Brief Genetics Report

The $Gly^{972} \rightarrow Arg$ IRS-1 Variant Is Associated With Type 1 Diabetes in Continental Italy

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The Arg^{972} insulin receptor substrate-1 (IRS-1) variant has been hypothesized to play a role in pancreatic β-cell stimulus-coupled insulin secretion and survival. We analyzed the relations between type 1 diabetes and the Arg^{972} IRS-1 variant. The frequency of the IRS-1 Arg^{972} variant was investigated in two independent sets of unrelated patients: a case-control study and a collection of type 1 diabetes simplex families. In the former group, frequency of the IRS-1 Arg^{972} variant was significantly increased in the patients ($P = 0.0008$), conferring an OR of 2.5. Transmission disequilibrium analysis of data obtained from the family set revealed that the Arg^{972} IRS-1 variant was transmitted from heterozygous parents to affected probands at a frequency of 70.2% ($P < 0.02$). Arg^{972} IRS-1 frequency showed no significant correlation with HLA genotypic risk for type 1 diabetes. Arg^{972} IRS-1 type 1 diabetic patients also had lower fasting plasma concentrations of C-peptide at the time of diagnosis with respect to patients carrying the wild-type IRS-1 (0.49 ± 0.058, $n = 34$, and 0.76 ± 0.066, $n = 134$, respectively [means ± SE]; $P = 0.051$). Our findings suggest a role for Arg^{972} IRS-1 in conferring risk for the development of type 1 diabetes. ***Diabetes*** **52:887–890, 2003**

Genetic variance in the insulin receptor substrate-1 (IRS)-1 is thought to play a key role in the insulin resistance that characterizes type 2 diabetes (1–6). Transfection studies have demonstrated that the most common IRS-1 variant, Arg^{972}, which involves a Gly 224 Arg substitution at codon 972, impairs insulin signaling via the phosphatidylinositol-3 (PI3)-kinase pathway (7), and in some (but not all) studies, this variant has been found with an increased frequency among type 2 diabetic patients (1–6).

Interestingly, carriers of the Arg^{972} substitution have been found to have lower fasting insulin and C-peptide levels than noncarriers (8,9), suggesting that this IRS-1 variant might also play a role in the secretory capacity of the β-cells themselves. Indeed, impaired insulin secretion has also been observed in rat insulinoma (RIN) cells overexpressing the Arg^{972} IRS-1 polymorphism (10) in human islets naturally carrying the variant (11), and even in normal glucose-tolerant subjects with the Arg972 variant (9). These observations raise the intriguing hypothesis that genetic defect in the IRS-1/PI3 kinase pathway might also be involved in the inadequate insulin secretion that characterizes type 2 diabetes.

More recent studies suggest that the Arg^{972} IRS-1 variant also plays a role in β-cell survival (12). We have found that human Arg^{972} islets contain a significantly higher number of apoptotic cells than their wild-type counterparts, and they are also resistant to the antiapoptotic effects of insulin (12). It has been speculated that apoptosis plays a crucial role in the autoimmune destruction of β-cells characterizing type 1 diabetes (13). An increase in apoptosis might have pathological consequences in diabetes-prone individuals, who have an autoreactive T-cell repertoire that can be mobilized by the exposed β-cell antigens. These considerations prompted us to examine the possible relations between the Arg^{972} IRS-1 variant and type 1 diabetes.

We evaluated the frequency of the IRS-1 Arg^{972} variant in 307 unrelated type 1 diabetic patients (age at onset: 0–14 years) and 243 unrelated control subjects from a single region in central Italy. Control subjects had no history of type 2 diabetes, type 1 diabetes, or other autoimmune diseases. The results of this case-control analysis are shown in Table 1. Arg^{972} was significantly more common in type 1 diabetic patients than in control subjects ($P = 0.0008$), conferring an OR of 2.5. The frequency of IRS-1 Arg^{972} among patients with HLA genotypes representing moderate-to-high risk for type 1 diabetes was not significantly different from that of the low-risk genotype subgroup of patients. The Arg972 IRS-1 variant was associated with a positive predictive value (PPV) for onset of type 1 diabetes, among children 15 years of age, of 0.32%, and it increased the PPV of the high-risk genotype DR3/DR4-

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IRS-1, insulin receptor substrate-1; PI3, phosphatidylinositol-3; PPV, positive predictive value; TDT, transmission disequilibrium test.

