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Human glucokinase gene: Isolation, characterization, and identification of two missense mutations linked to early-onset non-insulin-dependent (type 2) diabetes mellitus

(glucose/metabolism/phosphorylation/structure–function/chromosome 7)

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ABSTRACT **DNA polymorphisms in the glucokinase gene have recently been shown to be tightly linked to early-onset non-insulin-dependent diabetes mellitus in ≈80% of French families with this form of diabetes. We previously identified a nonsense mutation in exon 7 in one of these families and showed that it was the likely cause of glucose intolerance in this dominantly inherited disorder. Here we report the isolation and partial sequence of the human glucokinase gene and the identification of two missense mutations in exon 7, Thr-228 → Met and Gly-261 → Arg, that cosegregate with early-onset non-insulin-dependent diabetes mellitus. To assess the molecular mechanism by which mutations at these two sites may affect glucokinase activity, the crystal structure of the related yeast hexokinase B was used as a simple model for human β-cell glucokinase. Computer-assisted modeling suggests that mutation of Thr-228 affects affinity for ATP and mutation of Gly-261 may alter glucose binding. The identification of mutations in glucokinase, a protein that plays an important role in hepatic and β-cell glucose metabolism, indicates that early-onset non-insulin-dependent diabetes mellitus may be primarily a disorder of carbohydrate metabolism.**

Non-insulin-dependent diabetes mellitus (NIDDM) is a major public health problem that affects 5–7% of the world population. Although most forms of NIDDM do not exhibit simple Mendelian inheritance, the contribution of heredity to the development of NIDDM has been recognized for many years (1–4). Early-onset NIDDM or maturity-onset diabetes of the young (MODY) shares many features with the more common form(s) of NIDDM whose onset occurs in midlife (4). In addition, its early age of onset, clear mode of inheritance (autosomal dominant), high penetrance, and the availability of multigenerational pedigrees make MODY an attractive paradigm for genetic studies of NIDDM. Genetic studies have shown tight linkage of early-onset NIDDM and DNA markers on chromosome 20 (5) and the glucokinase gene (*GCK*) on chromosome 7 (6). Although the diabetes-susceptibility gene on chromosome 20 has not been identified, *GCK* is a candidate for the susceptibility gene on chromosome 7.

Glucokinase (ATP:D-hexose 6-phosphotransferase, EC 2.7.1.1) is expressed only in liver and pancreatic β cells and plays a key role in the regulation of glucose homeostasis (7–11). In the hepatocyte, the phosphorylation of glucose by glucokinase facilitates the uptake and metabolism of glucose by maintaining a gradient for glucose transport into these cells thereby regulating hepatic glucose disposal. In β cells, glucokinase is believed to be part of the glucose-sensing mechanism and to be involved in the regulation of insulin secretion.

Previously we reported a nonsense mutation in exon 7 that is linked to early-onset NIDDM in one family (12). Here we describe the partial sequence of the human glucokinase gene†† and the identification of two missense mutations in exon 7, T228M and G261R, that cosegregate with early-onset NIDDM.

MATERIALS AND METHODS

Families with Early-Onset NIDDM. Family history and clinical data were collected in three French families having MODY (6). In kindreds F388, F390, and F391, the mean age of apparent onset (diagnosis) of diabetes was 29, 23, and 12 years in the generations born after 1930, 1950, and 1970, respectively. Most of the affected individuals have mild fasting hyperglycemia with plasma glucose values ranging from 6.2 to 8.5 mM, and 2-h post-glucose load concentrations ranging from 8.3 to 14.4 mM. One patient (2105) has mild fasting hyperglycemia but normal glucose tolerance. The fasting serum insulin levels in the MODY patients are normal, ranging from 10 to 17 × 10^{-3} unit/liter. Two-hour post-glucose load insulin concentrations are much higher in families F388 and F390 [range 45–80 × 10^{-3} unit/liter] than in family 391 [range 15–51 × 10^{-3} unit/liter]. One nondiabetic individual (2025) is insulin-resistant with fasting serum insulin values of 88 × 10^{-3} unit/liter. All the affected individuals in families F388 and F390 are treated by diet alone. Three patients in kindred F391 (individuals 2070, 2041, and 2086) are presently being treated with sulfonylurea drugs. With the exception of individual 2044 in family F388, none of the diabetic individuals are obese or have reported ocular, renal, or vascular complications.

