

Brief Report

NEONATAL DIABETES MELLITUS
DUE TO COMPLETE GLUCOKINASE
DEFICIENCY

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DIABETES mellitus is a heterogeneous disorder that can occur at any age.¹ Neonatal diabetes mellitus, defined as insulin-requiring hyperglycemia within the first month of life, is a rare disorder that is usually associated with intrauterine growth retardation.² Like diabetes in general, neonatal diabetes is heterogeneous and can be either transient or permanent. Transient neonatal diabetes is associated with abnormalities of chromosome 6,^{2,3} whereas mutations in insulin promoter factor 1 result in pancreatic agenesis and permanent neonatal diabetes.⁴ We describe two patients in whom complete deficiency of the glycolytic enzyme glucokinase, a key regulator of glucose metabolism in pancreatic beta cells that couples extracellular glucose to insulin secretion, caused permanent neonatal diabetes.⁵

CASE REPORTS

Subject 1

A baby girl of Norwegian ancestry was delivered by cesarean section at 36 weeks' gestation (birth weight, 1670 g; length, 42 cm) because of poor fetal growth (Subject III-7 in Family 1 in Fig. 1).⁶ Her parents were first cousins, and both had glucose intolerance. In addition to being small for gestational age (less than the 3rd percentile), the infant had total situs inversus. On the first day of life, her blood glucose concentration was 145 mg per deciliter (8.1 mmol per liter), and on day 2 it was 300 mg per deciliter (17 mmol

per liter), at which time treatment with insulin was started. The initial insulin requirement was 0.75 U per kilogram of body weight per day. Blood glucose control was difficult to achieve, and there were large variations in blood glucose concentrations (range, 35 to 630 mg per deciliter [2 to 35 mmol per liter]), but no ketosis. Tests for antibodies against insulin, glutamic acid decarboxylase, and protein tyrosine phosphatase-like molecule IA-2 (a major target antigen of cytoplasmic islet-cell antibodies) were negative. Basal and glucagon-stimulated serum C-peptide concentrations were nearly undetectable on several occasions. Plasma glucagon concentrations were within the normal range. The girl had no digestive problems.

When the girl was five years old, epilepsy developed, probably as a sequela of a neonatal brain abscess, and she subsequently had mild learning and behavioral difficulties. Her motor development was normal. At the age of 15 years, the glycemic response to glucagon was normal. Her sister (Subject III-6 in Fig. 1) presented with typical type 1 diabetes at the age of seven years. Her mother (Subject II-7 in Fig. 1) was given a diagnosis of gestational diabetes at the age of 25 years. Her father (Subject II-6 in Fig. 1) had impaired fasting glycemia that was treated with diet.

Subject 2

An eight-year-old girl of Italian ancestry (Subject III-1 in Family 2 in Fig. 2) had had hyperglycemia (blood glucose, 715 mg per deciliter [40 mmol per liter]) and marked growth retardation when she was born at 38 weeks' gestation (birth weight, 1650 g — less than the 3rd percentile). She had been treated with insulin since birth, initially with a dose of 2 U per kilogram per day and currently with a dose of 1.4 U per kilogram per day. No diabetes-related antibodies (i.e., against insulin or glutamic acid decarboxylase) were detected. Basal serum C-peptide concentrations were low at birth, declined further with age, and did not increase in response to glucagon. The girl had no evidence of diabetic complications. Her mother had impaired fasting glycemia, and her father had impaired glucose tolerance.

METHODS

The studies were approved by the ethics committees of each institution and performed according to the Declaration of Helsinki. Written informed consent was obtained from all subjects or their parents.

Molecular Genetic Studies

The exons, flanking introns, and promoter regions of the genes encoding hepatocyte nuclear factors 1 α and 4 α , insulin promoter factor 1, the NK-2 (drosophila) homeobox homologue 2, neurogenic differentiation factor 1–beta-cell E-box transactivator 2, and glucokinase were screened for mutations in members of Family 1 by direct sequencing of polymerase-chain-reaction (PCR) products. The genes for insulin promoter factor 1, NK-2 homeobox homologue 6, and glucokinase were screened for mutations in Subject III-1 in Family 2 by single-strand conformation polymorphism analysis (in the case of insulin promoter factor 1) or denaturing gradient gel electrophoresis (in the case of the other two genes) and sequencing of any PCR products with altered mobility.

