

Brief Report

FAMILIAL HYPERINSULINISM CAUSED BY AN ACTIVATING GLUCOKINASE MUTATION

BENJAMIN GLASER, M.D., PREBAKARAN KESAVAN, PH.D.,
MOZHGAN HEYMAN, ELIZABETH DAVIS, M.B., B.S.,
ANTONIO CUESTA, M.D., PH.D., ANDREAS BUCHS, M.D.,
CHARLES A. STANLEY, M.D.,
PAUL S. THORNTON, M.B., B.Ch.,
M. ALAN PERMUTT, M.D., FRANZ M. MATSCHINSKY, M.D.,
AND KEVAN C. HEROLD, M.D.

SPONTANEOUS hyperinsulinemic hypoglycemia in adults is most frequently caused by sporadic, solitary pancreatic beta-cell tumors, whereas hyperinsulinemic hypoglycemia in childhood is commonly caused by generalized beta-cell dysfunction.¹ Mutations in the beta-cell sulfonylurea-receptor (*SURI*) gene or inward-rectifying potassium-channel (*Kir6.2*) gene were found in some patients.²⁻⁷ A distinct syndrome of hyperinsulinism with hyperammonemia was recently described,^{8,9} apparently caused by mutations in the glutamate dehydrogenase gene.¹⁰ However, many sporadic and familial cases of hyperinsulinism remain unexplained. Some may be due to somatic mutations in other genes, as suggested by reports of autosomal dominant familial hyperinsulinism that was not genetically linked to the *SURI* or *Kir6.2* locus.^{11,12}

Glucokinase, a hexokinase with a low affinity for glucose, controls the rate-limiting step of beta-cell glucose metabolism and is responsible for glucose-mediated regulation of insulin secretion.¹³ Loss-of-function mutations in this gene are associated with maturity-onset diabetes of the young,^{14,15} which is characterized by decreased glucose phosphorylation and decreased insulin secretion.¹⁶

In this report, we describe a unique mutation in the glucokinase gene that caused autosomal dominant fa-

miliar hyperinsulinism. These findings confirm the importance of glucokinase as the primary regulator of glucose-controlled insulin secretion in beta cells.

CASE REPORT

Preliminary clinical and genetic data on the study family have been reported previously.¹² The proband (Subject II-3 in Fig. 1), a 31-year-old white man, was seen after losing consciousness after breakfast; his plasma glucose concentration was 38 mg per deciliter (2.1 mmol per liter). During the preceding year he had noted tiredness, weakness, hunger, and shakiness in midmorning that were relieved by eating foods containing carbohydrates. Hypoglycemia with hyperinsulinemia was documented while the patient was fasting (Fig. 1). Counterregulatory hormone responses to hypoglycemia (plasma glucose, 31 mg per deciliter [1.7 mmol per liter]) were normal.¹⁷ The results of clinical and laboratory evaluations were otherwise normal, as were those of pancreatic computed tomography and magnetic resonance imaging.

The patient had two children (Subjects III-3 and III-4), both of whom had nonketotic hypoglycemic seizures with inappropriate hyperinsulinemia (Fig. 1). Measurements of urinary amino acids and urinary and plasma carnitines were normal, as were the results of pancreatic ultrasonography.

The proband's sister (Subject II-2), who was 36 years old and had multiple sclerosis, had been given a diagnosis of hypoglycemia at the age of 15 years. During fasting she had hypoglycemia and inappropriately elevated plasma insulin and C-peptide concentrations (Fig. 1). Her children (Subjects III-1 and III-2) were asymptomatic and normoglycemic.

The proband's father (Subject I-1) reported symptoms of hypoglycemia controlled by diet throughout adolescence and early adulthood. At the age of 48 years insulin-requiring diabetes mellitus developed. None of the subjects had evidence of multiple endocrine neoplasia.

All the affected family members were treated with diazoxide (100 to 300 mg per day), with complete resolution of hypoglycemia and hypoglycemia-related symptoms.

