonymous single nucleotide polymorphism; rpart, recursive partitioning; SBT, sequencing-based typing; SNP, single nucleotide polymorphism; STR, short tandem repeat.

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used primers CCTTTTTTTCAGGGAAAGTGC (FAM labeled) and CCTTACCATCTCCAGAAACTG.

SBT of *MICA* and *MICB*. Our aim was to maximize throughput in order to enable analysis of large sample sets; we therefore used a system that gave the most genotype information while minimizing the amount of sequencing and the cost. For this reason, we limited ourselves to the information that could be captured with a single pair of external primers. In both genes, exon 1 is ~8.5 kb from exon 2, exons 2–5 are in close proximity to each other in an approximately 2-kb segment, and exon 6 is ~2.25 kb centromeric of exon 5. Our *MICA* primers amplified a 1.9-kb fragment containing exons 2, 3, and 4; the *MICA-STR* is at the beginning of the exon 5 of *MICA*, and therefore it was not possible to sequence through exon 5. Our *MICB* primers amplified a 2.1-kb fragment containing exons 2, 3, 4, and 5. PCR (Note 1 of the online appendix) was done using *MICA* primers CCCCCTTCTTCTGTTCATCA (forward) and TGACTCTGAAGCACCAGCAC (reverse) and *MICB* primers GGACAGCAGAC CTGTGTGTTA (forward) and AAAGGAGCTTTCCCATCTCC (reverse); *MICA* sequencing primers: TCCTGCCCAGGAAGGTT and CCTGCTGAGTTCCACT GAC for exon 2, AGGAATGGGGGTCAGTGGAA and GAGGGTTTCCCTGGA CACAT for exon 3, and CTGTTCCTCTCCCCTCCTTA and CCATCCC TGCTGTCCCTAC for exon 4; *MICB* sequencing primers: GGACAGCAGA CCTGTGTGTTA and GCCTCCCTGACCCTATTCC for exon 2, GAGTAA TGGGAGGCCTTCT and TGCATCCATAGCACAGGG for exon 3, and CAGG AGTCCACCCTTGACAT, CGTTGACTCTGAAGCACCAG, and AAAGGAGCTT TCCCATCTCC for exons 4 and 5. For sequencing conditions, see online appendix Note 1.

We aligned and scored sequence reads using the Gap4 program from the Staden package (http://www.mrc-lmb.cam.ac.uk/pubseq/). To increase throughput we included a consensus trace in the alignment. Sequence data are not phased, and therefore a method was needed to determine which pair of alleles was present. While there are many nsSNPs in the MIC genes, they give rise to relatively few alleles per gene. The common alleles are listed on the IMGT/HLA Sequence Database (http://www.anthonynolan.org.uk/HIG/data.html) (18). Since the possible genotypes were limited and known, we used in-house software similar to the Helmberg Score software (19). Positions that varied were extracted from the static alignments of the IMGT/HLA Sequence Database. The alleles were reduced to just those SNPs that are present in the regions we sequenced. A file was then constructed of all possible pairs of *MICA* or *MICB* alleles. Our sequence data were extracted in the same format, and the two files were compared. Ambiguity created by missing SNP values was clarified by examining familial relationship and the *MICA-STR* data.

We validated this method in a reference panel of 40 cell lines received from the International Histocompatibility Working Group (IHWG) International Cell and Gene Bank (http://www.ihwg.org/cellbank/dna/refpan.html) and eight artificial heterozygotes made by pooling DNA from different homozygous cell lines. We obtained 100% concordance with the published *MICA* genotypes.

Sample populations. Using our SBT method, we genotyped both *MICA* and *MICB* in 818 type 1 diabetes–affected families. These families were from two collections: the Diabetes UK Type 1 Diabetes Warren collection (478 four member families comprised of an affected sib-pair and both parents) (20) and the USA Human Biological Data Interchange (HBDI) collection (340 four- and five-member families including more than one affected sibling and both parents) (21). In addition, we genotyped the *MICA-STR* in 2,023 U.K. type 1 diabetic case subjects and 1,748 geographically matched control subjects (22). All collections comprised subjects of white European origin. *HLA-DRB1* and *HLA-DQB1* had been genotyped previously in these subjects using Dynal RELI SSO assays (Invitrogen, Paisley, U.K.) (2).

Statistical analysis. Statistical analyses were carried out in the statistical package STATA v9 (http://www.stata.com), and the recursive partitioning (rpart) library in R was used for the recursive partitioning (23,24).