Kinetic Analysis of Recombinant Wild-Type and Mutant Glucokinase

Wild-type and mutant forms of human beta-cell glucokinase were expressed in *Escherichia coli*, and the kinetic properties of the purified proteins in the presence of 2 mmol of dithiothreitol per liter of reaction mixture were determined as described previously.⁵ We used nonlinear kinetics according to the Hill equation. The relative-activity index was used as a measure of the glucose phosphorylation capacity of the enzyme.⁵ We normalized this relative-activity index to a value of 90 mg of basal glucose per deciliter (5 mmol per liter) with the use of the expression coefficient $(5^b \times 2) \div (5^b + S_{0.5}^b)$, where b is the Hill coefficient (a coefficient that characterizes the sigmoidal glucose dependency of glucokinase) and $S_{0.5}$ is the glu-

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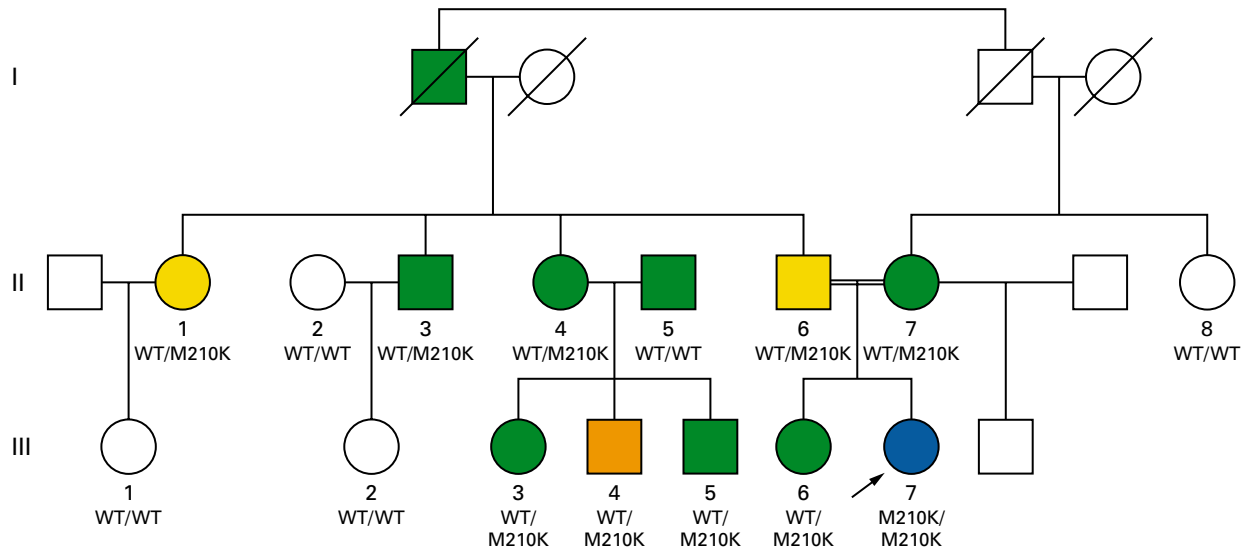


Figure 1. Pedigree of Family 1.

The subjects who were studied in each generation are numbered. Multiple forms of diabetes were present, including glucokinase-related maturity-onset diabetes of the young (those who were heterozygous for the M210K mutation in the glucokinase gene), type 1 diabetes (Subjects III-6 and II-7), and type 2 diabetes (Subject II-5). The proband (Subject III-7), who had permanent neonatal diabetes and total situs inversus (blue symbol), is indicated by the arrow. Diabetes (green symbols), impaired fasting glycemia (yellow symbols), and impaired glucose tolerance (orange symbol) were defined according to the criteria of the World Health Organization.¹ None of the subjects with diabetes had evidence of nephropathy. WT denotes the wild-type allele.

