Permanent Neonatal Diabetes Caused by Glucokinase Deficiency

Inborn Error of the Glucose-Insulin Signaling Pathway

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Neonatal diabetes can be either permanent or transient. We have recently shown that permanent neonatal diabetes can result from complete deficiency of glucokinase activity. Here we report three new cases of glucokinase-related permanent neonatal diabetes. The probands had intrauterine growth retardation (birth weight <1,900 g) and insulin-treated diabetes from birth (diagnosis within the first week of life). One of the subjects was homozygous for the missense mutation Ala378Val (A378V), which is an inactivating mutation with an activity index of only 0.2% of wild-type glucokinase activity. The second subject was homozygous for a mutation in the splice donor site of exon 8 (intervening sequence 8 [IVS8] + 2T→G), which is predicted to lead to the synthesis of an inactive protein. The third subject (second cousin of subject 2) was a compound heterozygote with one allele having the splice-site mutation IVS8 + 2T→G and the other the missense mutation Gly264Ser (G264S), a mutation with an activity index of 86% of normal activity. The five subjects with permanent neonatal diabetes due to glucokinase deficiency identified to date are characterized by intrauterine growth retardation, permanent insulin-requiring diabetes from the first day of life, and hyperglycemia in both parents. Autosomal recessive inheritance and enzyme deficiency are features typical for an inborn error of metabolism, which occurred in the glucose-insulin signaling pathway in these subjects. *Diabetes* 52: 2854–2860, 2003

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AI, relative activity index; $^{ATP}K_m$, ATP concentration required for glucokinase activity to be half maximum when glucose is in excess; BGPR, β-cell glucose phosphorylation rate; GSIR, glucose-stimulated insulin release; GST, glutathione S-transferase; IA-2, insulinoma-associated protein 2; I_{GKB}, relative activity index; IUGR, intrauterine growth retardation; IVS, intervening sequence; k_{cat}, turnover rate; MODY, maturity-onset diabetes of the young; nH, Hill coefficient for cooperativeness with glucose; PNDM, permanent neonatal diabetes; $S_{0.5}$, the concentration of glucose needed to achieve the half-maximal rate of phosphorylation.

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Neonatal diabetes, insulin-requiring hyperglycemia occurring within the first month of life is often associated with intrauterine growth retardation (IUGR) and can be either transient or permanent (1). Transient neonatal diabetes is associated with abnormalities of chromosome 6, including paternal uniparental disomy and paternal duplications of 6q24, with loss of imprinting (1,2) and increased risk of diabetes later in life. Mutations in the insulin promoter factor-1, a transcription factor implicated in pancreatic development and the regulation of insulin gene expression, result in permanent neonatal diabetes (PNDM) caused by pancreatic agenesis (3). We have recently shown that complete deficiency of the glycolytic enzyme glucokinase is another cause of PNDM (4). Two patients presented with IUGR, permanent insulin requirement from shortly after birth and homozygosity for mutations in the glucokinase gene (*GCK*). Here we present the results of screening eight cases of PNDM for mutations in glucokinase. Three of these had glucokinase-related PNDM, including a subject who inherited different inactivating mutations from each parent.

RESEARCH DESIGN AND METHODS

Subjects. The screening included eight cases of PNDM, defined as a diagnosis of permanent diabetes before age 1 month. The probands of families 1–3 were identified by one of the authors (P.R.N.) by a PubMed literature search, after which the corresponding authors (N.S. and S.U.S.) were contacted. The other PNDM patients were from the Department of Pediatrics, University of Bergen (N89-1) or referred to us by other clinics (BR1, R1826, AA, and US4-1). We have not performed a systematic, population-based screening program for this study. Informed consent was obtained from the subjects or their parents. The studies were performed according to the Declaration of Helsinki and approved by ethical committees.

Genetic studies. The exons, flanking introns, and minimal promoter regions of the gene encoding glucokinase were screened for mutations by direct sequencing of the PCR products. In vitro mutagenesis was performed as described in Bjørkhaug et al. (5). The primers 5′-CGTGTCTACGCGCG*T*TGCGCACATGTGCTCG-3′ (F1), 5′-CGAGCACATGTGCGCA*A*CGCGCGTAGACACG-3′ (R1), 5′-GCCTTCGGGGACTCC*A*GCGAGCTGGACGAGTTCC-3′ (F2), and 5′-GGAACTCGTCCAGCTCGC*T*GGAGTCCCCGAAGGC−3′ (R2) were used to introduce the appropriate nucleotide changes corresponding to the mutations A378V (F1 and R1) and G264S (F2 and R2).

