

# Update on Mutations in Glucokinase (*GCK*), Which Cause Maturity-Onset Diabetes of the Young, Permanent Neonatal Diabetes, and Hyperinsulinemic Hypoglycemia

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**ABSTRACT:** Glucokinase is a key regulatory enzyme in the pancreatic beta-cell. It plays a crucial role in the regulation of insulin secretion and has been termed the glucose sensor in pancreatic beta-cells. Given its central role in the regulation of insulin release it is understandable that mutations in the gene encoding glucokinase (*GCK*) can cause both hyper- and hypoglycemia. Heterozygous inactivating mutations in *GCK* cause maturity-onset diabetes of the young (MODY) subtype glucokinase (*GCK*), characterized by mild fasting hyperglycemia, which is present at birth but often only detected later in life during screening for other purposes. Homozygous inactivating *GCK* mutations result in a more severe phenotype presenting at birth as permanent neonatal diabetes mellitus (PNDM). A growing number of heterozygous activating *GCK* mutations that cause hypoglycemia have also been reported. A total of 620 mutations in the *GCK* gene have been described in a total of 1,441 families. There are no common mutations, and the mutations are distributed throughout the gene. The majority of activating mutations cluster in a discrete region of the protein termed the allosteric activator site. The identification of a *GCK* mutation in patients with both hyper- and hypoglycemia has implications for the clinical course and clinical management of their disorder. *Hum Mutat* 30:1512–1526, 2009. © 2009 Wiley-Liss, Inc.

**KEY WORDS:** MODY; permanent neonatal diabetes; PNDM; hyperinsulinemic hypoglycemia; glucokinase; *GCK*

## Introduction

Glucokinase is one of four members of the hexokinase family of enzymes. It operates as a monomer and phosphorylates glucose on

carbon 6 with MgATP as a second substrate to form glucose-6-phosphate (G6P). Under physiological conditions glucose is its preferred substrate; hence, its widely accepted name glucokinase rather than hexokinase IV or D. Glucokinase is a key regulatory enzyme in the pancreatic  $\beta$ -cell and catalyses the first reaction of the glycolytic pathway, the conversion of glucose to G6P. It plays a crucial role in the regulation of insulin secretion and is termed the glucose sensor in pancreatic  $\beta$ -cells because of its kinetics, which allows pancreatic beta cells to change glucose phosphorylation rate over a range of physiological glucose concentrations (4–15 mmol/l) [Matschinsky, 2002]. These kinetic characteristics are the enzyme's low affinity for glucose ( $S_{0.5}$  7.5–10 mmol/l), cooperativeness with glucose (Hill number of  $\sim 1.7$ ) and lack of inhibition by its product G6P.

Given its central role in the regulation of insulin release it is understandable that mutations in the gene encoding glucokinase (*GCK*; MIM# 138079; GenBank NM\_000162.2, NM\_033507.1, NM\_033508.1) can cause both hyper- and hypoglycemia. Heterozygous inactivating mutations in *GCK* cause a subtype of maturity-onset diabetes of the young (MODY), subtype glucokinase (*GCK*), an autosomal dominant mild fasting hyperglycemia. This subtype (*GCK*-MODY) is present at birth but is often subclinical and only detected later in life (MODY2; MIM# 125851) [Froguel et al., 1992; Hattersley et al., 1992]. Homozygous or compound heterozygous inactivating *GCK* mutations result in a more severe phenotype presenting at birth as permanent neonatal diabetes mellitus (PNDM; MIM# 606176) [Njolstad et al., 2001, 2003; Porter et al., 2005; Rubio-Cabezas et al., 2008; Turkkahraman et al., 2008]. A growing number of heterozygous activating *GCK* mutations have been reported, which cause Hyperinsulinemic Hypoglycemia (HH or HI; MIM# 601820) [Christesen et al., 2002, 2008; Cuesta-Munoz et al., 2004; Glaser et al., 1998; Gloyn et al., 2003; Sayed et al., 2009; Wabitsch et al., 2007].

The glucokinase gene (*GCK*) on chromosome 7p15.3–p15.1 consists of 12 exons that span 45,168 bp and encode for a 465-amino acid protein with a molecular weight of 52,191 Da, which is expressed in the pancreas, liver, brain, and the endocrine cells of the gut [Jetton et al., 1994]. The presence of tissue specific promoters allows differential regulation and transcription of different transcripts giving rise to three different sized exon 1 (a,b,c); the upstream promoter is functional in the pancreas and brain, whereas the downstream promoter is used only in the liver.

Additional Supporting Information may be found in the online version of this article.

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Exons 1b and 1c are expressed in the liver and exon 1a is expressed in the pancreatic  $\beta$ -cell [Lynedjian, 1993; Magnuson, 1990]. Glucokinase is also thought to play a role in glucose sensing in the hypothalamus [Spyer et al., 2000], anterior pituitary cells [Sorenson et al., 2007; Zelent et al., 2006] and gut endocrine cells [Jetton et al., 1994]. Recent studies suggest, however, that glucokinase does not function as the main gut glucose sensor in K and L cells that secrete the incretin hormones glucagon-like peptide (GLP-1) and glucose-dependent insulinotropic peptide (GIP), which both play a role in the regulation of glucose-stimulated insulin release by pancreatic  $\beta$ -cells [Murphy et al., 2009]. Glucokinase may still play a permissive role as a modifying glucose sensor in the process of L-cell sensing of sugars, amino acids and fatty acids present in the intestine.

This review will concentrate on the effect of *GCK* mutations on the pancreatic  $\beta$ -cell isoform of GCK. It is not meant to minimize the critical role of the liver and hepatic glucose metabolism and homeostasis. It has indeed been shown that GCK-MODY patients have a hepatic glucose phosphorylation deficit [Velho et al., 1996]. However, in contrast to the  $\beta$ -cells, the liver does not seem to have a threshold response to glucose, which would explain the predominant impact of the latter on the glucose set point of the organism.

## Glucokinase Regulation

Glucokinase is regulated by both transcriptional and post-translational mechanisms, and both of these differ between the liver and pancreatic  $\beta$ -cells [Lynedjian, 1993]. In the liver, glucokinase enzyme activity is inhibited by the binding of glucokinase regulatory protein (GKRP), which functions as a nuclear receptor that sequesters GCK in an inactive state in the nucleus at low glucose concentrations [Alvarez et al., 2002; Shiota et al., 1999; van Schaftingen and Viegas da Cunha, 2004]. Increased extracellular glucose concentrations leads to translocation of GCK to the cytoplasm, which enables rapid stimulation of glucose phosphorylation. Translocation of GCK between the nucleus and cytoplasm is also dependent on the bifunctional enzyme 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (PFKFB1, also known as PFK-2/FBPase-2) which is expressed in the pancreatic  $\beta$ -cell and liver [Baltrusch et al., 2001]. The bifunctional enzyme functions as an intracellular binding partner for GCK, and plays an important role in its activation through stabilizing a specific conformation of the GCK enzyme that results in higher catalytic activity [Baltrusch et al., 2001; Massa et al., 2004]. This process has been proposed as a posttranslational mechanism of GCK regulation, because these proteins interact at a glucose concentration relevant for the activation of GCK and regulation of insulin secretion [Massa et al., 2004].

Glucokinase interacts with other proteins such as the pro-apoptotic B-cell leukemia/lymphoma 2 (BCL2) family member BCL2-antagonist of cell death (BAD) [Danial et al., 2003]. BAD has been shown to be necessary for maximal glucokinase activity [Danial et al., 2003] and to play a role in the maintenance of glucose homeostasis and in glucose-stimulated insulin secretion by the pancreatic  $\beta$ -cell [Danial et al., 2008]. Purified free penta-ubiquitin chains have also been reported to interact and allosterically activate GCK, potentially representing yet another physiological regulatory mechanism for GCK-dependent insulin secretion in pancreatic  $\beta$ -cells [Bjorkhaug et al., 2007]. Defects in the regulation and interaction of glucokinase with these and other regulatory molecules represents a potential mutational

mechanism that could have an important impact on glycemic control.