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TABLE 1
Frequencies of IRS-1 Gly972Arg dimorphism in type 1 diabetic patients and control subjects

	Control subjects	Type 1 diabetic patients	Type 1 diabetic patients with high/moderate risk HLA genotype*	Type 1 diabetic patients with low risk HLA genotypes†
Genotypes‡				
n	243	307	192	115
Gly/Gly	223 (92)	250 (81)	156 (81)	94 (82)
Gly/Arg	20 (8)	54 (18)	35 (18)	19 (16)
Arg/Arg		3 (1)	1 (1)	2 (2)
Phenotypes§				
Arg positive	20 (8)	57 (19)	36 (19)	21 (18)
Arg negative	223 (92)	250 (81)	156 (81)	94 (82)

Data are *n* (%). *DR3/DR4-DQB1 *0302, DR3/DR3 and DR4-DQB1 *0302/X; †DR3/DRX, DRX/DRX DRX = neither DR3 or DR4-DQB1 *0302; ‡2 × 3 contingency table $P = 0.016$ (control subjects vs. type 1 diabetic subjects). §2 × 2 contingency table $P = 0.0008$; OR = 2.5; 95% CI = 1.5–4.3 (control subjects vs. type 1 diabetic subjects).

DQB1*0302 by 3% (from 4 to 7%). Transmission disequilibrium test (TDT) of 140 type 1 diabetes simplex families from the same area revealed that the Arg^{972} IRS-1 variant was transmitted from heterozygous parents to affected probands at a frequency of 70.2% ($P < 0.02$), whereas the rate of transmission to unaffected siblings was not significantly different from the expected 50% (Table 2).

This surprisingly strong association between the Arg^{972} IRS-1 variant and type 1 diabetes is at variance with previous reports (14,15), including that of Cox et al. (16), which involved genome screening of 767 type 1 diabetes multiplex families. In fact, the latter study revealed no linkage between the disease and region 2q36, where the IRS-1 gene has been mapped. It is important to recall, however, that at the present time single genes with limited effects are more likely to be identified by population-based case-control studies and linkage disequilibrium analysis within families, which were used in the present study. The fact that the Caucasian population studied by Cox et al. was ethnically mixed, whereas our analysis was confined exclusively to continental Italians, may also have contributed to the discrepancy between our findings.

The Arg^{972} variant also displayed marginal correlation ($P = 0.051$) with fasting insulin levels (reflected by C-peptide concentrations) at the time of diagnosis. These data were available for only 168 of the 307 patients, and it is possible that a stronger correlation might have emerged if this variable had been evaluated in a larger population. Nevertheless, our finding is compatible with previous observations indicating that the Arg^{972} variant negatively influences the secretory function of the β-cell (9–12).

The significance of a defect of this type in type 1 diabetes is difficult to determine. Whereas type 2 diabetes is clearly characterized by gradual loss of insulin secretion, the insulin deficit in type 1 diabetes is the result of more or less massive and rapid autoimmune destruction of the β-cell mass (1,13). However, a recent report indicates that in the early stages of the disease, certain type 1 diabetic patients show some degree of β-cell dysfunction, manifested primarily by hyperglycemia after glucose loading due to a defective insulin secretion (17). Interestingly, a similar impairment in insulin secretion has also been observed in the IRS-1 null mice, both in vivo and in vitro (18). Our findings confirm the growing body of evidence reflecting the negative effects of IRS-1 Arg972 on the ability of pancreatic β-cells to secrete insulin. This impairment might be related to the increased apoptosis and defective maturation of insulin granules, which we have recently documented in human pancreatic islets with the Arg972 IRS-1 variant (11,12). In fact, some investigators have suggested that increased β-cell apoptosis might function as a trigger for the autoimmune response in type 1 diabetes (13). Insulin has potent antiapoptotic effects that may depend on activation of the PI3 kinse/Akt pathway, and it is reasonable to assume that any significant reduction in insulin secretion could lead to an increase in "physiological" β-cell death. Alternatively, accumulation of immature insulin granules (as a result of the defect mentioned above) could function as an independent stimulus of apoptosis by provoking endoplasmic reticular stress, as recently observed (19). Regardless of it's cause(s), however, a substantial increase in the rate of β-cell apoptosis might exceed the capacities of the macrophage clearance system, and, in a genetically predisposed individual, exposure of β-cell antigens released from the remaining cells might be sufficient to trigger an autoimmune reaction (13).