Kinetic analysis of glucokinase. Wild-type and mutant forms of human β-cell glucokinase were generated and expressed as glutathionyl S-transferase (GST) fusion proteins in *E. coli*; the kinetic properties of the purified proteins were determined as previously described (6). We performed four serial experiments. The wild-type GST-glucokinase preparation was newly made for the current study. Kinetic data may vary as a function of the chemical nature

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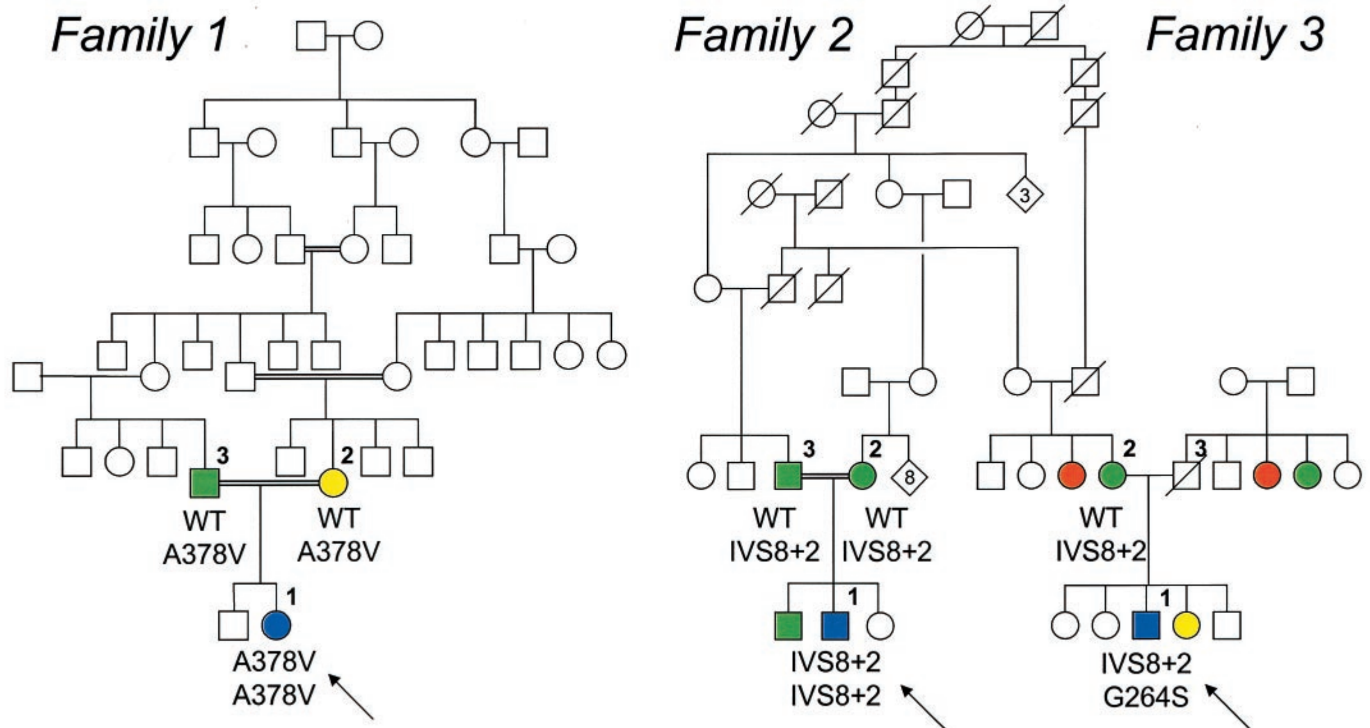

FIG. 1. Pedigrees of three families with PNDM. The subjects who were studied are numbered and their clinical features are described in RESEARCH DESIGN AND METHODS and Table 1. The probands with PNDM (●, ■) are indicated by the arrows. Impaired fasting glucose (○), impaired glucose tolerance (●), and diabetes (●, ■) were defined using the diagnostic criteria of the World Health Organization (19). A378V, the mutation A378V in glucokinase; G264S, the mutation G264S in glucokinase; IVS8 + 2, the mutation IVS8 + 2T→G in the glucokinase gene; WT, wild-type glucokinase.

and concentration of the sulfhydryl reagent used in the kinetic analysis. In our studies, 2 mmol/l dithiothreitol was used in the standard kinetic assay. Nonlinear kinetics using the Hill equation were applied. The relative activity index (AI) was calculated as previously described with some modification as AI = $(k_{cat}/S_{0.5}nH)(2.5/2.5 + {}^{ATP}K_m)(5^{nH} \times 2/5^{nH} + S_{0.5}nH)$, where k_{cat} is the turnover rate, $S_{0.5}$ is the concentration of glucose needed to achieve the half-maximal rate of phosphorylation, nH is Hill coefficient for cooperativeness with glucose, and ${}^{ATP}K_m$ is the ATP concentration required for glucokinase activity to be half maximum when glucose is in excess (6). This number indicates the in situ phosphorylation capacity of the enzyme at 5 mmol/l blood glucose. An intracellular ATP concentration of 2.5 mmol/l was assumed. The relative activity index was normalized to a basal blood glucose of 5 mmol/l to account for glucokinase expression.

