HLA-DQA1 and DQB1 Gene Polymorphisms in Type I Diabetic Patients From Central Italy and Their Use for Risk Prediction

RAFFAELLA BUZZETTI, LORENZA NISTICÒ, JOHN F. OSBORN, CLAUDIO GIOVANNINI, ALBERTO CHERSI, AND ROSA SORRENTINO

Susceptibility to type I diabetes has been shown to be highly correlated with the presence of an amino acid other than Asp at position 57 of the DQ β-chain (non-Asp^{57}) and also with the presence of an Arg at position 52 of the DQ α-chain (Arg^{52}). In this study we analyzed the DQA1 and DQB1 gene polymorphisms in 65 patients from central Italy and 93 randomly selected control subjects. Polymerase chain reaction amplification of DNA encoding the first polymorphic domain of the DQB1 and DQA1 chains was performed, and DQB1 gene polymorphism was evaluated by dot blot analysis using 11 sequence-specific oligonucleotide probes. For DQA1 typing, a new simple procedure based on allele-specific amplification and analysis of heteroduplex DNA molecules formed by the annealing of mismatched allelic strands was used. This technique allows the discrimination of Arg^{52} and non-Arg^{52} DQA1 alleles. We then calculated by logistic regression the contribution of these genetic markers to the development of diabetes. Frequencies and odds ratios relative to the amino acid in position 57 of the DQ β-chain and the amino acid in position 52 of the DQ α-chain showed that the highest odds ratio (odds ratio = 161; 95% confidence interval 19–1386) was that of the homozygous combination of the two susceptibility markers (non-Asp^{57} and Arg^{52}). Based on the incidence estimates of type I diabetes in the continental Italian population, the annual incidence rate of the disease was estimated for the different genotypes grouped according to the number of potentially formed susceptible heterodimers as 212.53, 12.60, 3.24, and 1.33/100,000 individuals per yr for the 4, 2, 1, and 0 susceptible heterodimers groups, respectively. *Diabetes* 42:1173–78, 1993

Type I diabetes is a complex, multifactorial, and heterogeneous disease. Its incidence in different ethnic groups is extremely variable, suggesting the involvement of environmental as well as genetic factors (1,2). To date, the only well-established genetic marker is represented by genes within the HLA system (3,4). Important insights into the genetics of type I diabetes have been gained through its association with the HLA-DQB1 gene, and the presence of the disease has been shown to be associated with an amino acid other than Asp at position 57 of the DQ β-chain (non-Asp^{57}) (5). Several studies with Caucasian populations have confirmed this association (6–11). More recently Khalil et al. (12) have suggested that the disease also correlates with those alleles of the DQA1 gene that carry an Arg at residue 52 (Arg^{52}) of the DQ α-chain. The presence, in *cis* or in *trans*, of DQB non-Asp^{57} and DQA Arg^{52} alleles seems to be a more specific marker of susceptibility, and the absolute risk of the disease appears to correlate positively with the number of DQ α/β-susceptible heterodimers expressed on the cell surface. This observation has been confirmed in the Spanish population, where the absolute risk varies from 1.9 to 101.7/100,000 per yr according to the possibility of generating 0, 1, 2, or 4 heterodimers (13). In this study, type I diabetic patients from central Italy were analyzed at the molecular level for both markers, and the absolute risk associated with the number of possible susceptible heterodimers was compared with the available data from other populations. We then estimated the incidence rate of the disease in each category of susceptible heterodimers.

From the Institute of Cell Biology, Italian National Research Council, Rome; Institute of Clinica Medica II, Institute of Igiene and Department of Cell Biology and Development, University of Rome La Sapienza; and Institute Regina Elena, Rome, Italy.

Address correspondence and reprint requests to Dr. Raffaella Buzzetti, Institute of Clinica Medica II, University of Rome La Sapienza Policlinico Umberto I, Viale del Policlinico 155, 00161, Rome, Italy.

Received for publication 30 December 1992 and accepted in revised form 12 March 1993.

Type I diabetes, insulin-dependent diabetes mellitus; WHO, World Health Organization; PCR, polymerase chain reaction; SSPE, sodium chloride-sodium phosphate-EDTA buffer; SDS, sodium dodecyl sulfate; bp, base pair; OR, odds ratio; CI, confidence interval; df, degrees of freedom.

