Glucokinase Gene Variants in Subjects With Late-Onset NIDDM and Impaired Glucose Tolerance

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OBJECTIVE — To investigate the frequency of variants of the glucokinase (GCK) gene in subjects with late-onset non-insulin-dependent diabetes mellitus (NIDDM) and in subjects with late-onset impaired glucose tolerance (IGT).

RESEARCH DESIGN AND METHODS — The study population included 36 Finnish patients with late-onset NIDDM who were treated with diet for >8 years or who were newly diagnosed and 40 subjects with late-onset IGT who had low or normal insulin levels when tested by an oral glucose tolerance test. All exons, exon-intron junctions, and islet and liver promotor regions of the GCK gene were amplified with the polymerase chain reaction and screened for mutations using single-strand conformation polymorphism analysis.

RESULTS — A silent third-base substitution (TAC→TAT) in codon 215 of exon 6 was found in 2.8% of NIDDM patients and in 5.0% of IGT subjects. Polymorphisms were found in islet exon 1 at nucleotide 403 (C→G) in 16.7% of NIDDM patients and in 17.5% of IGT subjects and in the noncoding region of the islet promotor at nucleotide −30 (G→A) in 13.9% of NIDDM patients and in 25.0% of IGT subjects. Furthermore, in liver intron 1 a variant (C→T), 12 base pairs upstream from the splice acceptor site, was found in 5.6% of NIDDM patients and in 7.5% of IGT subjects.

CONCLUSIONS — These results indicate that the mutations in the coding region of the GCK gene are not likely to play a major role in the pathogenesis of late-onset NIDDM or IGT in the Finnish population.

Glucokinase (GCK) (ATP: D-hexose-6-phosphotransferase, EC 2.7.1.1) is the major enzyme responsible for the phosphorylation of glucose in the liver and pancreas (1). GCK controls the rate-limiting step of glycolysis in pancreatic β-cells at physiological glucose levels. Decreased hepatic GCK could potentially impair hepatic glucose uptake, and decreased β-cell GCK could decrease glucose-induced insulin secretion by β-cells (2).

Over 20 different mutations of GCK have been identified in subjects with maturity-onset diabetes of the young (MODY) (3). Furthermore, mutations of this gene have been reported also in subjects with gestational diabetes (4). However, the frequency of GCK mutations in the coding region of the GCK gene in typical late-onset non-insulin-dependent diabetes mellitus (NIDDM) patients has been uncommon (5,6), but in mild forms of late-onset NIDDM or impaired glucose tolerance (IGT), defects in the GCK gene could play a more significant role. To address this question we screened 36 diet-treated or newly diagnosed Finnish patients with NIDDM and 40 Finnish subjects with late-onset IGT for mutations of the GCK gene.

RESEARCH DESIGN AND METHODS

Diabetic patients

Diabetic patients for this study were selected from a population study aiming to investigate cardiovascular risk factor levels and the prevalence of atherosclerotic vascular disease in the relatives of middle-aged diabetic and nondiabetic subjects (7). The baseline study was conducted in 1984–1986; the follow-up study was conducted in 1992–93. Thirty-six patients (17 men, 19 women), aged 61 ± 2 years (mean ± SE), were included in this study. All were diabetic patients who were treated with diet only at both examinations (n = 10) or who had developed previously undiagnosed NIDDM (n = 26) according to the World Health Organization criteria (8) during the 8-year follow-up, detected by an oral glucose tolerance test at the second survey.

Subjects with IGT

IGT subjects were selected for this study from a population study in which cardio-

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bp, base pair; GCK, glucokinase; IGT, impaired glucose tolerance; MODY, maturity-onset diabetes of the young; NIDDM, non-insulin-dependent diabetes mellitus; PCR, polymerase chain reaction; SSCP, single-strand conformation polymorphism.

Table 1—*Frequency of variants of the GCK gene in subjects with NIDDM and IGT*

	Location	Change	Frequency (%) NIDDM	IGT
n			36	40
Islet exon 1	Nucleotide 403	C→G	6 (16.7)	7 (17.5)
Islet promotor	Nucleotide −30	G→A	5 (13.9)	10 (25.0)
Liver intron 1	12 bp upstream from the splice acceptor site	C→T	2 (5.6)	3 (7.5)
Exon 6	Codon 215	TAC→TAT	1 (2.8)	2 (5.0)
Intron 9	8 bp from the splice donor site	C/C	13 (36.1)	22 (55.0)
		C/T	20 (55.6)	14 (35.0)
		T/T	3 (8.3)	4 (10.0)

vascular risk factor levels and the prevalence of diabetes and atherosclerotic vascular disease were investigated in elderly Finnish subjects, aged 65–74 years at baseline examination (9). The baseline study was conducted in 1986–1988; the follow-up study was conducted in 1990–1991. Of 892 subjects who did not have diabetes at baseline and from whom the results of the complete oral glucose tolerance test were available, 83 subjects had IGT at both examinations. Forty of the 83 IGT subjects (15 men, 25 women) who had the lowest insulin response at the second examination were selected for this study to maximize the probability of finding the variants of the GCK gene.

Informed consent was obtained from all subjects. The study was approved by the ethics committee of Kuopio University.

Identification of variants of the GCK gene

Exons 1a (islet exon 1), 1b (liver exon 1), and 2–10 and the islet and liver promoter regions of the GCK gene were amplified with the polymerase chain reaction (PCR) and screened for mutations using single-strand conformation polymorphism (SSCP) analysis (10). PCR amplification was conducted using primers and conditions previously published (5). Abnormally migrating DNA segments were sequenced to determine the DNA base substitution responsible for the abnormal SSCP pattern as previously described (11).

RESULTS — Table 1 summarizes the findings of the SSCP screening. A polymorphism in islet exon 1 at nucleotide 403 (C→G) was found in 6 of the 36 NIDDM patients and in 7 of the 40 IGT subjects. A polymorphism in the noncoding region of the islet promotor at nucleotide −30 (G→A) was found in 5 NIDDM patients and in 10 IGT subjects. Furthermore, in liver intron 1 a variant (C→T), 12 base pairs (bp) upstream from the splice acceptor site, was found in two NIDDM patients and in three IGT subjects. Only one NIDDM patient and two IGT subjects had a variant in exon 6, which upon sequencing was found to be a silent substitution TAC→TAT in the third position of codon 215. The most common polymorphism in intron 9 was C/T in NIDDM patients and C/C in IGT subjects (NS between subjects with NIDDM and IGT).

CONCLUSIONS — Previous studies have shown that mutations of the GCK gene are frequent in MODY (3) but uncommon in late-onset NIDDM (5,6). Because GCK gene mutations appear to cause a relatively mild form of NIDDM, we screened only NIDDM patients who were either newly diagnosed or treated with diet only and subjects with late-onset IGT.

Most of the variants in NIDDM and IGT subjects were found within the noncoding regions of the GCK gene. The variants of islet exon 1, liver intron 1, intron 9, and the islet promotor region (at nucleotide −30) have been previously reported (5). Only one NIDDM subject and two IGT subjects had a variant within the coding region of the GCK gene. This was located in exon 6 and was due to a silent substitution in codon 215 (TAC→TAT). In general, similar GCK variants were found in subjects with NIDDM and IGT, and furthermore, the frequency of these variants did not differ between NIDDM and IGT subjects. No previous study has included a systematic screening of GCK variants in late-onset IGT. Our NIDDM and IGT subjects were identified from population-based studies in order to get representative samples from affected individuals.

We conclude that mutations of the GCK gene do not play a major role in the pathogenesis of late-onset NIDDM and IGT in the Finnish population.

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