

Brief Genetics Report

New Amino Acid Substitutions in the *IRS-2* Gene in Finnish and Chinese Subjects With Late-Onset Type 2 Diabetes

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We investigated the significance of the variants of the *IRS-2* gene in patients with type 2 diabetes. The entire coding part of the *IRS-2* gene was screened by single-strand conformation polymorphism analysis in 40 Chinese and 40 Finnish patients with late-onset type 2 diabetes. The association of the variants of the *IRS-2* gene with type 2 diabetes was studied in 85 Finnish diabetic patients and 82 Finnish control subjects and in 100 Chinese diabetic patients and 85 Chinese control subjects. The four variants predicting structural changes in the insulin receptor substrate (IRS)-2 protein included an insertion of AAC (Asn) in the Asn repeat sequence centered around codons 29–36 (allele frequencies of 0 vs. 0.6% and 1.5 vs. 0%), the Ala157Thr substitution (0 vs. 0% and 0.5 vs. 0%), the Leu647Val substitution (0.6 vs. 0% and 0 vs. 0%), and the Gly1057Asp polymorphism (31 vs. 31% and 35 vs. 30%) ($P = \text{NS}$ for all comparisons). Furthermore, six silent variants were observed (CGC147CGG, CCC155CCG, GCC156GCT, AGT723AGC, TGT816TGC, and CCC829CCT). The Gly1057Asp polymorphism was not associated with insulin resistance or impaired insulin secretion in Finnish subjects with normal glucose tolerance ($n = 295$) or impaired glucose tolerance ($n = 38$). These data indicate that structural variants of the *IRS-2* gene were uncommon in Finnish and Chinese patients with type 2 diabetes. Thus, the variants in the coding part of the *IRS-2* gene are unlikely to have a major role in the development of type 2 diabetes in Finnish or Chinese subjects. *Diabetes* 50:1949–1951, 2001

Insulin receptor substrates (IRSs) represent the key proteins that transmit the insulin signal from the insulin receptor to downstream molecules (1,2). Because the disruption of the *IRS-2* gene may critically interfere with glucose homeostasis in mice (3),

structural defects in the *IRS-2* gene could potentially predispose type 2 diabetes in mice as well as in humans. Only three variants (Leu647Val, Gly897Ser, and Gly1057Asp substitutions) (4,5) have been previously reported in the *IRS-2* gene in Caucasian subjects. In this study, we investigated the coding part of the *IRS-2* gene for variants in Finnish and Chinese subjects with late-onset type 2 diabetes.

We identified four variants predicting structural changes and six silent variants in the coding region of the *IRS-2* gene (Table 1). The four variants predicting structural changes in the *IRS-2* gene included an insertion of AAC (Asn) in the asparagine repeat sequence centered around codons 29–36 (allele frequencies in Finnish and Chinese diabetic patients versus their respective control subjects: 0 vs. 0.6% and 1.5 vs. 0%), Ala157Thr substitution (0 vs. 0% and 0.5 vs. 0%), Leu647Val substitution (0.6 vs. 0% and 0 vs. 0%), and Gly1057Asp polymorphism (31 vs. 31% and 35 vs. 30%) ($P = \text{NS}$ for all comparisons). Furthermore, six silent variants were observed (CGC147CGG, CCC155CCG, GCC156GCT, AGT723AGC, TGT816TGC, and CCC829CCT). In both the Finnish and Chinese diabetic patients and control subjects, the Gly1057Asp polymorphism used in the association study was in Hardy-Weinberg equilibrium, thus indicating a representative sampling of subjects.