METHODS

Metabolic Studies

Plasma glucose, insulin, C-peptide, and proinsulin responses to 75 g of oral glucose were measured in the proband and his sister. For the C-peptide-suppression test, insulin was infused (0.1 U per kilogram of body weight per hour) and plasma glucose was measured every five minutes until the concentration was below 40 mg per deciliter (2.2 mmol per liter) and glycomenic symptoms appeared. Blood was drawn for measurement of plasma C peptide, and glucose was administered intravenously. The rates of diurnal insulin secretion were determined as previously described.¹⁸

All studies were approved by the institutional review committee at the University of Illinois at Chicago, and written informed consent was given by the study subjects or their parents.

Clinical Biochemical Measurements

Plasma glucose was measured by a glucose analyzer (Yellow Springs Instrument, Yellow Springs, Ohio), C peptide by radioimmunoassay,¹⁹ insulin by a double-antibody radioimmunoassay,²⁰ and proinsulin by sandwich enzyme-linked immunosorbent assay.²¹ The lower limit of detection was 3.3 μ U per milliliter (20 pmol per liter) for insulin and 0.02 ng per milliliter (0.007 nmol per liter) for C peptide.

Identification of Mutations

Linkage to the glucokinase locus was established with two known microsatellite markers flanking the glucokinase gene.²² Single-strand conformational polymorphism (SSCP) analysis of all 10 exons and the adjacent intron-exon boundaries was per-

From the Department of Endocrinology and Metabolism, Hebrew University Hadassah Medical School, Jerusalem, Israel (B.G., M.H., A.B.); the Department of Biochemistry and Biophysics and the Diabetes Research Center (P.K., E.D., A.C., F.M.M.) and the Department of Pediatrics (C.A.S.), University of Pennsylvania School of Medicine, Philadelphia; the National Metabolic Unit, Children's Hospital, Dublin, Ireland (P.S.T.); the Division of Endocrinology, Diabetes and Metabolism, Washington University School of Medicine, St. Louis (M.A.P.); and the Department of Internal Medicine, University of Illinois at Chicago, Chicago (K.C.H.). Address reprint requests to Dr. Glaser at the Department of Endocrinology and Metabolism, Hadassah University Hospital, Jerusalem, Israel, or to Dr. Herold at the University of Illinois at Chicago, 1819 W. Polk St., M/C640, Chicago, IL 60612.

©1998, Massachusetts Medical Society.

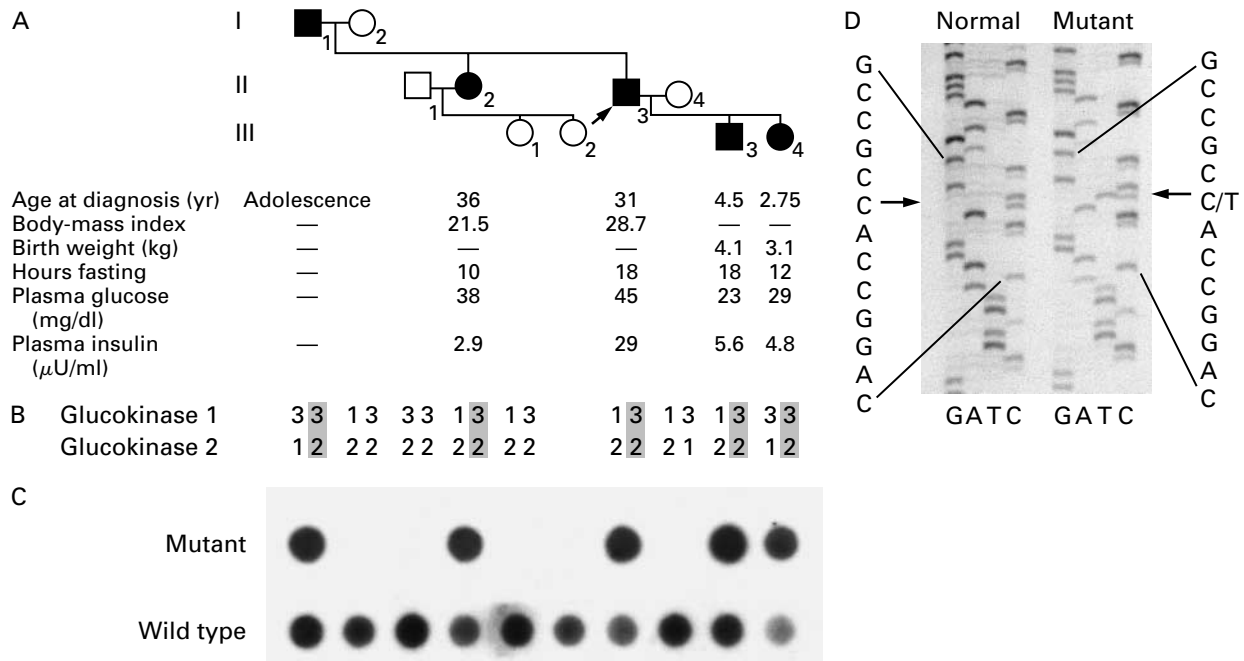


Figure 1. Studies in a Family with Hyperinsulinism.