TABLE 2
TDT of G972R IRS-1 variant to diabetic probands in 140 Italian simplex families

	IRS-1				
	T	NT	% T	χ^2	*P*
Affected siblings					
Arg972 allele	26	11	70.2	6.08	<0.02
Nonaffected siblings					
Arg972 allele	19	17	53	0.11	NS

T, transmitted; NT, not transmitted; % T, percentage transmission.

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The hypotheses discussed above are highly speculative, and the association we have found between IRS-1 Arg972 is obviously a preliminary finding that will have to be verified in other populations. The Arg972 variant of the IRS-1 seems to play a complex role in the pathogenesis of type 2 diabetes, affecting both peripheral insulin sensitivity and the functional capacities of the pancreatic β-cells themselves. In light of our findings, it is possible to speculate that the same mechanisms—in the presence of a genetically determined predisposition—might also result in or contribute to the clinical manifestations of type 1 diabetes.

RESEARCH DESIGN AND METHODS

Unrelated patients (145 men and 162 women) with type 1 diabetes (age at diagnosis 1–14 years) were recruited from diabetes care centers in the Lazio region (Central Italy).

Healthy unrelated control subjects, (135 men and 115 women aged 20–35 years) were randomly selected from a population of free-living individuals with negative screening for diabetes and referred for blood collection either to Atherosclerosis Clinic of Tor Vergata University or the Blood Transfusion Service of the University of Rome "La Sapienza." TDT analysis was performed on a separate data set consisting of 140 type 1 diabetes simplex families (unrelated to the patients enrolled in the case-control study). All subjects investigated had been born and were currently living in the Lazio region. The study was approved by the local ethics committee, and informed written consent for all procedures was obtained from each subject.

Genomic DNA was extracted from peripheral EDTA-treated blood cells using the QIAamp DNA Blood Kit (QIAGEN Genomics, Bothell, WA). The Arg972 substitution was detected by digestion of PCR products with restriction enzyme MvaI (Roche Applied Science, Milan, Italy). Briefly, PCR amplification was carried out on 100 ng genomic DNA with a final volume of 25 μl. The assay consisted of 2.5 mmol/l $MgC1_2$, 0.2 mmol/l DNTP, PCR buffer, 0.6 units AmpliTaq DNA polymerase (Perkin Elmer, Foster City, CA), and 0.2 μmol/l of the two oligonucleotide primers: forward 5′-CTT CTG TCA GGT GTC CAT CC-3′ and reverse 5′~TGG CGA GGT GTC CAC GTA GC-3′. Cycling program using a Perkin Elmer 9600 (Perkin Elmer) was as follows: initial denaturation at 95° for 5 min, followed by 37 cycles of denaturation at 95°C for 45 s, annealing at 58°C for 45 s, elongation at 72°C for 45 s, and a final elongation step at 72°C for 5 min. Restriction enzyme digestion was carried out in 30-μl reactions containing 25 μl precipitated secondary PCR product, 0.1 μl 10 × New England Biolabs buffer-2, and 2 units of the restriction enzyme MvaI. The reactions were incubated at 37°C for 16 h before electrophoresis. Electrophoresis was carried out on a 3.5% agarose gel and visualized after staining with ethidium bromide.

HLA-DRB1 and DQB1 loci were typed by PCR followed by a reverse line blot using labeled sequence-specific oligonucleotide probes kindly provided by H.A. Erlich and T.L. Bugawan (20). In 168 of the type 1 diabetic cases enrolled at the time of diagnosis, C-peptide levels was measured by radioimmunoassay using a commercially available kit (BioRad). The normal range (previously established based on findings in 150 control subjects) was 0.35–1.0 nmol/l with intracoefficients and intercoefficients varying between 10 and 15%.

The data were evaluated using the χ^2 test (with Yates' continuity correction) or Fisher's exact test when the criteria of χ^2 test were not fulfilled. The PPV for developing type 1 diabetes for the IRS-1 variant and for the high-risk HLA genotype were calculated based on their frequencies observed in our case-control study and the estimated prevalence of type 1 diabetes among children at 15 years of age in Italy (0.15%). This latter figure was extrapolated from previously published data showing a yearly incidence of 8–12 per 100,000 children in the Italian population (21).

Comparisons of C-peptide level among genotypes were made using the unpaired Student's test. The TDT was used to assess the transmission of the IRS-1 Arg972 variant to type 1 diabetic patients as described by Spielman et al. (22). The control population was in Hardy-Weinberg equilibrium for the tested locus.

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