Mathematical modeling. A minimal mathematical model was used to assess the impact of the G264S, IVS8 + 2T→G, and A378V mutations of glucokinase, in both the homozygous and heterozygous state, on the glucose-stimulated insulin secretion rate (GSIR) (6,16). The modeling was modified to account for adaptation of both alleles in homozygous and heterozygous cases by using the theoretically plausible expression $(S\text{nH} \times 2)/(S\text{nH} + S_{0.5}{}^{\text{nH}})$, rather than an empirical factor of 0.2 per mmol/l glucose change. S is the glucose level at threshold and "2" indicates that half-maximal induction is achieved at glucose $S_{0.5}$.

Structural analysis. A predicted model of the three dimensional structure of wild-type human glucokinase (1 glk; Rasmol Windows version 2.7.2.1) was used to inspect the spatial relations for the previously published homozygous missense mutations causing PNDM (M210K and T228M) (4,9) together with the present missense mutations G264S and A378V.

RESULTS

Clinical data.

Family 1. Family 1 is presented in Fig. 1, Table 1, and a preliminary report (7). The Turkish proband (T1-1) of central Anatolian (Caucasian) ancestry was a girl born at 33 weeks of gestation after a pregnancy complicated with oligohydramnios. Her birth weight was 1,550 g (10th centile) and her birth length was 43.5 cm (25th−50th centile). As a neonate, this girl suffered from respiratory failure that required intermittent mechanical ventilation. Hyperglycemia (13.4 mmol/l [241 mg/dl]) was present on the first day of life and increased rapidly to 24.6 mmol/l (450 mg/dl), after which insulin was administrated intravenously in a dosage of 2.4 units/kg daily. She had no digestive problems. Serum C-peptide was not detectable and neither markers for type 1 diabetes (islet cell antibodies and HLA type) nor transient neonatal diabetes (uniparental disomy for chromosome 6) were found (7). At present, this 4-year-old girl weighs 16.8 kg, her height is 98.5 cm, and she is being treated with insulin (0.60 units/kg) daily. Her recent fasting serum glucose varied between 3.0 mmol/l (54 mg/dl) and 10.3 mmol/l (185 mg/dl) and her HbA_{1c} was 8.6% (reference value for HbA_{1c} in the analytic laboratory was 4–5.7%). She shows no signs of diabetic complications, and her psychomotor development has been normal. Her parents were first cousins. The mother was diagnosed with gestational diabetes at age 33 years. The mother's recent fasting serum glucose was 6.9 mmol/l (125 mg/dl) and her HbA_{1c} was 5.5%. She is presently not taking any antidiabetic medication. The father had mild diabetes diagnosed at age 39 years. His recent fasting serum glucose was 8.6 mmol/l (155 mg/dl) and his HbA_{1c} was 7.6%. His illness is treated with diet only.

Family 2. Family 2 is presented in Fig. 1, Table 1, and a preliminary report (8). These Israeli parents of Arabic ancestry were related, as the mother was the father's first cousin once removed. The male proband (IS1-1) was child number two of three siblings. He showed IUGR and was

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TABLE 1
Clinical characteristics and genotypes of subjects with permanent neonatal diabetes screened for mutations in the glucokinase gene

	Neonatal history							Present history		
ID	Parents (glucose intolerance)	Birth weight (g)	Birth weight (centile)	Gestation age (weeks)	Age at diagnosis (days)	Blood glucose* (mmol/l)	Insulin treatment (units · kg^{-1} · day^{-1})	Insulin treatment (units · kg^{-1} · day^{-1})	Glucokinase mutation	Ref.
N17-1	Both	1,670	<3	36	1	16.8	0.8	1.1	M210K/M210K	4
TO/nd-1	Both	1,650	<3	38	1	39.6	2	1.4	T228M/T228M	4
T1-1	Both	1,550	10	33	1	13.4	2.4	0.6	A378V/A378V	TR
IS1-1	Both	1,900	<3	40	11	57.0	1.2	TDU	IVS8 + 2/IVS8 + 2	TR
IS2-1	Mother*	1,870	<3	38	2	12.0	TDU	0.9	IVS8 + 2/G264S	TR
BR1	Father only	3,100	—	—	30	29.7	TDU	0.4	None	TR
R1826	Mother only	—	—	38	Birth	—	TDU	TDU	None	TR
AA	Mother only	2,400	—	38	Birth	—	—	TDU	None	TR
US4-1	Mother only	2,100	10–25	36	5	13.3	TDU	1.0	None	TR
N89-1	Father only	1,440	<3	40	1	16.1	TDU	0.6	None	TR

Blood glucose measurement given is for first measurement. *Father diseased, thus unavailable for analysis. ID, identity used in the original publication; TDU, treated but dose unknown; TR, this report; —, unknown.

