Association of MHC Class I chain-related A (*MIC-A*) gene polymorphism with Type I diabetes

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Abstract

Aims/hypothesis. A distinct family of MHC genes has been identified in the class III region and denominated MHC Class I chain-related genes (*MIC*). The *MIC-A* gene is located between the *TNFA* and the *HLA-B* genes. The aim of our study was to test the association of the polymorphism of the *MIC-A* gene with Type I (insulin-dependent) diabetes mellitus and evaluate the interaction between *MIC-A* and *TNFA*, *HLA-B*, *HLA-DR* and *HLA-DQ* gene polymorphism.

Methods. Type I diabetic ($n = 95$) and healthy ($n = 98$) Italian subjects were typed for exon 5 of *MIC-A* and for *HLA-DRB1*, *HLA-DQA1*, *HLA-DQB1* and *TNFA* alleles. All subjects were also typed for the presence of *HLA-B8* or *HLA-B15*.

Results. The frequency of *MIC-A5* was increased in diabetic subjects (53 % vs 15 %) ($OR = 6.1$) (corrected $p, p_c < 0.0005$). Among HLA class II haplotypes, both *HLA-DRB1*03-DQA1*0501-DQB1*0201* (*DR3-DQ2*) and *DRB1*04-DQA1*0301-DQB1*0302* (*DR4-DQ8*) (“at-risk class II haplotypes”) were positively associated with diabetes ($OR = 6.7$ and 6.0 , respectively) ($p_c < 0.003$). Also *HLA-B8* was more frequent among Type I diabetic subjects than among

healthy control subjects ($OR = 2.8$, $p = 0.01$). None of the *TNFA* alleles were statistically significantly associated with Type I diabetes. The *MIC-A5* exon was negatively associated with age at clinical onset of diabetes ($p = 0.012$). Thus, 68 % diabetic subjects younger than 25 years and 29 % older than 25 years were carrying this allele. Both *MIC-A5* and the at-risk class II haplotypes were independently associated with Type I diabetes and the combined association of the two markers had the highest relative risk ($OR = 172$). In subjects younger than 25 years, the OR of *MIC-A5* was as high as 21.7 and was more than twofold that of at-risk class II haplotypes ($OR = 9.5$). The *MIC-A5* exon was not in linkage disequilibrium with any of the HLA-class I, class II or *TNFA* alleles studied.

Conclusions/interpretation. The *MIC-A* gene polymorphism is associated with genetic risk for Type I diabetes and the combination of *MIC-A5* and at-risk class II haplotypes is now to be seen as the strongest genetic marker for this disease. [Diabetologia (2000) 43: 507–514]

Keywords Autoimmunity, genetic risk, HLA, insulin-dependent diabetes, major histocompatibility complex, *TNFA* gene.

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Abbreviations: *MIC*, Class I chain-related gene; GCT, trinucleotide repeat; ICA, islet cell antibodies; IA-2, tyrosine phosphatase; OR , odds ratio; p_c , corrected p value.

Type I (insulin-dependent) diabetes mellitus is an autoimmune disease characterised by the presence of circulating islet autoantibodies and by T-lymphocyte infiltration of the islets of Langerhans with consequent destruction of pancreatic beta cells [1]. A permissive genetic background is required for the development of the islet autoimmune process. Type I diabetes is a complex genetic disease and a long series of genes has been found to be linked with disease

risk [2–12]. More specifically, the human leucocyte antigens (HLA) *B8* [13] and *B15* [14] increase the risk for Type I diabetes. The strongest genetic association so far identified, however, is with HLA class II genes located on the short arm of chromosome 6. It is well known that both HLA DRB1*04-DQA1*0301-DQB1*0302 (*DR4-DQ8*) and DRB1*03-DQA1*0501-DQB1*0201 (*DR3-DQ2*) (referred to as “at-risk class II haplotypes”) are positively, and DRB1*15-DQA1*0102-DQB1*0602 is negatively, associated with Type I diabetes in the Caucasian population [2, 3, 15, 16]. The relative risk of the DQA1*0501-DQB1*0201/DQA1*0301-DQB1*0302 genotype is higher than 20 [17]. Only a minority of the subjects carrying the high risk haplotypes/genotypes develops the disease, however, which suggests that additional genes (as well as environmental factors) play a crucial part in conferring either protection or susceptibility to Type I diabetes.

