Identification of Glucokinase Mutations in Subjects With Gestational Diabetes Mellitus

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Recent studies have shown that mutations in the glucokinase gene on chromosome 7 can cause an autosomal dominant form of NIDDM with a variable clinical phenotype and onset during childhood. The variable clinical phenotype includes mild fasting hyperglycemia (i.e., a plasma glucose value of >110 mg/dl, a value that is at least 2–3 SDs above normal), impaired glucose tolerance, gestational diabetes mellitus, as well as overt NIDDM as defined using National Diabetes Data Group or World Health Organization criteria. Because gestational diabetes mellitus was a clinical feature associated with glucokinase mutations, we have screened a group of women with gestational diabetes who also had a first-degree relative with diabetes mellitus for the presence of mutations in this gene. Among 40 subjects, we identified two mutations, suggesting a prevalence of ~5% in this group. Extrapolating from this result, the prevalence of glucokinase-deficient NIDDM among Americans may be ~1 in 2500. *Diabetes* 42:937–40, 1993

GDM is a heterogeneous group of disorders whose common feature is "glucose intolerance of varying severity with onset or first recognition during pregnancy (1)." GDM occurs in ~2% of all pregnancies and is commonly diagnosed in the late 2nd or early 3rd trimester when insulin requirements peak. In most women, the pathophysiology of GDM involves impaired insulin release, not decreased insulin sensitivity, as compared with normal gravidae. However, women with previous histories of GDM may show abnormal insulin release and insulin resistance after pregnancy. Because GDM is heterogeneous with regard to clinical and phenotypic characteristics, as well as progression to overt diabetes in the nongravid state, it is likely that the molecular defects underlying GDM differ among women with varying phenotypes.

A role for genetic factors in susceptibility to GDM was suggested by observations of increased prevalence of diabetes mellitus in first-degree relatives of GDM (2). Moreover, studies of candidate genes in GDM have revealed significant associations with RFLPs at the INSR locus in African-American GDM, and RFLPs at the INSR locus and IGF-II locus in Caucasians (3). Neither INSR nor IGF-II RFLPs were associated with GDM in Hispanic women. Obesity, gravidity, and maternal age confer varying degrees of risk for GDM among the three racial groups. These data support the hypothesis that GDM is genetically heterogeneous.

Recent studies have shown that mutations in the GCK can lead to the development of a dominantly inherited form of NIDDM with onset in childhood (4–6) termed MODY or, perhaps more appropriately, glucokinase-deficient NIDDM (7). Glucokinase mutations are believed to contribute to the development of NIDDM by a gene-dosage mechanism (4–6,8,9) with the reduced levels of cellular glucokinase activity that result from mutations causing an increase in the threshold for glu-
cose-stimulated insulin secretion (10). Because several subjects from families with GCK mutations were diagnosed as having NIDDM during pregnancy (5), we undertook this study to determine whether mutations in GCK are associated with GDM.

RESEARCH DESIGN AND METHODS

Study population. Subjects were participants in an earlier study of genetic markers in GDM (3). Glucose tolerance testing was performed in all women with glucose values of ≥130 mg/dl 1 h after a 50-g glucose load. Diagnosis of GDM was made on the basis of a 100-g OGTT according to the criteria of O'Sullivan and Mahan (11). All subjects were interviewed to obtain personal and family history information. To ascertain information on family history of diabetes, subjects were asked if either parent, siblings, or children have or have had diabetes. If diabetes was present in a relative, information regarding age of onset, duration, and treatment was elicited. From among a sample of 96 GDM patients who participated in our earlier study, stored DNA samples from 40 GDM patients who reported having a first-degree relative with diabetes were selected for this study. This sample included 18 Hispanic, 13 African-American, and 9 Caucasian subjects. No follow-up of these subjects was possible, and their present clinical status is unknown.

Screening of GCK for mutations. Exons 1a, 1b, 2–10 of GCK were screened for mutations after amplification with the PCR and electrophoresis of the denatured PCR product on a nondenaturing 5% polyacrylamide with or without 10% glycerol at 4°C (SSCP analysis) as described previously (4–6,8,9). Exons showing SSCP bands were cloned into M13mp18 and sequenced.

Functional characterization of GCK mutations. Mutagenesis of native human β-cell glucokinase and expression of the native and mutant enzyme in E. coli was conducted as described previously (9). Recombinant native and mutant glucokinase was purified to homogeneity, and V_{max} and K_{m}s for glucose and ATP were determined.