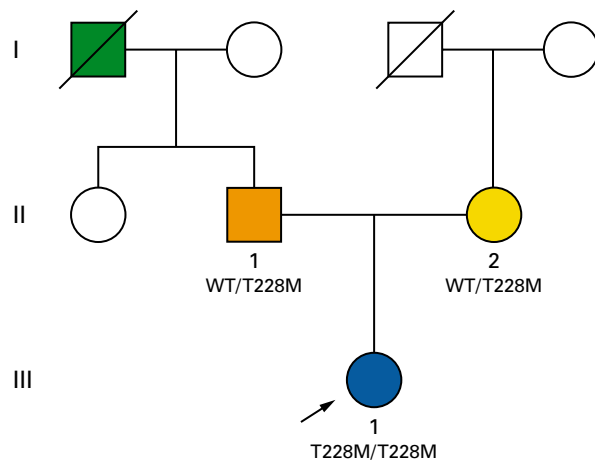


Figure 2. Pedigree of Family 2.

The subjects who were studied in each generation are numbered. The proband (Subject III-1), who had permanent neonatal diabetes (blue symbol), is indicated by the arrow. Her paternal grandfather had diabetes (green symbol), her mother had impaired fasting glycemia (yellow symbol), and her father had impaired glucose tolerance (orange symbol), all of which were defined according to the criteria of the World Health Organization.¹ The proband was homozygous for the T228M mutation in the glucokinase gene, and her parents were heterozygous. WT denotes the wild-type allele.

cose concentration required for glucokinase activity to be half maximal in the presence of 5 mmol of ATP per liter in the case of wild-type glucokinase and 10 mmol of ATP per liter in the case of mutant glucokinase.

Mathematical Modeling

We used a minimal mathematical model to assess the effect of the substitution of lysine for methionine at position 210 (M210K) and the substitution of methionine for threonine at position 228 (T228M) of glucokinase, in both the homozygous and heterozygous state, on the glucose-stimulated rate of insulin secretion.⁵ We modified the model to account for the adaptation of both mutant proteins in the homozygous and the heterozygous state to a change in the basal glucose concentration by using the theoretically plausible expression $(G_B^h \times 2) \div (G_B^h + S_{0.5}^h)$, where G_B is the basal glucose concentration (i.e., 90 mg per deciliter [5 mmol per liter] for the controls, 125 mg per deciliter [7 mmol per liter] for persons with one affected allele, and 360 mg per deciliter [20 mmol per liter] for the patients with permanent neonatal diabetes mellitus).

RESULTS

The clinical features of the two subjects with permanent neonatal diabetes (Subject III-7 in Family 1 and Subject III-1 in Family 2) suggested that they had a profound defect in beta-cell function. We therefore screened genes known to have a key role in the development and function of beta cells.⁷ In Subject III-7, we found no mutations in the genes encoding hepatocyte nuclear factor 1 α or 4 α , insulin promoter

factor 1, neurogenic differentiation factor 1, or the NK-2 homeobox homologue 2. However, we did detect a novel missense mutation in exon 6 (codon 210 and complementary DNA nucleotide 629; from ATG to AAG; Genbank accession number, M88011) of the glucokinase gene that resulted in the substitution of lysine for methionine at amino acid residue 210 (M210K). The proband was homozygous for this mutation and her parents and sister were heterozygous. The mutation cosegregated with diabetes or hyperglycemia in other family members (Fig. 1), and it was not found in 50 normal adults of Norwegian ancestry. These results suggested that in this family, a heterozygous M210K mutation caused the type 2 or glucokinase-related form of maturity-onset diabetes of the young and a homozygous M210K mutation caused permanent neonatal diabetes.

Subject III-1 in Family 2 was homozygous for a missense mutation in exon 7 (codon 228 and complementary DNA nucleotide 683; from ACG to ATG) of the glucokinase gene that resulted in the substitution of methionine for threonine at amino acid 228 (T228M). This mutation has been found previously in a family with glucokinase-related maturity-onset diabetes of the young.⁸ The girl's parents, although not known to be related, were heterozygous for this mutation (Fig. 2). Preliminary molecular genetic studies suggest that there was a founder effect for this mutation in the parents.