RESEARCH DESIGN AND METHODS

Sixty-five unrelated patients (30 males, 35 females, mean age 14.1 yr) affected by type I diabetes were randomly selected among those attending the outpatient department of the Clinica Medica II of the University of Rome La Sapienza and other district hospitals in Rome (Lazio region). Patients were diagnosed according to WHO criteria, and all had been treated with insulin since diagnosis. They were unrelated and 6 of them had a first-degree relative with type I diabetes. The patients (all Caucasian) belong to the Lazio region with a population of 5.8 million (including the city of Rome) with a 6.5/100,000 per yr incidence of type I diabetes in those <15 yr of age (2). Ninety-three unrelated blood donors (53 males, 40 females) were randomly selected as control subjects.

DQA1 and DQB1 gene amplification. Whole peripheral blood (14) or purified DNA (0.5 μg) was used as a source of template for DQA1 and DQB1 gene PCR amplification, as suggested by Saiki et al. (15). The second exon of DQA1 and DQB1 genes, each encoding the most polymorphic first external domain of their respective chain, were amplified using primers (25 pmol) recommended by the XIth International Histocompatibility Workshop: DQA-ampA 5′-GGTGTAAACTTGTACCAG-3′ (aa 12–17), DQA-ampB 5′-GGTAGCAGCGGTAGAGTTG-3′ (aa 86–80), DQB-ampA 5′-GCATGTGCTACTTCACCAAC G-3′ (aa 13–20), DQB-ampB 5′-CTGGTAGTTGTGTCT GCACAC- 3′ (aa 84–78). Annealing temperatures and magnesium chloride concentrations were 54°C and 1.5 mM for DQA1 gene and 57°C and 2.5 mM for DQB1 gene. Taq polymerase and the Mod. 9600 Thermocycler, both from Perkin Elmer (Emeryville, Ca), were used.

DQB1 gene polymorphism analysis. Aliquots of each DQB1 amplified product were spotted on a hybridization transfer membrane (Gene Screen Plus, DuPont-NEN, Boston, MA), denatured with 0.4 N NaOH and neutralized with 10 × SSPE (1.5 sodium chloride, 0.1 M sodium phosphate, 10 mM EDTA). All amplified products were hybridized with a DQB1 nonallele-specific probe (5′-CCACGTCGCTGTCGAAGC-3′ aa 45–39) and then diluted to obtain the same spot intensity. Filters were then prehybridized at 42°C with 6 × SSPE, 0.5% SDS, 5% Denhardt's solution, and 100 μg/ml herring sperm DNA, and hybridized using 11 DQB1 ^{32}P-labeled sequence-specific oligonucleotide probes as described by Ronningen et al. (7). The blots were probed at Td = 4° × (number of GC bp) + 2° × (number of AT bp) for 1 h, washed twice in 2 × SSPE, 0.1% SDS at room temperature and twice in 6 × SSPE, 1% SDS at the temperatures suggested elsewhere (7).

Discrimination between DQA1 Arg^{52} and non-Arg^{52} alleles. Following the nonallele-specific amplification performed with the primers described above, a recently reported technique based on group-specific amplification and DNA heteroduplex analysis was used (16). Briefly, the non-Arg^{52} alleles (*0101, *0102, and *0103) were separately amplified from the remaining alleles (*0201, *0301, *0401, *0501, and *0601) by using two specific 3′-end primers. The first group was not further analyzed. The second group includes a non-Arg^{52} allele (*0201). The latter was discriminated by heteroduplex analysis as follows: the amplified samples were mixed with a reference DNA (*0301), heat-denaturated, and renaturated at room temperature for 5 min. The mixture was then separated by electrophoresis on 12% polyacrylamide gel. The patterns obtained allow a clear distinction of the allele *0201 from *0401, *0501, and *0601. The allele *0301 also can be distinguished by the absence of heteroduplex bands.

Statistical analysis. Evaluation of different markers as risk predictors was performed by logistic regression (17). The OR estimates the risk of having the disease in the presence of a given marker relative to the risk in the absence of the marker. If a and b are the numbers of cases and control subjects with the marker, and c and d are the numbers of cases and control subjects without the marker, the OR is ad/bc (equation 1). Its statistical significance can be tested by χ^2 analysis. The 95% CI for OR can be estimated by using Woolf's formula for the SE of log OR:

$$\text{SE (log OR)} = 1/a + 1/b + 1/c + 1/d.$$

The annual incidence of the disease (I/100,000 per yr) is related to the prevalence of the disease (P) and the average duration of the disease (T) by the approximate relationship $P = IT$. For example, if in a population <15 yr of age the incidence rate was 6.5/100,000 per yr, and the mean age at diagnosis was 10.5 yr, the mean duration of the disease at <15 yr of age would be 15–10.5 = 4.5 yr and P would be 6.5 × 4.5 = 29/100,000. Thus, given an estimate of the incidence rate I, it is possible to estimate P. Similarly, given the prevalence, the incidence can be estimated as P/T. If P_x is the prevalence among those with a marker and P_0 is the prevalence among those without that marker, the OR_x is $P_x(1 - P_0)/[P_0(1 - P_x)]$, and rearrangement of this expression gives the prevalence P_x in terms of OR_x and P_0 as follows:

$$P_x = OR_xP_0/[1 + (OR_x - 1)P_0] \text{ (equation 2)}.$$

Thus, if the OR_x and the prevalence P_0 are known, the prevalence of the disease among those with the marker P_x can be calculated. If f_0, f_1, f_2, and f_4 are the fractions of the population with 0, 1, 2, or 4 heterodimers, respectively, and P_0, P_1, P_2, and P_4 are the population prevalences of the disease among those with 0, 1, 2, or 4 heterodimers, the overall population prevalence, P is as follows:

$$P = f_0P_0 + f_1P_1 + f_2P_2 + f_4P_4 \text{ (equation 3)}.$$

Because from equation 2, the prevalence among those in the population with $x = 1$, 2, or 4 heterodimers can be expressed in terms of OR_1, OR_2, OR_4, and P_0, substitution in equation 3 relates the overall population prevalence P to the fractions f_0, f_1, f_2, and f_4 to OR_1, OR_2, and OR_4 and to the prevalence of the disease among those with 0 heterodimers P_0.

Equation 3 then becomes rather clumsy, but when rearranged P_0 can be expressed in terms of overall prevalence P, OR_1, OR_2, and OR_4, and the fractions f_0, f_1, f_2, and f_4. Thus, given the prevalence P in the population,

TABLE 1
Prevalences and ORs of DQA and DQB alleles in Italian type I diabetic patients and control subjects

Alleles	Diabetic patients (n = 65) n (%)	Control subjects (n = 93) n (%)	P value	OR
DQA1*				
0101				
0102	22 (33.8)	53 (66.7)	0.007	0.39
0103				
0201	11 (16.9)	27 (29)	NS*	0.5
0301	29 (44.6)	12 (12.9)	<0.001	5.44
0401				
0501	47 (72.3)	73 (78.5)	NS*	0.72
0601				
DQB1*				
0201	47 72.3)	37 (39.8)	<0.001	3.95
0301	10 (15.4)	48 (51.6)	<0.001	0.17
0302	23 (35.4)	11 (11.8)	<0.001	4.06
0303	2 (3.1)	1 (1.1)	NS*	2.92
0402	1 (1.5)	11 (11.8)	0.036	0.12
0501	7 (10.8)	18 (19.4)	NS*	0.5
0502	7 (10.8)	10 (10.8)	NS*	1
0503	2 (3.1)	4 (4.3)	NS*	0.71
0601	—	3 (3.2)	NS*	0.2
0602	4 (6.2)	10 (10.8)	NS*	0.54
0603	1 (1.5)	10 (10.8)	NS*	0.13
0604	5 (7.7)	3 (3.2)	NS*	0.69

Bold, Arg^{52} and non-Asp^{57} alleles; OR, ratio of the odds on having the disease when the allele is present to the odds when it is absent.
*$P > 0.05$.

OR_1, OR_2, and OR_4 are estimated in the study using equation 1 and f_0, f_1, f_2, and f_4 are estimated by the fractions in the control group with 0, 1, 2, or 4 heterodimers, the value P_0 can be calculated.

Substituting this value of P_0 in equation 2 and using the study estimates of OR_1, OR_2, and OR_4 enables the prevalences of the disease P_1, P_2, and P_4 among those with 1, 2, or 4 heterodimers to be estimated. Finally, by using the relationship $P = IT$, it is possible to estimate the annual incidence rates I_0, I_1, I_2, and I_4 from P_0, P_1, P_2, and P_4.

RESULTS

Asp^{57} and non-Asp^{57} DQB1 alleles. Data obtained in 65 type I diabetic patients and 93 control subjects from central Italy are shown in Table 1. A significant increase of both DQB1*0201 (DR3-associated) and *0302 (DR4-associated) was found among the patients. The remaining three non-Asp^{57} alleles (*0501, *0502, and *0604) did not show any significant association (notably, these alleles are in linkage disequilibrium with non-Arg^{52} DQA alleles). A significant protective effect was seen only for DQB1*0301 (DR5-associated), whereas the protective effect of the DR2 haplotype was not detectable in accordance with previous results (18,19).

