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Identification of 14 new glucokinase mutations and description of the clinical profile of 42 MODY-2 families

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Summary Mutations in glucokinase are associated with defects in insulin secretion and hepatic glycogen synthesis resulting in mild chronic hyperglycaemia, impaired glucose tolerance or diabetes mellitus. We screened members of 35 families with features of maturity-onset diabetes of the young for mutations in the glucokinase gene and found 16 different mutations. They included 14 new mutations in the glucokinase gene: 9 missense mutations (A53S, G80A, H137R, T168P, M210T, C213R, V226M, S336L and V367M); 2 nonsense mutations (E248X and S360X); a deletion of one nucleotide resulting in a frameshift (V401del1); a substitution of a conserved nucleotide at a splice acceptor site (L122-1G → T); and a 10 base pair deletion that removed the GT of the splice donor site and the following eight nucleotides (K161 + 2del10). In addition, we found two previously identified mutations: R186X and G261R. Study of 260 subjects with glucokinase-deficient hyperglycaemia from 42 families with 36 different *GCK* mutations made it possible to define the clinical profile of this subtype of non-insulin-dependent diabetes mellitus (NIDDM). Hyperglycaemia due to glucokinase deficiency is often mild (fewer than 50 % of subjects have overt diabetes) and is evident during the early years of life. Despite the long duration of hyperglycaemia, glucokinase-deficient subjects have a low prevalence of micro- and macro-vascular complications of diabetes. Obesity, arterial hypertension and dyslipidaemia are also uncommon in this form of NIDDM. [Diabetologia (1997) 40: 217–224]

Keywords Diabetes mellitus, MODY, glucokinase mutations, insulin secretion, genetics.

Maturity-onset diabetes of the young (MODY) is a form of non-insulin-dependent diabetes mellitus (NIDDM) characterized by early onset, usually before 25 years of age and often in adolescence, and by autosomal dominant inheritance [1]. Although commonly thought to be a relatively rare form of NIDDM, recent studies suggest that it may not be that uncommon and 2–5 % of patients with NIDDM may in fact have MODY [2]. Mutations in genes on chromosomes 20, 7 and 12, designated *MODY1*, *MODY2/glucokinase (GCK)* and *MODY3*, respectively, can cause this form of diabetes [3–5]. Moreover, there are likely to be additional MODY genes since there are families in which MODY does not cosegregate with markers tightly linked to the three known MODY loci [5].

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Abbreviations: NIDDM, Non-insulin-dependent diabetes mellitus; MODY, maturity-onset diabetes of the young; *GCK*, glucokinase gene; IGT, impaired glucose tolerance; IDDM, insulin-dependent diabetes mellitus; bp, base pair.

Table 1. New glucokinase mutations in MODY families

Family	Location of mutation		Nucleotide change	Amino acid change	Designation
	Exon	Codon			
F585	2	53	GCC → TCC	Ala → Ser	A53S
F557	3	80	GGT → GCT	Gly → Ala	G80A
F540	intron 3	122	AG → AT at splice acceptor site		L122-1G → T
GF	4	137	CAT → CGT	His → Arg	H137R
F553	intron 4	161	Del T of GT at splice donor site and 9 bp[a]		K161 + 1del10
F587	5	168	ACC → CCC	Thr → Pro	T168P
HO	5	186	CGA → TGA	Arg → Stop (OP)	R186X
F586	6	210	ATG → ACG	Met → Thr	M210T
F583	6	213	TGC → CGC	Cys → Arg	C213R
F541	6	226	GTG → ATG	Val → Met	V226M
LZ	7	248	GAG → TAG	Glu → Stop (AM)	E248X
F588	7	261	GGG → AGG	Gly → Arg	G261R
BO	8	336	TCG → TTG	Ser → Leu	S336L
F630	9	360	TCG → TAG	Ser → Stop (AM)	S360X
F629	9	367	GTG → ATG	Val → Met	V367M
SA	9	401	GTA → _TA/A	Val → Stop (OC)	V401del1