born by Caesarian section after 40 gestational weeks at a birth weight of 1,900 g (<3rd centile) and a length of 45.0 cm (<3rd centile). At age 11 days, he was admitted to the hospital in a very severe condition with fever, dehydration, and a serum glucose of 57 mmol/l (1,026 mg/dl), which varied initially between 52 and 77 mmol/l (945 and 1,400 mg/dl). He was subsequently treated with insulin (1.2 units/kg) daily. No ketoacidosis was present. His further psychomotor development has been normal. GAD and insulinoma-associated protein 2 (IA2) antibodies were not detectable. The father was initially regarded as healthy. During the investigation, however, it was revealed that he had mild diabetes with fasting serum glucose of 8.4 mmol/l (151 mg/dl). His HbA_{1c} was 6.3% (reference value for HbA_{1c} in the analytic laboratory was 4–6.3%). During her pregnancies, the mother had gestational diabetes that was treated with diet alone. Presently, she has diabetes with a fasting serum glucose of 7.7 mmol/l (139 mg/dl) and an HbA_{1c} of 6.2%. The proband's elder brother (36 weeks' gestation, birth weight 1,950 g [<3rd centile]) had a recent fasting serum glucose of 7.2 mmol/l (130 mg/dl), whereas his sister (born at term, birth weight 4,300 g [>97th centile]) had a recent fasting serum glucose of 5.3 mmol/l (95 mg/dl).

Family 3. The male proband (IS2-1) was number three of five siblings (8). His mother was both first and third cousin of the father of the proband in Family 2, and she was also the third cousin once removed of the mother of the proband in Family 2 (Fig. 2). The proband's parents were not known to be related. The proband presented with IUGR and was born by vaginal delivery at a gestational age of 38 weeks. His birth weight was 1,870 g (<3rd centile) and his birth length was 44 cm (3rd centile) (Table 1). At age 2 days, he had hyperglycemia (12.0 mmol/l [216 mg/dl]) without ketoacidosis. His psychomotor development has been normal. He was treated with insulin from day 3 of life. At present, he is age 18 years and is treated with insulin daily (0.9 units/kg). His HbA_{1c} is presently 9.4% (reference value for HbA_{1c} in the analytic laboratory was 4–6.3%). The father died at age 49 years from hepatic failure, but had no history of diabetes. His mother was diagnosed with gestational diabetes during her pregnancies. She is now age 56 years and has developed manifest diabetes (fasting serum glucose 10.1 mmol/l [182 mg/dl]; HbA_{1c} 7.0%). Her diabetes is being treated with diet. The siblings had birth weights of 3,550 (55th centile), 4,300 (96th centile), 2,450 (6th centile), and 3,260 g (45th) centile), and their recent fasting serum glucose levels were 5.6, 5.5, 6.8, and 6.0 mmol/l (101, 99, 122, and 108 mg/dl), respectively.

Screening for the glucokinase gene. The clinical features of the probands (Table 1) could suggest a diagnosis of glucokinase-related PNDM, so we screened them for mutations in this gene.

Family 1. In this family, we screened for *GCK* by direct sequencing and found a novel missense mutation in exon 9 (nucleotide 1,139: G*C*T to G*T*T) of *GCK*, resulting in the substitution of alanine for valine at amino acid residue 378 of the glucokinase protein (designated c.1,139 C→T, A378V). Residue 378 is strictly conserved among glucokinase enzymes from man to *Drosophila*. The proband was homozygous, whereas her parents were heterozygous for the mutation. The mutation was not found in 91 individuals of Norwegian ancestry.

Families 2 and 3. Because of the known relationship between families 2 and 3 (Fig. 1), we first performed a linkage analysis using four microsatellite markers for the *GCK* gene (Fig. 2). Because the haplotype pattern suggested a homozygous and a heterozygous *GCK* mutation in the probands of families 2 and 3, respectively; we therefore sequenced this gene in all available samples. In family 2, we identified a splice site mutation in the second nucleotide of the donor splice site of exon 8, designated IVS8 + 2T→G. The proband was homozygous and the parents were heterozygous for this mutation. Family 3 was related to family 2 through the mother of the proband in family 3. The mother had inherited family 2's specific mutation IVS8 + 2T→G. The proband shared this mutation in addition to another *GCK* mutation in exon 7 (nucleotide 790: −*G*GC to −*A*GC), resulting in the substitution of glycine for serine at amino acid residue 264 of the glucokinase protein (designated c.790 G→A, G264S). Residue 264 is strictly conserved from man to *Drosophila*.