By subdividing the DQB1 alleles into the Asp^{57} and the non-Asp^{57} groups, the frequencies and ORs were obtained (Table 2 and Fig. 1*A*). Seventy percent of patients were non-Asp/non-Asp. This genotype had an OR of 32.7. The frequency of Asp/non-Asp genotype was not significantly different in patients and control subjects, whereas the Asp/Asp genotype was clearly protective, although it was found in 2 patients.

TABLE 2
ORs and CIs of genotypes relative to the amino acids in position 57 of the DQ β-chain, in position 52 of the DQ α-chain, and to the number of susceptible heterodimers

	Diabetic patients	Control subjects	OR	95% CI
Non-Asp/non-Asp	45	22	32.7	7.2–149.2
Asp/non-Asp	18	39	7.4	1.6–34.2
Asp/Asp	2	32	1.0	—
Arg/Arg	32	19	5.1	1.7–14.9
Arg/non-Arg	27	56	1.4	0.5–4.1
Non-Arg/non-Arg	6	18	1.0	—
Susceptible heterodimers				
4	23	1	161	18.7–1386
2	27	20	9.4	3.5–25.2
1	8	23	2.4	0.8–7.5
0	7	49	1.0	—

Arg^{52} and non-Arg^{52} alleles. The subdivision of DQA1 alleles for Arg^{52} and non-Arg^{52} combines group-specific amplification with DNA heteroduplex analysis. Based on this technique, the DQA1 alleles were subdivided into four groups (Table 1). One group includes the three non-Arg^{52} *01 alleles (*0101, *0102, and *0103), and another group includes the Arg^{57} alleles *04, *05, and *06. Discrimination between the single alleles inside these groups was not performed. Therefore, single allele associations could be calculated only for *0201 and *0301. The *0301 allele (DR4-associated) showed a highly significant association with the disease.

Figure 1*B* and Table 2 show the DQA1 data grouped according to presence or absence of Arg^{52} alleles. Of the patients, ~50% possessed the Arg/Arg genotype with an OR of 5.1, which is markedly lower than that of DQB1 non-Asp^{57}/non-Asp^{57} genotype. The frequency of non-Arg^{52}/non-Arg^{52} genotype was significantly decreased in the patients. Nevertheless, 6 patients possessed two protective DQA1 alleles.

Haplotype combinations of DQA1 and DQB1 alleles. Considering the different combinations of DQA and DQB alleles (Table 3), it appears that ~90% of the patients have at least one susceptible allele at each locus and that >33% of the patients possess all four susceptible alleles. When the different genotypes are grouped according to the number of susceptible heterodimers potentially formed, a distribution as shown in Fig. 1*C* can be obtained. The highest OR, 161, was observed by the group with four susceptible heterodimers, which concurs with the well-known predominance in diabetic patients of DR3/DR4 heterozygotes. Interestingly, a significant difference in the OR exists between the genotypes capable of forming four or two ($P < 0.05$) and two or one ($P < 0.01$) susceptible heterodimers. The difference between one and zero heterodimers was not statistically significant ($P > 0.10$). Notably, 7 (10.8%) patients belong