Isolation and Characterization of the Human Glucokinase Gene. Standard methods were carried out as described by Sambrook *et al.* (13). The insert from the full-length β-cell glucokinase cDNA clone pGEM-hGK20 (14) was labeled by nick translation and used to screen a male Caucasian placenta

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Abbreviations: NIDDM, non-insulin-dependent diabetes mellitus; SSCP, single-strand conformation polymorphism; MODY, maturity-onset diabetes of the young.

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††The sequence reported in this paper has been deposited in the GenBank data base (accession no. M93280).

Table 1. Sequences of primer pairs for PCR–SSCP analysis of human *GCK*

Exon	Upstream primer	Downstream primer	Size, bp
1a	5'-TCCACTTCAGAAGCCTACTG	5'-TCAGATTCTGAGGCTCAAAC	195
1b	5'-AGCAGGCAGGAGCATCTCTG	5'-GCTGCTCTCCCAGTGCAAAG	149
1c	5'-CCAGACTCTCCTCTGAACTC	5'-GAAGAAGAGGTTCCATCTGA	145
2	5'-TGCAGATGCCTGGTGACAGC	5'-CACAGCTGCTTCTGGATGAG	290
3	5'-TAATATCCGGCTCAGTCACC	5'-CTGAGATCCTGCATGCCTTG	295
4	5'-TAGCTTGGCTTGAGGCCGTG	5'-TGAAGGCAGAGTTCCTCTGG	272
5	5'-GCAGCCACGAGGCCTATCTC	5'-GAGAAAGGCAGGCAGTGCTG	195
6	5'-CCAGCACTGCAGCTTCTGTG	5'-GAGCCTCGGCAGTCTGGAAG	176
7	5'-AGTGCAGCTCTCGCTGACAG	5'-CATCTGCCGCTGCACCAGAG	285
8	5'-TGCCTGCTGATGTAATGGTC	5'-TGAGACCAAGTCTGCAGTGC	263
9	5'-ACTGTCGGAGCGACACTCAG	5'-CTTGGAGCTTGGGAACCGCA	367
10	5'-GTCGACTGCGTGCAGGGCGC	5'-TGTGGCATCCTCCCTGCGCT	263

genomic library (catalogue no. 946203, Stratagene). The exons were isolated and sequenced (15).

Single-Strand Conformation Polymorphism (SSCP) Analysis. For SSCP analysis (16), 100 ng of DNA was mixed with 10 pmol of human *GCK*-specific primer pairs (Table 1). The PCR (17) was carried out using GeneAmp reagents (Perkin–Elmer/Cetus) and 1.5 mM $MgCl_2$. The reaction volume was 10 μl and included 0.2 μl of [α-^{32}P]dCTP (3000 Ci/mmol; 1 Ci = 37 GBq; Amersham) and 1 unit of *Taq* DNA polymerase. The PCR conditions were initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 60°C for 1 min, and extension at 72°C for 2 min, with a final extension of 10 min. Exons 5 and 6 were coamplified using the 5′ exon 5 and 3′ exon 6 primers to yield a 379-base-pair (bp) fragment that was digested with *Pst* I prior to SSCP analysis. The resulting 214- and 166-bp fragments contained exons 5 and 6, respectively. Similarly exon 9 was digested with *Sph* I to yield 205- and 162-bp fragments representing the 5′ and 3′ halves of exon 9, respectively. The amplified PCR products were diluted 1:10 in formamide buffer, denatured at 95°C for 5 min, quick-cooled, and electrophoresed on glycerol-free and 10% (vol/vol) glycerol-containing 40-cm nondenaturing 5% polyacrylamide gel [acrylamide/*N*,*N*′-methylene bisacrylamide, 24:1 (wt/vol)] at room temperature and/or 4°C. The gels were electrophoresed at 6–20 W for 5–10 h, then dried, and autoradiographed overnight at −80°C with intensifying screens.

