Brief Genetics Report

IL12B Polymorphism and Type 1 Diabetes in the Italian Population

A Case-Control Study

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A polymorphism in the interleukin 12B gene was recently reported to be strongly associated with type 1 diabetes in 422 Australian and British families. We analyzed the same polymorphism in 470 Italian type 1 diabetic patients and 544 matched control subjects and found no evidence of association with the disease. ***Diabetes*** **51:1649–1650, 2002**

Type 1 diabetes is caused by the immune-mediated destruction of the insulin-secreting pancreatic β-cells, which is under polygenic control. Several putative diabetes loci have been sorted by whole genome–linkage analyses (1–2) or by directly testing an a priori hypothesis on selected genes or genomic regions (3–4).

Linkage with chromosome 5q33–34 was recently detected in 249 sib-pairs from 187 British and Australian multiplex families (5). Two noncoding polymorphisms of the interleukin 12B (*IL12B*) gene, mapping in the linked region, were strongly associated ($P = 10^{-5}$) with the disease in the subset of siblings sharing two 5q33 identical-by-descent alleles. The polymorphisms were an AT repeat in intron 4 and an A-C substitution in the 3′ untranslated region (UTR). The preferential transmission of the A allele (allele 1 in the original study) of the 3′ UTR variation was replicated in an independent sample of 235 Australian type 1 diabetic simplex families ($P = 10^{-4}$). Moreover, differences in the *IL12B* mRNA levels were related to the 3′ UTR genotype (5).

Given the relevance of this finding for prediction and preventive measures, it is important to test whether these results can be replicated in other populations.

We performed a large case-control study in the continental Italian population. The A-C 3′ UTR variation was typed in 470 unrelated type 1 diabetic patients and 544 unrelated control subjects.

Allele, phenotype, and genotype frequencies did not significantly differ between type 1 diabetic patients and control subjects (Table 1). A slight, not significant, increase of the C allele (+1.9%) was observed in patients compared with unaffected subjects. This result goes in the opposite direction of that reported by Morahan et al. (5), who showed a preferential transmission to diabetic patients of the more frequent A allele. No difference was detected in the frequencies of the 3′ UTR alleles when the patients were stratified for each of the HLA high-risk (DR3/DR4, DR4/DR4, DR3/DR3, and DR4/DRX) and low-risk (DR3/DRX and DRX/DRX) genotypes (Table 1). Also, sex or age at onset (before or after 15 years) did not affect the IL12B frequencies (data not shown).

To rule out the possible influence of population admixture, we typed the parents of 121 diabetic patients from the case-control panel for the 3′ UTR poymorphism and inferred the affected family–based control subjects (AFBACs). AFBAC allele frequencies (A = 71.2% and C = 28.8%) did not significantly differ from those of population control subjects.

In addition, to assign intragenic haplotypic combinations, we typed the intron 4 microsatellite in 16 diabetic families (64 chromosomes). We detected two alleles (frequencies 73.4 and 26.6%), which were combined in only two haplotypes with the 3′ UTR locus. This corresponds to the linkage disequilibrium (LD) pattern previously described (5). Therefore, the lack of association in the Italian population was not due to a population-specific pattern of LD in the region of interest.

It is unlikely that our failure to detect an association was the result of a lack of statistical power of the test because the size of our diabetic and control subject panel had >80% probability to detect a significant ($P = 0.05$) association, with a relative risk of 1.4 for the A/A or the A/C genotypes. This risk is lower than the genotypic relative risk (~1.9) that can be calculated from the genotype

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AFBAC, affected family–based control subject; LD, linkage disequilibrium; UTR, untranslated region.

TABLE 1
Frequencies of *IL12B* 3′ UTR A-C polymorphism in Italian type 1 diabetic patients and control subjects

	Alleles				Genotypes						Phenotypes			
	A		C		AA		AC		CC		A positives		A negatives	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%
Control subjects (n = 544)	787	72.3	301	27.7	287	52.8	213	39.1	44	8.1	500	91.9	44	8.1
Type 1 diabetic patients (n = 470)	662	70.4	278	29.6	226	48.1	210	44.7	34	7.2	436	92.8	34	7.2
Type 1 diabetic patients with high-risk HLA genotypes* (n = 209)	288	68.9	130	31.1	98	46.9	92	44.0	19	9.1	190	90.9	19	9.1
Type 1 diabetic patients with low-risk HLA genotypes† (n = 133)	188	70.7	78	29.3	65	48.9	58	43.6	10	7.5	123	92.5	10	7.5

Frequencies were compared in 2 × 2 or 2 × 3 contingency tables, and none of the comparisons were statistically significant ($P < 0.05$). *DR3/DR4, DR4/DR4, DR3/DR3, and DR4/DRX; †DR3/DRX and DRX/DRX.

frequencies, estimated assuming Hardy-Weinberg equilibrium, in diabetic children and AFBACs of simplex families, as described by Morahan et al. (5).

Our data are in agreement with a recent report showing no association of the *IL12B* polymorphism with type 1 diabetes risk in 387 Norwegian families (6) and with another family-based study, published while this manuscript was being revised, that was performed on five populations of European descent (7). Heterogeneity among association studies is frequent for genes of multifactorial diseases, and the reasons that may explain why the first published studies often overestimate the genetic effect have been thoroughly discussed (8).

In conclusion, it is unlikely that the *IL12B* 3′ UTR polymorphism has an impact on type 1 diabetes risk in the Italian population similar to that described in the British and Australian populations.

RESEARCH DESIGN AND METHODS

Italian type 1 diabetic patients were recruited mainly through diabetes care centers of central Italy. Mean age of onset was 10.5 years (SD ± 8.4). There were 256 men and 214 women. Control subjects were unrelated blood donors, medical students, and laboratory staff. Informed consent was obtained from all the participants in the study. Admixture with people of Sardinian heritage was estimated in subsets of patients and control subjects for which both parental surnames or birth places were known. Approximately six percent of diabetic patients and <1% of control subjects had one or both parents of Sardinian origin. These subjects were excluded from the analyses, although their *IL12B* frequencies were not different from continental Italians. *IL12B* 3′ UTR A-C variation was typed by PCR/restriction fragment–length polymorphism using primers previously described (9). The presence of the C nucleotide (allele 2 in the original study [5]) creates a *TaqI* restriction site. The intron 4 AT microsatellite was typed by denaturing bis-acrylamide electrophoresis of fluorescent PCR products. PCR primers for this locus were previously described (9). Genotypes were in Hardy-Weinberg equilibrium in patients and control subjects.

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