Each *MIC* locus was first tested for association with type 1 diabetes without conditioning on the MHC class II loci. Sets of case and matched pseudo-control subjects were generated from the affected sib-pair families and analyzed using conditional logistic regression (25) with Huber-White sandwich estimators to correct for the nonindependence of siblings. In the case-control collection, case and control subjects were matched to 12 broad geographical regions across the U.K. (Northern, East and West Ridings, North Midland, Eastern, Southeastern, Southern, Southwestern, Wales, Midlands, Scotland, London, and Northwestern) (22) and analyzed using logistic regression (2). The alleles of the *MIC* loci were modeled assuming a multiplicative mode of inheritance.

To establish whether any observed associations of *MICA* and *MICB* were independent of, and not due to LD with, the class II genes *HLA-DRB1* and *HLA-DQB1*, the effects of these highly associated loci must be taken into account in the analysis of *MICA* and *MICB*. The overall number of class II alleles, which includes many rare alleles, and the well-established nonmultiplicative effects of these loci complicate their modeling. We found that rpart was an effective method to model the genotypes of *HLA-DRB1* and *HLA-DQB1* (2). This is a risk-based grouping method that categorizes individuals as affected or unaffected based on their MHC class II genotypes (2). The resulting groups of MHC class II genotypes define strata within which additional, non–class II associations can be tested, using either conditional logistic regression (families) or logistic regression (case/control) (2). The rpart method was performed both in the case/controls and in the families (cases/pseudo-controls). The additional MHC class II independent effect of each *MIC* locus was tested by adding the alleles of the test locus to the logistic model and testing their independent effects by a Wald test (families) or a likelihood ratio test (case/control). Note that all the alleles of *MICA* or of *MICB* were included in the logistic model, which allows testing of the conditional association of the locus and calculation of the conditional relative risks (RRs)/odds ratios (ORs) of the individual alleles simultaneously, relative to a common reference allele. For the most common allele of the *MICA-STR* (*MICA-STR**A5.1), which has a minor allele frequency of 0.5, our power to detect an effect size of OR 2 was >98% at $\alpha = 10^{-5}$.

TABLE 1
MICB novel nonsynonymous SNPs found in one parent and one or more children: ++ transmitted to both children, + transmitted to one child

Position*	Nucleotide change	Protein change	Transmitted
508	A > G	Lys > Glu	++
641	G > T	Ser > Ile	+
800	G > A	Trp > stop	+
813	C > G	Ser > Arg	+

*Nucleotide position at http://www.anthonynolan.org.uk/HIG/seq/nuc/text/micb_nt.txt

RESULTS

Alleles and haplotypes. In *MICA*, 40 SNPs plus the *MICA-STR* yielded 55 distinct alleles in IMGT/HLA release 2.8 (Jan 2005) (18). We limited ourselves to SNPs in exons 2–4 so that *MICA**009 and *MICA**049 were indistinguishable, as were *MICA**027 and *MICA**048. Genotypes of the three loci were in Hardy-Weinberg equilibrium in unaffected parents and control subjects ($P > 0.05$). Frequencies for the *MICA* and *MICB* alleles were consistent in the U.K. and U.S. families and similar to those reported in other European populations (9,10,15,16). In online appendix Table 1, we list the combinations of *MICA* gene alleles and *MICA-STR* alleles observed in this dataset.

Novel alleles. In *MICB* we found four novel alleles that were due to nsSNPs in common alleles: at position 800 in *MICB**004, 813 in *MICB**008, 508 in *MICB**010, and 641 in *MICB**014 (Table 1). These SNPs were, however, extremely rare (each was found in only one family). We also identified a synonymous SNP (in 64 individuals) in *MICB* (G > T at position 762), which is not present on the IMGT/HLA alignment.

Association with type 1 diabetes. We analyzed the *MICA* and *MICB* alleles for association with type 1 diabetes in the family collections and the *MICA-STR* in the family and case control collections. For each locus we calculated two statistics: the *P* value of association for each locus overall and the RR/OR for each individual allele. When the *MIC* loci are analyzed without conditioning on *HLA-DRB1* and *HLA-DQB1*, several alleles of both genes showed a strong association with type 1 diabetes (*MICB* $P = 4.21 \times 10^{-19}$, *MICA* $P = 3.74 \times 10^{-15}$, *MICA-STR* $P = 5.44 \times 10^{-9}$). However, if the effect of these genes is conditioned on *HLA-DRB1* and *HLA-DQB1*, the association with type 1 diabetes disappears (*MICB* $P = 0.03$, *MICA* $P = 0.08$, *MICA-STR* $P = 0.76$). Note that $P = 0.03$ cannot be considered as evidence of association because it is produced as a result of using a Wald test that