[a] Sequence of mutation at splice donor site in family F553: G[TGGGCCGGGT]GGAGGGGCA → GGGAGGGCA
The three classes of stop codons are amber (AM), ocher (OC) and opal (OP)

Mutations in the glucokinase gene are the most common cause of MODY in France with approximately 50 % of subjects with MODY having mutations in this gene [4]. These subjects are often characterized by mild persistent fasting and postprandial hyperglycaemia. Clinical studies have shown that patients with glucokinase-deficient diabetes have a defect in glucose-stimulated insulin secretion with a rightward shift in the dose-response curve relating glucose concentration and rate of insulin secretion [6, 7]. This defect results from impaired sensitivity of pancreatic beta cells to glucose as a consequence of decreased glucokinase activity in these cells. Glucokinase-deficient subjects also exhibit a reduction in postprandial hepatic glycogen synthesis and increased rates of gluconeogenesis following meals [8]. Although subjects with *GCK* mutations may present with peripheral insulin resistance, the decreased insulin sensitivity appears to be secondary to the chronic hyperglycaemia [9].

In this report, we describe 16 families, 14 of French and 2 of Brazilian ancestry, in which MODY results from mutations in *GCK*. Fourteen of the 16 mutations identified in these families have not been previously described. The study of 260 glucokinase-deficient subjects and 341 unaffected relatives from 42 families with 36 different *GCK* mutations [4, 10, 11 and this report] provide a clinical profile of this subtype of NIDDM.

Subjects and methods

Subjects. Probands of 35 families were studied, 31 of which were of French and 4 of Brazilian ancestry. These subjects were seen by one of us at the outpatient clinics, or referred to us by their doctors with a clinical diagnosis of MODY, including onset before 25 years of age and familial NIDDM consistent with an autosomal dominant inheritance. Clinical data were obtained for each available member ($n = 115$) of the 16 families with a *GCK* mutation, during the course of a standard clinical examination performed either by one of us or by the subject's personal physician. Neurological history was taken and a physical examination performed focussing on symptoms and signs of distal symmetric sensorimotor polyneuropathy and autonomic neuropathy. Eye fundus examination was performed by the subject's personal ophthalmologist. Evidence for nephropathy was based on the presence of proteinuria tested by strips (lower limit of detection: 0,025–0,03 g/l). Subjects who were not overtly diabetic underwent an oral glucose tolerance test (OGTT) and a diagnosis of diabetes or impaired glucose tolerance (IGT) was made according to the criteria of the World Health Organization [12]: diabetes, fasting plasma glucose level greater than 7.8 mmol/l or 2-h post oral glucose load greater than 11.1 mmol/l; and IGT, 2 h-post oral glucose load greater than 7.8 mmol/l. Subjects were considered to have mild fasting hyperglycaemia if they had a fasting plasma glucose level between 6.1 and 7.7 mmol/l on two separate occasions.

Screening for mutations in the glucokinase gene. Mutations in *GCK* were identified by single-strand conformational polymorphism analysis and sequencing of bands with abnormal mobility as previously described [11, 13, 14].

Statistical analysis

Results are expressed as means ± SD unless otherwise stated. The Shapiro-Wilk W-test was used to test the Gaussian distribution of clinical and biological parameters. Group differences were assessed with Student's two-tailed unpaired *t*-test, using log-transformed data when appropriate. Qualitative traits were analysed using a contingency table. Statistics were performed with the JMP software (SAS Institute, Cary, N.C., USA).