The *TNFA* gene is located within the HLA class III region and its polymorphism influences the concentrations of TNF alpha produced [18]. In some studies, heterozygosity for *TNFA*-2 seems to increase the risk for Type I diabetes in subjects positive for DQA1*0501-DQB1*0201/DQA1*0301-DQB1*0302 [19]. The association of the *TNFA* gene polymorphism with Type I diabetes seems, however, to be due to a linkage disequilibrium with HLA class II genes [19, 20].

A distinct family of MHC genes has recently been identified within the class III region and has been denominated MHC class I chain-related genes (*MIC*) [21, 22]. The *MIC* family consists of three pseudogenes, *MIC-C*, *MIC-D* and *MIC-E* and two functional genes, *MIC-A* and *MIC-B* [22]. The *MIC-A* and *MIC-B* genes are located telomeric to the *TNFA* gene between the B-associated transcript (*BAT-1*) and the *HLA-B* genes [21–23]. The *MIC-A* and *MIC-B* genes contain long open reading frames encoding for MHC class I molecules with three distinct extracellular domains (alpha 1, 2 and 3), a transmembrane segment, and a cytoplasmatic tail, each encoded by a separate exon. Sequence analysis of the *MIC-A* gene showed a trinucleotide repeat (GCT) microsatellite polymorphism within the transmembrane region [24, 25]. So far, five alleles of the exon 5 of the *MIC-A* gene, which consist of 4, 5, 6 and 9 repetitions of GCT, or five repetitions of GCT with an additional nucleotide insertion (*GGCT*), have been identified [24]. These alleles have been accordingly named *A4*, *A5*, *A6*, *A9* and *A5.1* and the sizes are, respectively, 179 bp, 182 bp, 185 bp, 194 bp and 183 bp.

The polymorphism of the *MIC-A* gene [24–26] and its location in the HLA region warrant studies aimed at identifying an association with the risk for autoimmune diseases. The *MIC-A* gene has already been found to confer genetic risk for Behçet’s disease [27, 28] and ankylosing spondylitis [29]. We have recently

shown additionally that the *MIC-A5.1* allele is statistically significantly increased in patients with autoimmune Addison’s disease and that this association is not due to a linkage disequilibrium with HLA class II genes [30].

In this study, we evaluated the frequencies of *MIC-A* alleles and genotypes in Italian Type I diabetic and healthy subjects and we tested the interaction between *MIC-A* and *TNFA*, *HLA-B*, *HLA-DR* and *HLA-DQ* gene polymorphism.

Materials and methods

Subjects and samples. Genomic DNA was obtained from EDTA-treated peripheral blood samples from 95 Type I diabetic subjects (age at onset 1–37 years, median 25 years; 40 females and 55 males) and 98 healthy subjects (age 3–64 years, median 33 years; 48 females and 50 males) resident in central Italy. The DNA samples were purified by standard phenol-chloroform extraction, dissolved in sterile double-distilled water and stored at 4°C. Diagnosis of diabetes was made according to the criteria defined by the National Diabetes Data Group [31], and classification as Type I diabetes was based on the presence of ketosis, low body mass index and need for insulin therapy. In 93% of the subjects, the diagnosis of Type I diabetes was also confirmed by the presence of at least one of the three major islet autoantibodies: antibodies to islet-cell (ICA), GAD65 and tyrosine phosphatase-like molecules ICA512 (IA-2). None of the healthy control subjects enrolled in this study was positive for islet autoantibodies.

Neither the patients nor the healthy control subjects were related to each other and no sib pairs were included in this study.