Sequencing of PCR Products. The amplified PCR products were sequenced directly using an Applied Biosystems DNA sequencer, model 373A, a dideoxynucleotide-cycle-sequencing protocol, and fluorescent-labeled dideoxynucleotide terminators as described by Applied Biosystems. PCR products were also cloned into the *Hin*cII site of M13mp18 DNA and sequenced using a Sequenase DNA sequencing kit (United States Biochemical).

Modeling of Human Glucokinase Structure. The crystal structure of yeast hexokinase isozyme B was used as a simple model for the structure of human glucokinase. The atomic coordinates for the B isozyme were refined to an *R* factor of 0.203 with 2.1-Å x-ray data by using the correct amino acid sequence (18). This structure was examined on an ESV10 Evans and Sutherland computer graphics system using the program FRODO (19). The assumption is that the structural core and the active site residues are conserved in yeast hexokinase and human glucokinase. The elements of secondary structure, α-helices and β-strands, were located by visual examination and plotted on the amino acid sequence of yeast hexokinase. Then, the amino acid sequence of human glucokinase was aligned to that of yeast hexokinase by allowing deletions and insertions only between the elements of secondary structure. The positions of the mutations were then located on the aligned structure and in the hexokinase crystal structure in relation to the glucose binding site.

RESULTS

Isolation and Partial Sequence of Human Glucokinase Gene. Four clones (λhGK-1, -2, -5, and -7) were isolated by screening a human genomic library with a cDNA clone encoding human β-cell glucokinase (Fig. 1). The partial sequences of these clones (Fig. 2) indicated that human *GCK* has 12 exons. The exon–intron organization of human *GCK* is similar to that reported for the rat gene (10, 11) including the positions of each of the introns. However, human *GCK* has an additional exon, termed exon 1c (Fig. 2). In the human gene, exons 1a and 2–10 encode the β-cell glucokinase mRNA (14). Two glucokinase transcripts have been identified in human liver RNA (21); the major transcript is encoded by exons 1b and 2–10, and the minor transcript is encoded by exons 1b, 1c, and 2–10. As shown in Fig. 2, the sequences of the N termini of the three proteins encoded by the human glucokinase gene differ as do their sizes because of the use of alternative promoters and alternative splicing. The β-cell and liver, liver^{1b} and liver^{1c}, transcripts encode proteins of 465, 466, and 464 amino acids, respectively. In this report, amino acids are designated relative to the sequence of human β-cell glucokinase.

Identification of Nucleotide Alterations in Three Families With Early-Onset NIDDM. All 12 exons of the glucokinase gene of two affected and one unaffected family member were scanned for mutations using SSCP. Abnormally migrating bands, as well as bands of normal mobility, were observed in exon 7 in affected individuals of kindreds F388, F390, and F391 (Fig. 3). The abnormal conformers were not seen in any of the nondiabetic members of these kindreds. We also screened 89 unrelated French Caucasian nondiabetic individuals and 115 NIDDM patients (80 of whom were French Caucasians) for mutations in exon 7 by using SSCP, and none showed abnormal bands. In addition, a common conformer was seen on SSCP analysis of exon 9. This common polymorphism is located 8 nucleotides from the splice donor site in intron 9 (Fig. 2) and is present in normal individuals and subjects with NIDDM.

Identification of Missense Mutations in Exon 7. Sequencing of exon 7 revealed mutations (underlined) in codon 228 [A<u>C</u>G^{Thr} → A<u>T</u>G^{Met}] in kindred F391 and the same mutation in codon 261 (<u>G</u>GG^{Gly} → <u>A</u>GG^{Arg}) in kindreds F388 and F390 (Fig. 4). These mutations were found in all the affected subjects and were not present in any of the normal members of these kindreds. Both mutations occur within the context of a CpG dinucleotide, suggesting that these sites may represent

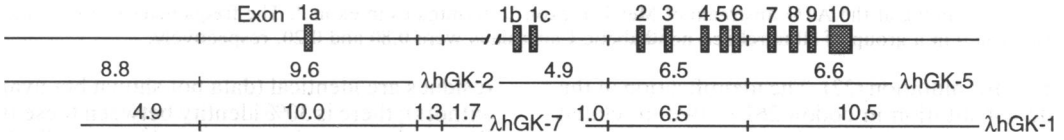

FIG. 1. Organization of the human glucokinase gene. The exons are indicated by shaded boxes. The map was derived from the four partially overlapping clones indicated. The *Eco*RI sites and the sizes of the corresponding fragments are indicated. Three microsatellite DNA polymorphisms useful for genetic studies have been identified in *GCK*. Two polymorphisms (refs. 6 and 14; M.S., unpublished data) are upstream of exon 1a, and one (6, 20) is downstream of exon 10.