Results

Identification of mutations in the GCK gene. Fourteen new and two previously described mutations were identified (Table 1) in 16 families (Fig. 1), out of the 35 MODY families. The new mutations included nine missense mutations (A53S, G80A, H137R, T168P, M210T, C213R, V226M, S336L and V367M), two nonsense mutations (E248X and S360X), a deletion of one nucleotide resulting in a frameshift (V401del1), a substitution of a conserved nucleotide at a splice acceptor site (L122-1G → T), and a 20 base pair (bp) deletion that removed the GT of the splice donor site and the following eight nucleotides (K161 + 2del10). The two previously identified mutations were R186X and G261R. The R186X mutation has been found in one other French family [4], a Japanese family [15], and a black African family from the Congo [11]. The G261R mutation has been found in two other French families [4] and a Japanese family [16].

Available family members were screened for the presence of the mutation identified in the proband, and in each case the mutation was present in all subjects with hyperglycaemia and in none of the normoglycaemic relatives, except for families SA and F553. In family SA, the V401del1 mutation was not detected in one of the proband's sisters (Fig. 1; II/36) or mother (I/35), both of whom presented with obesity and late age-of-onset NIDDM. No DNA or clinical data were available for the proband's deceased father. In family F553, the K161 + 1del10 mutation was also detected in a normoglycaemic 4-year-old child (III/3924). In family F540, the L122-1G → T mutation was detected in all subjects with MODY, and in the proband's brother (III/3588), a 13-year-old boy with a typical history of autoimmune insulin-dependent diabetes mellitus (IDDM). Diagnosis of IDDM was established at the age of 4 years by the presence of ketoacidosis and strongly positive islet-cell antibodies. The observation in this child of an HLA DRB1*04-13 genotype and a *GCK* mutation indicates that he had genetic susceptibility both to IDDM (allele 04) and MODY. Only the proband was available for study in families F585, F586 and HO, and only the proband and her mother in family BO. The H137R mutation found in family GF was observed in six hyperglycaemic subjects and four subjects for whom there was no information on glucose tolerance status. In the LZ family, the E248X mutation was found in the proband and three of his sons but was not detected in his parents or any of his siblings, all of whom were normoglycaemic. Based on the genotyping results with other polymorphic markers, there was no evidence for nonpaternity in the LZ family suggesting that the E248X mutation arose de novo. This is the second example of a de novo mutation in *GCK* that we have observed in our studies of 42 MODY families with glucokinase deficiency, the first being R36W in family F547 [11]. Both of these mutations occur within the context of a CpG dinucleotide, a hot spot for nucleotide substitutions in mammalian genes [17].

Clinical profile of glucokinase-deficient NIDDM. We have now studied 260 glucokinase-deficient subjects from 42 families with 36 different mutations (Table 2). They are characterized by a relatively mild form of diabetes, and in this large group, 42 % have overt diabetes, 24 % IGT, 31 % mild fasting hyperglycaemia, and 3 % normal glucose tolerance. Half of the subjects with normal glucose tolerance are children younger than 5 years, while the other half, aged 19 to 57 years, have had documented episodes of hyperglycaemia (pregnancy, steroid therapy). These data indicate that *GCK* mutations are highly penetrant.

The diagnosis of hyperglycaemia in the majority of the subjects was fortuitous, most often during the course of a routine check-up (school, military service, work), prospective testing because of a strong family history of diabetes, or the presence of gestational-onset diabetes. The average age at diagnosis of chronic hyperglycaemia was 25 ± 17 years. Fifty-six percent of the subjects were diagnosed before the age of 25 years. However, a decrease in the mean age of diagnosis was observed in consecutive generations (46 ± 16 years in patients older than 50 years, 26 ± 9 years in those aged 25–50 years, and 10 ± 5 years in those aged less than 25 years), suggesting that this parameter might have suffered ascertainment bias in the older generations. Prospective studies in family members younger than 25 years show that approximately 90 % of affected individuals present with hyperglycaemia before the age of 13 years. Furthermore, mild hyperglycaemia can be observed in infants as young as 12 months, suggesting that this condition may be present at birth. These observations are in agreement with reports showing that NIDDM with late age of onset is not associated with mutations in the coding regions of *GCK* [10, 18].

