Glucokinase Gene Variations in Japanese-Americans With a Family History of NIDDM

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OBJECTIVE — To determine if sequence variants in the glucokinase (GCK) gene contribute to the high risk of impaired glucose metabolism in Japanese-Americans and whether the gene sequence differs between Japanese-Americans and Caucasians.

RESEARCH DESIGN AND METHODS — Forty-seven unrelated Japanese-Americans with one or more first-degree relatives with non-insulin-dependent diabetes mellitus (NIDDM) were selected, irrespective of glucose tolerance status. By World Health Organization criteria, 13 had normal glucose tolerance, 11 had impaired glucose tolerance, and 23 had NIDDM. Variations in the GCK gene were identified by single-strand conformation polymorphism analysis and sequenced using standard techniques.

RESULTS — Six variants of the GCK gene were identified in a total of 21 subjects: *1*) a G→A substitution at nucleotide −30 in the β-cell–specific promoter; *2*) an A→G substitution at nucleotide 244 in the 5′-untranslated region (5′-UTR) of exon 1a; *3*) a C→G substitution at nucleotide 403 in the 5′-UTR of exon 1a; *4*) a G→A variant 13 base pair (bp) 5′ to the intron 3 exon 4 junction; *5*) a silent substitution in the third base of codon 145 in exon 4; and *6*) a C→T substitution 8 bp 3′ to the exon 9 intron 9 junction. None of these variations would be expected to affect the structure of the GCK enzyme. While none of these variants were significantly associated with IGT or NIDDM, a nonsignificant increase in the β-cell promoter variant was observed in subjects with abnormal glucose tolerance. No uniform sequence differences in the GCK gene were identified between Japanese-American and Caucasian-American subjects.

CONCLUSIONS — Mutations affecting the amino acid sequence of GCK do not account for the increased incidence of impaired glucose metabolism in Japanese-Americans, and the gene sequence does not uniformly differ from that in Caucasians.

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NIDDM, non-insulin-dependent diabetes mellitus; GCK, glucokinase; MODY, maturity-onset diabetes of the young; IGT, impaired glucose tolerance; OGTT, oral glucose tolerance test; UTR, untranslated region; bp, base pair; SSCP, single-strand conformation polymorphism; PCR, polymerase chain reaction; NGT, normal glucose tolerance; BMI, body mass index.

Non-insulin-dependent diabetes mellitus (NIDDM) is characterized by abnormal insulin secretion and insulin resistance. Glucokinase (GCK) plays a crucial role in the regulation of insulin secretion, and recently, mutations of the GCK gene have been identified and linked to maturity-onset diabetes of the young (MODY) (1).

GCK gene mutations have been identified in ~60% of French MODY families (1) but have been reported in only a few British and Japanese diabetic subjects (2–5). In one study of NIDDM in Japanese subjects under age 40, a single mutation was reported (4). It has also been reported that all Japanese differ from the originally reported nucleotide sequence in codons 107 (exon 3) and 446 (exon 10) (3). Further, using CA repeat polymorphisms, an association between the GCK gene locus and more typical NIDDM in Japanese has been found (6), supporting the possibility that GCK gene mutations may be involved in the etiology of NIDDM in this population.

Because Japanese-Americans are at a high risk of developing more typical NIDDM, with ~36% of second-generation middle-aged Japanese-American men having impaired glucose tolerance (IGT) and ~20% having NIDDM (7), we sought to determine if GCK mutations are associated with the high prevalence of abnormal glucose tolerance in Japanese-Americans. Further, since Japanese may differ in exons 3 and 10 of the GCK gene when compared with the originally published sequence, we sought to confirm this in our population.