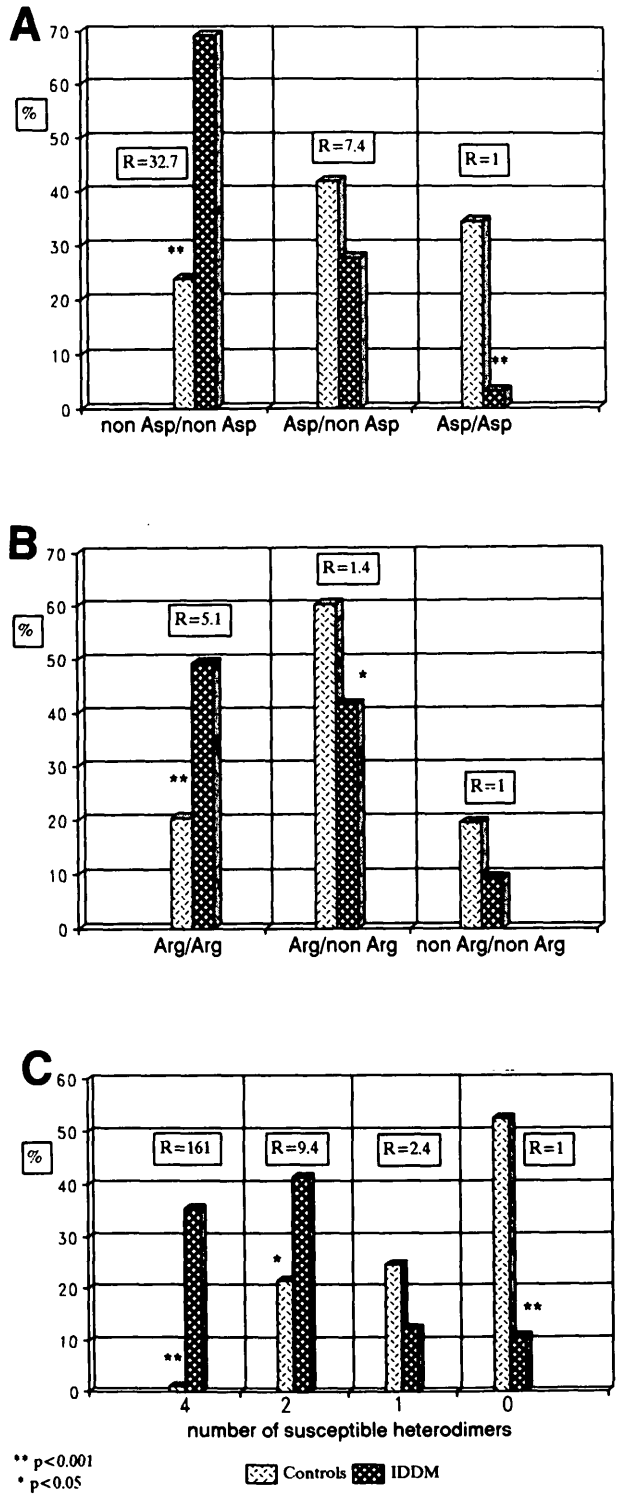

FIG. 1. Genotypic frequencies relative to the amino acid in position 57 of the DQ β-chain (*A*) and to amino acid in position 52 of the DQ α-chain (*B*) in type I diabetic patients and control subjects from central Italy. The distribution of susceptible heterodimers in type 1 diabetic patients and control subjects (*C*).

to the group of those genotypes unable to form any susceptible heterodimer.

Table 3 shows the number of patients and control subjects in each of the nine possible combinations of categories of DQA and DQB. Because DQA and DQB have three categories each, it is possible to fit a model using logistic regression, to investigate the ability of either DQA, DQB, both, or the number of heterodimers to explain differences in the distribution of patients and control subjects (Table 4) (17). In this case, χ^2, also known as scaled deviance in logistic regression, indicates how well the model fits the data. High values of χ^2 imply that the specified factor is unable to explain the observed data (Table 2), i.e., the factor is a poor predictor of risk. The number of susceptible heterodimers, calculated regardless of the *cis* or *trans* phase of the markers, appears to represent the most accurate risk predictor (Table 4).

Calculation of the estimated incidence according to the number of heterodimers. Using the fractions of controls with 0, 1, 2, and 4 heterodimers as estimates of the corresponding fractions in the populations, the values of f_0, f_1, f_2, and f_4 are 0.527, 0.247, 0.215, and 0.011, respectively. From equation 2 and using the values of the ORs from Table 2, the prevalence of the disease among individuals with 1, 2, or 4 heterodimers is as follows:

$$P_1 = 2.4\ P_0/(1 + 1.43\ P_0)$$
$$P_2 = 9.4\ P_0/(1 + 8.4\ P_0) \quad \text{(equation 4)}$$
$$P_4 = 161\ P_0/(1 + 160\ P_0).$$

The incidence of type I diabetes in the Lazio region is estimated to be 6.5/100,000 per yr among children <15 yr of age (2). If the mean age at diagnosis is 10.5 yr of age, the average duration of the illness in those <15 yr of age is 4.5 yr, and P is estimated as 29.25/100,000. Substituting the values f_0, f_1, f_2, and f_4, P_1, P_2, and P_4, and P in equation 3 and solving for P_0 yields P_0 = 6/100,000. Substitution of P_0 = 6.0/100,000 in equation 4 gives the following estimates of P_x of type I diabetes among 100,000 subjects with x heterodimers:

$P_0 = 6.0$; $P_1 = 14.6$; $P_2 = 56.7$; $P_4 = 956.4$.

Using the relationship incidence = prevalence/average duration of illness, those prevalence rates correspond to annual incidence rates as follows:

$I_0 = 1.33$; $I_1 = 3.24$; $I_2 = 12.6$; and $I_4 = 212.53/100{,}000$.