Kinetic Analysis of Recombinant Glucokinase

We prepared recombinant wild-type glucokinase, glucokinase with the M210K mutation, and glucokinase with the T228M mutation in *E. coli* and compared the kinetic properties of the purified proteins (Fig. 3).^{5,9} The relative activity of the protein with the M210K mutation and the protein with the T228M mutation was 0.16 and 0.05 percent, respectively, of that of wild-type glucokinase. The turnover rate of the glucokinase with the M210K mutation was 32 percent of that of the wild-type enzyme, the $S_{0.5}$ was increased by a factor of 5, and the ATP concentration required for glucokinase activity to be half maximal when glucose is in excess was increased by a factor of 3.9. The catalytic activity of the glucokinase with the T228M mutation was 0.008 percent of that of the wild-type enzyme, the $S_{0.5}$ was 72 percent of that of the wild type, and the ATP concentration required for glucokinase activity to be half maximal when glucose is in excess was increased by a factor of 1.8. Thus, M210K and T228M are both inactivating mutations, suggesting that they are the cause of diabetes in Families 1 and 2, respectively.

Mathematical Modeling and Pathophysiologic Implications

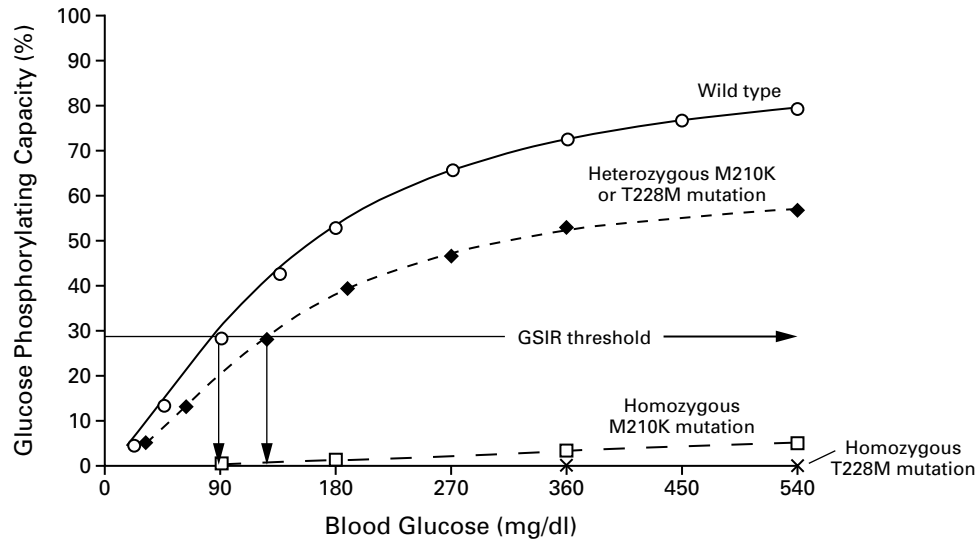
We used a modified minimal mathematical model⁵ to assess the effect of the M210K and T228M mu-

tations on the glucose-stimulated rate of insulin secretion and glucose homeostasis (Fig. 3). According to this model, 29 percent of the total glucokinase phosphorylating capacity of the beta cell is necessary to initiate insulin secretion in normal subjects, with the threshold defined as 90 mg per deciliter (5 mmol per liter). In the proband who was homozygous for the M210K mutation and the proband who was homozygous for the T228M mutation, this critical threshold is not reached even when the beta cell has adapted to a glucose concentration of 360 mg per deciliter. The parents of these subjects are heterozygous for either the M210K or the T228M mutation and had predicted thresholds for the glucose-stimulated rate of insulin secretion of about 125 mg per deciliter (7 mmol per liter), which is characteristic of patients with glucokinase-related maturity-onset diabetes of the young.

DISCUSSION

We found that permanent neonatal diabetes can result from a complete deficiency of glucokinase activity. This finding is perhaps not surprising, considering the key role of glucokinase in the regulation of insulin secretion in humans with glucokinase-related maturity-onset diabetes of the young and in mice that lack one or both glucokinase alleles.^{3,9-12} Other mammalian hexokinases, such as hexokinase I, II, and III, which are characterized by a high affinity for glucose and unlike glucokinase are subject to feedback control, are no substitute for glucokinase, primarily because they are kinetically unsuited to serve as a glucose sensor for pancreatic beta cells.¹³ In contrast to patients with glucokinase-related maturity-onset diabetes of the young, who have a partial deficiency of glucokinase resulting in mild fasting hyperglycemia, our two subjects with permanent neonatal diabetes as a result of a complete deficiency of glucokinase had severe hyperglycemia and required insulin treatment soon after birth. In this regard, mice lacking glucokinase have growth retardation and hyperglycemia at birth and die soon thereafter. These mice also have hypertriglyceridemia, hepatic steatosis, and reduced stores of hepatic glycogen, abnormalities that were not present in the two subjects we studied. Subject III-7 in Family 1 had no obvious defect of hepatic glycogen storage and had a normal glycemic response to the administration of glucagon, a measure of the ability of the liver to store glycogen and to mobilize glucose.