Panel A shows the pedigree of the family and clinical data at the time of the study. The proband is indicated by the arrow. Squares denote male family members, circles female family members, and solid symbols subjects with symptomatic hypoglycemia. The body-mass index is calculated as the weight in kilograms divided by the square of the height in meters. To convert values for glucose to millimoles per liter, multiply by 0.056, and to convert values for insulin to picomoles per liter, multiply by 6. In Panel B, haplotype analysis of the glucokinase locus with the use of flanking microsatellite polymorphisms demonstrates the cosegregation of a single glucokinase allele (shaded) with the clinical syndrome. In Panel C, allele-specific hybridization of oligonucleotide probes specific for the mutant and wild-type enzymes documents the cosegregation of the mutation with the clinical syndrome. In Panel D, sequence analysis of the antisense strand of exon 10 of the glucokinase gene in one affected subject shows a heterozygous mutation causing the substitution of T for C at codon 455 (Val455Met).

formed with published oligonucleotide primers.²³ Samples with mobility shifts were cycle-sequenced (SequiTherm Excel, Epicentre Technologies, Madison, Wis.) after gel purification.

Allele-Specific Oligonucleotide Hybridization

Genomic DNA was amplified by the polymerase chain reaction (PCR), denatured in 0.4 M sodium hydroxide and 0.025 M EDTA, and transferred to a Zeta-Probe GT blotting membrane (Bio-Rad, Hercules, Calif.). Then, ³²P-labeled oligonucleotides, ACAGGCCACCGCCGAGA (wild-type) and TCTCGGCGATGGCCTGT (mutated), were individually hybridized to the membrane-bound DNA at 43°C, washed at 54°C, and exposed to x-ray film overnight at -80°C.

Site-Directed Mutagenesis of Glucokinase Complementary DNA

Two methods were used to introduce the mutation into the wild-type islet glucokinase. The first used splicing by overlap extension.²⁴ An internal primer was annealed at position 1814, introducing an A in place of G at base 1822. The PCR product containing the mutation was ligated to the 5' coding fragment of wild-type islet glucokinase. In the second procedure, performed by Dr. M. Magnuson (Vanderbilt University, Nashville), the Val455Met mutation was introduced into human glucokinase complementary DNA with the method of Kunkel et al.²⁵ The mutated 21-bp oligonucleotide had one altered base at position 11. After mutagenesis and sequence confirmation, the wild-type and

mutant fragments were expressed as fusion products with a 26-kD *Streptomyces japonicum* glutathione S-transferase C-terminal protein fragment and purified to near-homogeneity by single-step affinity chromatography with glutathione-agarose (Sigma, St. Louis). This approach proved reliable in a study of glucokinase mutations in patients with maturity-onset diabetes of the young.¹⁶

Kinetic Analysis of Recombinant Wild-Type and Mutant Glucokinase

Kinetic constants were determined spectrophotometrically as described previously.^{16,26} We applied nonlinear kinetics using the Hill equation.²⁶ The effect of glucokinase regulatory protein was studied with methods described by Vandercammen and Van Schaftingen.²⁷ Because there were no apparent differences in the kinetic constants of samples of recombinant glucokinase prepared by the two procedures described above, the data were combined.