The prevalence of late complications of diabetes in 25 of the families studied here have been reported previously [19]. In the 42 families that we have now studied, proliferative retinopathy has been observed in less than 4 % of the glucokinase-deficient subjects with hyperglycaemia of more than 5 years duration, while proteinuria was detected in 6 %, and peripheral neuropathy in 4 % of these subjects. Obesity (BMI $\geq$ 27 kg/m^2) was present in less than 10 % of subjects at diagnosis of hyperglycaemia and hypertension was observed in less than 15 % of the glucokinase-deficient subjects older than 35 years. A low prevalence of dyslipidaemia was also observed: high triglycerides (> 1.8 mmol/l): 5.8 %; high LDL

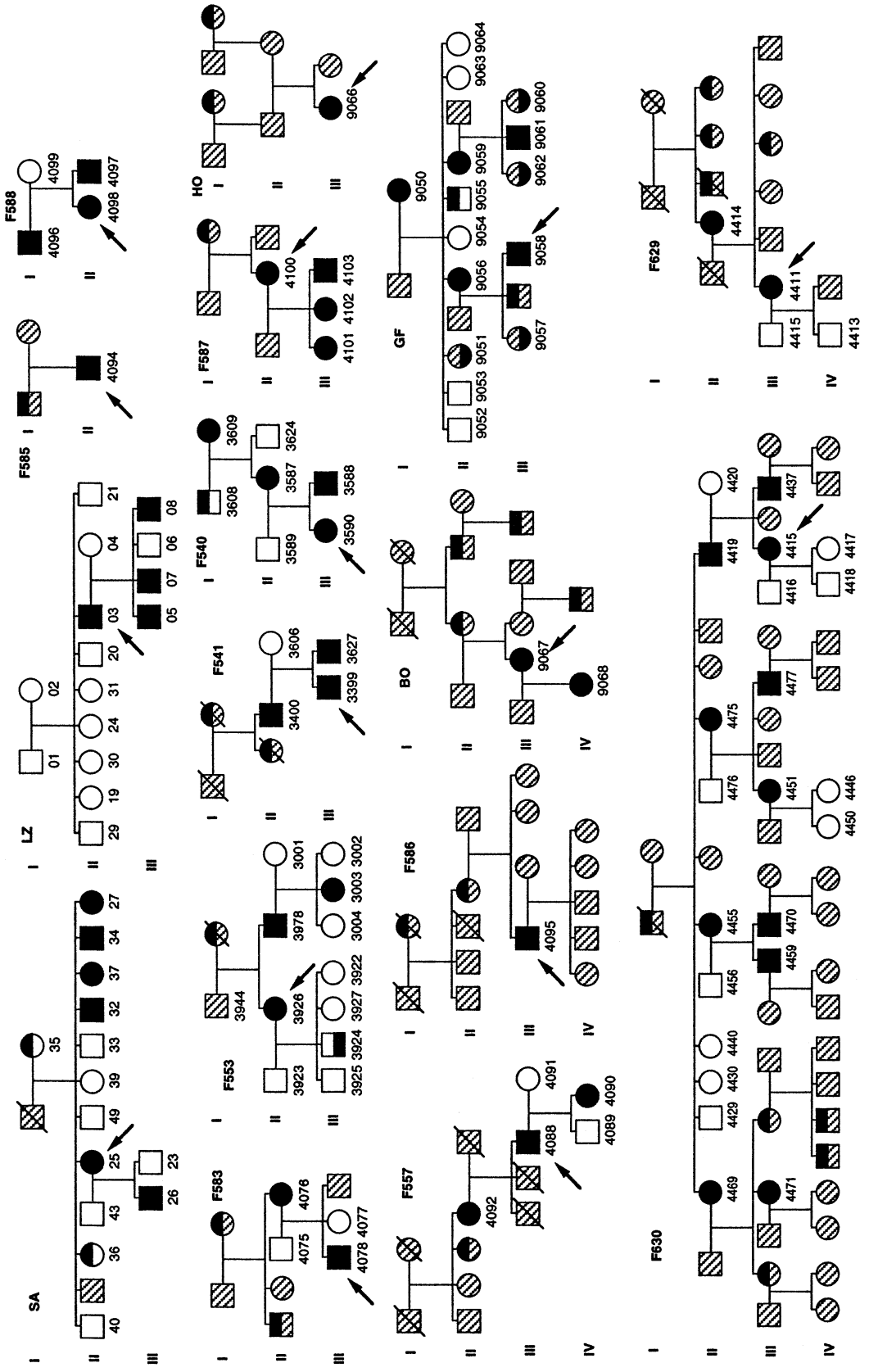

Fig. 1. Family trees of 16 pedigrees with glucokinase mutations and MODY. Kindreds F540, F541, F553, F557, F583, F585, F586, F587, F588, GF, HO, BO, F629 and F630 are of French ancestry, and kindreds SA and LZ of Brazilian ancestry. An arrow indicates the proband in each family.

Table 2. Clinical and biological profile of family members

	Glucokinase-deficient subjects	Unaffected relatives[a]	*p* value
Men/women	128/132	167/174	0.95
Age (years)	36 ± 21 (2–96)[b]	33 ± 19 (1–83)	0.14
Age at diagnosis (years)	25 ± 17 (1–80)[c]	–	–
Known duration of hyperglycaemia (years)	11 ± 10 (1–62)		