In 295 subjects with normal glucose tolerance (NGT) and 38 subjects with impaired glucose tolerance (IGT), the Gly1057Asp substitution of the *IRS-2* gene was not associated with sex, age, BMI, or glucose or insulin levels at 0, 1, and 2 h in an oral glucose tolerance test (OGTT) ($P = \text{NS}$, data not shown). Similarly, in the intravenous glucose tolerance test (IVGTT), the peak insulin level at 4 min did not differ significantly in subjects with and without the Gly1057Asp polymorphism, i.e., the insulin area under the insulin response curve during the first 10 min after the intravenous glucose bolus was GG $2,462 \pm 147$ (mean \pm SE), GA $2,682 \pm 138$, and AA $2,756 \pm 297$ pmol/min ($P = 0.72$) in the NGT group and GG $2,802 \pm 400$, GA $1,704 \pm 236$, and AA $2,591 \pm 705$ pmol/min ($P = 0.16$) in the IGT group; and the insulin sensitivity index was GG 4.3 ± 0.2 , GA 4.4 ± 0.2 , and AA $3.8 \pm 0.4 \times 10^{-5}$ ($\text{min} \cdot \text{pmol}^{-1} \cdot \text{l}^{-1}$) ($P = 0.36$) in the NGT group and GG 1.9 ± 0.4 , GA 3.1 ± 0.7 , and AA $3.0 \pm 1.0 \times 10^{-5}$ ($\text{min} \cdot \text{pmol}^{-1} \cdot \text{l}^{-1}$) ($P = 0.23$) in the IGT group. Furthermore, the Gly1057Asp substitution was not associated with age at onset of diabetes in 85

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IGT, impaired glucose tolerance; IRS, insulin receptor substrate; IVGTT, intravenous glucose tolerance test; NGT, normal glucose tolerance; OGTT, oral glucose tolerance test; PI3K, phosphatidylinositol 3-kinase.

TABLE 1

Allele frequencies (number of alleles) of the variants observed in the *IRS-2* gene in Chinese and Finnish patients with type 2 diabetes or in the respective control subjects

Nucleotide	Codon	Nucleotide change	Amino acid change	Chinese patients		Finnish patients	
				Control subjects (n = 85)	Type 2 diabetic patients (n = 100)	Control subjects (n = 82)	Type 2 diabetic patients (n = 85)
85	29	Insertion AAC	Asn8→Asn9	0	0.015 (3)	0.006 (1)	0
441	147	CGC→CGG	—	ND	0.005 (1)*	ND	0*
465	155	CCC→CCG	—	ND	0.005 (1)*	ND	0*
468	156	GCC→GCT	—	ND	0.005 (1)*	ND	0*
469	157	GCC→ACC	Ala→Thr0		0.005 (1)	0	0
1939	647	CTG→GTG	Leu→Val	0	0	0	0.006 (1)
2169	723	AGT→AGC	—	ND	0.560 (112)*	ND	0.359 (61)*
2448	816	TGT→TGC	—	ND	0.450 (90)*	ND	0.641 (109)*
2487	829	CCC→CCT	—	ND	0.450 (90)*	ND	0.512 (87)*
3170	1057	GGC→GAC	Gly→Asp	0.300	0.350 (70)	0.305 (50)	0.312 (53)

*Determined in 40 subjects with type 2 diabetes. ND, not determined.

Finnish patients with type 2 diabetes or insulin resistance (estimated with a euglycemic clamp) or in 82 healthy Finnish control subjects ($P = \text{NS}$) (data not shown).

The 5' half of the *IRS-2* gene contains the highly conserved PH and PTB sequences that target *IRS-2* to the cell membrane and insulin receptor (IR), respectively (1). The IR-*IRS-2* interaction results in phosphorylation of several tyrosine sites throughout *IRS-2*, which creates binding sites for downstream SH2-containing molecules. Low frequency of the variants in the 5' half of the *IRS-2* gene (allele frequencies ~1% in diabetic patients) may reflect the functional significance of this part of the protein. Although these infrequent variants are not located at the known active sites of *IRS-2*, effects on the stereochemical structure of the molecule are possible. The novel insertion variant of the asparagine repeat sequence in codons 29–36 (Asn8/Asn9) (two nucleotides upstream from the beginning of the PH domain) was observed in three Chinese diabetic patients and in one Finnish control subject, and thus was not specific to diabetic subjects. The novel Ala157Thr substitution observed in one Chinese diabetic patient predicts a nonconservative substitution of the hydrophobic alanine to the hydrophilic threonine at the conserved site of the IRSs. The previously reported Leu647Val substitution (5) was found in one Finnish diabetic patient. In functional tests, the binding of *IRS-2* to IR or p85 α of phosphatidylinositol 3-kinase (PI3K) was not altered in *IRS-2* carrying the Leu647Val variant (5), which argues against altered metabolic signaling through PI3K but does not exclude altered signaling through other downstream molecules.