DISCUSSION

According to Khalil's hypothesis (12), the combination of two markers (Arg^{52} in the DQA1 gene and non-Asp^{57} in the DQB1 gene) determines the degree of susceptibility to type I diabetes. This is related to the possibility of forming susceptible heterodimers composed of an α-subunit with Arg in position 52 and a β-subunit with an amino acid other than Asp in position 57. Assuming that this combination is not appreciably affected by the *cis* or *trans* position of the two markers, the different genotypes allow the generation of 4, 2, 1, or 0 susceptible heterodimers, respectively. Following this hypothesis, the number of possible heterodimers are expected to parallel the ORs associated with the different genotypes.

Two separate issues must be considered: first, the validity of this theory for explaining adequately the HLA association with type I diabetes; second, whether a more accurate estimate of the disease risk can be obtained by

TABLE 3
HLA-DQ α- (position 52) and HLADQ β- (position 57) genotypes in type I diabetic patients and control subjects

Genotypes		Susceptible heterodimers	Diabetic patients (n = 65)	Control subjects (n = 93)	P values
DQA	DQB		n (%)	n (%)	
Arg/Arg	**non-Asp/non-Asp**	4	23 (35.4)	1 (1.1)	<0.001
Arg/non-Arg	**non-Asp/non-Asp**	2	19 (29.2)	13 (14)	0.03
Arg/Arg	**non-Asp**/Asp	2	8 (12.3)	7 (7.5)	NS
Arg/non-Arg	**non-Asp**/Asp	1	8 (12.3)	23 (24.7)	NS
non-Asp/non-Asp	**non-Asp/non-Asp**	0	3 (4.6)	7 (7.5)	NS
Arg/Arg	Asp/Asp	0	1 (1.5)	12 (12.9)	0.024
non-Arg/non-Arg	**non-Asp**/Asp	0	2 (3.1)	12 (12.9)	NS
Arg/non-Arg	Asp/Asp	0	—	17 (18.3)	<0.001
non-Arg/non-Arg	Asp/Asp	0	1 (1.5)	1 (1.1)	NS

Bold, susceptible alleles.

considering the two markers together than non-Asp^{57} alone.

With regard to the first point, several data suggest that the HLA association of type I diabetes cannot be fully explained by the Arg^{52}/non-Asp^{57} theory, mainly for three reasons: *1*) some patients do not possess susceptible heterodimers; this was observed in almost all studies in different populations; *2*) the degree of the association of the different genotypes does not agree perfectly with the number (4, 2, 1, or 0) of expected susceptible heterodimers, for instance DR3/DR3, DR4/DR4, and DR3/DR4 should confer the same risk of disease because all these genotypes can generate four susceptible heterodimers; however DR3/DR4 has a much higher OR than either DR3/DR3 or DR4/DR4; *3*) in non-Caucasian populations, the HLA-association of type I diabetes follows different rules.

That the theory does not provide a full explanation of the existing observations is also borne out by these results, which show that ~10% of patients with type I diabetes possess DQ genotypes that do not allow the formation of any susceptible heterodimers. This is comparable with values varying from 3 to 8% observed in other populations (Table 5). However, the theory can still be tenable if provision is made for some additional assumptions, i.e., different pathogenic forms of the disease may exist and may follow different rules of HLA association, or may be not HLA associated. Other amino acid residues, apart from Arg^{52} and non-Asp^{57} in the same molecules, or even in different molecules controlled by genes in linkage disequilibrium, such as DR3 and DR4, may instead play a role. Whether the presence of this heterodimer is part of the pathogenetic process of type I diabetes or is a marker in linkage disequilibrium with the disease cannot be confirmed until the other partner of this process, i.e., the antigen, is identified with some certainty.

This study addresses the use of two markers for predicting the risk of diabetes, which is especially important for possible preventive measures in healthy relatives of type I diabetic patients. Considering together non-Asp^{57} and Arg^{52}, a much higher value of the OR is obtained for the highest risk group (OR = 161) compared with the values obtained either considering non-Asp^{57} alone (OR = 32.7) or Arg^{52} alone (OR = 5.1). The use of both markers enables the discrimination of a category of individuals with a very high disease risk.