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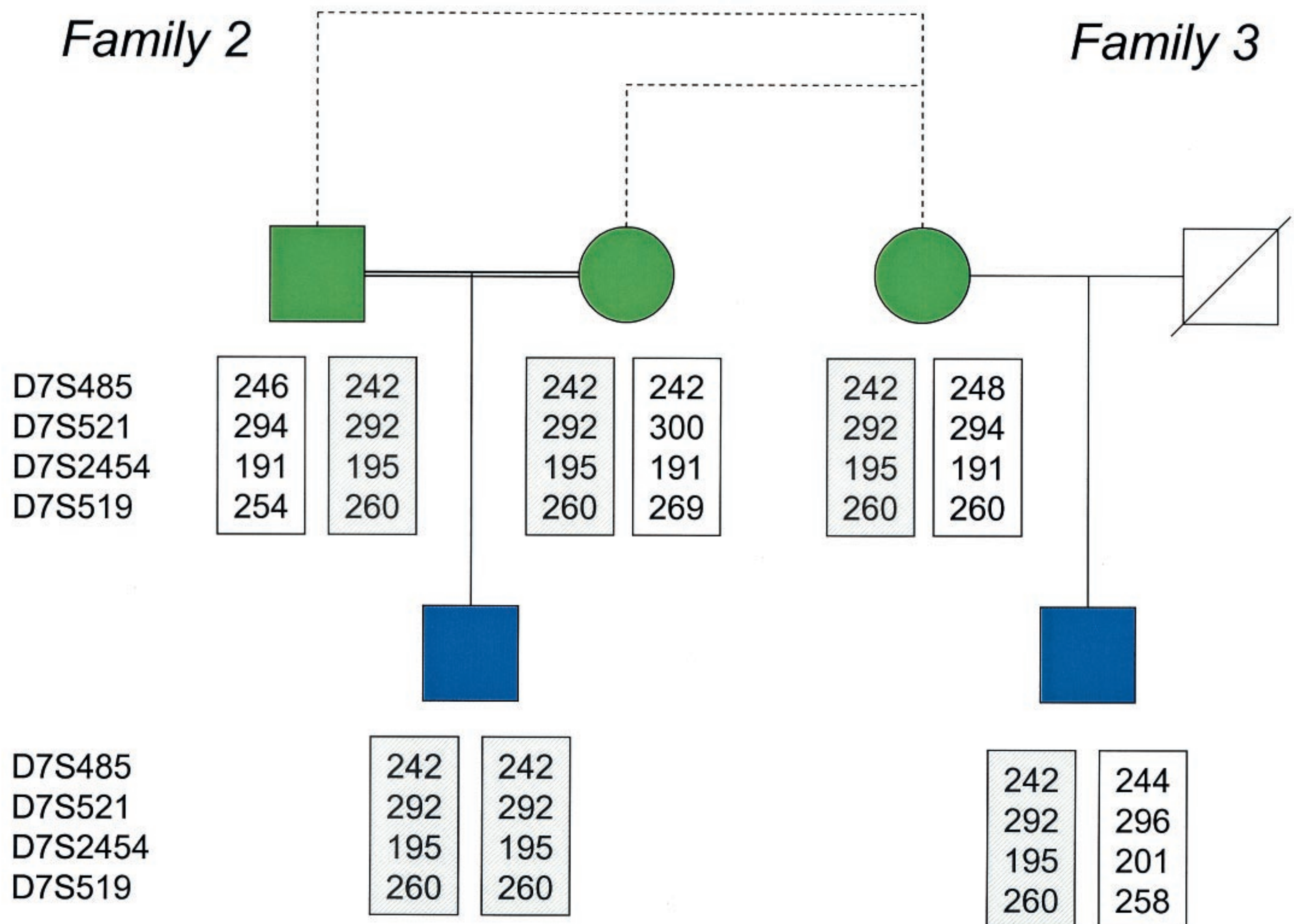

FIG. 2. Haplotypes from the *GCK* region of chromosome 7p15.3-p15.1, determined as allele sizes of microsatellite markers, are listed for the probands and their living parents of families 2 and 3. The haplotype distribution of the chromosomal segment defined by the markers *D7S485*, *D7S521*, *D7S2454*, and *D7S519* shows that families 2 and 3 share a chromosomal region, the proband of family 2 is homozygous, and the proband of family 3 is heterozygous for this chromosomal segment. Circles, females; squares, males; green, diabetes (WHO criteria); blue, PNDM.

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Neither IVS8 + 2T→G nor G264S were identified in 91 Norwegian subjects.

Other cases of neonatal diabetes. We screened five other patients with PNDM (clinical details given in Table 1). Pathogenic mutations in *GCK* were not identified in any of these patients.

