Insulin Receptor Substrate-1 Variants in Non–Insulin-dependent Diabetes

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Abstract

Insulin receptor substrate-1 (IRS-1) plays an important role in insulin-stimulated signaling mechanisms. Therefore, we investigated the frequency and clinical significance of variants in the coding region of this gene in patients with non–insulin-dependent diabetes (NIDDM). Initial screening included a population-based sample of 40 Finnish patients with typical NIDDM. Applying single strand conformation polymorphism analysis the following amino acid substitutions were found among the 40 NIDDM patients: Gly818Arg, Ser892Gly, and Gly971Arg. The first two variants have not been previously reported. Additional samples of 72 patients with NIDDM and 104 healthy control subjects with completely normal oral glucose tolerance test and a negative family history of diabetes were screened. The most common polymorphism was the Gly971Arg substitution which was found in 11 (9.8%) of 112 NIDDM patients and in 9 (8.7%) of 104 control subjects. The Gly818Arg substitution was found in 2 (1.8%) of NIDDM patients and in 2 (1.9%) of control subjects, and the Ser892Gly substitution was found in 3 (2.7%) NIDDM patients and in 1 (1.0%) control subject. The Gly971Arg substitution was not associated with an impairment in insulin secretion capacity (estimated by insulin responses in an oral glucose tolerance test or by the hyperglycemic clamp) or insulin action (estimated by the euglycemic clamp). Of the three amino acid substitutions observed Ser892Gly is the most interesting one since it abolishes one of the potential serine phosphorylation sites (SPGE) which is located immediately NH_2-terminal to the only SH2 binding site of growth factor receptor-bound protein (GRB2), and thus could potentially influence some aspects of signal tranduction and metabolic response to insulin. (*J. Clin. Invest.* 1994. 94:1141–1146.) Key words: insulin receptor substrate-1 • non-insulin-dependent diabetes

Introduction

Non–insulin-dependent diabetes mellitus (NIDDM)[1] is one of the most common metabolic disorders, affecting ~ 3–5% of Western populations (1). NIDDM is characterized by disturbances in insulin action and insulin secretion (2, 3), and hederity plays a significant role in the development of the disease. Several studies have demonstrated a familial aggregation of this disease, high concordance rate in identical twins, and a high risk of subsequent NIDDM in offspring of diabetic parents (4, 5).

Although basic metabolic disturbances in NIDDM have been characterized in detail (2, 6), the genetic basis of this disease remains almost completely unsolved. The etiology of NIDDM is known only in a subset of well-defined families with maturity-onset diabetes of the young, where mutations of the glucokinase gene have been found (7, 8). These defects in glucokinase cause a mild form of insulin deficiency. Although mutations in the genes encoding insulin (9), insulin receptor (10), and a mitochondrial tRNA (11) have been described, these mutations account only a minor fraction of the etiology of insulin resistance and NIDDM.

Insulin initiates its action on target tissues by binding to the α subunit of the insulin receptor (12). This results in autophosphorylation of the β subunit and in activation of tyrosine kinase of insulin receptor (13). In the cascade of insulin action, the first step after activation of insulin receptor is phosphorylation of a cytoplasmic protein, insulin receptor substrate-1 (IRS-1) (14, 15). IRS-1 contains 14 potential tyrosine phosphorylation sites and 52 potential threonine and serine phosphorylation sites. IRS-1 cDNA from human hepatocellular carcinoma (16) and human skeletal muscle (17) have been recently cloned and characterized. Human skeletal muscle IRS-1 cDNA encodes a protein of 1242 amino acids. The IRS-1 gene contains the entire 5′-untranslated region and protein coding region in a single exon.

Since post-receptor defects are characteristic features in NIDDM and since IRS-1 plays a central role in intracellular insulin signaling, studies on genetic variation in the coding region of the IRS-1 gene in NIDDM are of particular interest. Indeed, a recent report has suggested that the Ala512Pro and Gly971Arg polymorphisms of the IRS-1 gene are common in Danish patients with NIDDM (18). In this report we investigate the prevalence and clinical significance of the IRS-1 gene variants in typical Finnish NIDDM patients and describe two previously unreported amino acid substitutions of the IRS-1 gene.