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TABLE 2
Association of *MICA* and *MICB* alleles with type 1 diabetes, without conditioning on *HLA-DRB1* and *HLA-DQB1* ($P = 3.74 \times 10^{-15}$ and 4.21×10^{-19}, respectively) and with conditioning ($P = 0.08$ and 0.03, respectively)

	Frequency*†	Not conditioned on class II	Conditioned on class II
MICA alleles			
*001	0.03	2.63 (1.76–3.92)	1.55 (0.99–2.43)
*002	0.08	0.77 (0.57–1.04)	1.01 (0.72–1.43)
*004	0.05	0.49 (0.34–0.70)	0.69 (0.48–1.00)
*007	0.04	1.04 (0.72–1.48)	0.92 (0.61–1.37)
*008	0.48	1.00 (ref.)	1.00 (ref.)
*009/ *049	0.05	0.78 (0.55–1.11)	1.17 (0.81–1.70)
*010	0.09	1.87 (1.41–2.48)	1.08 (0.79–1.47)
*011	0.02	0.66 (0.35–1.25)	0.83 (0.43–1.61)
*012	0.01	0.47 (0.25–0.89)	0.58 (0.27–1.24)
*016	0.01	0.80 (0.37–1.74)	0.60 (0.21–1.68)
*017	0.02	0.23 (0.12–0.44)	1.00 (0.54–1.86)
*018	0.02	0.50 (0.28–0.92)	0.61 (0.29–1.27)
*019	0.01	0.98 (0.65–1.47)	1.46 (0.85–2.48)
*021	0.01	0.80 (0.21–2.99)	0.98 (0.37–2.60)
*027/ *048	0.01	0.44 (0.24–0.81)	0.81 (0.36–1.83)
*051	0.01	2.00 (0.12–32.06)	0.28 (0.04–1.87)
MICB alleles			
*002	0.16	1.48 (1.19–1.84)	0.83 (0.64–1.09)
*003	0.02	0.21 (0.10–0.42)	0.68 (0.33–1.41)
*004	0.17	0.99 (0.80–1.23)	1.06 (0.83–1.37)
*005	0.31	1.00 (ref.)	1.00 (ref.)
*008	0.17	2.15 (1.74–2.66)	1.04 (0.81–1.35)
*013	0.01	0.70 (0.33–1.49)	1.05 (0.37–2.98)
*014	0.01	0.74 (0.46–1.19)	0.64 (0.38–1.06)

Data are RR (95% CI). *Frequencies in unaffected parents. †Only alleles with frequency ≥0.01 shown.

is artificially biased toward the alternative hypothesis with rare data (rare alleles, below 0.01, were grouped together, the resulting group had frequency 0.014). Use of a likelihood ratio test without robust variance estimates produces a less significant P value ($P = 0.1$).

In Table 2 we present the RR for the alleles of *MICA* and *MICB* in the U.K. and U.S. families using the most common alleles (*MICA**008 and *MICB**005) as reference, as they give the tightest 95% CIs. Since none of the other alleles were significantly different to the reference, the reference alleles themselves are also not associated. *MICA-STR* was also not associated with type 1 diabetes after conditioning on *HLA-DRB1* and *HLA-DQB1* in the case/control collection ($P = 0.65$ for the individual alleles and $P = 0.91$ for the genotypes) or in the families ($P = 0.76$) (Table 3). We also tested the association of *MICA-STR**A5.1 genotypes against all the other genotypes grouped together. They were not associated with type 1 diabetes risk after conditioning (Table 4). We found that all *MICA* and *MICB* alleles were in strong LD (D′ >0.9) with one or more *HLA-DRB1* or *HLA-DQB1* alleles (online appendix Tables 2 and 3).

DISCUSSION

Our SBT method allows for large-scale and detailed genotyping of the full *MICA* and *MICB* alleles. SBT also allows discovery of novel alleles and, indeed, we found four new rare variants for *MICB*. These results illustrate the high level of allelic variability found in the *MICA* and *MICB* genes, implying that this variability has yet to be fully characterized.