Body mass index (kg/m^2)	22.0 ± 4.1	22.1 ± 4.3	0.94
Systolic blood pressure (mm Hg)	125 ± 17	123 ± 17	0.39
Diastolic blood pressure (mm Hg)	71 ± 11	70 ± 13	0.16
Fasting glucose (mmol/l)	7.0 ± 1.1	5.0 ± 0.5	0.0001
2-h glucose (mmol/l)	9.4 ± 3.0	5.1 ± 1.3	0.0001
Fasting insulin (pmol/l)	66 ± 42	60 ± 48	0.34
Fasting insulin/glucose (pmol/mmol)	8.70 ± 5.52	11.94 ± 9.6	0.002
2-h Insulin (pmol/l)	174 ± 150	198 ± 180	0.25
2-h Insulin/glucose (pmol/mmol)	18.66 ± 13.02	37.56 ± 32.10	0.0001
Creatinine (μmol/l)	83 ± 19	80 ± 19	0.24
Triglycerides (mmol/l)	0.97 ± 0.54	1.01 ± 0.60	0.39[d]
Total cholesterol (mmol/l)	5.49 ± 1.19	5.39 ± 1.23	0.19[d]
HDL cholesterol (mmol/l)	1.42 ± 0.36	1.41 ± 0.37	0.61[d]
LDL cholesterol (mmol/l)	3.63 ± 1.05	3.49 ± 0.99	0.13[d]
Apo A1 (mg%)	167 ± 31	164 ± 29	0.66[d]
Apo B (mg%)	111 ± 33	108 ± 31	0.36[d]
Lp(a) (mg%)	25.5 ± 20.0	27.5 ± 21.5	0.37[d]

Data expressed as mean ± SD and (range). Results partially reported in references [9, 19].

[a] Mutation negative, normo-glucotolerant first-degree relatives;

[b] age distribution; younger than 25 years: 93 (39 %); between 25 and 45 years: 74 (31 %); older than 45 years: 73 (30 %);

[c] age of diagnosis distribution: < 25 years: 142 (56 %); 25 to 45 years: 81 (32 %); > 45 years: 29 (12 %);

[d] age-adjusted comparisons.