Methods

Subjects

All subjects participating in this study were Finnish. Finnish population is genetically quite homogenous descending mainly from a small number of founders of Baltic Finnish and German origin (19).

Initial screening. The subjects with NIDDM screened for IRS-1 variants were selected from a previous population study (20, 21). Altogether 40 diabetic patients (18 men, 22 women) from this study were randomly selected for the initial analysis of the IRS-1 gene. Their age was 66.5±0.9 yr, body mass index 28.5±0.8 kg/m^2, fasting blood glu-

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1. *Abbreviations used in this paper:* GRB2, growth factor receptor-bound protein 2; IRS-1, insulin receptor substrate-1; NIDDM, non-insulin-dependent diabetes mellitus; SSCP, single strand conformation polymorphism.

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cose 10.0±0.4 mmol/l, duration of diabetes 13.2±1.5 yr, and the age of onset of diabetes 52.3±1.8 yr.

Additional screening. Screening for amino acid substitutions observed in the initial screening was performed on an additional 49 patients with NIDDM selected randomly from the epidemiological study described above (20) and on 23 NIDDM patients subsequently recruited from the diabetes clinic of the Kuopio University Hospital. The 104 subjects with normal glucose tolerance were selected randomly from two previous population studies (22, 23). None of control subjects had any chronic disease, any drug treatment which could influence carbohydrate metabolism, any abnormality in an oral glucose tolerance test (impaired glucose tolerance or diabetes according to the criteria of the World Health Organization) (24), or hypertension (use of antihypertensive drugs, or systolic/diastolic blood pressure > 160/95 mmHg). Each control subject had a negative family history of diabetes. Every diabetic and control subject had normal liver, kidney, and thyroid function tests, and no history of excessive alcohol intake. Diabetic patients fulfilled the criteria for diabetes and NIDDM according to the criteria of the World Health Organization (24).

Methods

Study protocol. Every control subject participating in this study underwent an oral glucose tolerance test (75 g of glucose in 10% solution). Oral glucose tolerance test was not performed on insulin-treated patients with NIDDM, instead the fasting C-peptide level was measured to exclude insulin-dependent diabetes. In each insulin-treated patient no history of ketoacidosis was recorded and their fasting C-peptide level exceeded 0.20 nmol/l. Therefore, it is quite unlikely that our study population included a significant number of patients with insulin-dependent diabetes (25). A subset of diabetic (n = 23) and control subjects (n = 70) were admitted to the metabolic ward for 2 d. All these diabetic patients were treated with diet only or oral antidiabetic drugs.

Informed consent was obtained from all subjects after the purpose and potential risks of the study were explained to them. The protocol was approved by the Ethics Committee of the University of Kuopio and was in accordance with the Helsinki declaration.

Hyperglycemic hyperinsulinemic clamp. This test was performed in 23 patients with NIDDM treated with diet or oral drugs to evaluate insulin secretion capacity under maximum glucose stimulation. On day 1, immediately following an oral glucose tolerance test at 120 min, blood glucose level was acutely increased to 20 mmol/l by glucose infusion (20% solution) and kept at 20 mmol/l until 180 min by infusing 20% glucose at varying rates according to blood glucose measurements performed at 5-min intervals. Mean blood glucose level during hyperglycemic clamp for the period from 160 to 180 min was 20.6±0.2 mmol/l. At 160, 170, and 180 min samples were drawn for plasma C-peptide measurements. The mean value of these C-peptide concentrations represents the maximum insulin secretion capacity of diabetic patients.

Euglycemic clamp. On day 2, the degree of insulin resistance was evaluated with the euglycemic hyperinsulinemic (insulin infusion of 80 mU/m^2/min (480 pmol/m^2/min)) clamp technique (26) as previously described in detail (27). [3-^{3}H]glucose was infused in patients with NIDDM as a primed (40 μCi) constant (0.40 μCi/min) infusion for 180 min before initiating the insulin infusion. Blood glucose was clamped at 5.0 mmol/l for the next 180 minutes by infusing 20% glucose at varying rates according to blood glucose measurements performed at 5-min intervals (mean coefficient of variation of blood glucose was < 4% both in patients with NIDDM and normal controls). In patients with NIDDM the rates of glucose appearance (R_a) and disappearance (R_d) during euglycemic hyperinsulinemic clamp studies were quantified from serum [3-^{3}H]glucose specific activities and calculated using Steele's equations in their modified derivative form because the tracer exhibit non-steady-state kinetics under these conditions (28). The rate of hepatic glucose output during euglycemic clamp was calculated as a difference between R_a and exogenous glucose infusion rate. Negative numbers of hepatic glucose output, largely due to a model error emerging at high rates of glucose metabolism (29), were taken to indicate completely suppressed hepatic glucose output. The data were calculated for each 20-min interval; the mean value for the period 120 to 180 min was used to calculate the rates of whole body glucose uptake. In subjects with normal glucose tolerance [3-^{3}H]glucose was not infused because hepatic glucose production is completely suppressed under these conditions according to our experience (27) and findings of other investigators (30). In control subjects the rate of whole body glucose uptake equals the glucose infusion rate.

