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Association between *HLA-DQB1* Alleles and Type 1 Diabetes in a Case-Parents Study Conducted in Santiago, Chile

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The human leukocyte antigen (HLA) system plays a crucial role in the autoimmune process leading to childhood diabetes. The purpose of this study was to evaluate the association between type 1 diabetes and the polymorphism encoded by the *HLA-DQB1* gene by using case-parents trios. The study area was the metropolitan region of Santiago, Chile, and cases were ascertained from March 1997 to August 1998. Genotyping was performed in 94 trios comprising incident cases less than 17 years of age at the time of diagnosis and their parents. The transmission/disequilibrium test was used to detect differential transmission in the *HLA-DQB1* locus. The authors found that alleles *DQB1*0302* and *DQB1*0201* were strongly associated with the disease. By using 1:3 matched sets of cases-pseudosibs and conditional logistic regression models, allelic relative risks were estimated for *DQB1*0302* (r = 7.2, 95% confidence interval: 2.8, 18.5) and *DQB1*0201* (r = 4.7, 95% confidence interval: 1.9, 11.6); *DQB1*0301* was considered the baseline allele. When case-parents trios were used, alleles *DQB1*0302* and *DQB1*0201* were strongly associated with a higher risk of type 1 diabetes in the population of Santiago. *Am J Epidemiol* 2001;153:794–8.

alleles; association; case-control studies; diabetes mellitus, insulin-dependent; genetics; HLA antigens; parents

Both genetic and environmental factors participate in the autoimmune process leading to type 1 diabetes (1). As a result of the multifactorial etiology of this disorder, incidence rates vary greatly when different countries are compared (2). The WHO DiaMond Molecular Epidemiology Sub-Project Group tested the hypothesis that geographic differences in the incidence of type 1 diabetes vary according to the frequency of susceptible alleles in the human leukocyte antigen (HLA) region (3). This study and others (4–7) support the hypothesis that HLA polymorphism contributes to the genetic basis of variation in the incidence of type 1 diabetes. The differences among countries and ethnic groups can be explained by a variety of factors, such as disparate exposure to environmental risk factors, distinct frequencies of susceptible alleles, and different types of gene-environment or gene-gene interactions. With respect to genetic factors, the frequencies of high-risk genotypes in the HLA loci diverge across ethnic groups because of the enormous variability of this genetic system (3). With regard to environmental risk factors, viral infections and dietary components have been implicated in the etiology of type 1 diabetes (8).

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Abbreviations: HLA, human leukocyte antigen; TDT, transmission/disequilibrium test.

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The Chilean population constitutes a melting pot of persons from different parts of the world, including Europeans originating from different countries, mainly Spain, and Amerindians (9). The Mapuche population is the major aboriginal group. According to the self-identification question on ethnicity from the 1992 Chilean census, 928,385 Chilean subjects older than 14 years of age declared that they belonged to the "Mapuche culture" (total population older than 14 years of age in Chile: 9,660,367) (10). However, this figure was estimated to be notably lower on surveys in which the self-identification question referred to "Mapuche population" instead of "Mapuche culture" (11). Following the first Spanish military expeditions to Chile in the 16th century, new migrations from Europe contributed to the development of the modern Chilean population. In the 20th century, economic and social changes forced large numbers of persons to migrate from rural areas to big urban nuclei, such as the city of Santiago and its surrounding areas. Therefore, a heterogeneous mixture of people and migration processes generated the population of the city of Santiago. This phenomenon should be taken into account when the association between HLA polymorphism and type 1 diabetes in this population is studied, since both the incidence of type 1 diabetes and the frequencies of HLA alleles differ markedly among populations and ethnic groups (12).

The incidence of type 1 diabetes in the metropolitan region of Santiago (the main urban nucleus of Chile) has been estimated to be as low as 2.36 per 100,000 inhabitants less than 15 years of age per year (13). Among the aboriginal Mapuche population living in the south of Chile, the incidence rate is even lower (0.42 cases per 100,000 inhabitants less than 15 years of age per year) (14). The incidence

rates of type 1 diabetes in Chile strongly contrast with the moderate-high incidence rates estimated in Spain or southern Europe (15). In other Latin-American countries with a Spanish or Portuguese heritage, there also seems to be a large geographic variation in the risk of developing type 1 diabetes (2).