***MIC-A* genotyping.** The exon 5 of the *MIC-A* gene was PCR amplified using 5′-CCTTTTTTTCAGGGAAAGTGC-3′ as forward primer and 5′-CCTTACCATCTCCAGAACTGC-3′ as reverse primer [24]. The reverse primer was labelled at 5′ end with fluorescent reagent 6-HEX (Amersham-Pharmacia Biotech, Uppsala, Sweden). The reactions were done in a Perkin Elmer programmable thermal cycler (Perkin Elmer, Norwalk, Conn., USA). Following amplification, the number of the GCT triplet repeat units was determined using an ABI prism (Perkin Elmer) automated DNA sequencer.

HLA class II genotyping. The polymorphic second exon of the *DQA1*, *DQB1* and *DRB1* genes was amplified in a Perkin Elmer programmable thermal cycler and the amplified products were manually dotted onto nylon membranes (Amersham, Arlington, Ill., USA), under denaturing conditions. The membranes were hybridised with sequence specific oligonucleotides (SSOs), 3′ end labelled with [32]P-deoxy cytosine triphosphate (dCTP) and washed in stringent conditions before exposure to x-ray film, as described previously [32]. The membranes were stripped of the labelled probe under alkaline conditions and reused for probing with other oligonucleotides.

HLA-B8 and HLA-B15 genotyping. Because Type I diabetes is associated with *HLA-B8* and *HLA-B15*, typing for these two alleles was done by PCR-SSP technique as described previously [33]. As positive controls, we used genomic DNA samples from four healthy subjects, identified as either *B8*-positive or *B15*-positive by low-resolution typing method (Biotest, Drei-

Table 1. *MIC-A* alleles and genotypes in Italian Type I diabetic and healthy subjects

<i>MIC-A</i> alleles and genotypes	Patients (n = 95)	Healthy subjects (n = 98)	OR (95 % CI)	p value	p_c
A5	50 (53 %)	15 (15 %)	6.1 (3.2–11.7)	< 0.0001	< 0.0005
A4	18 (19 %)	22 (22 %)	0.80 (0.28–2.3)	NS	NS
A5.1	33 (35 %)	34 (35 %)	1.0	NS	NS
A6	46 (48 %)	54 (54 %)	0.76 (0.45–1.4)	NS	NS
A9	26 (27 %)	37 (37 %)	0.62 (0.29–1.2)	NS	NS
A5/A6	24 (25 %)	3 (3 %)	10.7 (3.6–31.8)	< 0.0001	< 0.0015
A5/A5	17 (18 %)	5 (5 %)	4.1 (1.4–11.5)	0.02	NS
A5/A5.1	3 (3 %)	1 (1 %)	3.2 (0.04–234)	NS	NS
A5/A9	4 (4 %)	3 (3 %)	1.4 (0–> 1000)	NS	NS
A5/A4	2 (2 %)	3 (3 %)	0.68 (0–> 1000)	NS	NS

95 % CI = 95 % confidence interval; p_c = corrected p value (correction factor was 5 for *MIC-A* alleles and 15 for *MIC-A* genotypes)

eich, Germany). Each PCR test included an internal positive control primer pair amplifying a segment of the human growth hormone gene.

***TNFA* microsatellite typing.** Amplification by PCR of the *TNFA* gene was done as described [34]. The 5' primer was labelled with HEX fluorescent dye. Fragment sizes were identified in an ABI prism automated DNA sequencer. According to the fragment size, 13 alleles of the *TNFA* gene have been identified [34].