## A Structural Model of Human Glucokinase

The crystal structure of human GCK was solved in 2004 by Kamata and colleagues [2004] and has helped enormously in the understanding of the abnormal biochemistry of various GCK mutations. Glucokinase has a large and a small domain separated by a deep cleft where glucose binds. This cleft is composed of residues of the large domain (E256 and E290), the small domain (T168 and K169), and the connecting region I (N204 and D205).

Upon binding to substrates glucose and adenosine triphosphate (ATP), GCK undergoes a conformational change that brings the large and the small domains physically closer, resulting in a closed, active conformation. Glucokinase exists in three structural conformations: closed, open and "superopen," which define two catalytic cycles (slow and fast). The ratio between these two catalytic cycles is responsible for the characteristic sigmoidal response to glucose [Kamata et al., 2004].

The  $\alpha$ 13 and  $\alpha$ 5 helices, within the small domain, play a critical role in the conformational change that occurs between the active and inactive forms of the enzyme [Kamata et al., 2004]. Substitutions within these helices can improve the flexibility of the region or the interaction with surrounding residues, which enhances the affinity of the enzyme for glucose and its glucose phosphorylating performance [Pedelini et al., 2005].

Previously, the most widely used model was based on homology to human brain hexokinase I, which shares 54% amino acid identity with glucokinase [Mahalingam et al., 1999]. This model had been used extensively to locate many of the GCK-MODY and GCK-HH mutations [Christesen et al., 2002; Gloyn et al., 2003; Mahalingam et al., 1999] prior to the clarification of the crystal structure.

## A Mathematical Model to Predict the Threshold for Glucose Stimulated Insulin Secretion in Patients with GCK Mutations

A minimal mathematical model has been developed that predicts the threshold for glucose stimulated insulin release (GSIR) of activating and inactivating mutations of GCK using calculated kinetic parameters generated from functional studies of mutant glucokinase enzymes [Gloyn et al., 2004a; Matschinsky, 2002]. This model takes into account adaptation of both the wild-type and mutant alleles to the patient's blood glucose concentration and the compensatory effect of the wild-type allele of individuals with GCK disease, which has been shown to effect levels of expression of the GCK gene by a proposed posttranslational mechanism [Liang et al., 1992]. Additionally, the basal glucose level of individuals with GCK disease can be predicted without involving altered intermediary metabolism and/or insulin signaling [Gloyn et al., 2004a; Matschinsky, 2002].

Relatively small changes in the kinetic parameters can have pronounced effects on the threshold for GSIR and basal blood glucose levels. The minimal model predicts a threshold for GSIR for GCK-MODY mutations of  $6.71 \pm 0.1$  mmol/l, which compares well with the reported clinical data 6.8–7.3 mmol/l [Davis et al., 1999].

## Lessons from Animal Models

Various transgenic rodent models with global, isoform-specific or tissue specific *gck*-gene knockouts have been generated to help understand the role of GCK in glucose homeostasis at the cellular

level. Global homozygous *gck* knockout mice result in perinatal death from severe diabetes [Grupe et al., 1995; Postic et al., 1999], whereas global heterozygous *gck* knockout mice develop early mild-onset diabetes resembling the GCK-MODY phenotype [Grupe et al., 1995; Postic et al., 1999]. Rodent models have also been created with *gck*  $\beta$ -cell specific knockouts, and these studies have confirmed that islet  $\beta$ -cell *gck* expression is essential because any homozygous *gck* beta-cell knockout has been shown to result in severe hyperglycemia and death by postnatal day 4 [Bali et al., 1995; Grupe et al., 1995; Postic et al., 1999; Terauchi et al., 1995], whereas heterozygous  $\beta$ -cell specific *gck* knockout recapitulates the GCK-MODY phenotype in humans with mild diabetes and diminished insulin secretory response [Postic et al., 1999; Terauchi et al., 1995].

A total lack of *gck* expression in the liver alone results in only moderate hyperglycemia but with a pronounced defect in hepatic glucose utilization and glycogen synthesis [Postic et al., 1999]. In contrast, overexpression of *gck* in liver has been shown to cause an increase in hepatic glucose metabolism with a decrease in blood glucose levels, without an increase in insulin secretion [Jackerott et al., 2002; Niswender et al., 1997]. It has also been shown that *gck* overexpression in the liver causes a dramatic increase in triglycerides and fatty acids [O'Doherty et al., 1999], and may lead to insulin resistance [Ferre et al., 2003].

More recently there has been interest in the role of GCK in  $\beta$ -cell mass. Glucokinase haploinsufficient mice have demonstrated impaired GSIR and decreased  $\beta$ -cell replication in response to a high fat diet [Terauchi et al., 2007]. This is consistent with data from studies on mice fed on a high-fat diet who have been treated with glucokinase activators (GKAs), which have been shown to improve GSIR [Gorman et al., 2008] and increase  $\beta$ -cell proliferation in haploinsufficient *gck* mouse models [Nakamura et al., 2009].

Several mouse lines with heritable hyperglycemia have been produced through phenotype-driven mutagenesis programs using the chemical mutagen *N*-ethyl-*N*-nitrosourea (ENU), which have identified 13 GCK mutations associated with mild fasting hyperglycemia (Table 1) [Inoue et al., 2004; Toye et al., 2004]. Six of the 13 ENU GCK mutations have also been found in patients with GCK-MODY and/or PNDM [Aigner et al., 2008]. Some of the ENU *gck* mutant mouse lines displayed impaired glucose-responsive insulin secretion and the mutations had different effects on GCK mRNA levels and/or the stability of the GCK protein [Toye et al., 2004]. The collection of *gck* ENU mutant mouse and other animal models is valuable for understanding

glucokinase function and for the development of GCK activators. Recently, a knock-in strategy has been used to create the first mouse model of GCK-HH with a *gck* allele encoding the activating mutation (A456V), which results in an enzyme with increased affinity for glucose [Pino et al., 2007].

## GCK-MODY formerly known as MODY2

MODY is an autosomal dominantly inherited form of diabetes or hyperglycemia that is characterized by an early age of onset (at least one affected family member with an onset before 25 years of age) and pancreatic  $\beta$ -cell dysfunction. Glucokinase was the first MODY gene to be identified. Linkage between microsatellite markers and disease was described in French and UK pedigrees in 1992 [Froguel et al., 1992; Hattersley et al., 1992]. The demonstration of linkage to GCK was quickly followed by characterization of the human gene and the detection of mutations (Supp. Table S1). The first mutation was reported in 1992, and now over 600 mutations of the GCK gene have been described in many populations, with the majority found in the UK, France, and Spain. All inactivating mutations are associated with mild fasting hyperglycemia. The prevalence of GCK-MODY is difficult to assess as the mild hyperglycemia and the absence of symptoms means that patients are frequently not diagnosed. Large-scale population studies to assess the prevalence have not been done. In Caucasian populations approximately 2% of the population will be diagnosed as having gestational diabetes, and of these, approximately 2–5% will have a GCK mutation [Ellard et al., 2000]. This would suggest a population prevalence of 0.04–0.1%. There is some discrepancy in the literature on the prevalence of GCK-MODY in European Caucasian MODY families. In the UK, the prevalence is reported as 10–20% [Thomson et al., 2003], in France as 46–56% [Froguel et al., 1993], 41–61% in Italy [Mantovani et al., 2003; Massa et al., 2001], 25–80% in Spain [Barrio et al., 2002; Costa et al., 2000; Estalella et al., 2007], 31% in the Czech Republic [Pruhova et al., 2003], 12% in Norway [Sagen et al., 2008], and 10% in Denmark [Johansen et al., 2005]. The considerable variation in prevalence can be attributed to the way in which patients were ascertained and recruited for the various studies [Massa et al., 2001]. The average age of the cohort studied is likely to be crucial. For example, by studying adult populations such as the Norwegian cohort, GCK mutations were less frequently observed (12%) than

