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Rapid communication

Lack of association between the Gly40Ser polymorphism in the glucagon receptor gene and NIDDM in Finland

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Summary A heterozygous polymorphism changing GGT^{40} (Gly) to AGT^{40} (Ser) (Gly40Ser) in the glucagon receptor gene was reported to be associated with non-insulin-dependent diabetes mellitus (NIDDM). A possible involvement of this polymorphism in impaired glucose tolerance was also suggested in a French population. To replicate this finding we screened 311 unrelated NIDDM patients, 101 unrelated individuals with impaired glucose tolerance and 306 control subjects for the presence of the Gly40Ser polymorphism by use of polymerase chain reaction-restriction fragment length polymorphism in a Finnish population. None of the NIDDM or impaired glucose tolerant patients had this polymorphism. Instead, four of the control subjects (1.3 %) were heterozygous carriers of the polymorphism (NS). The age, body mass index, 2-h blood glucose level, 2-h insulin level, and incremental insulin area of the four subjects with this polymorphism were similar to those of the control subjects homozygous for the wild type. Taken together, the data do not support the suggested involvement of the Gly40Ser polymorphism in impaired glucose tolerance and the hypothesis of an association between NIDDM and the glucagon receptor gene in this population. [Diabetologia (1995) 38: 1246–1248]

Key words Glucagon receptor gene, non-insulin-dependent diabetes mellitus, impaired glucose tolerance.

Although family and twin studies have shown that non-insulin-dependent diabetes mellitus (NIDDM) is a strongly inherited disease [1, 2], the mechanisms and genes involved in the pathogenesis of the major form of the disease are still unknown [3]. Recently, a single heterozygous Gly to Ser polymorphism (Gly40Ser) was found in exon 2 of the glucagon receptor (GCG-R) gene and was shown to be associated with late-onset NIDDM in a French population [4]. A role of this polymorphism in the early stage of NIDDM, i.e. in patients with impaired glucose tolerance (IGT), was suggested [4]. To test this hypothesis we studied a representative population of NIDDM, IGT, and control subjects from two different regions in Finland for the presence of this polymorphism.

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Abbreviations: GCG-R, Glucagon receptor; IGT, impaired glucose tolerance; NIDDM, non-insulin-dependent diabetes mellitus; PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism.

Subjects and methods

We randomly selected 311 unrelated NIDDM patients, 101 patients with IGT and 306 unrelated control subjects without a family history of NIDDM from the Botnia region on the west coast of Finland [5] (201 NIDDM patients, 101 IGT patients and 195 control subjects) and from the Helsinki region on the south coast of Finland (110 NIDDM patients and 111 control subjects). All subjects were further characterized by a 75-g oral glucose tolerance test with the measurement of blood glucose and serum insulin concentrations at –5, 0, 5, 30, 60, and 120 min after the glucose load. HbA_{IC} was measured by high performance liquid chromatography. Clinical characteristics of the study subjects are shown in Table 1. The NIDDM patients were diagnosed according to the World

Table 1. Characteristics of study subjects

	NIDDM	IGT	Control subjects
n Subjects (females/males)	311 (147/164)	101 (44/57)	306 (174/132)
Age (years)	61 ± 1	48 ± 1[a]	54 ± 1
BMI (kg/m^2)	28.8 ± 0.3	28.2 ± 0.5	26.1 ± 0.3
Fasting blood glucose (mmol/l)	8.7 ± 0.2[b]	5.4 ± 0.1[b]	5.0 ± 0.1
2-h blood glucose of the OGTT (mmol/l)	14.3 ± 0.4[b]	8.5 ± 0.1[b]	5.1 ± 0.1
Fasting serum insulin (pmol/l)	15.3 ± 1.0[b]	12.0 ± 1.1[b]	7.9 ± 0.4
2-h insulin of the OGTT (pmol/l)	67.1 ± 5.1[b]	92.0 ± 7.2[b]	38.9 ± 2.3
Incremental insulin area (pmol · l^{-1} · min^{-1})	4839 ± 430	7853 ± 580	4925 ± 284
HbA_{1c} (%)	7.9 ± 0.1[b]	5.8 ± 0.1[b]	5.5 ± 0.1
Gly40Ser mutation	0[c]	0[d]	4 (1.3 %)

Data are means ± SEM. [a] $P < 0.01$ (t-test and Mann-Whitney test, compared with NIDDM). [b] $P < 0.01$ (t-test and Mann-Whitney test, compared with control subjects). [c] $P = 0.124$. [d] $P = 0.573$ (Fisher's Exact Test, compared with control subjects)

Health Organization criteria. The control subjects were defined as individuals with normal glucose tolerance and without family history of diabetes.