Statistical analysis. The odds ratio (OR) was calculated as described previously [35, 36]. When all the patients or the control subjects were negative for a particular allele or haplotype, Haldane's correction was used [37]. Differences in allele or haplotype frequencies between the diabetic subjects and the control group were tested by the chi-squared method. Yates' correction or the Fisher's exact tests were used when necessary. The strongest HLA association was tested using a method described previously [38]. The probability values were corrected (p_c) for the number of comparisons, according to the number of alleles, haplotypes or genotypes observed among healthy subjects: 5 for *MIC-A* alleles, 15 for *MIC-A* genotypes, 26 for *HLA-DQA1-DQB1-DRB1* haplotypes and 13 for *TNFA* alleles. The probability value for *B8* and *BI5* was not corrected, as they were the only *HLA-B* alleles tested in our study. The dependency of the frequency of *MIC-A* or *HLA-DR* and *HLA-DQ* alleles on sex and age at onset was assessed by logistic regression analysis. A p (or p_c) value less than 0.05 was considered significant.

Results

The frequency of the *MIC-A5* allele was increased in our cohort of Italian Type I diabetic subjects compared with the healthy control subjects and this allele had an OR of 6.1 (95 % CI: 3.2–11.7) ($p_c < 0.0005$) (Table 1). None of the other *MIC-A* al-

les was found to be associated, either positively or negatively, with the risk for the disease. Among the *MIC-A* genotypes, only A5/A6 and A5/A5 were significantly and positively associated with Type I diabetes.

Among *HLA-DR* and *DQ* haplotypes, the risk for Type I diabetes was significantly associated with both *DRB1*03-DQA1*0501-DQB1*0201* (*DR3-DQ2*) and *DRB1*04-DQA1*0301-DQB1*0302* (*DR4-DQ8*) (Table 2) ($p_c < 0.003$). The odds ratios of *DR3-DQ2* (6.7, 95 % CI: 3.4–13.3) and of *DR4-DQ8* (6.0, 95 % CI: 2.6–13.9) were similar to that observed for *MIC-A5*. The *DR3-DQ2/DR4-DQ8* genotype was absent in the 98 healthy control subjects but present in 11 of 95 (12 %) diabetic subjects (OR > 14.0, 95 % CI: 2.7–70.2).

We found *HLA-B8* was more frequent among Type I diabetic subjects than among healthy control subjects (Table 2) (OR = 2.8 $p = 0.01$). The frequency of *BI5* was slightly increased also in the diabetic cohort but the difference from the healthy control subjects was not statistically significant.

In our cohort of Type I diabetic and healthy subjects, no *TNFA* allele was positively or negatively associated with disease risk (Table 2). Although *TNFA-2* was more frequent in diabetic subjects (40 %) than in healthy control subjects (32 %), the difference did not reach a statistical significance. Similarly, the presence of *TNFA-2* did not increase the OR for Type I diabetes in either *HLA-DR3*- or *HLA-DR4*-positive subjects. The absence of *DR3-DQ2/DR4-DQ8*-positive subjects in our group of healthy control subjects did not allow us to test the hypothesis that *TNFA-2* was increased in diabetic subjects carrying this genotype.

Table 2. HLA class I and class II and *TNFA* gene polymorphism in Italian Type I diabetic and healthy subjects