Indirect calorimetry. Indirect calorimetry was performed with a computerized flow-through canopy-gas analyzer system (DELTATRAC; TM Datex, Helsinki, Finland) (31) as previously described (32) in connection of euglycemic clamp studies. Gas exchange (oxygen consumption and carbon dioxide production) was measured for 30 min after a 12-h fast before the clamp and during the last 30 min of the euglycemic clamp. The first 10 min of each set of data were discarded, and the mean value of the remaining 20 min was used in calculations. Protein, glucose, and lipid oxidation rates were calculated according to Ferrannini (33). The rate of carbohydrate nonoxidation during the euglycemic clamp was estimated by subtracting the carbohydrate oxidation rate (determined by indirect calorimetry) from the rates of whole body glucose disposal (determined by the euglycemic clamp).

Analytical methods. Blood glucose in the fasting state and during glucose clamp studies and plasma glucose in an oral glucose tolerance test were measured by the glucose oxidase method (Glucose Auto & Stat HGA-1120 analyzer; Daiichi Co., Kyoto, Japan). Plasma insulin and C-peptide concentrations were determined by radioimmunoassay (Phadeseph Insulin RIA 100; Pharmacia Diagnostics AB, Uppsala, Sweden; and C-peptide of insulin by 125J RIA kit, Incstar Co., Stillwater, MN). Nonprotein urinary nitrogen was measured by an automated Kjeldahl method (34). [3-^{3}H]glucose specific activity in plasma was determined as previously described (32).

Single-strand conformation polymorphism (SSCP) analysis. DNA was prepared from peripheral blood leucocytes. The single exon of the IRS-1 gene was amplified in 10 overlapping fragments ranging in size from 334 to 566 bp. Each fragment was amplified with the polymerase chain reaction (PCR) using primers shown in Table I and the products digested with the indicated restriction enzymes to obtain fragments of ~ 150–250 bp. SSCP analysis was performed according to Orita et al. (35). PCR amplification was conducted in a 15–20 μl volume containing 100 ng genomic DNA, 7.5–10 pmol of each primer, 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 0.3–1 U of Amplitaq DNA polymerase (Perkin-Elmer Cetus, Norwalk, CT), 1.5–2 μCi of (α-^{32}P)dCTP, dNTP (62.5–200 μM), and $MgCl_2$ (1–1.5 mM). For amplification of fragments 3, 5, and 9, 5% DMSO was included. PCR conditions were: denaturation at 94°C for 2–4 min, followed by 35 cycles of denaturation at 92–94°C for 45–60 s, annealing at 62–66°C for 1 min and extension at 72°C for 45–60 s with a final extension at 72°C for 4 min. The extension step was eliminated when the annealing temperature was over 64°C. Before SSCP analysis PCR fragments were digested with the restriction enzymes given in Table I. After enzyme digestion PCR products were first diluted 3–10-fold with 0.1% SDS 10 mM EDTA and then mixed (1:1) with loading dye mix (95% formamide, 20 mM EDTA, 0.05% bromphenol blue, 0.05% xylene cyanol). After denaturation at 98°C for 3 min, samples were immediately placed on ice. 2 μl of each sample were loaded onto a 5% (PCR products ≥ 200 bp) or 6% (PCR products < 200 bp) non-denaturating polyacrylamide gel (acrylamide/*N,N'*-methylene-bis-acrylamide ratio 49:1) containing 10% glycerol. Each sample was run at two different gel temperatures: at 45 W with fan cooling for ~ 5 h at gel temperature of 30–32°C, and at 55 W for ~ 4 h at a gel temperature of 40–42°C. These conditions have been shown to detect all known mutants of the lipoprotein lipase gene which have been found by direct sequencing in our laboratory (36, and unpublished observations). The gel was dried and autoradiographed overnight at −70°C with intensifying screens.