**Table 1. Mouse Models of GCK-MODY and GCK-HH**

Nucleotide change	Protein effect		Allele/mutant mouse line	Phenotype	Reported in patients	Reference
c.379T>C	S127P	Ser127Pro	<i>gck</i> <sup>S127P</sup> /M100960	MODY	N	<a href="http://gsc.riken.go.jp/mouse">http://gsc.riken.go.jp/mouse</a> [Herbach et al., 2007]
c.544G>A	V182M	Val182Met	<i>gck</i> <sup>V182M</sup> /Rgsc392	MODY	Y	[Inoue et al., 2004]
c.617C>T	T206M	Thr206Met	<i>gck</i> <sup>T206M</sup> /Rgsc735	MODY	Y	[Inoue et al., 2004]
c.659G>A	C220Y	Cys220Tyr	<i>gck</i> <sup>C220Y</sup> /Rgsc341	MODY	N	[Inoue et al., 2004]
c.671T>G	M224R	Met224Arg	<i>gck</i> <sup>M224R</sup> /Rgsc272	MODY	Y	[Inoue et al., 2004]
c.682A>G	T228A	Thr228Ala	<i>gck</i> <sup>T228A</sup> /Rgsc236	MODY	Y	[Inoue et al., 2004]
*	I366F	Ile366Phe	<i>gck</i> <sup>I366F</sup> /Gena348	MODY	N	[Toye et al., 2004]
c.1240A>G	K414E	Lys414Glu	<i>gck</i> <sup>K414E</sup>	MODY	Y	[Pino et al., 2007]
c.1255T>C	F419L	Phe419Leu	<i>gck</i> <sup>F419L</sup> /Rgsc552	MODY	Y	[Inoue et al., 2004]
c.1367C>T	A456V	Ala456Val	<i>gck</i> <sup>A456V</sup>	HH	Y	[Pino et al., 2007]
c.819T>G	Y273X	Tyr273X	<i>gck</i> <sup>Y273X</sup> /Rgsc475	MODY	N	[Inoue et al., 2004]
*	R345X	Arg345X	<i>gck</i> <sup>R345X</sup> /Rgsc702	MODY	N	[Inoue et al., 2004]
c.45+1G>T	IVS1A+1G>T	IVS1A+1G>T	<i>gck</i> <sup>IVS1A+1G&gt;T</sup> /Rgsc210	MODY	Y	[Inoue et al., 2004]
c.364-3C>A	IVS3-3C>A	IVS3-3C>A	<i>gck</i> <sup>IVS3-3C&gt;A</sup> /Rgsc149	MODY	N	[Inoue et al., 2004]
c.1019+2T>C	IVS8+2T>C	IVS8+2T>C	<i>gck</i> <sup>IVS8+2T&gt;C</sup> /Rgsc553	MODY	N	[Inoue et al., 2004]

\*Nucleotide change not given in the reference. Y = yes, N = no.

the high prevalence found in young children observed in the two Italian series (61 and 41%) [Mantovani et al., 2003; Massa et al., 2001] and a recent Chilean study (50%) [Codner et al., 2009]. The high prevalence of *GCK* mutations in French MODY families probably reflects that glucose tolerance testing was performed in asymptomatic young relatives in type 2 diabetes (T2DM) families recruited by media appeal direct to patients [Froguel et al., 1991]. The UK studies were predominantly of symptomatic patients attending hospital outpatients, so patients with *GCK* mutations are less prevalent [Thomson et al., 2003].

A recent study in the UK that investigated the prevalence of *GCK* mutations in overweight adult patients ascertained for fasting hyperglycemia identified mutations in <1% of patients [Gloyn et al., 2009]. These results are again in contrast to studies performed in children and adolescents with asymptomatic hyperglycemia where *GCK* mutations were identified in 43% of individuals [Feigerlova et al., 2006], suggesting once again that the age and clinical selection criteria of the target population plays a role in the prevalence of *GCK* mutation detection.

### Permanent Neonatal Diabetes Mellitus Subtype Glucokinase (PNDM-GCK)

PNDM is a rare form of diabetes diagnosed within the first 6 months of life [Edghill et al., 2006], with an estimated incidence of 1 in 260,000 live births [Slingerland et al., 2009]. Mutations in the genes encoding for *GCK* and the  $\beta$ -cell potassium ATP ( $K_{ATP}$ ) channel genes *ABCC8* (*SUR1*; MIM# 600509) and *KCNJ11* (*Kir6.2*; MIM# 600937), and the insulin gene (*INS*; MIM# 176730) are the most common cause of PNDM [Babenko et al., 2006; Gloyn et al., 2004b; Stoy et al., 2007]. Only eight isolated cases of PNDM attributable to *GCK* mutations have been reported, either homozygous or compound heterozygous for a missense, frameshift, or nonsense mutation, resulting in complete deficiency of glucokinase activity [Njolstad et al., 2003; Njolstad et al., 2001; Porter et al., 2005; Rubio-Cabezas et al., 2008; Turkkahraman et al., 2008].

Six of the eight *GCK*-PNDM cases to date have been in consanguineous Arabic or European *GCK*-MODY families where the parents are distantly related [Njolstad et al., 2001, 2003; Porter et al., 2005; Rubio-Cabezas et al., 2008; Turkkahraman et al., 2008]. Studies in European collections of PNDM have suggested that complete glucokinase deficiency is not a common cause of PNDM but should be considered in families with a history of glucose intolerance, or MODY in first degree relatives, especially when consanguinity is suspected [Gloyn et al., 2002; Vaxillaire et al., 2002].

### Hyperinsulinemic Hypoglycemia Subtype Glucokinase (GCK-HH)

HH, also known as persistent hyperinsulinemic hypoglycemia of infancy (PHHI), hyperinsulinemia of infancy (HI), and congenital hyperinsulinemia of infancy (CHI), is characterized by inappropriate over secretion of insulin despite hypoglycemia. The estimated incidence of HH in populations without a founder effect is 1 in 37,000 to 1 in 50,000 per year [Glaser et al., 2000]. HH is a heterogeneous disorder with mutations reported in the  $\beta$ -cell potassium ATP ( $K_{ATP}$ ) channel genes *ABCC8* (*SUR1*) and *KCNJ11* (*Kir6.2*), the genes encoding the mitochondrial enzymes glutamate dehydrogenase (*GLUD1*; MIM# 606762), the short-chain L-3-hydroxyacyl-CoA dehydrogenase (*HADH*; MIM# 609975), and glucokinase (*GCK*). Glucokinase-HH (GCK-HH) is caused by activating mutations in *GCK*. In a combined Danish, Norwegian, and UK study, the prevalence of GCK-HH was

estimated to be 1.2% of all HH cases [Christesen et al., 2008]. Activating mutations in *GCK* result in an increased affinity of glucokinase for its substrate glucose and inappropriate insulin secretion at low blood glucose levels [Matschinsky, 2002]. Eleven activating *GCK* mutations have been reported: S64Y, T65I, G68V, W99R, W99L, M197I, Y214C, V452L, V455M, and A456V, p.Ala454dup (reported as ins454A) (Supp. Table S1) [Christesen et al., 2002, 2008; Cuesta-Munoz et al., 2004; Glaser et al., 1998; Gloyn et al., 2003; Meissner et al., 2009; Sayed et al., 2009; Wabitsch et al., 2007]. Interestingly, most of these mutations, with the exception of M197I [Sayed et al., 2009], cluster in a discrete region of *GCK* which is remote [ $\sim 20$  Å] from the substrate-binding site, termed the allosteric activator site, which is where small synthetic molecular activators bind [Christesen et al., 2002; Gloyn et al., 2003] (Fig. 1). The severity of hypoglycemia is variable depending upon the mutation. For example, the mutations Y214C and p.Ala454dup (reported as ins454A) cause severe and possibly fatal hypoglycemia [Cuesta-Munoz et al., 2004; Sayed et al., 2009], whereas the majority of mutations described to date result in a phenotype of mild hypoglycemia, which is often asymptomatic and is responsive to pharmacological treatment [Christesen et al., 2002; Glaser et al., 1998; Gloyn et al., 2003; Meissner et al., 2009; Sayed et al., 2009; Wabitsch et al., 2007]. There is some preliminary evidence that islet morphology may vary according to the severity of the mutation, with normal-appearing islets in some cases [Gloyn et al., 2003; Wabitsch et al., 2007] and enlarged islet size in others [Cuesta-Munoz et al., 2004].