	Patients (<i>n</i> = 95)	Controls (<i>n</i> = 98)	OR (95 % CI)	<i>p</i> value	<i>p_c</i>
<i>DR3-DQ2</i>	48 (51 %)	13 (13 %)	6.7 (3.4–13.3)	< 0.0001	< 0.0026
<i>DR4-DQ8</i>	30 (32 %)	7 (7 %)	6.0 (2.6–13.9)	< 0.0001	< 0.0026
<i>DR3-DQ2/DR4-DQ8</i>	11 (12 %)	0	> 14.0 (2.7–70.2)	0.001	0.026
Genotype <i>B8</i>	25 (26 %)	11 (11 %)	2.8 (1.2–6.3)	0.01	0.01
<i>B15</i>	14 (15 %)	10 (10 %)	1.5 (0.5–4.4)	NS	NS
<i>TNFA1</i>	8 (8 %)	3 (3 %)	2.9 (0.6–14.5)	NS	NS
<i>TNFA2</i>	38 (40 %)	31 (32 %)	1.3 (0.8–2.1)	NS	NS
<i>TNFA3</i>	5 (5 %)	1 (1 %)	5.3 (0.41–68)	NS	NS
<i>TNFA4</i>	11 (11 %)	12 (13 %)	0.94 (0.11–7)	NS	NS
<i>TNFA5</i>	20 (21 %)	14 (14 %)	1.6 (0.66–3.9)	NS	NS
<i>TNFA6</i>	14 (15 %)	21 (21 %)	0.63 (0.22–1.6)	NS	NS
<i>TNFA7</i>	22 (23 %)	23 (23 %)	1.0	NS	NS
<i>TNFA8</i>	1 (1 %)	5 (5 %)	0.20 (0.01–2.7)	NS	NS
<i>TNFA9</i>	1 (1 %)	8 (8 %)	0.12 (0.01–0.95)	0.04	NS
<i>TNFA10</i>	36 (38 %)	40 (41 %)	0.88 (0.61–1.3)	NS	NS
<i>TNFA11</i>	10 (10 %)	9 (9 %)	1.1 (0.07–15.9)	NS	NS
<i>TNFA12</i>	1 (1 %)	2 (2 %)	0.51 (0–> 100)	NS	NS
<i>TNFA13</i>	4 (4 %)	11 (11 %)	0.35 (0.06–1.4)	NS	NS

95 % CI = 95 % confidence interval; *p_c* = corrected *p* value (correction factor was 13 for *TNFA* alleles and 26 for HLA haplotypes/genotypes)

The logistic regression analysis showed that the frequency of the *MIC-A5* allele in diabetic subjects, but not that of either *DR3-DQ2* or *DR4-DQ8*, was dependent on age at onset of diabetes (*p* = 0.012), but did not depend on sex. Thus, 39 of 57 (68 %) patients younger than 25 years were positive for the *MIC-A5* allele, as compared with 11 of 38 (29 %) patients older than 25 years (Fig. 1). In Type I diabetic subjects older than 25 years, the frequency of *MIC-A5* (11 of 38, 29 %) was not significantly higher than the frequency observed in healthy subjects with similar age (18 %).

To evaluate whether *MIC-A5* is independently associated with Type I diabetes, we then did the test for the strongest HLA association using a method described previously [38] (Tables 3, 4). This analysis confirmed that both *MIC-A5* and *HLA DR3-DQ2* and/or *HLA DR4-DQ8* were independently associated with Type I diabetes (Table 4 comparisons 1, 2, 3, 4, 5 and 6) and showed that the combined association of *MIC-A5* and at-risk HLA class II haplotypes was

associated with the highest odds ratio (Table 4, comparison 8). No linkage disequilibrium between *MIC-A* and HLA class II genes was detected in either patients or healthy control subjects (Table 4, comparisons 9 and 10). Similarly, the polymorphism of the *MIC-A* gene did not result in linkage disequilibrium with that of the *TNFA* gene.

These results show that *MIC-A5* is associated with Type I diabetes and that *MIC-A* and *HLA-DR-DQ* polymorphism interact in conferring genetic risk for the disease. When the analysis was repeated in subjects younger than 25 years, the presence of *MIC-A5* had an OR of 21.7 (95 % CI: 7.9–59.9) (*p_c* < 0.0005 vs healthy subjects of similar age), which was more than twofold that associated with the presence of at-risk class II haplotypes (OR = 9.5, 95 % CI: 3.9–23.4). In the same group of patients, the relative risk of the simultaneous presence of both *MIC-A5* and *DR3-DQ2* and/or *DR4-DQ8* was 775-fold that associated with the absence of both markers.

