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ORIGINAL ARTICLE

Familial adult onset hyperinsulinism due to an activating glucokinase mutation: implications for pharmacological glucokinase activation

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Summary

Context Glucokinase (GCK) phosphorylates and thereby "traps" glucose in cells, thus serving as a gatekeeper for cellular glucose metabolism, particularly in hepatocytes and pancreatic beta cells. In humans, activating GCK mutations cause familial hyperinsulinaemic hypoglycaemia (GCK-HH), leading to keen interest in the potential of small-molecule glucokinase activators (GKAs) as treatments for diabetes mellitus. Many such agents have been developed; however, observation of side effects including hypertriglyceridaemia and hepatic steatosis has delayed their clinical development.

Objective To describe the clinical presentation and metabolic profiles of affected family members in a kindred with familial hyperinsulinism of adult presentation due to a known activating mutation in *GCK*.

Design Clinical, biochemical and metabolic assessment, and *GCK* sequencing in affected family members.

Results In the 60-year-old female proband, hyperinsulinaemic hypoglycaemia (blood glucose 2·1 mmol/mol, insulin 18 pM) was confirmed following 34 h of fasting; however, abdominal computed tomography (CT), pancreatic MRI, endoscopic ultrasound, octreotide scintigraphy and selective arterial calcium stimulation failed to localize an insulinoma. A prolonged OGTT revealed fasting hypoglycaemia that was exacerbated after glucose challenge, consistent with dysregulated glucose-stimulated insulin release. A heterozygous activating mutation, p.Val389Leu, in the glucokinase gene (*GCK*) was found in the proband and four other family members. Of these, two had been investigated elsewhere for recurrent hypoglycaemia in adulthood, while the other two adult relatives were asymptomatic despite profound hypoglycaemia. All three of the available family members with the p.Val389Leu mutation had normal serum lipid profiles, normal rates of fasting hepatic *de novo* lipogenesis and had hepatic triglyceride levels commensurate with their degree of adiposity.

Conclusion Activating *GCK* mutations may present in late adulthood with hyperinsulinaemic hypoglycaemia and should be considered even in older patients being investigated for insulinoma. Normal circulating lipids, rates of hepatic *de novo* lipogenesis and appropriate hepatic triglyceride content for degree of adiposity in the patients we describe suggest that even lifelong *GCK* activation in isolation is insufficient to produce fatty liver and metabolic dyslipidaemia.

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Introduction

Glucokinase (GCK) is an important regulator of glucose homoeostasis that serves as both the blood glucose "sensor" in pancreatic β-cells and as the key gatekeeper of glucose disposal to promote glycogen and triglyceride synthesis within the liver. Given the fundamental role of GCK in both pancreatic and hepatic glucose metabolism, small-molecule GCK activators (GKAs) that lower the threshold for glucose-stimulated insulin secretion (GSIS) and increase hepatic glucose uptake have been developed as potential therapeutics for the treatment of type 2 diabetes mellitus. Initial reports, however, suggest that some GKAs are associated with an increased risk of hypoglycaemia and may accelerate the development of hepatic steatosis and dyslipidaemia, thereby calling into question the safety of long-term induction of GCK hyperactivation as a therapeutic strategy.[1]

In humans, genetic study of patients with Mendelian disorders of glucose homoeostasis has provided unequivocal evidence for the critical metabolic role of GCK. Heterozygous inactivating *GCK* mutations result in a subtype of maturity onset diabetes of the young (GCK-MODY), while homozygous

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inactivating mutations produce permanent neonatal diabetes. Conversely, rare, heterozygous activating mutations cause familial hyperinsulinaemic hypoglycaemia (GCK-HH).[2] GCK-HH is characterized by a spectrum of clinical phenotypes, most commonly identified in neonates but also sometimes unrecognized until later childhood or adulthood.[3] Adults with activating GCK mutations are most commonly identified during family screening after identification of an affected neonate.

Biochemical studies have demonstrated that, in general, naturally occurring GCK-HH mutations increase the affinity of GCK for glucose and lower the threshold for GSIS, reflecting aberrant activity of the pancreatic GCK isoform.[4,5] However, GCK is also expressed in nonpancreatic tissues including liver and enteroendocrine L cells that secrete GLP-1. Hepatocytes express the vast majority of total body GCK, and in these cells, the enzyme regulates intermediary metabolism, including the synthesis of glycogen, cholesterol, triacylglycerols and activation of *de novo* lipogenesis. In rodents, overexpression or pharmacological activation of hepatic GCK results in hypertriglyceridaemia and hepatic steatosis.[6–8] Similarly, in healthy human subjects, elevated hepatic GCK expression is associated with increased hepatic lipogenesis and liver triglyceride content and single nucleotide polymorphisms within the liver-specific glucokinase kinase regulatory protein (GKRP) are associated with elevated plasma triglyceride levels and hepatic steatosis.[9–11] Furthermore, chronic administration of an oral GKA induced hypertriglyceridaemia and increased incidence of hypoglycaemia in insulin-treated patients with type 2 diabetes mellitus.[12] Collectively, these data suggest that GCK hyperactivation, whether genetic or pharmacological, drives metabolic dyslipidaemia and fatty liver.