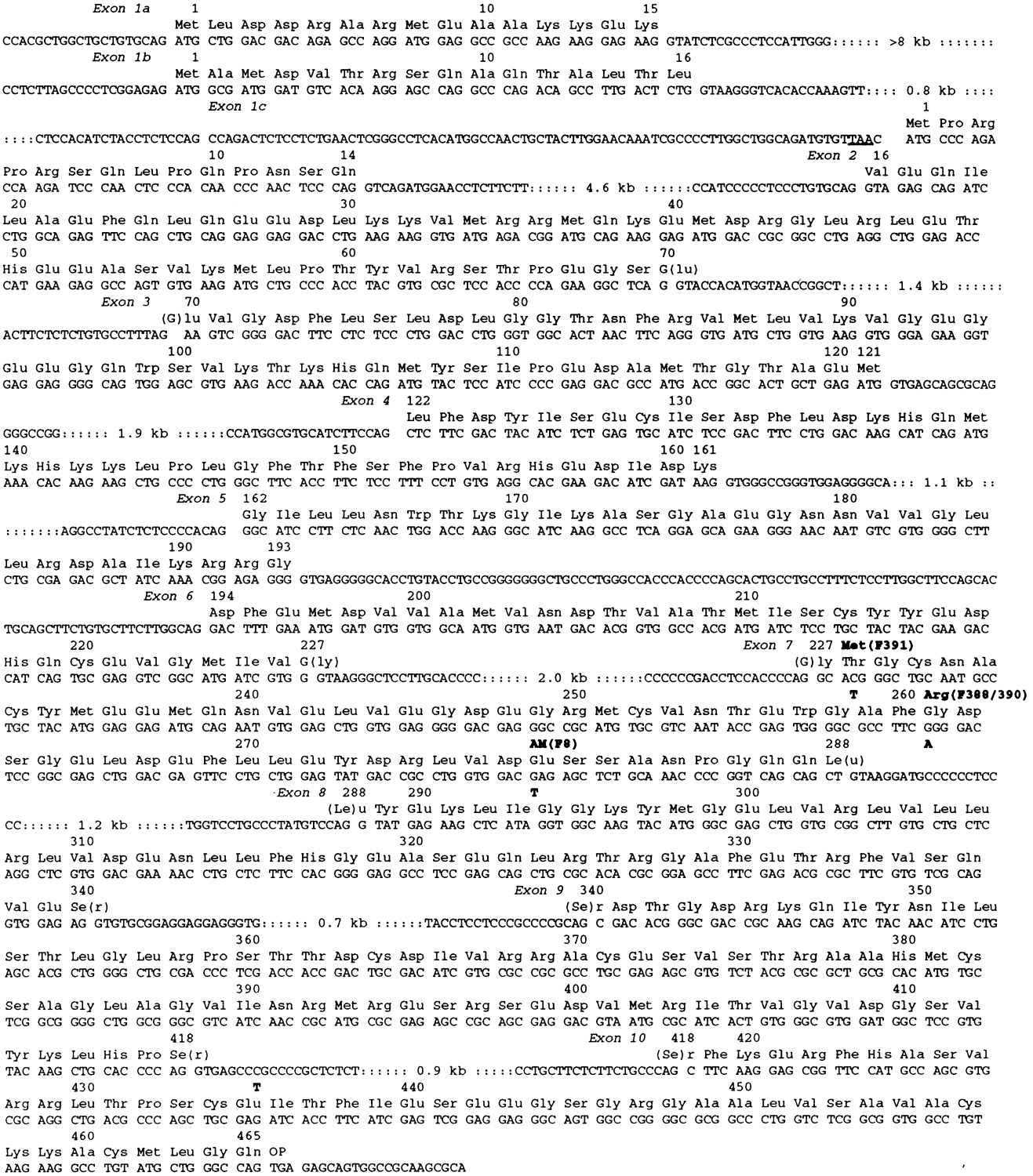

FIG. 2. Partial sequence of the human glucokinase gene. Nucleotide and predicted amino acid sequences are shown. The number of the amino acid at the beginning and the end of each exon is noted. Approximate sizes of the introns are indicated. The mutations in exon 7 and the polymorphism in intron 9 are shown in boldface type. Note that the minor liver glucose transcript liver^{1c} includes both exons 1b and 1c. However, translation initiated at the ATG/Met-1 in exon 1b would terminate at the stop codon (underlined) immediately upstream of Met-1 in exon 1c. Similarly, translation beginning at the ATG upstream of Met-1 in exon 1c terminates in exon 2. The frequencies of the C and T alleles of the polymorphism in intron 9 in a group of 30 unrelated nondiabetic Caucasians were 0.80 and 0.20, respectively.

potential hot spots for mutation (22). The identification of the same nucleotide substitution in codon 261 in two unrelated families is consistent with this hypothesis.