RESEARCH DESIGN AND METHODS

— Forty-seven (37 men and 10 women) unrelated Japanese-American individuals with one or more first-degree relatives with a history of NIDDM were selected from an ongoing epidemiological study of NIDDM and coronary heart disease in Japanese-Americans living in King County, Washington (7). Each subject's glucose tolerance sta-

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Table 1—*Demographic characteristics of the 47 Japanese-American subjects*

	NGT	IGT	NIDDM
n	13	11	23
Age (years)	56.6 ± 2.3 (38.3–67.4)	63.3 ± 2.5 (45.2–72.5)	62.4 ± 1.3 (46.1–72.0)
BMI (kg/m²)	25.7 ± 1.1 (20.0–33.7)	25.0 ± 0.9 (20.3–29.7)	25.1 ± 0.6 (19.4–30.0)

Data are means ± SE (range).

tus was determined by their most recent oral glucose tolerance test (OGTT) based on World Health Organization criteria (7). Three Caucasian-American subjects previously determined to have no GCK mutations were used as negative controls in the determination of whether sequence variations in exons 3 and 10 exist between Japanese-Americans and Caucasian-Americans. The study was approved by the human subjects review committee of the University of Washington.

Molecular methods

Exons 1a and 1b, including their 5′-untranslated regions (5′-UTRs), exons 2–9, the translated region of exon 10, the 3′ 177 base pair (bp) of the β-cell–specific GCK promoter, and the 3′ 248 bp of the hepatocyte-specific promoter of the GCK gene were screened for mutations by single-strand conformation polymorphism (SSCP) analysis. Primer sequences for polymerase chain reaction (PCR) amplification were provided by Dr. M. Alan Permutt (8,9). For regions >300 bp in length, either additional internal primers were used or restriction endonuclease digestion was used to optimize fragment size to between 150 and 275 bp. DNA (PCR) amplification of each region of the GCK gene was performed for 35 1-min cycles at 94°C, 1 min at the optimal annealing temperature for each primer pair (62–69°C) and 0.75–1 min at 72°C. Final extension was performed at 72°C for 7 min. SSCP was performed according to the method of Orita et al. (10) with the following modifications. [^{32}P]dCTP-labeled, single-stranded fragments were electrophoresed in 5% polyacrylamide gels containing 10% (vol/vol) glycerol at 40–55 W with and without a cooling fan to achieve measured gel temperatures of 28–30°C and 38–40°C, respectively. Electrophoresis was continued until the samples had advanced to the lower third of the gel to increase the likelihood of detecting variant alleles. We have previously shown that these SSCP conditions reveal all variants in the LPL gene that were identified by direct sequencing (11).

Variant conformers identified on SSCP were sequenced directly from PCR-generated double-stranded DNA using the dideoxy-chain termination method of Sanger (12) and the Sequenase DNA sequencing kit (United States Biochemicals, Cleveland, OH). Both strands of each DNA fragment were sequenced. Where direct sequencing of the PCR product did not result in definitive sequencing, allele-specific sequencing was performed after cloning of the PCR product using the TA Cloning System Version 1.3 (Invitrogen, San Diego, CA).

Statistical analysis

Statistical analysis to test for differences in polymorphism frequency between glucose tolerance groups was performed using χ^2 analysis. $P < 0.05$ was considered significant.

RESULTS — The GCK gene was examined in all 47 subjects whose demographic characteristics are listed in Table 1. Six of the 13 subjects classified as normal had abnormal glucose tolerance on previous testing.

Six sequence variants were identified. A summary of the polymorphisms identified and the frequency of each polymorphism based on glucose tolerance status is presented in Table 2. All variants were observed in the heterozygous form. One individual with NIDDM had both the β-cell–specific promoter polymorphism at −30 and the silent mutation in exon 4 at codon 145. Another diabetic individual had both the β-cell–specific promoter polymorphism and the poly-

Table 2—*GCK gene variations identified in Japanese-American subjects*

	Variation	Frequency (%)			
		Normal	IGT	NIDDM	All
n		13	11	23	47
β-cell promoter Nucleotide-30	G→A	1 (7.6)	3 (27.3)	4 (17.4)	8 (17.0)
Exon 1a Nucleotide 244	A→G	1 (7.6)	1 (9.1)	2 (8.7)	4 (8.5)
Exon 1a Nucleotide 403	C→G	2 (15.3)	1 (9.1)	6 (26.1)	9 (19.1)
Intron 3 13 bp 5′ to exon 4	G→A	—	1 (9.1)	—	1 (2.1)
Exon 4 Codon 145	C→G	—	—	1 (4.3)	1 (2.1)
Intron 9 8 bp 3′ to exon 9	C→T C/C	4 (33.3)	0 (0)	8 (66.7)	12 (26.7)
	C/T	6 (23.1)	9 (34.6)	11 (42.3)	26 (57.8)
	T/T	3 (42.9)	1 (14.3)	3 (42.9)	7 (15.6)

Data are *n* (%).