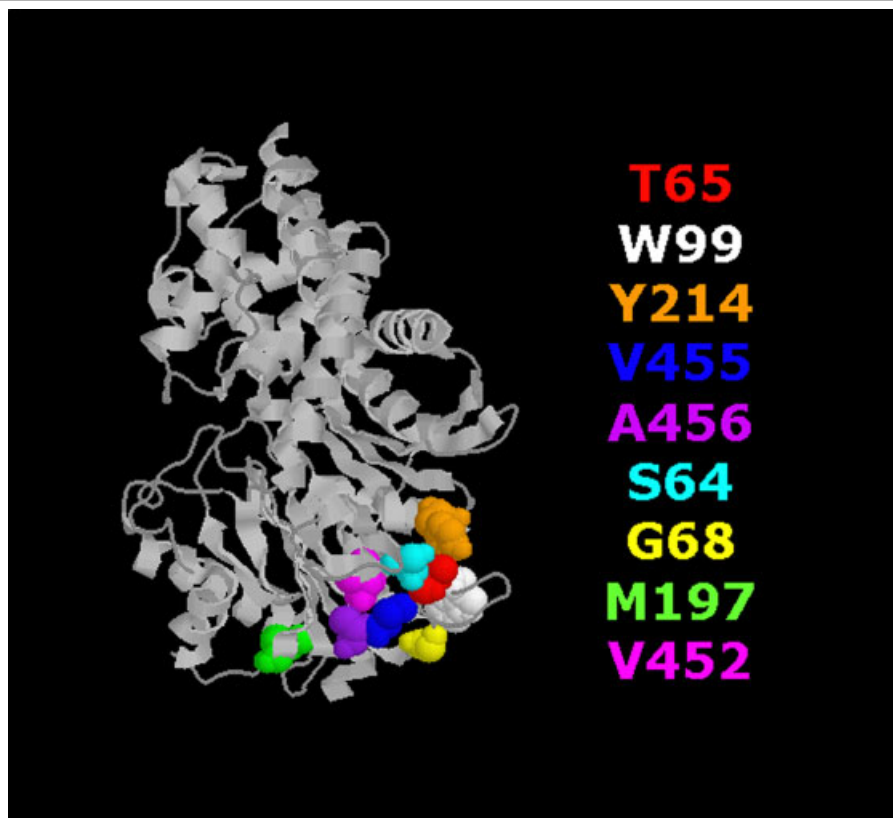
## Mutations and Polymorphisms

### Mutations

A total of 620 mutations have been reported in 1441 MODY, PNDM, and HH families (Supp. Table S1). The mutations are located throughout the 10 exons of the gene expressed in the pancreatic  $\beta$ -cell isoform of glucokinase (exons 1a, 2–10), although the number of mutations detected in exon 1a is low ( $n = 6$ ). This could be in part due to the fact that some laboratories only screen exons 2–10 of the *GCK* gene [Ellard et al., 2000; Gloyn et al., 2002; Thomson et al., 2003]. Recently, a novel *GCK* pancreatic islet promoter mutation  $-71G > C$  has been described in a total of six families (five from Slovakia and one from the UK) [Gasperiakova et al., 2009]. Missense, nonsense, frameshift, and splice site mutations have been reported and are distributed throughout the 10 exons. There are no mutation “hot spots,” most of the mutations are private, but 255 have been reported in more than one family, and of these 7.1% are at CpG dinucleotides (Supp. Table S1). Partial or whole gene deletions have been identified in a small number of UK *GCK*-MODY cases (3.5%) [Ellard et al., 2007] and have been shown to be a rare cause of *GCK*-MODY in Spain [Garin et al., 2008]. Gene dosage assays have also shown that overexpression of *GCK* due to gene duplication is not a common cause of HH [van de Bunt et al., 2008].

### Polymorphisms

A considerable number of polymorphisms have been reported in the literature or have been detected during routine sequencing of *GCK*; these are listed in Supp. Table S2. The most common polymorphisms are the IVS9+8T > C variant, which has a minor allele frequency in UK populations of 15% [Thomson et al., 2003] and the islet promoter variant  $-30G > A$ , which has a minor allele



**Figure 1.** Structural model of GCK illustrating the clustering of the naturally occurring activating mutations, which cause GCK-HH. All residues except M197 are clustered in the allosteric activator site.

frequency of 30% in UK populations [Weedon et al., 2005]. This promoter variant has been reproducibly shown to be associated with increased FPG levels and birth weight [Weedon et al., 2006, 2005]. Recent large-scale studies have shown that another *GCK* promoter polymorphism (rs4607517), which is in almost complete linkage disequilibrium (LD) with the *GCK* –30 variant, is also unsurprisingly associated with increased FPG levels but interestingly not with susceptibility to T2DM [Prokopenko et al., 2009].

## Biological Relevance

### *CpG dinucleotides*

A total of 446 point mutations (402 missense, 44 nonsense) within the coding region have been described. Sixteen of these are CG>TG and eight are CG>CA base substitutions, compatible with the deamination of methylcytosines in CpG dinucleotides. This represents 5.4% of *GCK* point mutations, a considerably lower proportion than the 30.4% observed in an analysis of 1,262 mutations in various genes by Cooper et al. [1995]. Therefore, deamination of methylcytosines at CpG dinucleotides does not appear to be an important cause of mutations within the *GCK* gene.

There are 13 reports of de novo mutations in the *GCK* gene. These mutations are R36W, Q38P, S64Y, T65I, G81S, W99R, W99L, R191W, M197I, Y214C, p.Ala454dup (reported as ins454A), IVS3-1G>T and E248X [Christesen et al., 2008; Cuesta-Munoz et al., 2004; Gloyn et al., 2003; Hager et al., 1994; Massa et al., 2001; Sayed et al., 2009; Velho et al., 1997]. Only two of these de novo mutations occur within the context of a CpG dinucleotide, a “hot

spot” for nucleotide substitutions in mammalian genes [Cooper et al., 1995]. Testing whether or not a mutation is de novo requires parental DNA, and as such, is not something that is routinely performed, and may partly explain why there are only 13 reports of de novo mutations. Many mutations may be indeed de novo, but they have not been classified as such.

### *Evidence for a founder effect*

Just over half (~59%) of the mutations detected have been private (365/620). There are four reports of a possible mutation founder effect [Dussoix et al., 1997; Estalella et al., 2007; Sagen et al., 2008; Saker et al., 1996]. The V203A mutation has been found in one French family [Velho et al., 1997] and four Swiss families [Dussoix et al., 1997], whereas the G299R mutation has been found in two pedigrees in the Oxford area of the UK [Saker et al., 1996]. Microsatellite analysis in the latter case suggested a founder effect [Saker et al., 1996]. Two mutations, E399X and A379V, have been found in four families from the same region of Spain [Estalella et al., 2007], while microsatellite analysis of six families in Norway with an E339G mutation was consistent with a common origin and is likely to be a founder mutation [Sagen et al., 2008].

### *Mutation detection*

Because there are no common mutations and the mutations are distributed throughout the gene, exons 1a–10 are usually routinely screened for mutations [Thomson et al., 2003]. Routine molecular

diagnostic testing for GCK-MODY does not currently always include the promoter region. Given the recent discovery of a GCK promoter mutation, it is recommended that screening the GCK  $\beta$ -cell promoter region be included in routine diagnostic testing for GCK-MODY, particularly in patients with a strong clinical phenotype [Gasperikova et al., 2009]. Mutation screening and detection are usually carried out by either a combination of denaturing high-performance liquid chromatography (DHPLC) analysis and direct sequencing or simply by direct sequencing.