Mutations in Glucokinase May Alter Glucose and ATP Binding. Structural alignment of the amino acid sequences of human β-cell glucokinase (465 amino acids) (Fig. 2) and yeast hexokinase B (486 amino acids) (23) indicated that 138 residues are identical (data not shown but available from the authors); there is 30% identity between these two sequences. We predict that human glucokinase will show the same arrangement of α-helices and β-strands as observed in the crystal structure of yeast hexokinase.

There are 11 introns within the glucokinase gene. Although many of the introns occur near the ends of secondary

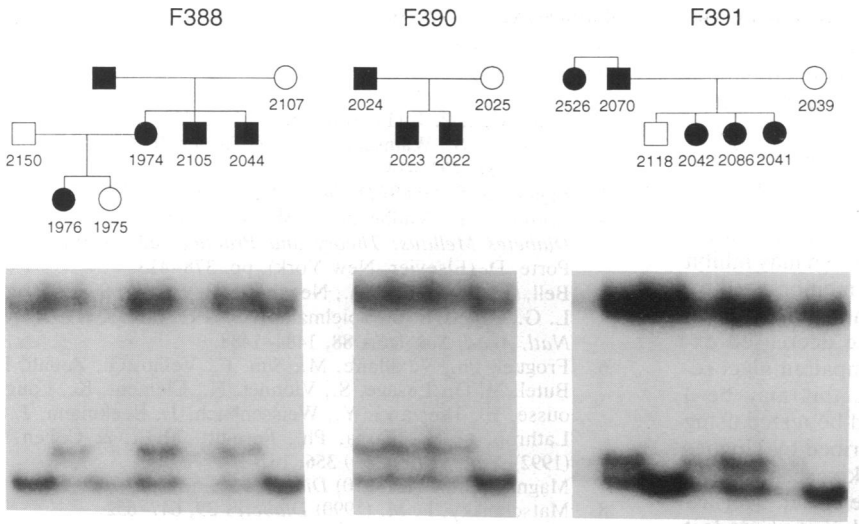

FIG. 3. SSCP analysis of exon 7 in kindreds F388, F390, and F391. Solid symbols indicate patients with early-onset NIDDM and the open symbols indicate nondiabetic individuals. Numbers under the symbols indicate the Centre d'Etude Polymorphisme Humain sample identification number. PCR/SSCP of exon 7 was carried out. The SSCP pattern shown here was obtained using a glycerol-free gel at room temperature. The unique conformer cosegregates without exception with the diabetic phenotype.

structural elements, it is not obvious that the exons represent separate functional modules of the enzyme.