Here we describe a 60 year-old woman who first presented with symptomatic hyperinsulinaemic hypoglycaemia in her fifth decade, leading to a prolonged search for a suspected insulin-secreting tumour before a heterozygous activating mutation in the *GCK* gene, p.Val389Leu, was identified in her and four other family members. We further report the wider metabolic phenotype of three family members available for study, including determination of hepatic triglyceride, hepatic *de novo* lipogenesis and serum lipid profiles.

Methods

Clinical studies were performed after approval of the National Health Service Research Ethics Committee United Kingdom. Each participant provided written informed consent, and all studies were conducted in accordance with the principles of the Declaration of Helsinki.

Genetic analyses

Genomic DNA was extracted from peripheral blood lymphocytes or saliva. The coding region and splice junctions of *GCK* were then amplified by PCR of genomic DNA and sequenced by conventional Sanger sequencing using the BigDye® Terminator v3·1 Cycle Sequencing Kit (Applied Biosystems, Paisley, UK) and analysed on an ABI 3730 automated sequencer (Applied Biosystems, Paisley, UK). Primer sequences are available on request.

Analytic methods

Insulin and C-peptide were measured by the Diasorin Liaison® XL automated immunoassay analyser using a one-step chemiluminescence immunoassay (Diasorin, Saluggia, Italy). Total GLP-1 was measured by a commercially available two-site immunoassay (Meso Scale Discovery Kit, Gaithersburg, MD, USA).

Oral glucose tolerance test

Whole-body insulin sensitivity was assessed with a 5-h, 75-g oral glucose tolerance test. After insertion of an antecubital intravenous line, blood samples were collected at 0, 10, 30, 60, 90, 120, 150, 180, 210, 240, 270 and 300 min. for determination of plasma glucose, insulin, C-peptide and total GLP-1.

Body composition

Body composition was measured by Lunar Prodigy dual-energy X-ray absorptiometry (GE Healthcare, Amersham, UK).

Proton magnetic resonance spectroscopy

Liver fat was measured using proton magnetic resonance spectroscopy on a Siemens Verio 3T MR scanner (Siemens Healthcare Diagnostics, Camberley, UK). A spectrum was obtained from a voxel, cube length 1·5 cm, located within the posterior aspect of the right lobe of the liver, using the point-resolved selective spectroscopy (PRESS) sequence. During this measurement, volunteers were given breathing instructions with a 7-s cycle, which was designed and gated such that participants were at the end of expiration during the localization and subsequent acquisition. Nonwater suppressed data were acquired with TR = 7 s, TE = 35 ms and 20 averages. The voxel was positioned to avoid blood vessels, the gall bladder and any proximity to fat surrounding the kidney using HASTE transaxial images that were also acquired in the same phase of respiration. T_2 relaxation times for CH_2 lipid and water were determined from similarly gated spectra using seven different TEs (30–130 ms). The spectra were analysed in jMRUI and fitted using the AMARES algorithm with prior knowledge.[23,24] Liver fat was quantified using T_2-corrected values and expressed as a percentage of (CH_2 lipid)/(CH_2 lipid + water).

De novo *lipogenesis*

To determine the rates of fasting hepatic *de novo* lipogenesis, the incorporation of deuterium into plasma TG during administration of deuterium-labelled water was used to determine the fractional synthetic rate of fatty acids and calculated as described previously.[25]