Fig. 1A–C. Frequency of *MIC-A5* (A), HLA-DRB1*03-DQA1*0501-DQB1*0201 (*DR3-DQ2*) (B) and HLA-DRB1*04-DQA1*0301-DQB1*0302 (*DR4-DQ8*) (C) in Italian Type I diabetic subjects, in relation to age at disease onset and in age-matched healthy control subjects. ■, Type I diabetic subjects; □, healthy control subjects

Table 3. Analysis of the strongest HLA association comparing *MIC-A5* with *DR3-DQ2* and/or *DR4-DQ8*. Basic data

Factor A (<i>MIC-A5</i>)	Factor B (HLA <i>DR3-DQ2</i> and/or <i>DR4-DQ8</i>)	Patients (<i>n</i> = 95)	Healthy subjects (<i>n</i> = 98)
+	+	35 (x1)	1 (y1)
+	–	15 (x2)	14 (y2)
–	+	32 (x3)	19 (y3)
–	–	13 (x4)	64 (y4)

x, y = reference alphabet for identification of healthy subjects

Furthermore, the test for the strongest HLA association also showed the absence of linkage disequilibrium between *MIC-A5* and *HLA-B8* and that *MIC-A5* and *B8* are independently associated with Type I diabetes (OR = 6.4 for *MIC-A5* in the absence of *HLA-B8*, $p_c < 0.0006$). Similarly, the association between *MIC-A5* and the risk for Type I diabetes was not influenced by the presence of *HLA-B15*.

Discussion

Our study shows that *MIC-A* gene polymorphism is associated with the risk for Type I diabetes. In our cohort of Italian subjects, the genetic susceptibility to the disease was best represented by the interaction between *HLA-DR3-DQ2* and/or *HLA-DR4-DQ8* and *MIC-A5* and did not depend on *TNFA* gene polymorphism. The *MIC-A5* exon seems to be the strongest genetic marker of susceptibility to Type I diabetes in subjects younger than 25 years.

The function of the *MIC-A* gene product is little understood. Furthermore, it is still not clear how the gene polymorphism could influence the biological importance of the *MIC-A* molecule. The crystal structure of the *MIC-A* molecule has been determined and showed a dramatically altered MHC class I fold [39]. More specifically, an unusual distribution of amino acid substitutions is present in the putative ligand-binding site of the *MIC* molecule [25]. The MHC class I homologue *MIC-A* is recognised by one subset of $\gamma\delta$ T-cells and could present invariant ligands such as glycolipids or carbohydrates [21, 25, 40]. The *MIC-A* protein is essentially expressed in the gut epithelium and no expression has been found in B-lymphocytes or T-lymphocytes [21, 41]. The expression of *MIC-A* is not under the control of the classical HLA molecules stimuli [21, 40] but under that of heat-shock promoter elements [41]. Its expression can be induced, however, in CD4⁺ and CD8⁺ cells, by stimulation with phytohemagglutinin [40]. These data suggest a putative role of *MIC-A* in inflammatory processes.

The existence of susceptibility genes for Type I diabetes within the segment of MHC between *TNFA* and *HLA-B* genes had already been predicted [42].

Table 4. Test of the strongest HLA association comparing *MIC-A5* with *DR3-DQ2* and/or *DR4-DQ8*

Comparison	Test	Entries in 2 x 2 table				OR (95 % CI) p	p_c
		a	b	c	d		
1. A vs non-A	Individual association	x1 + x2 50	x3 + x4 45	y1 + y2 15	y3 + y4 83	6.1 (3.2–11.7)	< 0.0001 < 0.0006
2. B vs non-B	Individual association	x1 + x3 67	x2 + x4 28	y1 + y3 20	y2 + y4 78	9.3 (5–17.4)	< 0.0001 < 0.0006
3. ++ vs --	Indiv. <i>MIC-A5</i> ass.	x1 35	x3 32	y1 1	y3 19	20.8 (3.8–114)	0.0005 0.003
4. +- vs - -	Indiv. <i>MIC-A5</i> ass.	x2 15	x4 13	y2 14	y4 64	5.3 (2–13.9)	0.0007 0.0042
5. ++ vs +- -	Indiv. HLA <i>DR3/DR4</i> ass.	x1 35	x2 15	y1 1	y2 14	32.7 (6.1–174)	< 0.0001 < 0.0006
6. -- vs - -	Indiv. HLA <i>DR3/DR4</i> ass.	x3 32	x4 13	y3 19	y4 64	8.3 (3.7–18.5)	< 0.0001 < 0.0006
7. +- vs - +	Diff. between ass.	x2 15	x3 32	y2 14	y3 19	0.6 (0.1–2.4)	NS NS
8. ++ vs - -	Combined ass.	x1 35	x4 13	y1 1	y4 64	172 (47.5–623)	< 0.0001 < 0.0006
9. Ass. A and B in patients	Linkage in patients	x1 35	x2 15	x3 32	x4 13	0.95 (0.34–2.6)	NS NS
10. Ass. A and B in controls	Linkage in controls	y1 1	y2 14	y3 19	y4 64	0.24 (0.01–3.2)	NS NS