The positions of the mutations in human glucokinase associated with early-onset NIDDM have been indicated on the ribbon backbone drawing of yeast hexokinase B in Fig. 5. The mutation of E279 to a stop codon (12) is predicted to generate an inactive enzyme. The two missense mutations T228M and G261R are located in or close to the active-site cleft between the two domains of the enzyme. The residue Thr-228 is invariant in all mammalian and yeast glucokinases and hexokinases (23–25). In yeast hexokinase B, the equivalent residue is Thr-234 and the hydroxyl side chain of this amino acid forms a hydrogen bond with a sulfate ion in the crystal structure. This sulfate site was predicted to represent one of the phosphate binding sites of ATP. Therefore, in glucokinase, the T228M mutation is proposed to eliminate a hydrogen bond interaction with the phosphate of ATP, which is expected to reduce the binding affinity for ATP and affect enzymatic activity. Gly-261 of glucokinase is equivalent to Asp-274 of hexokinase B. This residue forms part of a loop leading into the glucose binding site. Substitution with arginine introduces a much larger side chain that may interfere with the correct conformation of the enzyme near the active site. It should be noted that although Gly-261 is not conserved between human glucokinase and yeast hexokinase B, it is invariant in all mammalian hexokinases and glucokinases.

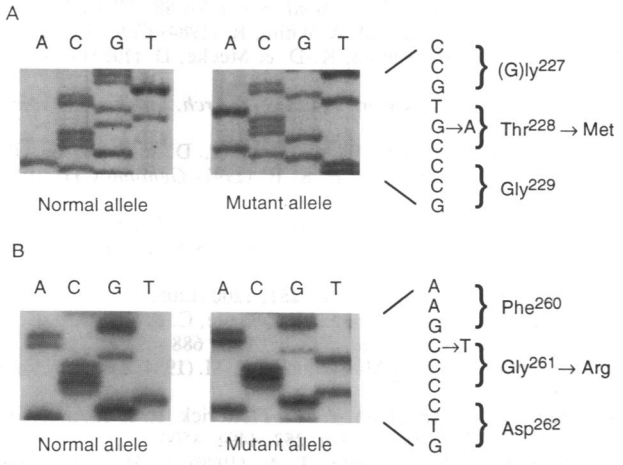

FIG. 4. Sequences of mutations in exon 7. (*A*) Sequence of point mutation in subject 2070, kindred F391. The C → T transition in codon 228 producing a Thr → Met mutation is shown. (*B*) Region of the missense mutation in subject 2044, kindred F388. The G → A transition in codon 261 resulting in the Gly → Arg mutation is indicated. Sequences were obtained after subcloning PCR fragments into M13mp18. Sequences shown here represent the antisense orientation.

DISCUSSION

We have identified two missense mutations in glucokinase that cosegregate with early-onset NIDDM. They were not seen on screening 89 nondiabetic and 115 typical NIDDM patients, implying that these mutations are the cause of the diabetes in the three families that we studied. The molecular mechanism by which mutations in glucokinase may lead to the development of an autosomal dominant inherited disease of glucose intolerance and diabetes mellitus is unknown. Glucokinase catalyzes the first step, and also the first rate-limiting reaction, of glycolysis in liver and pancreatic β cells and is believed to play an important role in glucose disposal