RESULTS

Clinical Studies

Oral glucose-tolerance tests in the proband and his sister (Table 1) showed hypoglycemia during fasting, normal glucose tolerance, and reactive hypoglycemia three hours after the ingestion of glucose. Three percent of the total plasma insulin immunoreactivity was due to proinsulin (normal, <30 percent). During

TABLE 1. RESULTS OF CLINICAL STUDIES IN THE PROBAND (SUBJECT II-3) AND HIS SISTER (SUBJECT II-2).*

TEST	SUBJECT II-2			SUBJECT II-3		
	SERUM GLUCOSE	PLASMA INSULIN	PLASMA C PEPTIDE	SERUM GLUCOSE	PLASMA INSULIN	PLASMA C PEPTIDE
	mg/dl	μ U/ml	ng/ml	mg/dl	μ U/ml	ng/ml
Oral glucose-tolerance test						
0 min	38	0.4†	0.12†	43	26.8†	0.36†
60 min	68	0.7	0.11	72	268	1.05
120 min	68	4.2	0.56	49	173	1.06
180 min	31	6.7†	0.76†	32	106†	0.83†
Prolonged fast						
1st symptoms‡	43 (22 hr)	2.0†	0.07†	45 (18 hr)	19.5†	0.37†
End of fast§	40 (25 hr)	4.9†	0.07†	40 (45 hr)	—	—

*To convert values for glucose to millimoles per liter, multiply by 0.056; to convert values for insulin to picomoles per liter, multiply by 6; and to convert values for C peptide to nanomoles per liter, multiply by 0.331.

†During hypoglycemia (plasma glucose, <45 mg per deciliter [2.5 mmol per liter]), plasma insulin and C-peptide concentrations in normal subjects are less than 1.5 μ U per milliliter (9 pmol per liter) and less than 0.03 ng per milliliter (0.01 nmol per liter), respectively.²⁸

‡Values in parentheses are the times at which the first symptoms appeared.

§Values in parentheses are the durations of the fast.

fasting (Table 1), both the proband and his sister had mildly symptomatic hypoglycemia after 18 to 22 hours; their plasma glucose concentrations did not decrease further despite continuation of the fast for 27 and 3 hours, respectively. During hypoglycemia, their plasma insulin and C-peptide concentrations were inappropriately elevated, and no sulfonylurea drugs were detected in the proband's urine.

In the proband, exogenous insulin administration resulted in a decrease in plasma glucose concentrations (from 43 to 35 mg per deciliter [2.4 to 1.9 mmol per liter]) and plasma C-peptide concentrations (from 0.21 to 0.08 ng per milliliter [0.07 to 0.03 nmol per liter]) at 35 minutes. The results in his sister were similar.

In the proband's sister, the dynamics of 24-hour insulin secretion were similar to those reported for normal subjects with similar body-mass indexes,¹⁸ the only abnormality being the low plasma glucose concentrations during fasting (49 mg per deciliter [2.7 mmol per liter]) and after meals (52 to 59 mg per deciliter [2.9 to 3.3 mmol per liter]).

Identification and Confirmation of Glucokinase Mutation

Allele segregation of polymorphic markers flanking the glucokinase gene was consistent with a dominant pattern of inheritance (Fig. 1B). An SSCP mobility shift was detected that was caused by a change in a single base, which resulted in the substitution of methionine for valine at codon 455 (Val455Met) (Fig. 1). The allele-specific oligonucleotide hybridization assay confirmed the mutation and demon-

strated cosegregation with hypoglycemia (Fig. 1C). This mutation was not found in 37 unrelated white families with hyperinsulinism, including 6 with an apparently autosomal dominant form.

Kinetic Analysis of Recombinant Glucokinase

Mutant-enzyme activity, when expressed in terms of half-maximal glucose concentration (the apparent K_m), was 65 percent lower than that of the wild-type enzyme (2.9 mM vs. 8.4 mM). In contrast, the mean (\pm SE) activity of the mutant enzyme, expressed in terms of moles of substrate phosphorylated per mole of enzyme per second (K_{cat}), was indistinguishable from that of the wild-type enzyme (54.4 ± 3.5 and 50.2 ± 4.9 mol per mole per second, respectively). Likewise, the mutant enzyme had no effect on the Hill coefficient (a measure of the effect of the interaction between glucokinase and its substrate on enzyme activity), the K_m for ATP, the glucose dependency of the K_m for ATP, and the inhibition of enzyme activity by stearyl-coenzyme A and glucokinase regulatory protein. The relative K_{cat} values and affinities with glucose, mannose, and fructose as substrates were the same for both enzymes. Thus, the Val455Met mutant resulted in only one singular abnormality: lowering of the K_m for glucose by 65 percent.