Kinetic analysis of recombinant glucokinase. We prepared recombinant wild-type, A378V and G264S glucokinase in *E. coli* and compared the kinetic properties of the purified GST fusion proteins (Table 2) by previously described methods (6,9). The A378V proteins had a relative activity index (I_{GKB}) that was only 0.2% of that of wild-type glucokinase. The k_{cat} of A378V glucokinase was practically the same as that of the wild-type, and the glucose $S_{0.5}$ was increased 76-fold. $^{ATP}K_m$ was increased 27-fold. In contrast, the I_{GKB} of recombinant G264S proteins was near normal (0.86 of wild-type). Hence, G264S glucokinase k_{cat} was nearly equal to that of wild-type, and the glucose $S_{0.5}$ was only moderately elevated (129% of wild-type). G264S glucokinase $^{ATP}K_m$ was 130% of wild-type glucokinase. Thus, A378V severely impairs in vitro glucokinase activity and is the likely cause of PNDM and maturity-onset diabetes of the young (MODY) in family 1. By contrast, G264S has only a modest effect on the enzyme in vitro, if any. We did not test the effect of the IVS8 + 2T→G on RNA expression or glucokinase activity, but if intron 8 is not removed there is an in-frame stop codon positioned at nucleotides 479–481 of intron 8. This would give rise to a mutant glucokinase protein of 455 amino acids lacking residues 340–465 of the normal protein, but with an addition of 160 residues encoded by intron sequences.

TABLE 2
Kinetic characteristics of wild-type and mutations A378V and G264S of glucokinase

Kinetic parameter	Wild-type	A378V	G264S
k_{cat} (s^{-1})	64.2 ± 3.06	55.4 ± 4.69	63.5 ± 2.70
$S_{0.5}$ (mmol/l)	7.56 ± 0.31	576 ± 12.1	9.76 ± 0.74
nH	1.77 ± 0.04	0.94 ± 0.02	1.57 ± 0.05
$^{ATP}K_m$ (mmol/l)	0.37 ± 0.01	9.92 ± 0.31	0.48 ± 0.05
Relative activity index	1	0.0023	0.86

Data are means ± SE and represent the means of the kinetic analyses of four independent expressions of wild-type and mutant GST-glucokinase. Note that the Hill coefficient (nH) and the relative activity index are unit less.

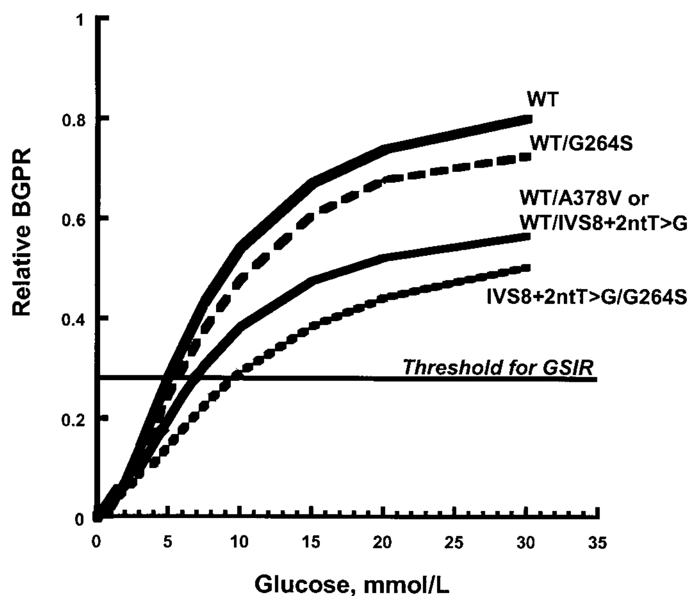

FIG. 3. Comparison of the functional properties of wild-type, G264S, IVS8 + 2T→G, and A378V glucokinase. Shown are the results of a mathematical model predicting the effect of the wild-type (WT) glucokinase, the G264S, IVS8 + 2T→G, and A378V glucokinase mutations in the heterozygous and homozygous state on BGPR and the apparent threshold of GSIR. In the homozygous disease-causing state, the threshold for GSIR cannot be achieved at the glucose concentrations that are observed with insulin treatment (i.e., based on adaptation of glucokinase to a fasting blood glucose of 20 mmol/l), thus leading to a total failure of GSIR. The compound heterozygous IVS8 + 2T→G/G264S (•••) shows a shift to the right and GSIR is achieved at a glucose concentration of 9.5 mmol/l. The heterozygous MODY2 state (—) shows the predicted rightward shift of GSIR from 5 to 7 mmol/l glucose typical for MODY2, but G264S (- - -) has only a subtle effect on GSIR compared to WT glucokinase (━). The horizontal line transects the different glucose dependency profiles of the relative BGPR at the respective thresholds for GSIR. The calculations are based on the kinetic data presented in Table 2.

Mathematical modeling and pathophysiological implications. A modified minimal mathematical model (9) was used to quantitate the impact of A378V and G264S on GSIR and glucose homeostasis (Fig. 3). According to this model and based on the results of the current control data set, 28.7% of the total β-cell glucose phosphorylation rate (BGPR) is necessary to initiate insulin secretion in the control subjects, with a threshold defined as 5 mmol/l. WT/IVS8 + 2T→G or WT/A378V had thresholds of 6.9 mmol/l, IVS8 + 2T→G/G264S had a threshold of 9.5 mmol/l, and WT/G264S had a threshold of 5.6 mmol/l. With 50 mmol/l glucose, there was practically no glucokinase-dependent glucose metabolism with the homozygous cases. The other affected heterozygous parents had predicted thresholds for GSIR of ~7 mmol/l, characteristic of patients with MODY2.