2-h glucose and insulin are values during an oral glucose tolerance test; other biological parameters are fasting values

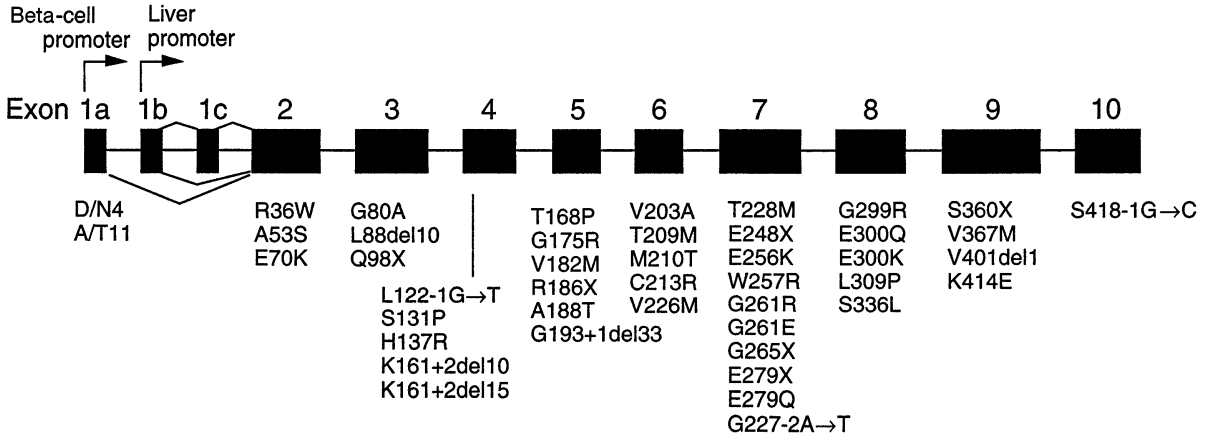

Fig. 2. Glucokinase mutations found in subjects with diabetes mellitus [4, 10, 11, 15, 16, 21, 23–25, 27, and this report]. The exon-intron organization of the human glucokinase gene is shown and the mutations found in each exon noted. Amino acid residues are numbered as in the beta-cell form of human glucokinase. The two amino acid polymorphisms in the unique NH_2-terminal portion of beta-cell glucokinase which is encoded by exon 1a are indicated: Asn/Gln 4 (D/N 4) and Ala/Thr 11 (A/T 11)

cholesterol (> 4.9 mmol/l): 5.7 %; low HDL cholesterol (< 0.8 mmol/l): 1 %; low apoprotein A1 levels (< 130 mg/ml): 6 %; high apoprotein B (> 130 mg/ml): 18 %; and high lipoprotein (a) (> 30 mg/ml): 19 %.

Discussion

Mutations in *GCK* are the most common cause of MODY identified to date. Forty-two different mutations and two amino acid polymorphism have now been reported (Fig. 2). They have been found in subjects of many different racial and ethnic backgrounds including Caucasians (Brazil [this report], France [4, 10, 11], Italy [20], Sweden [21], Switzerland [22], United Kingdom [23], and United States [24]), Asians (Japan [15, 16, 25]), blacks (Congo [11]), and admixed populations (African-American [21, 26] and Puerto Rican [24]). The majority of the mutations identified to date have been described in only a single family suggesting that the present list is not exhaustive and that new mutations will continue to be found.