Model Studies

The pathophysiologic effect of the change in the affinity of islet glucokinase for glucose from 8.4 to 2.9 mM is best illustrated by a simple model. If we

assume that the expression of the wild-type and mutated enzymes is the same, heterozygosity for the Val455Met mutation results in a marked shift to the left (increased sensitivity) of the glucose dependency of islet glucokinase (Fig. 2). The Val203Ala mutation associated with maturity-onset diabetes of the young has the opposite effect.²⁶ In estimating the glucose threshold for insulin release in the heterozygous proband and his sister, we assumed that the glucokinase mutation was the only change in the beta cells. We based the estimate on the Hill equation,²⁶ using the specific rates at the threshold for the two alleles at 20 to 30 percent of the K_{cat} , since previous studies established that insulin release is triggered when the glycolytic rate reaches 20 to 30 percent of capacity.²⁹ The threshold of a subject who is heterozygous for the Val455Met mutation is predicted to be 2 to 2.5 mM glucose, as compared with the threshold in a normal subject (wild type/wild type) of 4 to 5 mM and the threshold of approximately 8 mM in a subject who is heterozygous for the Val203Ala mutation associated with maturity-onset diabetes of the young.

DISCUSSION

We characterized the clinical abnormalities and the biochemical and genetic causes of a subtype of autosomal dominant familial hyperinsulinism expressed in three generations of one family. Analysis of the glucokinase gene revealed a conservative missense mutation (Val455Met) that cosegregated with the disease; Val455 is not thought to be part of the glucose-binding site,³⁰ and none of the missense mutations associated with maturity-onset diabetes of the young are located in this region.¹⁵ Therefore, comprehensive kinetic characterization of the mutant enzyme was essential to confirm that this mutation caused the clinical syndrome.

When expressed *in vitro*, the Val455Met mutation increased the affinity of glucokinase for glucose. The hypoglycemia in this family can be entirely explained by this change. The increased affinity for glucose results in higher rates of glycolysis at low glucose concentrations and therefore a higher rate of insulin secretion at any plasma glucose concentration.

The control of insulin secretion was otherwise appropriate in relation to changes in plasma glucose concentrations, as expected from the proposed defect. During prolonged fasting, the subjects' plasma glucose concentrations stabilized at about 40 mg per deciliter (2.2 mmol per liter), significantly lower than normal, and did not decrease further as happens in patients with insulinomas or those with familial hyperinsulinism caused by *SUR1* or *Kir6.2* mutations,³ in whom insulin secretion is uncoupled from glucose metabolism. Our model calculations predict a pathologically low glucose threshold of 2 to 2.5 mM (36 to 45 mg per deciliter) for the

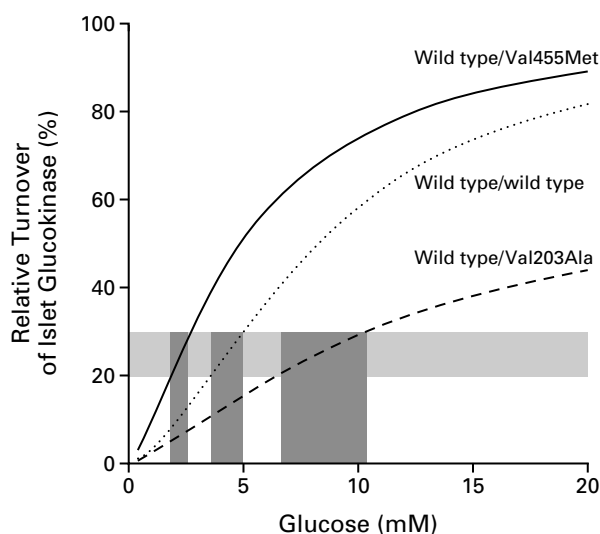


Figure 2. Model of the Glucose Dependency of Glucose Phosphorylation and of the Predicted Glucose Thresholds for Insulin Secretion in Normal Subjects (Wild Type/Wild Type), Heterozygotes with Familial Hyperinsulinism (Wild Type/Val455Met), and Heterozygotes with Maturity-Onset Diabetes of the Young (Wild Type/Val203Ala).