Direct sequencing. Genomic DNA from individuals with variant single strand conformers was used as a template in the amplification reaction as described above (total volume 100 μl containing 70 pmol of each primer and 5 U of Amplitaq DNA polymerase). Amplified segments were purified by electrophoresis on a 1% low-melting-point

Table I. Primers, Their Position, Size of the Amplified Fragment, Enzyme Digestion, and Fragment Size for Single Strand Conformation Polymorphism (SSCP)*

No	Sequence 5′ → 3′	Position	Size of amplified fragment	Cleavage enzyme	Restriction fragments
			bp		*bp*
1F	AGCCTCCCTCTGCTCAGCG	982	566	RsaI	132, 44, 183, 207
1R	GTCTGACCCAGGCCCTTGG	1547		HinfI	268, 298
2F	AGAGGTCTGGCAAGTGATCC	1503	442	TaqI	186, 256
2R	GATGCTCTCAGTGCGTGATC	1944			
3F	CCGATCACGCACTGAGAGC	1923	486	HinfI	139, 301, 46
3R	CTTAGCTCCTCCTCACCGC	2408		Sau 3AI	329, 157
4F	TTCCGCAGTGTCACTCCGG	2344	438	Sau 3AI	222, 216
4R	CAGACCCTCCTCTGGGTAG	2781			
5F	GCCGGGACACAGGCACTCC	2724	467	Sau 3AI	231, 236
5R	TACCACCGCTGCTCTCCAC	3190			
6F	CCACTCTCATGTCTTGCCTC	3138	334	Sau 3AI	188, 146
6R	ACCCAGGCTGTCGCTGCTG	3471			
7F	TCCACTAGCTCTGGTCGCC	3394	350	TaqI	153, 197
7R	TAGCCAGACTGATCACTCCC	3743			
8F	CAAGGCCAGCACCTTACCTC	3618	479	RsaI	224, 255
8R	GGCTCACCTCCTCTGCAGC	4096			
9F	GTGGACACCTCGCCAGCTG	4024	407	Sau 3AI	257, 150
9R	ATCCTCGCTGCTGCTGCTG	4430			
10F	ACCCGGGTGGGCAACACAG	4351	418	BstNI	128, 86, 87, 117
10R	GCTGTGATGTCCAGTTGAGCT	4768			

* According to Araki et al. (17).

agarose gel and directly sequenced using Sequenase (US Biochemical Corp.) as previously described (37).

Data analysis. All calculations were performed using the SPSS/PC+ programs (SPSS Inc., Chicago, IL). Data are presented as mean±SEM. Statistical significance between the two groups was evaluated with the X^2 test or unpaired Student's *t* test when appropriate. Insulin concentrations were log-transformed for statistical analyses.

Results

Clinical characteristics of the study groups. Table II gives clinical and metabolic characteristics of study subjects who participated in the initial and additional screening. The mean age of diabetic patients was 63.1 years. These diabetic patients represented typical Finnish patients with NIDDM. They were obese, hyperglycemic, and the mean age of onset of diabetes was > 50 years.

Initial screening for the IRS-1 gene variants. The sequence variants found among 40 NIDDM patients are shown in Table III. All variants were detectable under both the low and high temperature conditions of electrophoresis of single-stranded DNA segments on polyacrylamide gels. The most common polymorphism was a silent substitution CTC → CTT in codon

Table II. Clinical Characteristics of the Study Groups

	Control	NIDDM
Sex, M/F	88/16	56/56
Age, yr	55.2±0.5	63.1±0.9
Body mass index, kg/m^2	26.6±0.3	30.3±0.5
Fasting glucose, mmol/l	5.2±0.1	9.2±0.3
Duration of diabetes, yr	—	8.1±0.8
Age of onset of diabetes, yr	—	54.0±1.1
Treatment for diabetes, percent		
Diet	—	50
Oral drugs	—	28
Insulin	—	22