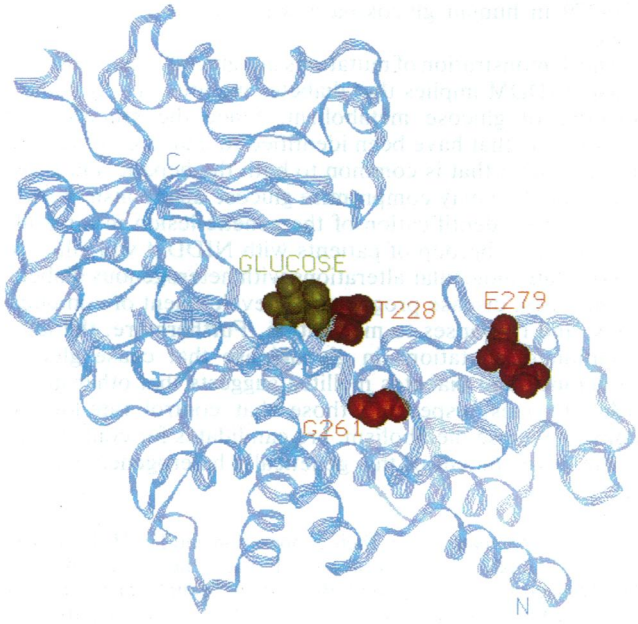

FIG. 5. Model for human glucokinase. A ribbon drawing of the α-carbon backbone of yeast hexokinase B is shown (in blue) and is predicted to represent a structural model for human glucokinase. The glucose molecule (green) was extracted from the inhibitor *O*-toluoylglucosamine present in the crystal structure of hexokinase B. The glucokinase mutations T228M, G261R, and E279AM (this mutation produces a truncated protein) are indicated in red. The N and C termini of the protein are noted by N and C.

by the liver and to participate in glucose sensing by the pancreatic β cell. The pattern of inheritance of early-onset NIDDM argues for the presence of both normal and mutant glucokinase molecules in the hepatocyte and β cell. Since glucokinase appears to act as a monomer (26), it seems unlikely that the mutant protein binds to the normal functional enzyme and acts as a dominant negative regulator of glucokinase activity. However, if glucokinase associates with GLUT2, the specific glucose transporter of pancreatic β cells and hepatocytes, perhaps the mutant protein may inhibit the transport of glucose across the plasma membrane; i.e., glucokinase mutations function as dominant-negative regulators of glucose transporter activity. Since decreased expression of GLUT2 is associated with an impaired glucose-stimulated insulin secretory response (27), this may be a reasonable hypothesis and, moreover, could be tested using the artificial insulin-secreting cell lines described by Hughes *et al.* (28). Heterozygous defects in glucokinase may also cause early-onset diabetes by a gene-dosage effect. Meglasson and Matschinsky (29) have suggested that a modest decrease in glucokinase activity may shift the threshold for insulin secretion in response to a physiological glucose challenge from 5 to 6 mM. Glucokinase mutations may alter the β-cell ATP/ADP ratios and thereby perturb the pathway of insulin secretion by an additional mechanism. Hexokinases and glucokinase catalyze the transfer of the phosphate group from ATP to water and to the hydroxyl group of the specific acceptor glucose (30–32). However, the rate of the reaction with glucose is 5×10^6 times as fast as the rate with water (33) because the enzyme undergoes a large conformational change that brings the phospho-acceptor residue near the γ-phosphate of ATP in a hydrophobic environment that excludes water from the active site. It is possible that mutations in the human glucokinase gene yield proteins that do not undergo the same conformational changes upon glucose binding and thus whose ATPase activity is significant compared to the phosphate group transfer to glucose. Analysis of site-directed mutations at Thr-228, Gly-261, and Glu-279 in human glucokinase will resolve some of these issues.

The demonstration of mutations in a glycolytic enzyme that cause NIDDM implies that diabetes mellitus may truly be a disorder of glucose metabolism. Since the mutations in glucokinase that have been identified to date are in that part of the protein that is common to both the hepatic and β-cell isoforms, they may compromise glucose metabolism in both tissues. The identification of the genetic lesion in a readily identifiable subgroup of patients with NIDDM will allow us to correlate molecular alterations with heterogenous diabetic phenotypes, disease progression, development of complications, and responses to medication. Furthermore, the identification of mutations in glucokinase that cause glucose intolerance and diabetes mellitus suggests that other glycolytic enzymes, especially those that control rate-limiting steps in glucose metabolism, are candidates for contributing to the development of this genetically heterogeneous disorder.