Structural analysis. A theoretical structural analysis of the residues of the inactivating mutations A378V and G264S, together with the previously described inactivation mutations M210K and T228 mol/l (4,9), was modeled. The residues are localized in the substrate-binding cleft (T228) or close to the active site (M210, A378) of the enzyme involved in binding glucose and MgATP (Fig. 4). The residue G264 is localized on the outer part of the enzyme and not in the immediate vicinity of the substrate binding site.

DISCUSSION

We have previously shown that PNDM can result from complete deficiency of glucokinase activity (4). That study and the results presented here indicate that glucokinase mutations leading to inactive enzyme can cause PNDM and support a description of this form of PNDM as a specific syndrome (Online Mendelian Inheritance in Man #606176). Glucokinase-related PNDM has now been described in patients from Europe (Norway and Italy) and the Middle East (Turkey and Israel) and it is therefore likely that similar cases will be found in other populations.

The clinical picture of our new patients is quite similar to that of the first two cases. The patients had moderate or severe IUGR, birth weights of 1,550–1,900 g, and severe hyperglycemia and required subsequent exogenous insulin shortly after birth. This profile fits with the key role played by glucokinase in the regulation of insulin secretion in humans with glucokinase-related diabetes (MODY2) and in mice lacking one or both *Gck* genes (10,11). That insulin is a potent fetal growth factor is illustrated by our three cases and $Gck^{-/-}$ mice, which are also born growth retarded (11). Our three patients also demonstrate that the fetal growth effect of insulin is most pronounced in the last trimester. Thus, the premature proband of family 1 had a birth weight at the 10th centile, whereas the probands of family 2 and 3 were born at term with birth weights <3rd centile. Although the missense mutation G264S had significant enzyme activity, we believe this mutation is pathogenic as the residue G264 is strictly conserved and there are other members of family 3 at risk with a MODY2 phenotype. Moreover, the compound heterozygous proband of family 3 had severe hyperglycemia at day 2 of life and a birth weight of only 1,870 g, characteristics that are compatible with two defective alleles.

In this regard, it is interesting to compare fetuses with two mutated *GCK* alleles with those having a *GCK* mutation on one allele only (12). If a normal fetus is subjected to a diabetic environment (mother heterozygous for a *GCK* mutation), the combination of intrauterine hyperglycemia and augmented fetal insulin secretion leads to a birth weight increase of ~0.5 kg. When the mother and the fetus are both heterozygous, the reduced insulin secretion in the fetus might in theory balance the effect of the maternal hyperglycemia. Hence, the fetus will have a birth weight in the normal range. Should the fetus, but not the mother, be heterozygous for a *GCK* mutation, the birth weight is lower by ~0.5 kg. If the mother is heterozygous and the infant is homozygous, as in our cases, a nonoperating glucokinase renders the infant insensitive to maternal hyperglycemia and severe IUGR may ensue (mean birth weight 1,728 g in the present three and previous two cases) (4).

Which cases of neonatal diabetes should be screened for glucokinase mutations? We would suggest screening primarily diabetic neonates with IUGR of moderate or severe degree who have glucose-intolerant parents. If these criteria are not fulfilled, the absence of glucokinase mutations would not be surprising (Table 1) (13–15). It is noteworthy that four of the five cases of glucokinase deficiency had hyperglycemia within the first 2 days of life, illustrating the insulin secretion defect subsequent to the glucokinase deficiency. The proband of family 2 was