PCR-RFLP screening for the Gly40Ser polymorphism. As the Gly40Ser polymorphism in exon 2 of GCG-R gene eliminates a *Bst*E II restriction site, this polymorphism can be detected by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The PCR primers were 5′ TGTCTGGTTGCTTGTGCATG-3′ (upstream) and 5′-GAAGAGAACTCAGGAAGTGC-3′ (downstream), which flank the whole exon 2 of GCG-R gene (sequence information kindly provided by Dr. P. Froguel, CEPH, Paris, France). Genomic DNA (100 ng) was amplified in a total volume of 20 µl. PCR conditions were: initial denaturation at 94 °C for 5 min, 30 cycles at 94 °C for 30 s, 60 °C for 30 s, 72 °C for 30 s, and a final extension at 72 °C for 10 min. We then incubated 10 µl of the PCR product for 3 h with 1 U of *Bst*E II at 60 °C in a final volume of 15 µl without further purification. The samples were then run on a 2.0 % agarose gel, stained with ethidium bromide and analysed under ultraviolet light. In the presence of the polymorphism, the restriction site for *Bst*E II is lost; therefore, the allele of this polymorphism corresponds to the 196 bp (base pair) undigested band.

Statistical analysis

Data are expressed as means ± SEM. Differences in allele frequencies and group means were tested by Fisher's Exact Test, t-test and Mann-Whitney test where appropriate.

Results and discussion

The allele frequencies of the Gly40Ser polymorphism of the GCG-R gene among NIDDM, IGT and control subjects are shown in Table 1. None of the NIDDM patients had this Gly40Ser polymorphism. In contrast to previous results [4] four of the control subjects (1.3 %) were heterozygous carriers of this polymorphism (NS). Direct sequencing confirmed the presence of the variant sequence changing GGT^{40} (Gly) to AGT^{40} (Ser) in these four subjects (data not shown). The age (60 ± 4 years), BMI (26.0 ± 1.4 kg/m^2), 2-h blood glucose level (5.4 ± 0.1 mmol/l), 2-h insulin level (45.1 ± 18.0 pmol/l), and incremental insulin area (6319 ± 3119 pmol · l^{-1} · min^{-1}) of the four control subjects heterozygous for the polymorphism were similar to those of the control subjects homozygous for the wild type (Table 1). The GCG-R with the reported substitution of Ser40 for Gly40 has 3 times lower binding affinity for glucagon than the wild-type receptor [4], and binding sites for glucagon have been found in the pancreatic islet beta cells [6]. Therefore, the polymorphism could theoretically influence insulin secretion. However, our findings of normal glucose tolerance and normal insulin secretion in the four control subjects carrying this polymorphism do not support this hypothesis.

The study performed in a French population used the transmission disequilibrium test to test for linkage based on the premise that a parent heterozygous for an associated allele will more often transmit this allele to affected offspring. It was found that the Gly40Ser mutation was transmitted in excess among affected members only when individuals with IGT were included in the analysis. However, on transmission disequilibrium test diabetes alone did not show any evidence for linkage to the GCG-R gene. Therefore, the authors proposed that this mutation may play a role in the early stage of NIDDM; i.e., in patients with IGT [4]. The implication of this hypothesis is that the frequency of the Gly40Ser polymorphism in younger individuals with IGT should be increased compared with the frequencies in patients with overt diabetes. To test this hypothesis we analysed a group of 101 unrelated individuals with IGT (mean age 48 ± 1 years) with a 2-h blood glucose value between 7.5 and 10.0 mmol/l. However, none of them were carriers of this polymorphism.

Taken together, the data challenge the hypothesis that a beta-cell defect would make the carriers of this polymorphism more prone to develop NIDDM and do not support the hypothesis that there is an association between the Gly40Ser polymorphism and NIDDM in this population.

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