In the 3' half of the *IRS-2* gene, which shows a weaker structural similarity with other members of the IRS family (4), we found three common silent variants along with the previously reported Gly1057Asp polymorphism (5) near the 3' end of the *IRS-2* gene. A previous study in Caucasian glucose-tolerant subjects (5) showed that homozygous subjects for the Gly1057Asp polymorphism had ~25% decreased fasting, 30- and 60-min insulin, and C-peptide concentrations in an OGTT ($P < 0.01$). In another study on Caucasian subjects, the Asp1057 allele of the variant represented a protective factor against type 2 diabetes in subjects with BMI $< 27 \text{ kg/m}^2$ ($P = 0.038$), and the Asp1057

allele was associated with a low fasting C-peptide level in healthy control subjects ($P = 0.02$) (6). However, we did not observe any influence of the Gly1057Asp polymorphism on insulin secretion (OGTT and IVGTT) or insulin sensitivity in 295 middle-aged subjects with NGT or in 38 subjects with IGT. Previously described Gly879Ser substitution was not observed in Finnish or Chinese diabetic patients (4).

In conclusion, two novel amino acid variants were described and identified in the coding region of the *IRS-2* gene. Because no associations of these variants with type 2 diabetes, impairment of insulin secretion, or insulin resistance were found, it is unlikely that the *IRS-2* gene plays a major role in the development of type 2 diabetes in Finnish and Chinese subjects. Our conclusion is in agreement with a recent study (7) showing that a polymorphic marker in the *IRS-2* gene was not linked to type 2 diabetes in Caucasian families, excluding the *IRS-2* gene as a major predisposing gene to this disease.

RESEARCH DESIGN AND METHODS

Subjects. The single exon of the *IRS-2* gene was initially screened in 40 Finnish (age at diagnosis 53 ± 1 years) and 40 Chinese (age at diagnosis 54 ± 1 years) patients with late-onset type 2 diabetes. The Finnish diabetic patients had reported a positive family history of type 2 diabetes. The variants predicting structural changes in the *IRS-2* gene were investigated in additional samples of 45 Finnish diabetic patients, 82 Finnish control subjects, 60 Chinese diabetic patients, and 85 Chinese control subjects. The expanded group of 85 Finnish unrelated diabetic subjects (43 men and 42 women, aged 67 ± 1 years, BMI $29.4 \pm 0.5 \text{ kg/m}^2$) (8) and 82 unrelated healthy control subjects (82 men, aged 54 ± 1 years, BMI $27.2 \pm 0.5 \text{ kg/m}^2$) (9) participating in this study were randomly selected from our previous population studies from eastern Finland. The expanded group of 100 Chinese unrelated diabetic patients (43 men and 57 women, aged 60 ± 1 years, BMI $25.3 \pm 0.3 \text{ kg/m}^2$) were randomly chosen from outpatient clinics in the southwestern part of China, and 85 control subjects (30 men and 55 women, aged 52 ± 1 years, BMI $26.6 \pm 0.3 \text{ kg/m}^2$) consisted of healthy unrelated subjects living in the same area. Furthermore, separate samples of 295 Finnish subjects with NGT (150 men and 145 women, aged 44 ± 1 years, BMI $25.6 \pm 0.2 \text{ kg/m}^2$) and 38 Finnish subjects with IGT (15 men and 23 women, aged 51 ± 2 years, BMI $28.4 \pm 0.8 \text{ kg/m}^2$) from our previous population study (10), diagnosed by an OGTT (75-g glucose) after a 12-h overnight fast (11), were used to investigate the effect of the Gly1057Asp polymorphism on insulin secretion. The study was approved by the local ethics committees and was in accordance with the Helsinki Declaration.

Methods. Insulin secretion was assessed with an OGTT and an IVGTT, and insulin sensitivity was estimated with the insulin sensitivity index based on

Bergman's Minimal Model, after a 12-h overnight fast (12). The *IRS-2* gene was screened by the single-strand conformational polymorphism analysis in ten overlapping segments (cut to 150- to 250-bp fragments, primer sequence available from authors), and the variants were directly sequenced.

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