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morphism at nucleotide 403 in exon 1a. The relatively common intron 9 polymorphism identified in African-American subjects (8) was also present in Japanese-American subjects (Table 2).

Katagiri et al. (3) reported that in all their Japanese subjects, nucleotides in codons 107 and 446 differed from the originally reported sequence in Caucasians (13). In Japanese-American subjects, exons 3 and 10 containing these codons did not differ by SSCP or direct sequencing when compared with three Caucasian-American control subjects.

CONCLUSIONS — Japanese-Americans are at a high risk of developing NIDDM. To determine whether mutations of the GCK gene could explain this increased risk, we screened this gene in Japanese-American subjects with a strong family history of NIDDM. In the entire coding regions of the gene, only one silent mutation in exon 4 was identified. The remaining substitutions occurred within nontranslated regions of the gene and therefore would not be predicted to affect the amino acid sequence or enzymatic function of GCK. Thus, we do not find mutations to account for the association between the GCK locus and NIDDM in Japanese observed by Noda et al. (6). Bearing in mind the possibility of a spurious association, this suggests the possibility of mutant alleles in important regulatory sequences of GCK or in another gene close to the GCK locus.

The only possible association with abnormal glucose tolerance was the variant identified at nucleotide −30 in the β-cell–specific promoter. This polymorphism was present in 17% of all subjects studied. Seven of the eight subjects with this polymorphism had either IGT or diabetes on their most recent OGTT. The other individual had IGT on one occasion but had normal glucose tolerance (NGT) on his most recent OGTT. The difference in frequency of the β-cell–specific promoter polymorphism between glucose tolerance groups, based on the most recent glucose tolerance status, was not significant. Similarly, if the analysis was performed with the six normal subjects who previously had abnormal glucose tolerance reclassified as either IGT or NIDDM, then the findings remained nonsignificant. Thus, the increased frequency of the promoter polymorphism is either a result of an unanticipated selection for abnormal glucose tolerance status, which was not observed for the other polymorphisms, or a result of the relatively small numbers in each group. Knowledge regarding the important regions regulating transcription of the human GCK gene is accumulating. A β-cell promoter construct with this single base variant at −30 was observed to have <10% of the activity of the wild-type construct (14), suggesting that this variant may be functionally significant. Thus, additional studies are warranted to further determine whether this promoter variant is associated with abnormal glucose tolerance in Japanese-Americans.

Katagiri et al. (3) reported a sequence difference in codons 107 (exon 3) and 446 (exon 10) in Japanese compared with the GCK sequence originally described by Tanizawa et al. (13). SSCP and direct sequence analysis in our Japanese-American subjects confirm the sequence of ATG (Met) at codon 107 as reported by Stoffel et al. (15) rather than ACG (Thr) as found by Tanizawa et al. (13). Japanese subjects also have the ATG sequence at codon 107 (Y. Oka, personal communication). At codon 446, we found the sequence GGC (Gly) as reported by both Tanizawa et al. (13) and Stoffel et al. (15) The difference reported at this site by Katagiri et al. (3) appears to have been related to a primer-associated sequencing error (Y. Oka, personal communication). Since the ancestors of our Japanese-American subjects emigrated from Japan, one would not expect a major genetic difference to exist between native Japanese and Japanese-Americans. Thus, we are confident that a sequence variation is not normally present in exons 3 and 10 in Japanese-Americans.

In summary, mutations affecting the amino acid sequence of the GCK enzyme are not a common cause of abnormal glucose tolerance in Japanese-American subjects with a strong family history of NIDDM.

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