Table III. Variants of the IRS-1 Gene in the Initial and Additional Screening in Control Subjects and in Patients with NIDDM (Percentage in Parentheses)

		Initial screening	Additional screening	
Codon*	Change	NIDDM (n = 40)	Control (n = 104)	NIDDM (n = 112)
422	GAT → GAC	2 (5.0)	ND	ND
762	CTC → CTT	10 (25.0)	ND	ND
804	GCA → GCG	1 (2.5)	ND	ND
818	GGG → CGG (Gly → Arg)	1 (2.5)	2 (1.9)	2 (1.8)
892	AGC → GGC (Ser → Gly)	2 (5.0)	1 (1.0)	3 (2.7)
893	CCG → CCC	0	0	1 (0.9)
971	GGG → AGG (Gly → Arg)	4 (10.0)	9 (8.7)	11 (9.8)

* According to Araki et al. (17). *ND,* not determined for additional 104 control subjects and 72 NIDDM patients.

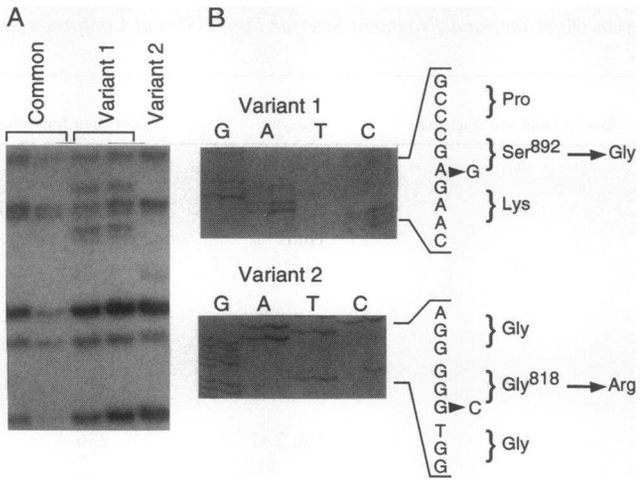

Figure 1. (*A*) Single strand conformation analysis of PCR fragment 7 (see Table I) of the IRS-1 gene from normal subject and carriers of sequence variants. (*B*) Sequence of the IRS-1 gene in the region of codons 892 and 818. The left lane is of the normal sequence and the right lane represents corresponding sequence from the carrier of the variant. *Variant 1,* the Ser→Gly substitution in codon 892. *Variant 2,* the Gly→Arg substitution in codon 818.

762 observed in 10 of the 40 subjects (25.0%). The GGG → AGG (Gly → Arg) substitution in codon 971 was found in 4 of the 40 subjects (10.0%). This Gly971Arg substitution creates a BstNI restriction site and therefore its presence was verified in addition of direct sequencing also by BstNI digestion of the PCR product followed by electrophoresis on a 8% nondenaturating polyacrylamide gel and visualized after staining with ethidium bromide. The GGG → CGG (Gly → Arg) substitution in codon 818 was found in 1 of 40 patients (2.5%), and the AGC → GGC (Ser → Gly) substitution in codon 892 in 2 of the 40 subjects (5%) (Fig. 1). The Ser892Gly substitution creates an Eco0109 restriction site and therefore its presence was verified by digestion of the PCR product with Eco0109 and electrophoresis on a 8% nondenaturating polyacrylamide gel. Furthermore, silent substitutions in codons 422 (GAT → GAC) and 804 (GCA → GCG) were found in 2 (5%) and in 1 (2.5%) of the 40 patients, respectively.

Additional screening for the IRS-1 gene variants. 72 additional NIDDM patients (a total of 112) as well as 104 subjects with completely normal glucose tolerance were screened for the amino acid substitutions in codons 818, 892, and 971. The Gly818Arg substitution was observed in 2 of 112 diabetic patients (1.8%) and in 2 of 104 control subjects (1.9%) (P = NS between the groups). All subjects were heterozygous for this amino acid substitution. The Ser 892 Gly substitution was found in 3 of 112 diabetic (2.7%) and in 1 of 104 control subjects (1.0%) (P = NS). All these subjects were heterozygous for this variant. The Gly971Arg substitution was found in 9 of 104 control subjects (8.7%) and in 11 of 112 diabetic patients (9.8%) (P = NS). One diabetic patient and two control subjects were homozygous for this substitution. Rare allele frequencies for the 971 polymorphism were similar in control and diabetic subjects (0.053 vs. 0.054, P = NS).