The total absence of basal insulin release in our subjects with glucokinase-related permanent neonatal diabetes is unexplained. Why does basal insulin release, which normally contributes as much as half of the daily insulin output, cease even though it could be stimulated by other fuels (e.g., amino acids and fatty acids) and potentiated by hormones and neurotransmitters (e.g., glucagon-like peptide 1, gastric inhibitory peptide, and acetylcholine)?¹³ Systematic studies of the insulin secretory response in the two subjects



VARIABLE	WILD TYPE	M210K MUTATION	T228M MUTATION
Turnover rate (sec ⁻¹)	52.4±5.7	16.7±1.6	0.004±0.0004
Glucose S _{0.5} (mmol/liter)	7.7±0.1	38.7±1.9	5.5±1.5
Hill coefficient	1.66±0.00	1.63±0.04	0.71±0.07
ATP _{K_m} (mmol/liter)	0.35±0.01	1.38±0.11	0.62±0.16

Figure 3. Comparison of the Modeled Functional Properties of Wild-Type Glucokinase, Glucokinase with the M210K Mutation, and Glucokinase with the T228M Mutation.

The graph shows the results of mathematical modeling to predict the effect of wild-type glucokinase and glucokinase with heterozygous and homozygous M210K and T228M mutations on the rate of phosphorylation of glucose by beta cells and the threshold for glucose-stimulated insulin release (GSIR). In the presence of a homozygous M210K mutation or a homozygous T228K mutation, the threshold for glucose-stimulated insulin release cannot be reached at the blood glucose concentrations achieved during insulin treatment (because of the limited adaptation of glucokinase to a blood glucose concentration of 360 mg per deciliter), thus leading to a total failure of the system of glucose-stimulated insulin release. In the heterozygous state, there is a rightward shift of the threshold for glucose-stimulated insulin release from 90 to 125 mg per deciliter (5 to 7 mmol per liter). All kinetic data are mean (±SE) values for three different enzyme preparations. The turnover rate was obtained by increasing the glucose concentration in a stepwise fashion in the presence of a constant concentration of ATP (5 mmol per liter in the case of wild-type glucokinase and 10 mmol per liter in the case of mutant glucokinase) and then calculating the maximal velocity. The Hill coefficient for cooperativity characterizes the sigmoidal glucose dependency of glucokinase. S_{0.5} denotes the glucose concentration required for glucokinase activity to be half maximal in the presence of 5 mmol of ATP per liter in the case of wild-type glucokinase and 10 mmol of ATP per liter in the case of mutant glucokinase, and ATP_{K_m} denotes the ATP concentration required for glucokinase activity to be half maximal when glucose is in excess.⁷ To convert values for glucose to millimoles per liter, multiply by 0.056.

may provide a better understanding of the role of glucose and other secretagogues in the regulation of insulin secretion.

The molecular basis for the situs inversus in Subject III-7 in Family 1 is unknown. The absence of situs inversus in Subject III-1 in Family 2 and in glucokinase-deficient mice suggests that it is not a consequence of glucokinase deficiency. Genetic factors have a role in determining left-right asymmetry,¹⁴ and Subject III-7 in Family 1 may have a mutation in a gene involved in this process.

Neonatal diabetes is a rare disorder with an estimated incidence of 1 in 400,000 live births.² Although the true prevalence of glucokinase mutations is un-

known, glucokinase-related maturity-onset diabetes of the young appears to be relatively common and underdiagnosed, because the majority of carriers do not have clinical diabetes.¹⁵ The frequency of glucokinase mutations may also be higher in some populations because of a founder effect.¹⁶ Thus, complete glucokinase deficiency could be the cause of a substantial proportion of cases of permanent neonatal diabetes. Mutations in this gene should be sought in infants with neonatal diabetes, especially if some first-degree relatives have glucose intolerance.

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