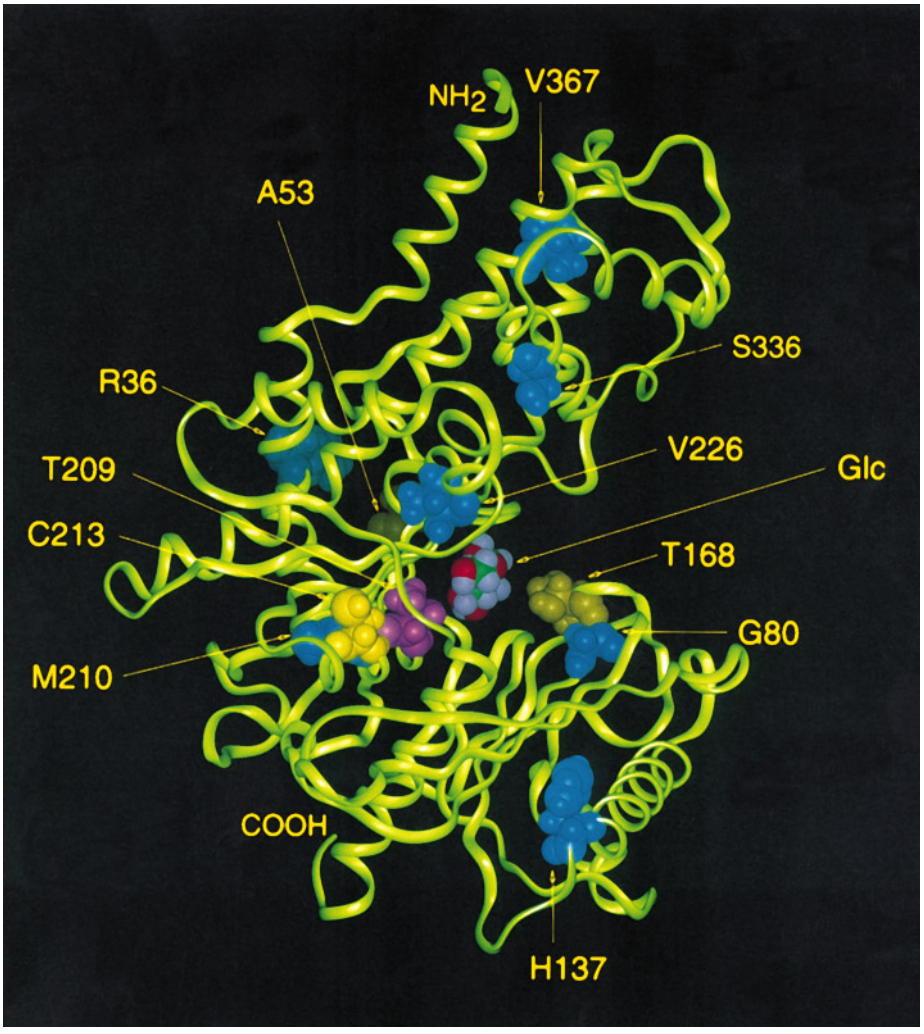

Fig. 3. Model of the human beta-cell form of glucokinase with a molecule of glucose in the active site cleft. The alpha-carbon backbone of glucokinase is represented as a ribbon and the locations of amino acid residues that are the site of missense mutations are shown by space-filling models in blue, green, pink and yellow. A space-filling model of glucose (Glc) in the active site cleft is shown with the individual atoms indicated: green, carbon; red, oxygen; and grey, hydrogen. Amino acid residues are noted by their single-letter abbreviation and residue number. This model was built in the Insight graphic environment (Biosym Technologies, Inc., San Diego, Calif., USA)

Those mutations found in more than one family include V182M (two French families [4]), R186X (two French [11 and this report], one Japanese [15], and one Black-African family [11]), A188T (one Japanese [25] and one Italian family [20]), V203A (one French [4] and four Swiss families [22] suggesting a possible founder effect in Switzerland), G261R (three French [4 and this report] and one Japanese family [16]), E279Q (two African-American families [21]), and G299R (two British families with a possible founder effect in Oxfordshire [23]).

Our previous studies have shown that missense mutations have variable effects on glucokinase activity ranging from a small change in affinity for glucose to complete inactivity [21, 27–29]. Based on a model of the structure of human beta-cell glucokinase [30], we have observed that the known missense mutations fall into three main categories: mutations of conserved active site residues – these generally have a drastic effect on catalytic activity; mutations predicted to distort the enzyme structure – these tend to show reduced activity; and mutations of surface residues that eliminate conserved interactions with other residues and may reduce the stability of the structure or affect the conformational change that is observed on binding of glucose – these often show a small reduction in activity. In addition to affecting enzyme activity or stability [21, 27, 31], mutations may alter the interaction of glucokinase with other proteins such as the glucokinase regulatory protein [32].