The simultaneous presence of several of these variants in the same individual was uncommon. However, all diabetic (n = 2) and control subjects (n = 2) who had the Gly818Arg substitution had also the Gly971Arg substitution.

Insulin sensitivity and insulin secretion in subjects with and without the Gly971Arg substitution. Table IV gives the results of euglycemic and hyperglycemic clamp studies. Total whole body glucose uptake, a measure of insulin sensitivity, did not differ in subjects with and without the Gly971Arg substitution in either group. Consequently, the rates of glucose oxidation and glucose nonoxidation, as well as lipid oxidation, were similar in subjects with and without the Gly971Arg variant. The results of the hyperglycemic clamp study in patients with NIDDM demonstrated that this substitution was not associated with an impairment in insulin secretion capacity measured by C-peptide or insulin concentrations under maximal glucose stimulation. Other amino acid substitutions were so uncommon that no statistical analysis with respect to insulin sensitivity and insulin secretion between those with and without a rare variant was possible to perform. Table V shows glucose and insulin levels in the fasting state and after a glucose load in subjects with and without the Gly971Arg substitution in control subjects and in patients with NIDDM. No statistically significant association between this substitution and glucose or insulin responses was found in either group.

Discussion

IRS-1 plays a central role in the signaling of insulin action in insulin-sensitive target cells, particularly in skeletal muscle and adipose tissue. In these tissues activation of the insulin receptor induces tyrosine and serine phosphorylation of the cytoplasmic protein, IRS-1 (15). Thus, IRS-1 seems to be a primary substrate of insulin receptor tyrosine kinase in vivo and its phosphorylation is linked to insulin action (14, 15). Tyrosine-phosphorylated sites within IRS-1 associate with high affinity to cellular proteins that contain Src homology 2 (SH2) domains (38). These include phosphatidylinositol (PI)-3 kinase (39), growth factor receptor-bound protein 2 (GRB2) (40), and Nck (41). GRB2 is a small widely expressed cytoplasmic protein whose entire sequence is composed of a single SH2 domain flanked by two SH3 domains (40). Recent studies have indicated that GRB2 couples the insulin receptor to the ras signaling pathway (42, 43).

Although IRS-1 is an essential component of the insulin signaling pathway, direct evidence that its expression is required for insulin action is missing. Consequently, the role of IRS-1 in the pathogenesis of NIDDM remains to be proven. However, the following findings support the notion that IRS-1 could potentially be a good candidate gene for NIDDM. First, several metabolic studies on NIDDM patients have indicated that both the rates of glucose oxidation and nonoxidation are significantly reduced in these patients (2, 6) suggesting that the defect in insulin action is likely to reside at a step proximal to the activation of key intracellular enzymes involved in glucose metabolism. Because IRS-1 is the first insulin signaling protein in the cascade of insulin action, defects in this protein could potentially lead to oxidative and non-oxidative defects in glucose metabolism. Second, IRS-1 is widely expressed and highly conserved across species and tissues (17). Since insulin resistance includes several tissues (skeletal muscle, fat, liver) defects in IRS-1 could lead to insulin resistance in these tissues.

Our study of 112 Finnish patients with NIDDM and 104 control subjects demonstrated that the most common variant in

Table IV. Association of the Gly971Arg Substitution of IRS-1 with Insulin Sensitivity and Insulin Secretion in Control Subjects and in Patients with NIDDM

	Control		NIDDM	
	Common (n = 62)	Variant (n = 8)	Common (n = 19)	Variant (n = 4)
Euglycemic clamp				
Total glucose uptake, μmol/kg/min	56.5±1.6	62.4±4.7	27.9±2.1	29.5±5.9
Glucose oxidation, μmol/kg/min	20.1±0.6	20.1±1.1	11.6±0.9	13.0±1.6
Glucose nonoxidation, μmol/kg/min	36.1±1.4	42.6±4.5	16.3±1.8	16.2±3.7
Lipid oxidation, μmol/kg/min	0.04±0.2	0.22±0.2	2.54±0.3	1.37±0.6
Hyperglycemic clamp				
Glucose, mmol/l	ND	ND	20.5±0.2	20.8±0.3
Maximum C-peptide, nmol/l	ND	ND	2.29±0.34	2.52±0.49
Maximum insulin, pmol/l	ND	ND	444±101	509±127

ND, not determined.