TABLE 4
Values of scaled deviance obtained after fitting various models in logistic regression to the data in Table 2

Marker	N° categories	Scaled deviance	df
Non-Asp^{57}	3	29.6	6
Arg^{52}	3	58.5	6
Non-Asp^{57} plus Arg^{52}	6	9.9	4
N° susceptible heterodimers	4	8.8	5

TABLE 5
ORs and incidence rates of type I diabetes for different numbers of HLA-DQ susceptible heterodimers in European populations

	Number of susceptible heterodimers				Global incidence*
	4	2	1	0	
Central Italy					6.5
Diabetic patients	23	27	8	7	
Control subjects	1	20	23	49	
OR	161	9.4	2.4	1	
I	212	12.6	3.2	1.3	
Belgium					9.8
Diabetic patients	126	95	24	19	
Control subjects	18	72	70	169	
OR	65.8	10.9	2.9	1	
I	116	19	5	1.7	
France					7.8
Diabetic patients	124	85	14	7	
Control subjects	6	28	65	116	
OR	399	59.4	4.2	1	
I	562	85	4.2	1.4	
Spain					10.6
Diabetic patients	66	27	1	8	
Control subjects	6	21	17	43	
OR	59.1	6.9	0.3	1	
I	117	13.8	0.6	2	

I, estimated incidence rate/100,000 per yr. Data for Belgium, France, and Spain are from Heimberg et al. (11), Khalil et al. (20), and Gutierrez-Lopez et al. (13).
*According to Green et al. (2).

Moreover, a category with a lower, but still significant risk also can be recognized, which corresponds to the ability to generate two susceptible heterodimers. Individuals with a single heterodimer possess a still lower risk, but it is higher than that of the protected group.

Table 4 shows the scaled deviance for each combination of markers. The χ^2 values, when only one marker is used in the regression model, are high, 29.6 and 58.5, respectively, with 6 df compared with the χ^2 value of 9.9 with 4 df when both are used. When the number of heterodimers is used for prediction, the χ^2 value is reduced slightly to 8.83 but with one more df, indicating that this is the best predictive model.

We calculated the absolute risks associated with 0, 1, 2, or 4 susceptible heterodimers in the Lazio region population, where the incidence of IDDM is 6.5/100,000 per yr in those <15 yr of age, as determined in the Eurodiab Ace Study (2). Individuals with "4 heterodimers genotypes" could have an annual incidence of 212.5/100,000 per yr as opposed to 1.3 for the individuals with "0 heterodimers genotypes." We also have made the same calculation for Asp^{57} and Arg^{52} data in different populations (11,13,20), where annual incidence estimates also are available. If the risk estimated by the number of heterodimers is predominant over the risks contributed by environmental factors and by genetic factors other than HLA, it may be expected that the annual incidence values for the different heterodimers groups are uniform in the different populations. In other words, if the above assumption is correct, an individual, given the number of his heterodimers, should have a similar probability of developing the disease regardless of the population to which he belongs. As shown in Table 5, comparison of the incidence rates for individuals with 0, 1, 2, and 4 heterodimers indicates consistent and progressive increases in each of the four population groups.

Given that the estimates of incidence are based on relatively small numbers of cases and control subjects and the assumptions under which they have been calculated, it is perhaps surprising that the results are so consistent.

This concurs with the concept, originally proposed by Dorman et al. (9), that population variation in the incidence of type I diabetes is largely explained by geographic variations of non-Asp^{57} alleles; this is now reinforced by our data on Arg^{52} alleles. However, to confirm this observation it is important that similar typing data are collected on widely different populations.

ACKNOWLEDGMENTS

This study was supported by targeted projects of the Italian National Research Council on Genetic Engineering and on Biotechnology and Bioinstrumentation, by the Italian Ministry of University and Scientific Research, and by the Juvenile Diabetes Foundation. L.N. was supported by a fellowship from the International Diabetes Immunotherapy Group and Neuroimmunology.

We are especially grateful to Dr. Roberto Tosi for many helpful discussions and valuable comments. We are indebted to Dr. Paolo Pozzilli for valuable discussion and advice. We also are indebted to the many physicians of the Lazio region who took part in the Eurodiab subarea A project and allowed patients to be included. We also thank Giuliana Falasca for synthesis of the oligonucleotides, and Eleuteria Lancia and Irene Pauselli for excellent technical assistance.