Previous and ongoing studies of type 1 diabetes associations, including the Type 1 Diabetes Genetics Consortium (www.t1dgc.org) and the IHWG (http://www.ncbi.nlm.nih-.gov/projects/mhc/ihwg.cgi?cmd=DS&ID=11), have either omitted *MICA* and *MICB* or focused on the *MICA-STR* because of the costs and complications associated with obtaining the full genotypes of *MICA* and *MICB*. Many

TABLE 3
Association of the *MICA-STR* alleles with type 1 diabetes in the family and the case/control collections, both without conditioning on *HLA-DRB1* and *HLA-DQB1* ($P = 5.44 \times 10^{-9}$ families and $P = 9.10 \times 10^{-9}$ case/control) and with conditioning on *HLA-DRB1* and *HLA-DQB1* ($P = 0.76$ families and $P = 0.65$ case/control)

	Family collection		Case/control	
Allele	Not conditioned on class II	Conditioned on class II	Not conditioned on class II	Conditioned on class II
*A4	1.02 (0.83–1.26)	1.01 (0.79–1.28)	1.05 (0.91–1.23)	1.08 (0.81–1.43)
*A5	1.24 (1.01–1.53)	0.87 (0.66–1.14)	1.47 (1.26–1.71)	0.85 (0.65–1.12)
*A5.1	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
*A6	0.62 (0.50–0.77)	0.94 (0.72–1.24)	0.60 (0.53–0.70)	0.90 (0.70–1.17)
*A9	0.59 (0.46–0.75)	1.11 (0.84–1.47)	0.71 (0.61–0.83)	1.03 (0.78–1.36)

Data are RR (95% CI) for family collection and OR (95% CI) for case/control.

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TABLE 4
Comparison of *MICA-STR**A5.1 genotypes with other *MICA-STR* genotypes

Genotypes	Not conditioned on class II	Conditioned on class II
X/X	1.00 (ref.)	1.00 (ref.)
X/*A5.1	1.22 (0.99–1.51)	0.94 (0.72–1.24)
*A5.1/*A5.1	1.43 (1.08–1.90)	1.05 (0.75–1.48)

Data are RR (95% CI).

published reports have identified one or more of the alleles or genotypes of the *MICA-STR* as being associated with type 1 diabetes. However, some of these studies did not condition adequately on the MHC class II genes (12–14), while others used subgroup analysis to analyze the *MICA-STR* in samples chosen to have particular class II genotypes (7–9,11). This subgrouping causes a multiple testing problem and reduces the sample set and hence the power to detect MHC class II–independent effects. We conclude that, in the European populations analyzed here, the common *MICA* and *MICB* alleles are unlikely to affect type 1 diabetes susceptibility, nor are they markers for the strong independent associations of the common alleles of *HLA-B* (*HLA*-B*39) and *HLA-A* (2).