Kinetic constants for Val203Ala were obtained from Kesavan et al.²⁶ The glucose thresholds for insulin release for each genotype, represented by the vertical bands, correspond to 20 to 30 percent of the composite glucokinase K_{cat} (horizontal band).

stimulation of insulin release, which is reasonably close to the clinically observed values. The proband, who was the heavier of the two subjects tested, had higher plasma insulin concentrations than his sister, despite having similar plasma glucose concentrations. This finding suggests the occurrence of normal beta-cell compensation for the insulin resistance associated with obesity.

The age at onset and severity of symptoms varied markedly in this family, and we have no explanation for this variation. The long-term effects of the Val455Met mutation on beta-cell function are also unclear, but it is noteworthy that diabetes mellitus developed later in life in the oldest affected family member. Thus, eventual beta-cell failure is a possible sequela of this mutation. It is also possible that an increased affinity for glucose in liver glucokinase, which is encoded by the same gene as islet glucokinase, may increase glycogen synthesis and decrease the efficiency of gluconeogenesis.

In summary, we describe a clinically distinct syndrome of autosomal dominant familial hyperinsulinism due to a mutation of glucokinase that results in increased affinity of the enzyme for glucose. The hypoglycemia resulting from this mutation underscores the essential role of glucokinase in regulating insulin secretion as the glucose sensor of beta cells.

Supported in part by grants (DK16746, to Dr. Permutt; RR-00240, to Dr. Stanley; and DK19525 and DK22122, to Dr. Matschinsky) from the National Institutes of Health; an American Diabetes Association Mentor-Based Fellowship (to Drs. Davis and Matschinsky); a grant (194164, to Dr. Permutt) from the Juvenile Diabetes Foundation; a grant (493/00191/2, to Drs. Glaser and Permutt) from the United States-Israel Binational Science Foundation; a grant (82677, to Dr. Glaser) from the Israel Ministry of Health; and grants from the Clinical Research Center (RR-00055) and the Diabetes Research and Training Center (DK-20595) at the University of Chicago.

We are indebted to Drs. Kenneth Polonsky and Jeppe Sturis for their help in performing the analysis of insulin secretory rates.