Based on these results, we can consider the possible effects on activity of the nine new missense mutations identified during the course of this study. The mutations G80A, T168P, M210T and V226M are predicted to alter residues in or near the active site cleft (Fig. 3). These mutations are expected to greatly reduce catalytic activity. The mutation C213R is predicted to result in distortion of the glucokinase structure due to the introduction of a large Arg residue in place of Cys and is expected to cause a reduction in catalytic activity. The mutations A53S, H137R, S336L and V367M affect residues located far from the active site and as such may have relatively little effect on catalysis. The mutations H137R and S336L are not predicted to alter the structure or stability of glucokinase and the mechanism by which they cause

MODY is unclear. However, since they are located on the surface of the glucokinase molecule, they could alter its interactions with other proteins such as the glucokinase regulatory protein [32]. The effects of these mutations on glucokinase structure and function must now be examined directly by expressing recombinant mutant glucokinase and measuring its kinetic properties, thermal stability, and interactions with other molecules. Change in any of these three aspects of the behaviour of glucokinase may affect its activity in vivo and result in MODY [21, 27, 31].

The study of 260 glucokinase-deficient subjects from 42 families with 36 different mutations demonstrates that this form of NIDDM is characterized by a relatively mild hyperglycaemia, with less than 50 % of subjects presenting with overt diabetes. Interestingly, we have observed that 65 % of the glucokinase-deficient subjects classified as having overt diabetes according to the W. H. O. diagnostic criteria fulfilled the 2-h criterium only and had fasting plasma glucose values lower than 7.8 mmol/l. Thus, decompensation of the postprandial hyperglycaemia rather than an increase in the fasting glucose levels seems to be associated with the transition from impaired glucose tolerance to overt diabetes in these subjects. The observation of decreased net glycogen synthesis and increased rates of gluconeogenesis following meals in glucokinase-deficient subjects, underscores the key role of the liver and of mutant hepatic glucokinase, in the pathophysiology of this form of NIDDM [8].

There was no evidence in this cohort of glucokinase-deficient subjects for the well-established association of NIDDM of IGT with a cluster of risk factors for macrovascular disease including hypertension, obesity, and dyslipidaemia. The low prevalence of these other disorders is consistent with the low frequency of coronary heart disease in subjects with glucokinase-deficient diabetes, none of whom had a previous history of myocardial infarction, and only eight subjects were being treated for angina or showed electrocardiographic evidence of myocardial ischaemia. However, it should be pointed out that the majority of these subjects were not tested to exclude silent coronary heart disease which is frequent in NIDDM.

The long-term chronic hyperglycaemia present in subjects with glucokinase deficiency is not associated with increased frequency of late complications of diabetes. We have observed a lower prevalence of proliferative retinopathy, proteinuria, and peripheral neuropathy in this cohort than in other subtypes of MODY and late-onset NIDDM [19]. For instance, the risk of proliferative retinopathy in glucokinase-deficient subjects is one-third that in MODY-3 subjects, and one-eighth that of subjects with late-onset NIDDM [19]. This may be a consequence of the relatively small increase in blood glucose levels which is possibly lower than the threshold above which the risk of diabetic complications increases, and of the low prevalence of hypertension in the subjects with glucokinase-deficient diabetes.

In conclusion, hyperglycaemia associated with *GCK* mutations is often mild, develops during the early years of life, and is not associated with increased frequency of micro- and macrovascular complications of diabetes. These mutations are highly penetrant as nearly all affected individuals have abnormalities of glucose homeostasis. Although it is clear that mutations in *GCK* can lead to overt diabetes, there is still a great deal that we do not know about the natural history of glucokinase-dificient diabetes, including the factors that trigger the progression from mild chronic hyperglycaemia to IGT to overt diabetes. Prospective studies of a large group of families such as those we have identified could lead to the identification of these factors, which may be genetic or environmental, and provide insight into the clinical progression of this and other forms of MODY as well as of the more common late-onset form(s) of NIDDM.

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