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1. Cambridge, P. J. (1928) *Br. Med. J.* **2**, 738–741.
2. O'Rahilly, S., Wainscoat, J. S. & Turner, R. C. (1988) *Diabetologia* **31**, 407–414.
3. Fajans, S. S. (1989) *Diabetes/Metab. Rev.* **5**, 579–606.
4. Rotter, J. I., Vadheim, C. M. & Rimoin, D. L. (1990) in *Diabetes Mellitus: Theory and Practice*, eds. Rifkin, H. & Porte, D. (Elsevier, New York), pp. 378–413.
5. Bell, G. I., Xiang, K.-s., Newman, M. V., Wu, S.-h., Wright, L. G., Fajans, S. S., Spielman, R. S. & Cox, N. J. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 1484–1488.
6. Froguel, Ph., Vaxillaire, M., Sun, F., Velho, G., Zouali, H., Butel, M. O., Lesage, S., Vionnet, N., Clément, K., Fougerousse, F., Tanizawa, Y., Weissenbach, J., Beckmann, J. S., Lathrop, G. M., Passa, Ph., Permutt, M. A. & Cohen, D. (1992) *Nature (London)* **356**, 162–164.
7. Magnuson, M. A. (1990) *Diabetes* **39**, 523–527.
8. Matschinsky, F. M. (1990) *Diabetes* **39**, 647–652.
9. Pilkis, S. J. & Granner, D. K. (1992) *Annu. Rev. Physiol.* **54**, 885–909.
10. Magnuson, M. A., Andreone, T. L., Printz, R. L., Koch, S. & Granner, D. K. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 4838–4842.
11. Magnuson, M. A. & Shelton, K. D. (1989) *J. Biol. Chem.* **264**, 15936–15942.
12. Vionnet, N., Stoffel, M., Takeda, J., Yasuda, K., Bell, G. I., Zouali, H., Lesage, S., Velho, G., Iris, F., Passa, Ph., Froguel, Ph. & Cohen, D. (1992) *Nature (London)* **356**, 721–722.
13. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY), 2nd Ed.
14. Nishi, S., Stoffel, M., Xiang, K., Shows, T. B., Bell, G. I. & Takeda, J. (1992) *Diabetologia*, in press.
15. Sanger, F., Coulson, A. R., Barrell, B. G., Smith, A. J. H. & Roe, B. A. (1980) *J. Mol. Biol.* **143**, 161–178.
16. Orita, M., Iwahana, H., Kanazawa, H., Hayashi, K. & Sekiya, T. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 2766–2770.
17. Saiki, R. K., Gelfand, D. H., Stoffel, S., Scharf, S. J., Higuchi, R., Horn, G. T., Mullis, K. B. & Erlich, H. A. (1988) *Science* **239**, 487–491.
18. Harrison, R. W. (1985) Ph.D. thesis (Yale Univ., New Haven, CT).
19. Jones, A. T. (1985) *Methods Enzymol.* **115**, 157–171.
20. Matsutani, A., Janssen, R., Donis-Keller, H. & Permutt, M. A. (1992) *Genomics* **12**, 319–325.
21. Tanizawa, Y., Koranyi, L. I., Welling, C. M. & Permutt, M. A. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 7294–7297.
22. Barker, D., Schafer, M. & White, R. (1984) *Cell* **36**, 131–138.
23. Frohlich, K.-U., Entian, K.-D. & Mecke, D. (1985) *Gene* **36**, 105–111.
24. Thelen, A. P. & Wilson, J. E. (1991) *Arch. Biochem. Biophys.* **286**, 645–651.
25. Griffin, L. D., Gelb, B. D., Wheeler, D. A., Davison, D., Adams, V. & McCabe, E. R. B. (1991) *Genomics* **11**, 1014–1024.
26. Holroyde, M. J., Allen, M. B., Storer, A. C., Warsy, A. S., Chesher, J. M., Trayer, I. P., Cornish-Bowden, A. & Walker, D. G. (1976) *Biochem. J.* **153**, 363–373.
27. Unger, R. H. (1991) *Science* **251**, 1200–1205.
28. Hughes, S. D., Johnson, J. H., Quaade, C. & Newgard, C. B. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 688–692.
29. Meglasson, M. D. & Matschinsky, F. M. (1984) *Am. J. Physiol.* **256**, E1–E13.
30. Steitz, T. A., Anderson, W. F., Fletterick, R. J. & Anderson, C. M. (1977) *J. Biol. Chem.* **252**, 4494–4500.
31. Bennett, W. S. & Steitz, T. A. (1980) *J. Mol. Biol.* **140**, 211–230.
32. Lin, S.-X. & Neet, K. (1990) *J. Biol. Chem.* **265**, 9670–9675.
33. Trayser, K. A. & Colowick, S. P. (1961) *Arch. Biochem. Biophys.* **94**, 161–168.