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Results

Case histories and genetic studies

A 63-year-old woman presented with a history of low mood, weight gain and recurrent episodes of dizziness which improved following food ingestion. She had no significant medical history and did not take any medications. Her father, with whom she had little contact, was said to have been diagnosed with an insulinoma. Her BMI was 35·4 kg/m^2, but physical examination was otherwise normal. Initial investigation revealed hypoglycaemia on a random blood glucose measurement (2·8 mM), low Hb_A1c of 26 mmol/mol (NR: 35–45 mM), negative plasma sulfonylurea screen and an IGF-2/IGF-1 ratio of 2·4 (NR: <10), thereby prompting further investigation for an insulin-secreting tumour. Hyperinsulinaemic hypoglycaemia (blood glucose 2·1 mmol/mol, insulin 18pM, C-peptide 250 pM) was provoked by 34 h of fasting; however, a notable feature of the fast was that plasma glucose remained between 2·1 and 2·9 mM throughout. Further investigation including abdominal computed tomography, pancreatic magnetic resonance imaging, endoscopic ultrasound, octreotide scintigraphy and selective arterial calcium stimulation failed to provide anatomical or biochemical evidence of a localized insulinoma. An extended oral glucose tolerance test revealed fasting hypoglycaemia that was exacerbated following a glucose challenge (Fig. 1). Subsequent analysis of the *GCK* gene revealed a previously characterized heterozygous activating mutation, p.Val389Leu, in the proband and four other family members, including her father (Fig. 2). The proband was intolerant of acarbose and diazoxide, and blood glucose levels did not improve with either subcutaneous octreotide or nifedipine. After explanation of her altered glucose set point, institution of a low-glycaemic-index diet and an exercise programme, glycaemia remains stable, although the Hb_A1c, at 26 mmol/mol, suggests persisting hypoglycaemia. Nevertheless, she is only occasionally symptomatic and has lost 10 kg in body weight over a 12-month period.

The proband's father (patient I.I) presented at the age of 77 with a history suggestive of hypoglycaemia and biochemical evidence of fasting hyperinsulinaemic hypoglycaemia (plasma glucose 2·2 mM, serum insulin 40 pM, serum C-peptide 757 pM). After failed attempts to localize a suspected insulin-secreting tumour by triple-phase abdominal computed tomography and endoscopic ultrasound, and in line with the patient's wishes, he was commenced on diazoxide but was lost to follow-up for 5 years. Now, at 84 years of age, he remains on diazoxide (50 mg daily) with subjective symptomatic improvement despite evidence of continuing chronic hypoglycaemia (Hb_A1c 28 mmol/mol).

The proband's son (patient III.7), although known within the family to suffer episodes of agitation that settled with food, had not had prior endocrine evaluation. At 34 years of age, he was identified as a heterozygote for the familial p.Val389Leu GCK mutation. He had a BMI of 37 kg/m^2 but had no abnormalities on general examination. Nevertheless, an Hb_A1c of 24 mmol/mol and plasma glucose of 2·6 mM with concomitant insulin of

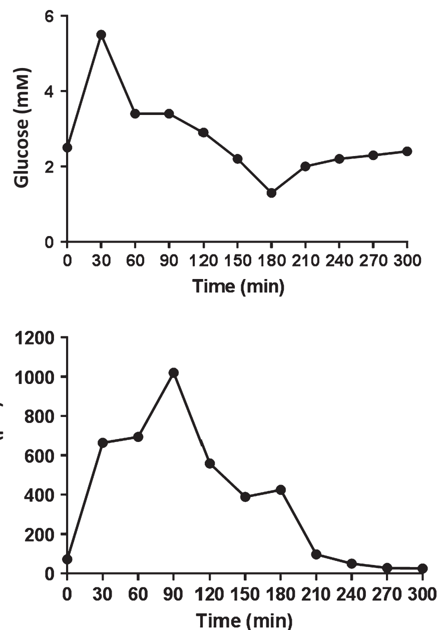

Fig. 1 Prolonged oral glucose tolerance test in the proband with corresponding plasma insulin measurements.

124 pM confirmed a diagnosis of hyperinsulinaemic hypoglycaemia. During a prolonged OGTT, postprandial hypoglycaemia was profound (plasma glucose 1·4 mM; insulin 182 pM at 210 min.) due to an exaggerated hyperinsulinaemic response (peak insulin 2950 pM; peak C -peptide 8043 pM). He is managed on a low glycaemic diet.

Patient III.2 had a history of symptoms suggestive of hypoglycaemia at the age of 20 years; however, despite formal documentation of hypoglycaemia, no further investigation was undertaken. A low random blood glucose (3·0 mM) and Hb_A1c

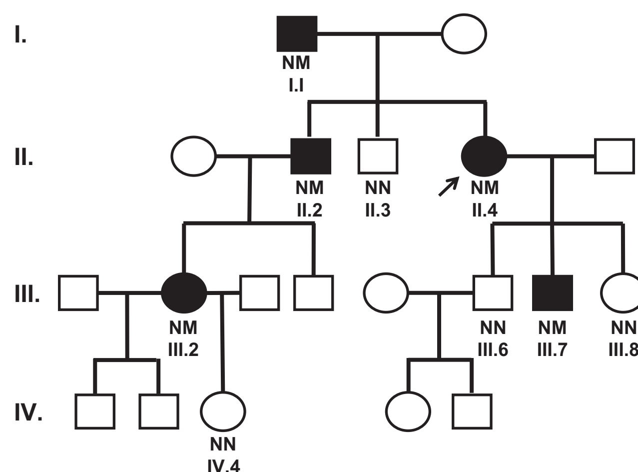

Fig. 2 Pedigree of family with the p.Val389Leu Glucokinase mutation. NM denotes heterozygous carriers of p.Val389Leu, and NN represents wild-type individuals. The proband is indicated by the arrow. Black symbols: p.Val389Leu. White symbols: normoglycaemic and/or not genetically tested. Circles: females. Squares: males.