95 % CI = 95 % confidence interval; p_c = corrected p value (correction factor was 6 for comparisons 1–8 and 9 for comparisons 9 and 10). ass. = association, Indiv. = individual, Diff. = difference, x, y = reference alphabet for identification of healthy subjects

Our study shows now that the polymorphism of the *MIC-A* gene, located in that MHC segment, indicates the genetic risk for Type I diabetes. This association could be the result of a linkage disequilibrium between the *MIC-A* gene and a still unidentified gene in the HLA region. In this respect, the absence of linkage disequilibrium with HLA class II haplotypes and with *TNFA* alleles is highly relevant. These results are in line with the absence of linkage disequilibrium between the *MIC-A* and the *MIC-B* genes [43, 44], that we have also confirmed in the Italian cohort [30], and this is presumably due to a recombination hot-spot between the two *MIC* genes [45]. The results of our paper are thus consistent with the hypothesis that the association of *MIC-A* polymorphism with Type I diabetes is not due to a linkage disequilibrium with genes located centromeric to the *MIC-A* gene, e.g. in the HLA class II and class III regions.

Some studies have tested the association of *TNFA* gene polymorphism with the risk for Type I diabetes [18–20]. The *TNFA* microsatellite alleles do not seem to be independently associated with the disease. Similarly, in our study, no *TNFA* allele was found to be statistically significantly increased in Type I diabetic subjects.

To evaluate whether the association of *MIC-A5* with Type I diabetes was influenced by *HLA-B* gene polymorphism, we estimated the frequency of *B8* and *B15* in our cohort of Italian subjects. Our results on class I association are in line with those of previous studies on Italian diabetic subjects [46] and show that

MIC-A5 is associated with the risk for Type I diabetes independently of *HLA-B* gene polymorphism.

Several lines of evidence support the hypothesis that different immune and genetic associations distinguish childhood Type I diabetes from the adult form of the disease. Thus, insulin autoantibodies, IA-2/ICA512Ab and ICA are less frequent in adult patients than in children [47–49] and *HLA-DR4-DQ8* is strongly associated with childhood Type I diabetes [50]. These different associations are likely correlated with the different clinical manifestations of the disease, more acute and pronounced in children and often slowly progressive in adult subjects. The inverse correlation between the frequency of *MIC-A5* and age at onset of the disease further supports the hypothesis that a distinct genetic background characterises childhood Type I diabetes. It is notable that APS II-Addison's disease, which is very rare in children, was found to be associated with *MIC-A5.1*, and not with *MIC-A5* [30]. Further studies on adult-onset, slowly progressive Type I diabetes, as well as on other organ-specific autoimmune diseases, are needed to address the question whether *MIC-A5.1* is a genetic marker of adult-onset endocrine autoimmunity, compared to *MIC-A5*, which might result as a specific marker of childhood Type I diabetes.

Our study shows that *MIC-A* gene polymorphism is strongly associated with risk for Type I diabetes. According to our data, the combination of *MIC-A5* and *HLA-DR3-DQ2* and/or *HLA-DR4-DQ8* is now to be seen as the most important genetic marker iden-

tified for this disease. The typing of the *MIC-A* gene will be instrumental in the future identification of subjects at high risk for Type I diabetes and in the planning of intervention trials.

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