A previous case-control study indicated a strong association between HLA class II alleles and type 1 diabetes in Chile (6). Because of the heterogeneous composition of the Chilean population, use of parents instead of nonrelated controls in a matched analysis would be expected to yield unbiased estimations of the strength of the association between type 1 diabetes and HLA class II polymorphism, since it avoids case-control differences that could otherwise arise from the selection of controls whose genetic backgrounds differ systematically from those of cases (16). The purpose of the present study was to evaluate the association between type 1 diabetes and the HLA class II polymorphism encoded by the *HLA-DQB1* locus in Chilean families comprising trios of cases and parents.

MATERIALS AND METHODS

Subjects

Families were ascertained through the probands, incident cases with type 1 diabetes who were less than 17 years of age at the time of diagnosis (mean, 8.3 years; standard deviation, 4.3 years; 55 boys and 39 girls). From March 1997 to August 1998, an active search for newly diagnosed children was carried out by using different sources, such as emergency services in hospitals, private clinics, pediatricians, and the voluntary registry of the Juvenile Diabetes Foundation of Chile. Initially, blood samples were obtained from 103 incident cases. Of these, the parents of 5 cases refused to take part in the study. Three other families did not participate because either one or both of the proband's parents were deceased prior to the diagnosis of diabetes. Another child was adopted, and the biologic parents were not accessible. In total, blood samples from 94 trios comprising the incident case and both parents were available for laboratory analysis. Each nuclear family considered in this research had a single affected child. The study was approved by the local ethics committee, and one parent in each family gave written informed consent.

Because the population-based registry of type 1 diabetes in the metropolitan region of Santiago includes only cases less than 15 years of age, the precise number of incident cases aged 17 years or less during the period March 1997 to August 1998 was unknown. However, through a retrospective search of different sources for cases who were less than 15 years of age, the registry ascertained a total of 74 cases in 1995, 58 cases in 1996, and 70 cases in 1997. Reliable data for 1998 were not yet available when we conducted this study. Therefore, according to the registry data from previous years, the 94 cases included in the present study (87 cases less than 15 years of age, 5 cases aged 15 years, and 2 cases aged 16 years) seemed to represent a very high proportion of all diagnosed cases less than 17 years of age in the metropolitan region of Santiago from March 1997 to August 1998.

Laboratory analysis

Genotyping of cases and parents for the *HLA-DQB1* gene was performed by using polymerase chain reaction and dot-blot analysis with sequence-specific oligonucleotide probes; seven alleles were distinguished (6): *DQB1*0201*, *DQB1*0301*, *DQB1*0302*, *DQB1*0303*, *DQB1*0402*, *DQB1*0501*, and *DQB1*0602*. Allelic variants *DQB1*0501*, *DQB1*0502*, and *DQB1*0503* were genotyped as *DQB1*0501*. Alleles *DQB1*0601* and *DQB1*0602* were genotyped as *DQB1*0602*. The alleles identified in this study represent only a subset of all possible alleles in the *DQB1* locus. These genetic variants were chosen on the basis of the known prevalence of these genetic polymorphisms in the Chilean population (6) and their hypothesized susceptibility to or protective effects against type 1 diabetes (17).

Statistical methods

The transmission/disequilibrium test (TDT) was used to assess transmission of *HLA-DQB1* alleles from heterozygous parents to diseased children (18). When affected children are sampled, associations between the genetic factor and the disease cause the probability of transmission to differ from the expected value (probability of transmission = 0.5). The TDT is a valid test of association even if population stratification is present (19). Exact and asymptotic multiple-allele versions of the TDT were conducted to assess the global association between *HLA-DQB1* alleles and type 1 diabetes in Chilean case-parents trios, and allele-specific p values for which an exact TDT was used were computed (20, 21). In the allele-specific TDT, Bonferroni correction can be applied to obtain p values adjusted for multiple comparisons (22).

Allelic relative risks (r) were estimated by using conditional logistic regression techniques with 1:3 matched sets of cases and pseudosibs (23, 24). With this method, each case-parents trio generates a case and three matched pseudosibs represented by all possible combinations of parental alleles not inherited by the case. A logistic model with $K - 1$ covariates was fitted, in which K was defined as the number of alleles examined. In this model, each covariate represents the number of copies of the allele of interest; *DQB1*0301* was considered the baseline allele. In this context, r denotes the allelic relative risk of disease for the allele of interest versus the baseline based on the assumption that the effects of alleles are multiplicative on the relative risk. Thus, r^2 estimates the relative risk of disease for those who are homozygous for the allele of interest in relation to those with a homozygous genotype for the baseline allele.