Earlier studies have used fluorescent single-stranded conformational polymorphism (F-SSCP) analysis, but this method has largely been replaced by DHPLC and sequencing due to its high false positive rate (5%) [Boutin et al., 2001]. A recent study that used DHPLC to screen for mutations in GCK reported an assay sensitivity of 98% based on the testing of 58 known GCK mutations distributed across the gene with a false positive rate of 4% [Gloyn et al., 2009]. However, direct sequencing remains the most sensitive method (>99%), particularly when used in conjunction with appropriate software for sequence analysis [Boutin et al., 2001; Ellard et al., 2009].

Gene dosage measured by Multiplex Ligation-dependent Probe Amplification (MLPA), a method for the detection of copy number changes in DNA sequences [Sellner and Taylor, 2004], has become an important tool adjunct to sequence analysis when a diagnosis of MODY is strongly suspected because large deletions encompassing whole exons may not be detected by sequencing.

There are now European best practice guidelines for molecular genetic testing in MODY [Ellard et al., 2008].

### Functional studies

There are 74 missense and two frameshift mutations that have been functionally characterized currently reported in the literature (Supp. Table S1 and Table 2); the majority of these alter the enzyme's kinetics. Many kinetic parameters can be altered, and they are usually done so in combination. For inactivating mutations (MODY, PNDM) the glucose  $S_{0.5}$  is usually raised, the ATP  $K_m$  and the Hill coefficient can be increased or decreased, and the maximal specific activity ( $K_{cat}$ ) decreased. The overall result for inactivating mutations is a decrease in the enzyme's phosphorylating potential, which extrapolates to a marked reduction in  $\beta$ -cell glucose usage and hyperglycemia for GCK-MODY mutations [Davis et al., 1999]. Meanwhile, for GCK-HH activation of glucokinase catalysis by the mutations S64Y, T65I, G68V, W99R, W99L, M197I, Y214C, V452L, p.Ala454dup (reported as ins454A), V455M, and A456V, the opposite changes to the kinetic parameters are seen [Christesen et al., 2002, 2008; Cuesta-Munoz et al., 2004; Glaser et al., 1998; Gloyn et al., 2003; Meissner et al., 2009; Sayed et al., 2009; Wabitsch et al., 2007]. These mutations result in the lowering of the glucose  $S_{0.5}$ , an increase or decrease of  $K_{cat}$ , and a reduction of the Hill coefficient for glucose either separately or in combination. These kinetic changes result in an enzyme which has an increased affinity for the substrate glucose (lower  $S_{0.5}$ ) and frequently is catalytically more active (increased  $K_{cat}$ ), which contrasts with the catalytically less active sigmoidal wild-type glucokinase and greatly reduced activity of the GCK-MODY mutant enzymes.

In some cases the sequence change alone cannot predict the clinical phenotype of a given mutation. In most cases the defect in glycemia can be explained by the enzyme kinetics, yet, some GCK-MODY mutations have near normal enzyme kinetics or even a mild increase in affinity for glucose. In these instances other

mutational mechanisms may be at play, for instance, mechanisms that effect enzyme stability [Burke et al., 1999] or binding with regulatory molecules such as GKRP [Arden et al., 2007; Gloyn et al., 2005] or the bifunctional enzyme PFK-2/FBPase-2 [Arden et al., 2007] or perhaps with an unknown endogenous allosteric activator [Gloyn et al., 2005]. For example, the V62M GCK-MODY mutation appears to be paradoxically kinetically activating as opposed to inactivating [Gloyn et al., 2005], and has been shown to have a lack of inhibition by liver GKRP and a lack of activation by pharmacological GCK small molecular activators [Gloyn et al., 2005], although studies in min6 cells have suggested that the V62M GCK mutation may result in catalytic instability [Arden et al., 2007]. There is evidence that at least nine other GCK-MODY mutations affect enzyme stability from recombinant mutant GCK protein studies [Burke et al., 1999; Davis et al., 1999; Galan et al., 2006; Garcia-Herrero et al., 2007; Gloyn et al., 2005; Kesavan et al., 1997; Sagen et al., 2006]. Furthermore, studies have shown that mutations in GCK can result in a loss of regulation by GKRP, which contributes to, but does not cause, glucose intolerance in patients with GCK-MODY and GCK-HH [Arden et al., 2007; Gloyn et al., 2005; Heredia et al., 2006a; Sagen et al., 2006].

Activating mutations causing GCK-HH appear to have different mutational mechanisms. Some activating mutations have been shown to increase glucose binding to the glucokinase enzyme (T65I, A456V), whereas others (W99R, Y214C, V455M) facilitate GCK isomerization into the active form [Heredia et al., 2006a,b]. Functional testing of GCK mutants will provide further insight into the complex mutational mechanisms and the regulation of the allosteric activator site, which may provide insights on novel approaches to target GCK pharmacologically.

The recently described GCK islet promoter mutation, -71G>C, has been shown to modulate GCK gene expression through loss of allele-specific Sp1 binding [Gasperikova et al., 2009]. This is currently the only mutation reported in the promoter region but with increased screening of the promoter additional mutations may be found that could shed light on the elements that are critical for human GCK transcription.

### Clinical relevance

#### Clinical management of GCK-MODY

The identification of a GCK gene mutation in a patient provides a definite diagnosis of MODY (GCK-MODY) and helps to predict the likely prognosis and clinical course. GCK-MODY mutations result in a discrete phenotype despite the wide variety of mutations characterized by elevated FPG with the majority of the patients having blood glucose values within a tight range of 6–8 mmol/l. This is in contrast to other subtypes of MODY, type 1 (T1DM) and type 2 diabetes (T2DM). Patients with GCK mutations have mild stable fasting hyperglycemia and rarely have osmotic symptoms. The vast majority will therefore be detected by screening either for routine medicals, during pregnancy or family screening when MODY is suspected. In most patients the age of diagnosis is therefore the age at which they are first tested.

Administration of a 2-hr oral glucose tolerance test (OGTT) and measuring FPG levels are helpful to determine the degree of hyperglycemia and to uncover the underlying genetic causes if MODY is suspected. Measuring FPG and OGTT testing have been shown to be good discriminators between *HNF1A* and GCK mutations in patients, and can be used as a guide to genetic diagnostic testing [Stride et al., 2002]. It is recommended that