REFERENCES

1. Yamagata K, Nakajima H, Hanofusa T, Noguchi T, Tanake T, Kono N, Tarui S: Aspartic acid at position 57 of DQ β-chain does not protect against type I diabetes in Japanese subjects. *Diabetologia* 32:762–64, 1989
2. Green A, Gale EAM, Patterson CC, Eurodiab Ace Study Group: Wide variation in the incidence of childhood-onset insulin-dependent diabetes mellitus in Europe: the Eurodiab Ace Study. *Lancet* 339:905–909, 1992
3. Svejgaard A, Platz P, Ryder L: HLA and disease 1982: a survey. *Immunol Rev* 70:193–98, 1983
4. Thompson G: HLA-DR antigens and susceptibility to insulin-dependent diabetes mellitus. *Am J Hum Genet* 36:1309–13, 1984
5. Todd JA, Bell JI, McDevitt HO: HLA-DQ β-gene contributes to susceptibility and resistance to insulin-dependent diabetes mellitus. *Nature* (Lond) 329:599–604, 1987
6. Morel PA, Dorman JS, Todd JA, McDevitt HO, Trucco M: Aspartic acid at position 57 of the HLA-DQ β-chain protects against type I diabetes: a family study. *Proc Natl Acad Sci USA* 85:8111–16, 1988
7. Ronningen LS, Iwe T, Halstensen TS, Spurkland A, Torsby E: The amino acid at position 57 of the HLA-DQ β-chain and susceptibility to develop insulin-dependent diabetes mellitus. *Hum Immunol* 26: 215–25, 1989
8. Baisch JM, Weeks T, Jiles R, Hoover M, Stastny P, Capra JD: Analysis of HLA-DQ genotypes and susceptibility in insulin-dependent diabetes mellitus. *N Engl J Med* 322:1836–41, 1990
9. Dorman JS, LaPorte RE, Stone RA, Trucco M: Worldwide differences in the incidence of type I diabetes are associated with amino acid at position 57 of the HLA-DQ β-chain. *Proc Natl Acad Sci USA* 87:7370–74, 1990
10. Ronningen KS, Spurkland A, Iwe T, Vartdal F, Thorsby E: Distribution of HLA-DRB1, HLA-DQA1, and HLA-DQB1 alleles and DQA1-DQB1 genotypes among Norwegian patients with insulin-dependent diabetes mellitus. *Tissue Antigens* 37:105–11, 1991
11. Heimberg H, Nagy ZP, Somers G, De Leeuw I, Schuit FC: Complementation of HLA-DQA and HLA-DQB genes confers susceptibility and protection to insulin-dependent diabetes mellitus. *Hum Immunol* 33:10–17, 1992
12. Khalil I, D'Auriol L, Gobet M, Morin L, Lepage B, Deschamps I, Park MS, Degos L, Galibert F, Hors J: A combination of HLA-DQ β Asp 57-negative and HLA-DQ α Arg 52 confers susceptibility to insulin-dependent diabetes mellitus. *J Clin Invest* 85:1315–19, 1990
13. Gutierrez-Lopez MD, Bertera S, Chantres MT, Vavassori C, Dorman JS, Trucco M, Serrano-Rios M: Susceptibility to type I diabetes in Spanish patients correlates quantitatively with expression of HLA-DQ α Arg 52 and HLA-DQ β non-Asp 57 alleles. *Diabetologia* 35:583–88, 1992
14. D'Amato M, Sorrentino R, Tosi R: Extremely simplified sample preparation for HLA genomic typing. *Tissue Antigens* 39:40–41, 1992
15. Saiki RK, Gelfand DH, Stoffel S, Scharf SJ, Higuchi R, Horn GT, Mullis KB, Erlich HA: Primer-directed enzymatic amplification of DNA with a thermo-stable DNA polymerase. *Science* 239:1350–54, 1988
16. Sorrentino R, Costanzi S, Cascino I, Tosi R: Probe-less genomic typing of Arg 52 (type I diabetes-associated) and non-Arg 52 (non-type I diabetes associated) HLA-DQA 1 alleles. *Autoimmunity* 13:269–74, 1992
17. Breslow N and Day N: Chapter 6. In *Statistical Methods in Cancer Research.* IARC Publication No. 32, Lyon, 1980
18. Vela M, Adorno D, Longo A, Papola F, Maccarone D, Centis D, Raponi MP, Candela A, Campea L, Orsini M, Ferrara GB: HLA typing data on 100 type I (insulin-dependent) diabetic patients from central Italy show a negative association with DR5 rather than with DR2. *Clin Immunol Immunopathol* 45:143–46, 1987
19. Sorrentino R, De Grazia U, Buzzetti R, Nisticò L, Iannicola C, Costanzi S, Tosi R: An explanation for the neutral effect of DR2 on IDDM susceptibility in central Italy. *Diabetes* 41:904–908, 1992
20. Khalil I, Deschamps I, Lepage V, Al-Daccak R, Degos L, Hors J: Dose effect of *cis*- and *trans*-encoded HLA-DQ α-β heterodimers in IDDM susceptibility. *Diabetes* 41:378–84, 1992