Another statistical model was fitted with binary indicators to code for genotypes. In this instance, the large number of genotypes and our modest sample size produced great instability in the estimation of the genotypic relative risk (data not shown). Alternatively, genotypic relative risks r_1 and r_2 were estimated by using indicator variables for groups of alleles reported as susceptible (*DQB1*0302* and *DQB1*0201*) or nonsusceptible to type 1 diabetes (25). In this model, r_1 and r_2 denoted the genotypic relative risk of

disease for those with one or two copies of the susceptible alleles, respectively, in relation to those with no copies of the susceptible alleles. All statistical analyses were carried out with STATA 6.0 statistical software (Stata Corporation, College Station, Texas).

RESULTS

Exact and asymptotic TDT for multiple alleles provided strong evidence for the association between *HLA-DQB1* alleles and type 1 diabetes ($p < 0.0001$). Table 1 shows the results of the genetic analysis of *DQB1* alleles. One exact TDT was conducted for each allele to produce uncorrected p values for association (adjusted p values can be obtained by using Bonferroni correction for multiple tests).

The major contribution to the multiple-allele chi-square statistic was from 21 transmissions of the *DQB1*0201* allele from 21 heterozygous *DQB1*0201*/*DQB1*0501* parents, 17 transmissions of the *DQB1*0302* allele from 17 heterozygous *DQB1*0302*/*DQB1*0402* parents, and 17 transmissions of the *DQB1*0302* allele from 17 heterozygous *DQB1*0302*/*DQB1*0602* parents. Therefore, the *DQB1*0201* and *DQB1*0302* alleles were strongly associated with susceptibility to type 1 diabetes. Among 8 heterozygous parents with *DQB1*0201*/*DQB1*0302* genotypes, 6 transmitted the *DQB1*0302* allele. On the other hand, alleles *DQB1*0301*, *DQB1*0303*, *DQB1*0402*, *DQB1*0501*, and *DQB1*0602* were transmitted with a frequency of less than 0.5 from heterozygous parents to their children. Only one diabetic case had the *DQB1*0602* allele, which was transmitted from a heterozygous *DQB1*0602*/*DQB1*0301* parent. None of the diabetic patients had the *DQB1*0402* allele.

Table 2 presents the allelic relative risks and 95 percent confidence intervals for the association between alleles in the *DQB1* locus and type 1 diabetes (baseline allele, *DQB1*0301*). Because *DQB1*0401* was found rather infrequently, table 2 does not include estimates for this particular allele. Table 3 shows estimates of the genotypic relative risks r_1 and r_2 for carriers who had one or two copies of the susceptible alleles, respectively, in relation to those with no copies of these alleles. We used as susceptible alleles those reported to be part of the main susceptible *HLA-DQ* haplotypes in most populations (*DQB1*0201* and *DQB1*0302*) (25).

TABLE 1. Transmission/disequilibrium test for the association between *HLA-DQB1* alleles and type 1 diabetes in case-parents trios, Santiago, Chile, 1997–1998

Allele	No. of heterozygous parents	Transmitted/ not transmitted	Uncorrected *p* value
*DQB1*0201*	52	40/12	0.0001
*DQB1*0301*	44	13/31	0.01
*DQB1*0302*	90	83/7	<0.0001
*DQB1*0303*	30	6/24	0.001
*DQB1*0402*	17	0/17	<0.0001
*DQB1*0501*	68	21/47	0.002
*DQB1*0602*	27	1/26	<0.0001

TABLE 2. Allelic relative risks and 95% confidence intervals for the association between *DQB1* alleles and type 1 diabetes in case-parents trios, Santiago, Chile, 1997–1998

Allele	Allelic relative risk*	95% confidence interval
*DQB1*0201*	4.65	1.86, 11.58
*DQB1*0302*	7.18	2.79, 18.49
*DQB1*0303*	0.19	0.04, 0.97
*DQB1*0501*	1.03	0.45, 2.39
*DQB1*0602*	0.03	0.003, 0.39

* Assumes that the effects of the alleles are multiplicative on the relative risk; baseline, the *DQB1*0301* allele.

DISCUSSION

Most studies of the association between genetic markers and type 1 diabetes have used a case-control design. There are several explanations for the statistically significant associations found in these case-control studies, including the following: 1) the allele under study is a sufficient cause of the disease or participates in a sufficient cause of the disease; 2) the allele is in linkage disequilibrium with alleles of the actual causative gene; 3) in a prevalence-type case-control study, associations may occur if the genetic marker modifies survival of cases; and 4) the allele-disease associations may arise as an artifact of population admixture.