**Table 2. Functional Characterization of Naturally Occurring *GCK* Mutations**

Region	Phenotype	Nucleotide and systematic name	Protein effect	References for functional characterization	Kinetic mutation effect	Mutation effect other
Islet promoter	MODY	c.-71G>C	–	[Gasperikova et al., 2009]	N/A	Allele specific loss of Sp1 transcription factor binding resulting in reduced promoter activity
Exon 2	MODY	c.106C>T	p.Arg36Trp	[Miller et al., 1999]	Decreased $S_{0.5}$ , Decreased $^{ATP}K_m$ , Decreased $K_{cat}$	
	MODY	c.130G>A	p.Gly44Ser	[Gloyn et al., 2004]	Increased $S_{0.5}$ , decreased hill number, decreased $^{ATP}K_m$ , Decreased $K_{cat}$	
	MODY	c.157G>T	p.Ala53Ser	[Davis et al., 1999] [Miller et al., 1999] [Gloyn et al., 2004]	Kinetics same as wild type Thermolabile Near normal kinetics	
	MODY	c.182A>C	p.Tyr61Ser	[Estellella et al., 2007]	Increased $S_{0.5}$ , decreased $^{ATP}K_m$	
	MODY	c.185T>C	p.Val62Ala	[Gloyn et al., 2004]	Increased $S_{0.5}$ , decreased hill number, decreased $K_{cat}$ , decreased $^{ATP}K_m$	
	MODY	c.184G>A	p.Val62Met	[Arden et al., 2007; Gloyn et al., 2004, 2005]	Decreased $S_{0.5}$ , decreased hill number, decreased thermostability, decreased $K_{cat}$	Catalytic instability demonstrated in min6 cells
	HH	c.191C>A	p.Ser64Tyr	[Christesen et al., 2008]	Decreased $S_{0.5}$ , decreased hill number, decreased $K_{cat}$ , increased $^{ATP}K_m$	
	HH	c.194C>T	p.Thr65Ile	[Gloyn et al., 2003, 2004; Heredia et al., 2006a]	Decreased $S_{0.5}$ , decreased hill number, decreased $K_{cat}$ , increased $^{ATP}K_m$	
	HH	c.203G>T	p.Gly68Val	[Wabitsch et al., 2007]	Decreased $S_{0.5}$ , decreased hill number, increased $K_{cat}$ , decreased $^{ATP}K_m$	
	MODY	c.208G>A	p.Glu70Lys	[Burke et al., 1999; Davis et al., 1999; Gloyn et al., 2004; Kesavan et al., 1997]	Increased $S_{0.5}$ , decreased hill number, decreased $K_{cat}$	Clonal cell line study showed mutant to be thermostable
Exon 3	MODY	c.214G>A	p.Gly72Arg	[Arden et al., 2007; Gloyn et al., 2004; Sagen et al., 2006]	Decreased $S_{0.5}$ , decreased hill number, slightly decreased thermostability, decreased $K_{cat}$ , increased $^{ATP}K_m$	Catalytic instability demonstrated in min6 cells
	MODY	c.239G>C	p.Gly80Ala	[Davis et al., 1999; Miller et al., 1999]	Increased $S_{0.5}$ , decreased hill number, decreased $K_{cat}$	
	HH	c.295T>A	p.Trp99Arg	[Gloyn et al., 2003, 2004; Heredia et al., 2006a; Zelent et al., 2008]	Decreased $S_{0.5}$ , decreased hill number, increased $K_{cat}$ , increased $^{ATP}K_m$	
	HH MODY	c.296G>T c.323A>G	p.Trp99Leu p.Tyr108Cys	[Sayed et al., 2009] [Gloyn et al., 2004]	Decreased $S_{0.5}$ , increased $K_{cat}$ Increased $S_{0.5}$ , decreased hill number, decreased $K_{cat}$ , decreased $^{ATP}K_m$	
Exon 4	MODY	c.391T>C	p.Ser131Pro	[Takeda et al., 1993]	Increased $S_{0.5}$ , decreased $K_{cat}$ , decreased $^{ATP}K_m$	
	MODY	c.410A>G	p.His137Arg	[Davis et al., 1999] [Gloyn et al., 2004]	Kinetics same as wild type Kinetics same as wild type	
	MODY	c.437T>G	p.Leu146Arg	[Gloyn et al., 2004; Sagen et al., 2006]	Increased $S_{0.5}$ , decreased hill number, decreased $K_{cat}$ , decreased $^{ATP}K_m$	
	MODY	c.480_482dupTAA	p.Asp160_Lys161 insAsn	[Garcia-Herrero et al., 2007]	Increased $S_{0.5}$ , decreased hill number, decreased $^{ATP}K_m$	
Exon 5	MODY	c.493C>T	p.Leu165Phe	[Galan et al., 2006]	Increased $S_{0.5}$ , decreased hill number, decreased $K_{cat}$ , decreased thermostability, increased $^{ATP}K_m$	
	PNDM MODY	c.502A>G	p.Thr168Ala	[Turkkahraman et al., 2008]	Increased $S_{0.5}$ , decreased hill number, decreased $K_{cat}$ , increased $^{ATP}K_m$	
	MODY	c.502A>C	p.Thr168Pro	[Davis et al., 1999; Gloyn et al., 2004; Miller et al., 1999]	Increased $S_{0.5}$ , decreased hill number, increased $^{ATP}K_m$ , decreased $K_{cat}$	

TABLE 2. Continued

Region	Phenotype	Nucleotide and systematic name	Protein effect	References for functional characterization	Kinetic mutation effect	Mutation effect other
Exon 6	MODY	c.523G>A	p.Gly175Arg	[Davis et al., 1999; Gidh-Jain et al., 1993; Gloyn et al., 2004]	Increased $S_{0.5}$ , decreased hill number, decreased $K_{cat}$	
	MODY	c.524G>A	p.Gly175Glu	[Davis et al., 1999]	Increased $S_{0.5}$ , decreased $K_{cat}$	
	MODY	c.544G>T	p.Val182Leu	[Estalella et al., 2008]	Increased $S_{0.5}$ , increased hill number, decreased $^{ATP}K_m$	
	MODY	c.544G>A	p.Val182Met	[Davis et al., 1999; Gidh-Jain et al., 1993; Gloyn et al., 2004]	Increased $S_{0.5}$ , decreased hill number, decreased $K_{cat}$ , decreased $^{ATP}K_m$	
	MODY	c.562G>A	p.Ala188Thr	[Takeda et al., 1993]	Increased $S_{0.5}$ , decreased $K_{cat}$ , decreased $^{ATP}K_m$	
	MODY	c.563C>A	p.Ala188Glu	[Gloyn et al., 2004]	Increased $S_{0.5}$ , decreased hill number, decreased $K_{cat}$ , decreased $^{ATP}K_m$	
	HH	c.591G>T	p.Met197Ile	[Sayed et al., 2009]	Decreased $S_{0.5}$ , decreased $K_{cat}$ , increased $^{ATP}K_m$	
	MODY	c.608T>C	p.Val203Ala	[Burke et al., 1999; Davis et al., 1999; Gidh-Jain et al., 1993; Gloyn et al., 2004]	Increased $S_{0.5}$ , decreased $K_{cat}$ , increased $^{ATP}K_m$	
	MODY	c.617C>T	p.Thr206Met	[Galan et al., 2006]	Increased $S_{0.5}$ , decreased hill number, decreased $K_{cat}$	
	MODY	c.622G>A	p.Ala208Thr	[Sagen et al., 2006]	Increased $S_{0.5}$ , decreased $K_{cat}$ , decreased hill number, increased $^{ATP}K_m$	
	MODY	c.626C>T	p.Thr209Met	[Miller et al., 1999]	Decreased $S_{0.5}$ , increased $^{ATP}K_m$ , decreased $K_{cat}$	
	PNDM MODY	c.629T>A	p.Met210Lys	[Gloyn et al., 2004; Sagen et al., 2006]	Increased $S_{0.5}$ , decreased hill number, increased $^{ATP}K_m$ , decreased $K_{cat}$	
	MODY	c.629T>C	p.Met210Thr	[Davis et al., 1999; Gloyn et al., 2004]	Increased $S_{0.5}$ , decreased hill number, decreased $K_{cat}$	
	MODY	c.637T>C	p.Cys213Arg	[Davis et al., 1999; Gloyn et al., 2004; Miller et al., 1999; Zelent et al., 2008]	Increased $S_{0.5}$ , decreased hill number, increased $^{ATP}K_m$ , decreased $K_{cat}$	
	HH	c.641A>G	p.Tyr214Cys	[Cuesta-Munoz et al., 2004; Gloyn et al., 2004; Heredia et al., 2006a]	Decreased $S_{0.5}$ , decreased hill number, increased $^{ATP}K_m$ , increased $K_{cat}$	
	MODY	c.676G>A	p.Val226Met	[Pedelini et al., 2005]	Decreased $^{ATP}K_m$	
	MODY	c.676G>A	p.Val226Met	[Davis et al., 1999; Gloyn et al., 2004; Miller et al., 1999]	Increased $S_{0.5}$ , decreased hill number, increased $^{ATP}K_m$ , decreased $K_{cat}$	
	MODY	c.682A>G	p.Thr228Ala	[Marotta et al., 2005]	Decreased $S_{0.5}$ , decreased hill number	
	MODY	c.697T>C	p.Cys233Arg	[Estalella et al., 2008]	Kinetics same as wild type	
	MODY	c.703A>G	p.Met235Val	[Garcia-Herrero et al., 2007]	Thermostable, decreased $K_{cat}$	
	MODY	c.704T>C	p.Met235Thr	[Gloyn et al., 2004]	Increased $S_{0.5}$ , decreased hill number, decreased $^{ATP}K_m$ , decreased $K_{cat}$	
	MODY	c.755G>A	p.Cys252Tyr	[Gloyn et al., 2004]	Increased $S_{0.5}$ , increased hill number, increased $^{ATP}K_m$ , decreased $K_{cat}$	
				[Zelent et al., 2008]	Decreased hill number, decreased $K_{cat}$	
	MODY	c.766G>A	p.Glu256Lys	[Gidh-Jain et al., 1993]	Decreased $S_{0.5}$ , decreased $K_{cat}$	
	MODY	c.769T>C	p.Trp257Arg	[Takeda et al., 1993]	Decreased $S_{0.5}$ , decreased $K_{cat}$	
	MODY	c.781G>A	p.Gly261Arg	[Davis et al., 1999; Gidh-Jain et al., 1993]	Increased $S_{0.5}$ , increased hill number, increased $^{ATP}K_m$ , decreased $K_{cat}$	
				[Gloyn et al., 2004]	Decreased hill number	
	MODY	c.787T>C	p.Ser263Pro	[Gloyn et al., 2004; Sagen et al., 2006]	Increased $S_{0.5}$ , decreased hill number, decreased thermostability, increased $^{ATP}K_m$	
	PNDM MODY	c.790G>A	p.Gly264Ser	[Gloyn et al., 2004; Njolstad et al., 2003; Sagen et al., 2006]	Near normal kinetics mild elevation of $S_{0.5}$ , slight decrease in hill number, slightly decreased thermostability	