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REFERENCES

1. Risch N: Assessing the role of HLA-linked and unlinked determinants of disease. *Am J Hum Genet* 40:1–14, 1987
2. Nejentsev S, Howson JM, Walker NM, Szeszko J, Field SF, Stevens HE, Reynolds P, Hardy M, King E, Masters J, Hulme J, Maier LM, Smyth D, Bailey R, Cooper JD, Ribas G, Campbell RD, The Wellcome Trust Case Control Consortium, Clayton DG, Todd JA: Localization of type 1 diabetes susceptibility to the MHC class I genes *HLA-B* and *HLA-A*. *Nature* 450:887–892, 2007
3. Nejentsev S, Gombos Z, Laine AP, Veijola R, Knip M, Simell O, Vaarala O, Akerblom HK, Ilonen J: Non-class II HLA gene associated with type 1 diabetes maps to the 240-kb region near HLA-B. *Diabetes* 49:2217–2221, 2000
4. Valdes AM, Wapelhorst B, Concannon P, Erlich HA, Thomson G, Noble JA: Extended DR3–D6S273-HLA-B haplotypes are associated with increased susceptibility to type 1 diabetes in US Caucasians. *Tissue Antigens* 65:115–119, 2005
5. Collins RW: Human MHC class I chain related (MIC) genes: their biological function and relevance to disease and transplantation. *Eur J Immunogenet* 31:105–114, 2004
6. Alizadeh BZ, Eerligh P, van der Slik AR, Shastry A, Zhernakova A, Valdigem G, Bruining JG, Sanjeevi CB, Wijmenga C, Roep BO, Koeleman BP: *MICA* marks additional risk factors for type 1 diabetes on extended HLA haplotypes: an association and meta-analysis. *Mol Immunol* 44:2806–2812, 2007
7. Ide A, Babu SR, Robles DT, Wang T, Erlich HA, Bugawan TL, Rewers M, Fain PR, Eisenbarth GS: Homozygosity for premature stop codon of the MHC class I chain-related gene A (*MIC-A*) is associated with early activation of islet autoimmunity of *DR3/4-DQ2/8* high risk DAISY relatives. *J Clin Immunol* 25:303–308, 2005
8. Van Autreve JE, Koeleman BP, Quartier E, Aminkeng F, Weets I, Gorus FK, Van der Auwera BJ: MICA is associated with type 1 diabetes in the Belgian population, independent of *HLA-DQ*. *Hum Immunol* 67:94–101, 2006
9. Nikitina-Zake L, Ghaderi M, Park Y, Babu S, Eisenbarth G, Sanjeevi CB: MICA gene polymorphism in HBDI multiplex families. *Ann N Y Acad Sci* 1037:150–156, 2004
10. Gupta M, Ludvigsson J, Sanjeevi CB: Frequency of *MICA* in all babies in southeast Sweden (ABIS) positive for high-risk *HLA-DQ* associated with type 1 diabetes. *Ann N Y Acad Sci* 1037:138–144, 2004
11. Allcock R, Cheong K, Christiansen F, Witt C: Comment to: Gambelunghe G, Ghaderi,: Cosentino A et al. (2000) association of MHC class I chain-related A (MIC-A) gene polymorphism with type I diabetes: Diabetologia 43:507–514. *Diabetologia* 44:514–516, 2001
12. Lee YJ, Huang FY, Wang CH, Lo FS, Tsan KW, Hsu CH, Huang CY, Chang SC, Chang JG: Polymorphism in the transmembrane region of the MICA gene and type 1 diabetes. *J Pediatr Endocrinol Metab* 13:489–496, 2000
13. Shtauvere-Brameus A, Ghaderi M, Rumba I, Sanjeevi CB: Microsatellite allele 5 of MHC class I chain-related gene a increases the risk for insulin-dependent diabetes mellitus in latvians. *Ann N Y Acad Sci* 958:349–352, 2002
14. Bilbao JR, Martin-Pagola A, Calvo B, Perez de Nanclares G, Gepv N, Castano L: Contribution of *MIC-A* polymorphism to type 1 diabetes mellitus in Basques. *Ann N Y Acad Sci* 958:321–324, 2002
15. Ahmad T, Marshall SE, Mulcahy-Hawes K, Orchard T, Crawshaw J, Armuzzi A, Neville M, van Heel D, Barnardo M, Welsh KI, Jewell DP, Bunce M: High resolution *MIC* genotyping: design and application to the investigation of inflammatory bowel disease susceptibility. *Tissue Antigens* 60:164–179, 2002
16. Gao X, Single RM, Karacki P, Marti D, O'Brien SJ, Carrington M: Diversity of *MICA* and linkage disequilibrium with *HLA-B* in two North American populations. *Hum Immunol* 67:152–158, 2006
17. Mizuki N, Ota M, Kimura M, Ohno S, Ando H, Katsuyama Y, Yamazaki M, Watanabe K, Goto K, Nakamura S, Bahram S, Inoko H: Triplet repeat polymorphism in the transmembrane region of the MICA gene: a strong association of six GCT repetitions with Behcet disease. *Proc Natl Acad Sci U S A* 94:1298–1303, 1997
18. Robinson J, Waller MJ, Parham P, Bodmer JG, Marsh SG: IMGT/HLA Database–a sequence database for the human major histocompatibility complex. *Nucleic Acid Res* 29:210–213, 2001
19. Helmberg W, Zahn R, Keller E, Weinmair B, Lanzer G, Albert E: Virtual DNA analysis as a platform for interlaboratory data exchange of HLA DNA typing results. *Tissue Antigens* 54:379–385, 1999
20. Bain SC, Todd JA, Barnett AH: The British Diabetic Association–Warren Repository. *Autoimmunity* 7:83–85, 1990
21. Lernmark A, Ducat L, Eisenbarth G, Ott J, Permutt MA, Rubenstein P, Spielman R: Family cell lines available for research. *Am J Hum Genet* 47:1028–1030, 1990
22. Clayton DG, Walker NM, Smyth DJ, Pask R, Cooper JD, Maier LM, Smink LJ, Lam AC, Ovington NR, Stevens HE, Nutland S, Howson JM, Faham M, Moorhead M, Jones HB, Falkowski M, Hardenbol P, Willis TD, Todd JA: Population structure, differential bias and genomic control in a large-scale, case-control association study. *Nat Genet* 37:1243–1246, 2005
23. R Development Core Team: *A Language and Envoronment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, 2006
24. Therneau TM, Atkinson EJ: *An Introduction to Recursive Partitioning Using the rpart Routine*. Minnesota, Mayo Clinic, Section of Statistics, 1997
25. Cordell HJ, Clayton DG: A unified stepwise regression procedure for evaluating the relative effects of polymorphisms within a gene using case/control or family data: application to HLA in type 1 diabetes. *Am J Hum Genet* 70:124–141, 2002

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