RESULTS

Identification of mutations in GCK in subjects with GDM. Twenty-three different mutations in GCK have been identified in subjects with NIDDM (4–6,8,9,12–14; P. Froguel et al., unpublished observations; this report). The mutations in GCK are not evenly distributed throughout the gene. Rather, they are clustered in exons 4–8 with 19 of 23 mutations occurring in these 5 exons. Of the other exons, no mutations associated with diabetes have been found in exons 1a, 1b, or 1c, and a single mutation each has been identified in exons 2, 3, 9, and 10.

Exons 1a, 1b, 2–10 were scanned for mutations in 40 subjects who were selected for study because of GDM and a first-degree relative with NIDDM. Five abnormal conformers in exons 3, 4, 6, and 7 were identified by PCR-SSCP analysis in 4 subjects. In addition, 11 of the subjects were heterozygous for the common C/T polymorphism in intron 9 (8). In exon 4, a nonsense mutation in codon 131 (relative to the sequence of the β-cell form of human glucokinase) resulted in a Ser (TCC) → Pro (CCC) substitution (Fig. 1A), and in exon 7, a polypeptide chain-terminating mutation in codon 265; Glu (GAG) → AM (TAG) (Fig. 1B). Both subjects were heterozygous for these mutations, and they had one normal and one mutant GCK allele. In addition, we identified a silent mutation in exon 6 and substitutions in introns 2 and 3: codon 200 in exon 6 (TGC → TGT); T → C substitution in intron 2 located 52 nucleotides upstream of exon 3; and an A → G substitution in intron 3 located 18 nucleotides upstream of exon 4.

Based on the results of previous studies (4,6), the Glu → AM mutation is likely to be the cause of GDM in the one subject and, as described below, the abnormal enzymatic properties of the Ser → Pro mutation imply that it is the cause of GDM in the second subject. By contrast, the silent substitution in codon 200 and the substitutions in introns 2 and 3 probably do not increase the risk of developing GDM, but further studies are needed to address this issue directly.

Clinical features of GDM subjects with GCK mutations. The patient who was heterozygous for the Ser → Pro (S131P) mutation was an obese 31-yr-old Puerto Rican woman who was gravidae 1 during the index pregnancy. She delivered a 3935-g female infant. Although she reported borderline elevated blood glucose levels at 26 yr of age, a diagnosis of NIDDM was not made. GDM was diagnosed on the basis of an OGTT at 20 wk gestation (0° = 149 mg/dl, 1° = 238 mg/dl, 2° = 260 mg/dl, 3° = 227 mg/dl) (white class B1). This subject's mother was diagnosed with diabetes mellitus in her late 20s and was treated with insulin; she died in her late 40s.

The subject who was heterozygous for the Glu → Stop (E265X) mutation was a thin 32-yr-old Caucasian woman who was gravidae 2 during the index pregnancy. She reported elevated fasting blood glucose levels (highest value was 130 mg/dl) since age 16 but normal 3-h OGTT. In a previous (untreated) pregnancy, she delivered a 3104-g female infant. GDM was diagnosed in the index pregnancy on the basis of an OGTT at 15 wk gestation (0° = 120 mg/dl, 1° = 169 mg/dl, 2° = 158 mg/dl, 3° = 140 mg/dl) (white class A2). She delivered a 3317-g infant. The subject's mother and two sisters had been diagnosed with diabetes mellitus. Her mother had been treated with diet only for >40 yr, one sister also had been treated with diet for ~15 yr, and one sister had been treated with oral hypoglycemic agents for 18 yr.

Effect of Ser → Pro mutation on glucokinase activity. The Ser → Pro mutation was constructed on the human β-cell glucokinase backbone and expressed in E. coli. Purification of the normal and mutant enzymes to homogeneity and kinetic analysis of glucokinase activity showed that this mutation had a significantly reduced activity and a much lower affinity for glucose than the native β-cell enzyme (Table 1). This result is consistent with this mutation causing GDM.
FIG. 1. Mutations in glucokinase gene in GDM. A: Ser^{131} → Pro mutation. The SSCP patterns and sequences of the normal and mutant alleles are shown. B: Glu^{265} → AM mutation. The SSCP patterns and sequences of the normal and mutant alleles are shown. The arrows indicate the abnormal SSCP conformers. Both abnormal conformers were identified by electrophoresis through a 5% polyacrylamide gel without glycerol at 4°C.