REFERENCES

1. Stanley CA, Baker L. Hyperinsulinism in infants and children: diagnosis and therapy. *Adv Pediatr* 1976;23:315-55.
2. Thomas PM, Cote GJ, Wohllk N, et al. Mutations in the sulfonylurea receptor gene in familial persistent hyperinsulinemic hypoglycemia of infancy. *Science* 1995;268:426-9.
3. Nestorowicz A, Wilson BA, Schoor KP, et al. Mutations in the sulfonylurea receptor gene are associated with familial hyperinsulinism in Ashkenazi Jews. *Hum Mol Genet* 1996;5:1813-22.
4. Nichols CG, Shyng S-L, Nestorowicz A, et al. Adenosine diphosphate as an intracellular regulator of insulin secretion. *Science* 1996;272:1785-7.
5. Thomas P, Ye Y, Lightner E. Mutation of the pancreatic islet inward rectifier Kir6.2 also leads to familial persistent hyperinsulinemic hypoglycemia of infancy. *Hum Mol Genet* 1996;5:1809-12.
6. Thomas PM, Wohllk N, Huang E, et al. Inactivation of the first nucleotide-binding fold of the sulfonylurea receptor, and familial persistent hyperinsulinemic hypoglycemia of infancy. *Am J Hum Genet* 1996;59:510-8.
7. Nestorowicz A, Inagaki N, Gono T, et al. A nonsense mutation in the inward rectifier potassium channel gene, *Kir6.2*, is associated with familial hyperinsulinism. *Diabetes* 1997;46:1743-8.
8. Zammarchi E, Filippi L, Novembre E, Donati MA. Biochemical evaluation of a patient with a familial form of leucine-sensitive hypoglycemia and concomitant hyperammonemia. *Metabolism* 1996;45:957-60.
9. Weinzimer SA, Stanley CA, Berry GT, Yudkoff M, Tuchman M, Thornton PS. A syndrome of congenital hyperinsulinism and hyperammonemia. *J Pediatr* 1997;130:661-4.
10. Stanley CA, Lieu Y, Hsu B, Ponz M. Hypoglycemia in infants with hyperinsulinism & hyperammonemia: gain of function mutations in the pathway of leucine-mediated insulin secretion. *Diabetes* 1997;46:Suppl 1:217A. abstract.
11. Kukuvitis A, Deal C, Arbour L, Polychronakos C. An autosomal dominant form of familial persistent hyperinsulinemic hypoglycemia of infancy, not linked to the sulfonylurea receptor locus. *J Clin Endocrinol Metab* 1997;82:1192-4.
12. Thornton PS, Satin-Smith MS, Herold K, et al. Familial hyperinsulinism with apparent autosomal dominant inheritance: clinical and genetic differences from the autosomal recessive variant. *J Pediatr* (in press).
13. Matschinsky F, Liang Y, Kesavan P, et al. Glucokinase as pancreatic beta cell glucose sensor and diabetes gene. *J Clin Invest* 1993;92:2092-8.
14. Froguel P, Zouali H, Vionnet N, et al. Familial hyperglycemia due to mutations in glucokinase: definition of a subtype of diabetes mellitus. *N Engl J Med* 1993;328:697-702.
15. Froguel P, Vaxillaire M, Velho G. Genetic and metabolic heterogeneity of maturity-onset diabetes of the young. *Diabetes Rev* 1997;5:123-30.
16. Liang Y, Kesavan P, Wang LQ, et al. Variable effects of maturity-onset diabetes-of-youth (MODY)-associated glucokinase mutations on substrate interactions and stability of the enzyme. *Biochem J* 1995;309:167-73.
17. Polonsky KS, Herold KC, Gilden JL, et al. Glucose counterregulation in patients after pancreatotomy: comparison with other clinical forms of diabetes. *Diabetes* 1984;33:1112-9.
18. Polonsky KS, Given BD, Hirsch L, et al. Quantitative study of insulin secretion and clearance in normal and obese subjects. *J Clin Invest* 1988;81:435-41.
19. Faber OK, Binder C, Markussen J, et al. Characterization of seven C-peptide antisera. *Diabetes* 1978;27:Suppl 1:170-7.
20. Morgan CR, Lazarow A. Immunoassay of insulin: two antibody system: plasma insulin levels of normal, subdiabetic and diabetic rats. *Diabetes* 1963;12:115-26.
21. Hartling SG, Dinesen B, Kappelgaard AM, Faber OK, Binder C. ELISA for human proinsulin. *Clin Chim Acta* 1986;156:289-97.
22. Tanizawa Y, Chiu KC, Province MA, et al. Two microsatellite repeat polymorphisms flanking opposite ends of the human glucokinase gene: use in haplotype analysis of Welsh Caucasians with type 2 (non-insulin-dependent) diabetes mellitus. *Diabetologia* 1993;36:409-13.
23. Chiu KC, Tanizawa Y, Permutt MA. Glucokinase gene variants in the common form of NIDDM. *Diabetes* 1993;42:579-82.
24. Buchs A, Wu L, Morita H, Whitesell RR, Powers AC. Two regions of GLUT 2 glucose transporter protein are responsible for its distinctive affinity for glucose. *Endocrinology* 1995;136:4224-30.
25. Kunkel TA, Roberts JD, Zakour RA. Rapid and efficient site-specific mutagenesis without phenotypic selection. *Methods Enzymol* 1987;154:367-82.
26. Kesavan P, Wang L, Davis E, et al. Structural instability of mutant beta-cell glucokinase: implications for the molecular pathogenesis of maturity-onset diabetes of the young (type-2). *Biochem J* 1997;322:57-63.
27. Vandercammen A, Van Schaftingen E. Competitive inhibition of liver glucokinase by its regulatory protein. *Eur J Biochem* 1991;200:545-51.
28. Turner RC. Hypoglycaemia. In: Weatherall DJ, Ledingham JGG, Warrell DA, eds. *Oxford textbook of medicine*. Vol. 2. Oxford, England: Oxford University Press, 1996:1505-12.
29. Matschinsky FM. Glucokinase as glucose sensor and metabolic signal generator in pancreatic beta-cells and hepatocytes. *Diabetes* 1990;39:647-52.
30. Pilkis SJ, Weber IT, Harrison RW, Bell GI. Glucokinase: structural analysis of a protein involved in susceptibility to diabetes. *J Biol Chem* 1994;269:21925-8.