IRS-1 was the Gly971Arg substitution, observed in about 10% of control subjects and diabetic patients. With respect to NIDDM patients our results are in accordance with a previous study of Danish population by Almind et al. (18) which showed that the prevalence of this amino acid substitution was 11.6% (10/86) in diabetic patients. However, in their study the frequency of this substitution was considerably lower in control subjects, only 4.0% (3/76) as compared to our study (8.7%). Furthermore, Almind et al. (18) reported an Ala512Pro substitution in 8 of 86 NIDDM patients (9.3%) and in 2 of 76 control subjects (2.6%). We did not observe this substitution in the Finnish population either using SSCP or specific enzyme digestion with DraIII as described previously (18). Instead, we observed the Gly818Arg substitution in 2 of 112 NIDDM patients and in 2 of 104 control subjects. This substitution occurred always with the Gly971Arg polymorphism indicating positive linkage disequilibrium (rare alleles on the same homolog).

In the study of Almind et al. (18) diabetic patients with the Gly971Arg substitution had similar degree of insulin sensitivity but lower levels of fasting plasma insulin and C-peptide levels than those without substitutions at codons 971 and 512. Our findings from the euglycemic clamp study (Table V) support the results of Almind et al. (18) that the Gly971Arg substitution is not associated with insulin resistance. In fact, both control and diabetic subjects with this variant were somewhat, albeit not significantly, more insulin sensitive than those without the Gly971Arg substitution. In contrast to the study of Almind et al. (18) we did not find any significant association of the Gly971Arg substitution with insulin levels in an oral glucose tolerance test (Table V) or maximum insulin secretion capacity in NIDDM patients treated with diet or oral antidiabetic drugs during hyperglycemic clamp study (Table IV).

Table V. Association of the Gly971Arg Substitution of IRS-1 and Plasma Glucose (mmol/l) and Insulin (pmol/l) Levels in an Oral Glucose Tolerance Test in Control Subjects and in Patients with NIDDM

	Control		NIDDM	
	Common (n = 95)	Variant (n = 9)	Common (n = 101)	Variant (n = 11)
Fasting glucose	5.1±0.2	5.5±0.3	9.4±0.3	10.5±1.1
1-h glucose*	6.6±2.2	7.4±3.0	16.4±0.6	17.6±1.1
2-h glucose*	5.0±0.1	5.3±0.3	15.9±0.7	15.9±1.3
Fasting insulin‡	56±4	44±6	131±9	156±32
1-h insulin*	482±43	352±62	507±57	545±128
2-h insulin*	246±26	186±29	571±69	622±193

* Available in all control subjects and in 57 patients with NIDDM (51 without and 6 with the variant). ‡Available in all control subjects and in 88 patients with NIDDM (78 without and 10 with the variant).

We found two previously unreported variants of the IRS-1 gene, the Gly818Arg substitution in 2 of 112 diabetic patients and in 2 of 104 control subjects, and the Ser892Gly substitution in 3 of 112 diabetic patients and in 1 of 104 control subjects. The Ser892Gly substitution is potentially interesting for the etiology of NIDDM since it abolishes one of the serine phosphorylation sites (Ser-Pro-Gly-Glu) which is conserved between human and rat IRS-1 sequences (17). Furthermore, this site is located immediately NH_2-terminal to the SH2 binding site of GRB2, a protein that associates with IRS-1 upon insulin-induced phosphorylation. Skolnik et al. (44) have identified a short sequence motif (YVNI) present in IRS-1 (amino acids 896–898) which specifically binds the SH2 domain of GRB2 with high affinity. The authors demonstrated that of all phosphopeptides tested only S-P-G-E-Y-V-N-I-E-F-G-S (amino acids 890–901 in IRS-1), which encopassed the amino acid sequence around Tyr896 of IRS-1, bound GRB2 with a high affinity (K_d = 35 nM). Therefore, the Ser892Gly substitution may influence the binding of GRB2 to IRS-1 and the activation of downstream insulin signaling proteins.

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