TABLE 2. Continued

Region	Phenotype	Nucleotide and systematic name	Protein effect	References for functional characterization	Kinetic mutation effect	Mutation effect other
Exon 8	MODY	c.793G>A	p.Glu265Lys	[Estalella et al., 2008; Galan et al., 2006]	Increased $S_{0.5}$ , decreased thermostability, increased $^{ATP}K_m$	
	MODY	c.823C>T	p.Arg275Cys	[Gloyn et al., 2004]	Near normal kinetics	
	MODY	c.835G>C	p.Glu279Gln	[Gidh-Jain et al., 1993]	Increased $S_{0.5}$ , decreased $K_{cat}$	
	MODY	c.893T>A	p.Met298Lys	[Gloyn et al., 2004]	Increased $S_{0.5}$ , decreased hill number, increased $^{ATP}K_m$ , decreased $K_{cat}$	
	MODY	c.895G>C	p.Gly299Arg	[Gidh-Jain et al., 1993]	Decreased $S_{0.5}$ , decreased $^{ATP}K_m$ , decreased $K_{cat}$	
	MODY	c.898G>C	p.Glu300Gln	[Gidh-Jain et al., 1993]	Increased $S_{0.5}$	
	MODY	c.898G>A	p.Glu300Lys	[Burke et al., 1999; Davis et al., 1999; Gidh-Jain et al., 1993; Kesavan et al., 1997]	Kinetics same as wild type Decreased thermostability	Clonal cell line study showed mutant to be unstable in vivo
				[Gloyn et al., 2004]	Increased $S_{0.5}$ , increased $^{ATP}K_m$	
	MODY	c.922A>T	p.Arg308Trp	[Garcia-Herrero et al., 2007]	Increased $S_{0.5}$ , decreased $K_{cat}$ , decreased thermostability	
	MODY	c.926T>C	p.Leu309Pro	[Davis et al., 1999; Gidh-Jain et al., 1993; Gloyn et al., 2004]	Increased $S_{0.5}$ , decreased hill number, increased $^{ATP}K_m$ , decreased $K_{cat}$	
Exon 9	MODY	c.1007C>T	p.Ser336Leu	[Davis et al., 1999; Gloyn et al., 2004; Marotta et al., 2005]	Decreased $S_{0.5}$ , decreased hill number, increased $^{ATP}K_m$ , decreased $K_{cat}$	
	MODY	c.1016A>G	p.Glu339Gly	[Gloyn et al., 2004; Sagen et al., 2006]	Increased $S_{0.5}$ , decreased hill number, increased $^{ATP}K_m$ , decreased $K_{cat}$	
	MODY	c.1099G>A	p.Val367Met	[Davis et al., 1999; Gloyn et al., 2004; Miller et al., 1999]	Kinetics same as wild type	
	MODY	c.1129C>T	p.Arg377Cys	[Gloyn et al., 2004; Sagen et al., 2006]	Increased $S_{0.5}$ , decreased $K_{cat}$	
	PNDM MODY	c.1139C>T	p.Ala378Val	[Gloyn et al., 2004; Njolstad et al., 2003]	Increased $S_{0.5}$ , increased $^{ATP}K_m$ , decreased hill number, decreased $K_{cat}$	
	MODY	c.1136C>T	p.Ala379Val	[Estalella et al., 2008]	Increased $S_{0.5}$ , increased $^{ATP}K_m$	
	MODY	c.1148C>T	p.Ser383Leu	[Gloyn et al., 2004]	Increased $S_{0.5}$ , increased $^{ATP}K_m$ , decreased hill number, decreased $K_{cat}$	
	PNDM MODY	c.1190G>T	p.Arg397Leu	[Garcia-Herrero et al., 2007]	Decreased thermostability	
	MODY	c.1232C>T	p.Ser411Phe	[Gloyn et al., 2004]	Decreased $S_{0.5}$ , increased $^{ATP}K_m$ , decreased hill number, decreased $K_{cat}$	
	MODY	c.1240A>G	p.Lys414Glu	[Davis et al., 1999; Pino et al., 2007]	Increased $S_{0.5}$ , increased hill number, increased $^{ATP}K_m$ , decreased thermostability, decreased $K_{cat}$	
Exon 10				[Gloyn et al., 2004]	Decreased $S_{0.5}$	
	MODY	c.1258A>G	p.Lys420Glu	[Estalella et al., 2008]	Increased $S_{0.5}$ , increased hill number	
	MODY	c.1358C>T	p.Ser453Leu	[Sagen et al., 2006]	Increased $S_{0.5}$ , increased $^{ATP}K_m$ , decreased hill number, decreased $K_{cat}$	
	HH	c.1361_1363dupCGG	p.Ala454dup	[Sayed et al., 2009]	Decreased $S_{0.5}$ , decreased hill number, decreased $K_{cat}$	
	HH	c.1363C>A	p.Val455Met	[Burke et al., 1999; Davis et al., 1999; Gloyn et al., 2004; Heredia et al., 2006a]	Decreased $S_{0.5}$ , increased $^{ATP}K_m$ , decreased hill number, decreased $K_{cat}$	Clonal cell line study showed mutant with decreased $S_{0.5}$
	MODY	c.1364T>A	p.Val455Glu	[Gloyn et al., 2008]	Increased $S_{0.5}$ , decreased $K_{cat}$	
	HH	c.1367C>T	p.Ala456Val	[Christesen et al., 2002; Gloyn et al., 2004; Heredia et al., 2006a; Pedelini et al., 2005; Pino et al., 2007]	Decreased $S_{0.5}$ , thermostable, decreased $^{ATP}K_m$ , decreased hill number, increased $K_{cat}$	

All sequence information is based on GenBank reference sequence NM\_000162.2. Nucleotide numbering reflects cDNA numbering with +1 corresponding to the A of the major start codon of exon 1a, the alternate exon 1 present in the pancreatic isoform. N/A; not applicable,  $K_{cat}$ ; maximal specific activity,  $S_{0.5}$ ; affinity for the substrate glucokinase.

<sup>a</sup>Details not given in original publication of the insertion or deletion or the nucleotide change.

GCK-MODY families only require FPG testing because a GCK mutation is not very likely if a subject has a FPG under 5.5 mmol/l (98% of GCK-MODY patients) [Stride et al., 2002].

Patients with GCK mutations rarely need any pharmacological treatment, and the majority are managed on diet alone. A recent study showed that mean HbA1c was unaltered by discontinuing insulin or oral hypoglycemic agents (OHAs) in 87% of GCK-MODY patients [Gill Carey et al., 2007]. Additionally, over-treatment with OHAs or insulin therapy has been reported [Sagen et al., 2008], and may be especially risky for patients with GCK mutations because they have an altered counterregulatory response to hypoglycemia [Guenat et al., 2000].