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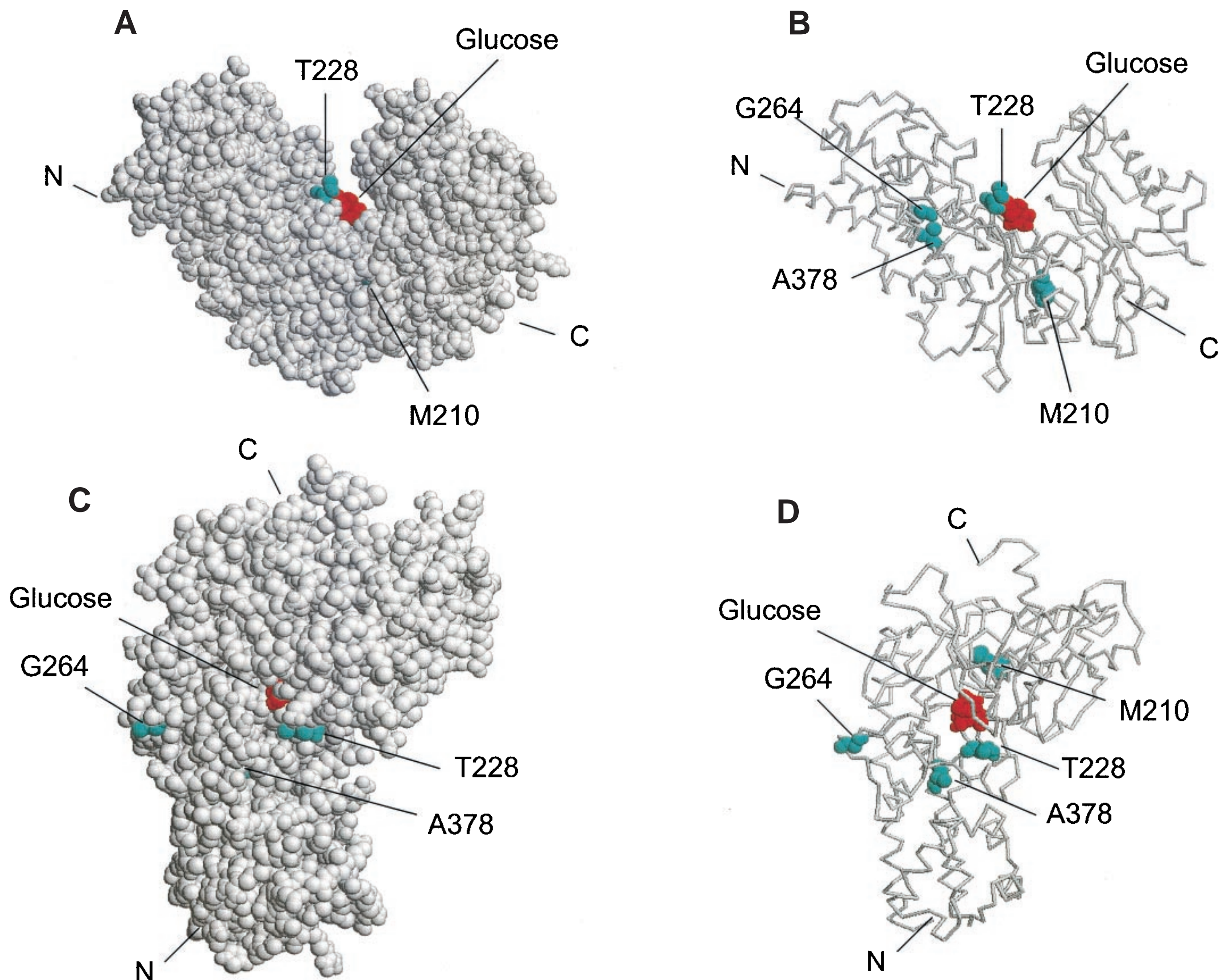

FIG. 4. Structural aspects of mutations associated with PNDM. In the models of the predicted glucokinase three-dimensional structure, the amino acid residues are represented by space-filled molecules (*A* and *C*) or backbone structures (*B* and *D*), whereas the ligand glucose is illustrated as space filled molecule (●). The residues of the three inactivation mutations (●, space fill) are localized in the substrate-binding cleft (T228, *A* and *C*), or close to the active site (M210 and A378, *B* and *D*), of the enzyme. The residue of the mutation G264S (●, space fill) is, however, present on the outer part of the enzyme suggesting near normal enzymatic activity (*C*). The NH_2- and COOH-terminals are indicated with N and C, respectively. *A* and *B* are oriented in the same way, as are *C* and *D*.

referred to the hospital in a very severe situation at day 11, suggesting he had hyperglycemia shortly after birth as well.

Is it possible to predict the phenotype of patients with glucokinase mutations from the genotypes? We believe our patients with homozygous or compound heterozygous *GCK* mutations are important for understanding more of the mechanisms for glucokinase as the glucose sensor. The corresponding residues of the missense mutations M210K, T228M, and A378V are localized either in the cleft leading to or close to the active site of the enzyme (Fig. 4). Thus the effect on the enzyme activity can be assumed to be dramatic and so the phenotype of the patient. In contrast, even though the proband of family 3 had PNDM, his recombinant glucokinase G264S had near normal enzyme activity. Because of its near normalcy, thermal stability tests (16) were also performed (data not shown). Thus, glucokinase G264S was indistinguishable from wild-type and A53S glucokinase (both thermostable under the test conditions) in contrast to the established thermolabile mutant E300K, which was used in parallel as a positive control (16). In this connection, it is puzzling why ~10% of the glucokinase mutations identified in diabetic patients are enzymatically normal (16). Why the patients have hyperglycemia at all given their near normal enzyme activities is not yet clear. Clearly, glucokinase may have other roles in the regulation of insulin secretion. There might be other unknown factors interacting with glucokinase that may be involved in the stimulation of insulin release from the granule (17). Alternatively, the mutations could indicate an impaired surface binding site to interacting proteins in this region.

Glucokinase deficiency may be regarded as a recessively inherited inborn error of metabolism, with heterozygous carriers having a mild phenotype (MODY2) (18) and homozygous carriers being associated with PNDM, as a particular severe phenotype.

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