With regard to the first explanation, a large quantity of literature suggests the involvement of HLA class II molecules in the autoimmune process leading to type 1 diabetes (26). Regarding the second explanation, HLA loci are in strong linkage disequilibrium, and it is difficult to ascertain specifically which alleles are associated more strongly than others with the disease. However, there is evidence supporting the finding that *HLA-DQ* alleles, compared with other HLA class I or II alleles, are more adequate epidemiologic markers of type 1 diabetes susceptibility (27). Some authors have

TABLE 3. Genotypic relative risks and 95% confidence intervals for the association between *DQB1*-susceptible genotypes and type 1 diabetes in case-parents trios, Santiago, Chile, 1997–1998

Genotype	Genotypic relative risk*	95% confidence interval
No susceptible alleles	Reference	
One susceptible allele	$r_1 = 6.00$	2.10, 17.18
Two susceptible alleles	$r_2 = 70.95$	20.59, 244.43

* Susceptible alleles are *DQB1*0201* and *DQB1*0302*; the reference group is composed of genotypes with no copies of the susceptible alleles.

suggested that *HLA-DQB1* polymorphisms are more important than variation in the *DQA1* gene on the basis of the risk conferred by allelic variants in the *DQB1* gene encoding different amino acids at position 57 of the peptide-binding site (25, 28). Moreover, rapid identification of groups of high-risk *DQB1* alleles has been proposed for large-scale screening projects as an efficient way to determine susceptibility to type 1 diabetes (29).

With respect to the third explanation, measures of association based on prevalent cases may be biased because of differential survival (30). In our case-parents study, recruitment of incident cases ensured the representativity of our patients with respect to all type 1 diabetes cases diagnosed during the study period. Concerning the fourth explanation, it is accepted that the case-parents design offers an adequate framework for study of the association between genetic polymorphisms and diseases since it eliminates the confounding effect of population stratification by ethnicity. This confounding effect is problematic in genetic epidemiology studies of mixed populations (31).

When we compared the present data with a previous case-control study conducted in Chile (6), the results of the case-parents study confirmed the identification of *DQB1*0302* and *DQB1*0201* as the main alleles of susceptibility in the *DQB1* locus. On the other hand, alleles *DQB1*0402*, *DQB1*0501*, *DQB1*0301*, *DQB1*0303*, and *DQB1*0602* appeared to be inversely associated with type 1 diabetes in our study. Their reduced transmissibility may have been the result of a potential protective role of these alleles. However, although the protective effect of some *HLA-DQB1* alleles (especially *DQB1*0602*) is widely accepted (17), the interpretation of the effect of these alleles on disease status is more difficult to evaluate than the effect of the high-risk alleles, since some of these allelic variants may be neutral and are not transmitted when paired with a high-risk allele such as *DQB1*0201* or *DQB1*0302*.

It is worth noting that our interpretation of the results of this case-parents study relies on the assumption that, conditional on parental genotypes, the proportions of offspring genotypes follow Mendelian probabilities in the population. Additionally, the allelic relative risks shown in table 2 were computed by assuming that the effects of alleles are multiplicative on the relative risk for the disease. Deviations from these assumptions would invalidate to a variable degree our interpretations regarding the transmission probabilities or the allelic relative risks estimated by means of case-parents trios. On the other hand, the genotypic relative risks shown in table 3 were computed as an overall assessment of the effect of *HLA-DQB1* polymorphisms, since comparison categories were composed of genotypes containing alleles with variable degrees of susceptibility to or protection against type 1 diabetes.

Some authors have argued that population-based, incident case-control designs in which candidate genes are used are more suitable than a case-parents design; they can assess the effect of environmental risk factors and better define gene-environment interactions in the population, a crucial step in disease prevention and health promotion (32, 33). However, family-based studies still may be useful if population stratification is present. A case-parents design identifies alleles associated with the disease, ruling out alleles that could have been spuriously associated with the disease in a case-control study. Therefore, the combination of results from case-control and case-parents studies may help to elucidate the causative and spurious associations between genetic polymorphisms and type 1 diabetes. In this context, it has been suggested that the bias generated by population stratification in case-control studies is usually small (34).

In conclusion, it can be inferred that the *DQB1*0302* and *DQB1*0201* alleles have strong positive associations with type 1 diabetes in Chile. In the near future, further epidemiologic research will evaluate the interaction between *HLA-DQB1* risk-alleles and environmental factors that trigger type 1 diabetes in the Chilean population.

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