Of particular importance is the fact that microvascular complications, which are a considerable cause of mortality and morbidity in diabetes, are rare in GCK-MODY patients. Although there are few studies published on the complications of GCK-MODY, it would be expected that complications in GCK-MODY patients would be rare as a consequence of the relatively small increase in blood glucose levels and the low prevalence of hypertension in the subjects with GCK mutations [Velho et al., 1997]. Data from a French series reported few cases (4–6%) of proliferative retinopathy, proteinuria, and peripheral neuropathy in GCK-MODY patients [Velho et al., 1997] as well as a Norwegian cohort reported few cases of retinopathy and macrovascular disease [Sagen et al., 2008]. Furthermore, there is a mild deterioration of fasting blood glucose with age, which is in line with the normal population [Stride et al., 2002]. These patients do not need to be followed up in the Diabetes Clinic but should have an HbA1c checked annually by their general practitioner.

Mutations in GCK may be suspected during pregnancy when fasting blood glucose levels are in the range of 5.5–5.8 mmol/l before, during, and after pregnancy, with a relative low increment during an OGTT [Ellard et al., 2000]. Moreover, women with GCK mutations may have babies that are large for gestational age if the baby has not inherited the GCK mutation [Hattersley et al., 1998; Singh et al., 2007], and babies that inherit a GCK mutation have lighter placentas than those without the mutation [Shields et al., 2008]. Fetal birth weight has been shown to be predominantly altered by fetal genotype and not by treatment of maternal hyperglycemia with insulin [Spyer et al., 2009] with no reduction in  $\beta$ -cell function or deterioration of glucose tolerance in offspring exposed to moderate hyperglycemia in the uterus as a result of maternal GCK mutations [Singh et al., 2007]. However, it is not known if fetal growth can be influenced by treating maternal hyperglycemia with insulin. Lowering maternal blood glucose in babies not harboring a GCK mutation may also potentially adversely affect fetal growth [Spyer et al., 2009]. It is suggested that mothers undergo serial fetal abdominal scans to identify babies showing excessive growth to thereby target insulin therapy to those at greatest risk, or alternatively, to induce delivery at 38 weeks to avoid the risks of delivering a macrosomatic baby [Kjos et al., 2001; Spyer et al., 2009].

### *Clinical management of GCK-PNDM*

Homozygous or compound heterozygous inactivating mutations of GCK are a rare cause of PNDM. Glycemic control has been shown to improve in response to oral sulphonylurea therapy in patients with  $K_{ATP}$  channel-activating mutations (*KCNJ11* & *ABCC8*) after being transferred from insulin injection therapy [Pearson et al., 2006; Rafiq et al., 2008]. Recently, treatment of a 4-year-old GCK-PNDM patient with the sulphonylurea,

glibenclamide, over a 3-month period was shown to be beneficial with an increase in both basal and stimulated insulin secretion (by 12-fold) [Turkkahraman et al., 2008]. Although insulin injections could not be stopped, there was a 1.3% reduction in HbA1c levels on a reduced insulin dose [Turkkahraman et al., 2008]. The authors suggested that insulin treatment could not be completely stopped because the ATP generated by glucose metabolism in the  $\beta$ -cells would be insufficient for continued insulin granule docking and exocytosis in the postprandial period when glucose levels rise [Turkkahraman et al., 2008]. It is possible that patients with a less severe clinical phenotype resulting from a milder GCK mutation, for example, R397L, may respond better to sulphonylureas than those with a more severe phenotype resulting from severe mutations such as the T168A mutation [Porter et al., 2005; Turkkahraman et al., 2008]. Although there are no current trials in progress, glucokinase activators would also be an ideal treatment in this patient subgroup, and in the future this may be a standard treatment for GCK-PNDM.

### *Clinical management of GCK-HH*

The identification of a GCK mutation in HH also provides a definite diagnosis, and, with the increasing number of reported cases, provides information on the prognosis and the likely clinical course of the disease. The vast majority of patients with HH resulting from a GCK mutation respond well or tolerate diazoxide [Christesen et al., 2002, 2008; Glaser et al., 1998; Gloyn et al., 2003; Meissner et al., 2009; Sayed et al., 2009; Wabitsch et al., 2007], and there is evidence suggesting that some of these patients may not actually require pharmacological intervention and may be able to control their symptoms by eating regularly [Gloyn et al., 2003; Wabitsch et al., 2007].

This is in contrast to the vast majority of patients with HH due to mutations in the  $K_{ATP}$  channel genes (*ABCC8*, *KCNJ11*) who respond poorly or are totally refractory to the potassium channel opener diazoxide, which is used to inhibit insulin secretion [Touati et al., 1998]. Due to the very high circulating blood levels of insulin, it is often necessary to perform a subtotal pancreatectomy to control the hypoglycemia in patients with  $K_{ATP}$ -HH because insulin secretion is unregulated and insulin levels are much higher [Gloyn et al., 2003]. However, this is not necessary with the majority of patients with GCK-HH as the defect is a “resetting” of the threshold for GSIR, and insulin secretion remains regulated and only high for the blood glucose levels. There are, however, reported cases of GCK-HH that are resistant to diazoxide therapy [Sayed et al., 2009], and one case where brain damage occurred even after treatment with diazoxide and subtotal pancreatectomy [Cuesta-Munoz et al., 2004].

There is evidence from at least one study to suggest that patients with neonatal hyperinsulinemia are more likely to develop T2DM later in life. A study carried out in an extended Finnish family has illustrated that mutations in *ABCC8* can present as hypoglycemia during infancy and diabetes in early adulthood [Huopio et al., 2003]. However, other larger case series have failed to detect an increase in T2DM prevalence in individuals with autosomal dominant *ABCC8* mutations causing HH [Pinney et al., 2008]. Studies have also shown that mutations in the *HNF4A* gene, which plays a key role in the regulation of pancreatic insulin secretion, can cause a transient form of HH that may evolve into decreased insulin secretion and diabetes (*HNF4A*-MODY) later in life [Pearson et al., 2007]. However, it is not known whether this is the case with GCK activating mutations. It has been suggested that

because the defect is in glucose sensing rather than excess insulin secretion this is unlikely to result in the “ $\beta$ -cell exhaustion” seen in  $K_{ATP}$  channel mutations [Huopio et al., 2003]. From the cases reported to date there is no evidence to support increased T2DM in patients with GCK-HH.

## Future Prospects

The identification of a *GCK* mutation in a patient with either MODY, PNDM, or HH provides a firm diagnosis of their disorder and also confirms the subtype. Given the implications for prognosis, treatment decisions, and inheritance, it is important to identify individuals with *GCK* mutations [Gloyn and Ellard, 2006]. As with any autosomal dominantly inherited disorder the identification of a disease causing mutation in a proband has implications for other family members. Unless the mutation is de novo the proband's sibling will have a 50% chance of having inherited the mutation. In the case of GCK-MODY and GCK-HH it is worth performing a fasting blood glucose test in family members as both disorders can be subclinical prior to undertaking mutation screening. The discovery of inactivating and activating *GCK* mutations and their functional characterization has led to important concepts that explain essential characteristics of GSIR. Work in this area has led to important insights into the biochemical activation and regulation of glucokinase and has provided a drug target for the treatment of T2DM. It is possible that, over the next 5–10 years, we will see patients with not only GCK-PNDM but also T2DM being treated by novel pharmacological agents that activate glucokinase by a similar mechanism to the mutations that result in GCK-HH. The identification of several classes of small molecule glucokinase activators (GKAs) that bind to the *GCK* allosteric activator site has drawn considerable recent interest [Bertram et al., 2008; Efanov et al., 2005; Grimsby et al., 2008; Iino et al., 2009; McKerrecher et al., 2006] and at least one *GCK*-targeted activator is undergoing Phase I clinical trials as a diabetes therapy [Grimsby et al., 2003; Guertin and Grimsby, 2006; McKerrecher et al., 2006]. A recent review has been published outlining the scientific basis and developmental progress of various GKAs [Matschinsky, 2009]. The search for an endogenous activator/regulator of *GCK* is also attractive, for it may hold the promise of providing a further molecular target for therapeutic intervention.

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