DISCUSSION
GDM occurs in ~2% of all pregnancies and is associated with increased perinatal morbidity and mortality and an increased frequency of loss of a viable fetus (1). Although GDM usually returns to a state of normal glucose tolerance after parturition, ~60% of women with a history of GDM subsequently develop diabetes (1,2). This study indicates that GDM can result from mutations in GCK. We identified mutations in GCK in 1 of 18 Hispanic subjects, 1 of 9 Caucasian, and 0 of 13 African-American subjects. The failure to identify mutations in African-American subjects is not because of their absence in this group, as we have previously identified a Glu^{279} → Gln mutation in two different unrelated African-American subjects with NIDDM (9, G. Bell et al., unpublished observations). The subjects selected for analysis in this study had a first-degree relative with diabetes, and as a group comprised ~42% (40 of 96) of subjects with GDM who were recruited into a previous study without consideration of family history (3). Our results suggest that GDM may be a consequence of glucokinase mutations in 5–10% of Hispanic and Caucasian women. This may be a minimum estimate of the frequency of mutations as PCR and SSCP may detect only ~90% of all nucleotide substitutions (15). It is also possible that there may be mutations in the promoter region that can lead to GDM, although we have not identified mutations in this region in other studies (4–6,8). The identification and functional characterization of mutations in the promoter region of GCK in this group, as well as in subjects with familial forms of NIDDM who do not have mutations in protein-coding regions of the gene, requires further study. Mutations in the promoter region that reduced glucokinase mRNA levels would be predicted to result in decreased cellular levels of glucokinase activity and thus increase the threshold for glucose-stimulated insulin secretion as proposed for missense and nonsense mutations (4–6,8,9).

Glucokinase mutations define a subtype of diabetes, glucokinase-deficient NIDDM, with autosomal dominant inheritance and a variable phenotype that includes mild fasting hyperglycemia with fasting plasma glucose values of >110 mg/dl (i.e., values that are 2–3 SDs above normal), impaired glucose tolerance, and less frequently overt NIDDM as diagnosed using criteria of the NDDG or the WHO (6). Because of this variable phenotype, sub-
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projects with glucokinase mutations may be misclassified as having classical late-onset NIDDM if strict diagnostic criteria (NDDG or WHO) are applied and family members are not tested. The potential for misclassification is also shown in this study. The subjects with GDM studied herein were recruited for genetic studies of GDM before the recognition of the relationship between GCK mutations and NIDDM. Retrospective analysis of the clinical features of the 2 patients identified with GCK mutations indicated that both had a history of borderline diabetes as well as first-degree relatives with diabetes, features consistent with their having undiagnosed glucokinase-deficient NIDDM. The identification of mutations in glucokinase, besides indicating the cause of NIDDM in some subjects, also provides information about the structure of this important regulatory enzyme of glycolysis (9).

The mutation Glu265 \( \rightarrow \) AM would result in the synthesis of a-carbon backbone of yeast hexokinase-B suggests a possible mechanism whereby mutation of this residue, which is quite distant from the active site, may lead to distortion of the structure of the active site in the mutant enzyme for ATP. Modeling of this mutation on the \( \alpha \)-carbon backbone of yeast hexokinase-B suggests a mechanism whereby mutation of this residue, which is quite distant from the active site, may lead to impaired enzymatic activity. Ser131 is located adjacent to Phe195 in the crystal structure, which connects by \( \beta \)-strand 8 to the active site residue Asp205. Thus, the Ser131 \( \rightarrow \) Pro mutation, by disrupting \( \alpha \)-helix 3, could lead to distortion of the structure of the active site in the region of Asp205, a residue that functions as a critical base catalyst in the enzymatic reaction (16).

GCK mutations have been identified in French, British, Swedish, Japanese, African-American, and Hispanic (Puerto Rican) subjects (4-6,8,9,12-14, and this study). They are present in \( \sim 60\% \) of French families with early-onset NIDDM or MODY, a group that may include \( \sim 5\% \) of all French subjects with NIDDM (6). This study suggests GCK mutations may occur in at least 2\% of GDM subjects without regard to family history of diabetes. The true prevalence of GCK mutations and their overall contribution to the incidence of NIDDM are unknown. Extrapolating from the results of this study, their prevalence in the U.S. may be \( \sim 1 \) of 2500 (2 of 40 [2 mutations in 40 GDM subjects selected on the basis of a positive family history] \( \times \) 40 of 96 [frequency of GDM patients with first-degree relative with NIDDM] \( \times \) 1 of 50 [frequency of GDM] \( = \) 1 of 2400; the prevalence of GCK mutations among women and men is expected to be similar). Although further studies are necessary to establish the actual prevalence of GCK mutations, glucokinase-deficient NIDDM may be as frequent as cystic fibrosis (17) in the American population.

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