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Table 1. Body composition, biochemistry, rates of *de novo* lipogenesis and hepatic triglyceride content in patients with the p.Val389Leu GCK mutation

	I.I	II.4 (proband)	III.2	III.7
Age at genetic diagnosis (years)	84	63	33	34
BMI (kg/m2)	28	40	23	37
% Body fat	ND	50	36·0	36·6
Hb_A1c (mmol/mol)	28	27	20	24
Fasting blood glucose (mM)	2·2	2·1	2·4	2·6
Fasting plasma insulin (pM)	40	18	33	124
Fasting C-peptide (nM)	0·76	0·25	0·52	1·1
Albumin (30–51 g/l)	46	41	36	40
Bilirubin (0–17 uM)	10	10	10	8
ALP (30–135 U/l)	178	130	59	80
ALT (0–50 U/l)	17	43	13	50
γGT (0–51 U/l)	ND	27	16	29
Cholesterol (mM)	ND	5·1	5·1	4·4
Triglycerides (mM)	ND	0·9	0·9	2
HDL (mM)	ND	1·35	1·4	0·59
LDL (mM)	ND	3·35	3·3	2·91
Hepatic TG content (%)	ND	16·5	0·09	17·7
Hepatic *de novo* lipogenesis (2–5%)[25]	ND	2·3	2·5	ND
Management	Diazoxide	Low-glycaemic-index diet	Low-glycaemia-index diet	Low-glycaemia-index diet

ALP, alkaline phosphatase; ALT, alanine aminotransferase; ND, not determined.

(20 mmol/mol) at the age of 33 years prompted re-evaluation. At that stage, she reported hypoglycaemic symptoms occurring 1–2 h postprandially, resulting in a fear of eating and consequent weight loss. Her BMI at presentation was 23·0 kg/m^2. Hyperinsulinaemic hypoglycaemia was documented on fasting (blood glucose 2·4 mM; insulin 33 pM; C-peptide 520 pM). Radiological investigations including abdominal computed tomography and pancreatic MRI were unremarkable. A prolonged oral glucose tolerance test revealed severe symptomatic hyperinsulinaemic hypoglycaemia (blood glucose 1·2 mM and insulin 289 pM after 150 min.). She is currently managed on a low glycaemic diet with some symptomatic improvement.

Patient II.2 was identified as a carrier of the p.Val389Leu GCK mutation through predictive genetic testing. He has never reported symptoms suggestive of hypoglycaemia nor has he been reviewed by a medical professional in this regard. Blood samples could not be obtained for further biochemical testing.

Biochemical characteristics of patients with the GCK p.Val389Leu mutation

To determine whether individuals with lifelong genetic GCK activation have evidence of deleterious hepatic effects such as increased lipogenesis, fatty liver and metabolic dyslipidaemia, serum indices of liver function, circulating plasma triglyceride and cholesterol levels, hepatic triglyceride content and rates of fasting hepatic *de novo* lipogenesis were assessed. As shown in Table 1, all but one subject with GCK p.Val389Leu had normal plasma levels of alanine aminotransferase (ALT), alkaline phosphatase (ALP) and gamma-glutamyl transpeptidase (γ-GT). Subject I.I had mildly elevated ALP levels with normal ALT and bilirubin measurements. Similarly affected individuals available for assessment had normal circulating lipid profiles, normal rates of fasting hepatic *de novo* lipogenesis, determined using deuterium incorporation into plasma palmitate, and hepatic triglyceride levels measured by magnetic resonance spectroscopy commensurate with their degree of adiposity, as determined by a previously published large, population-based study.[13]

GLP-1 levels in response to a prolonged OGTT in patients with hyperinsulinaemic hypoglycaemia due to activating GCK mutations

Glucokinase is expressed in the enteroendocrine L cells that secrete the incretin hormone GLP-1 in response to a luminal glucose load. To determine whether increased GCK activity in L cells potentiates GLP-1 secretion and contributes to a hyperinsulinaemic response to oral glucose, GLP-1 levels were determined in three individuals with the GCK p.Val389Leu mutation during a prolonged OGTT. Peak GLP-1 levels were not elevated in patients with the activating p.Val389Leu GCK mutation (data not shown) compared with previously published data obtained from control subjects and carriers of an inactivating GCK mutation.[14,15]

Discussion

Activating glucokinase mutations are associated with marked phenotypic heterogeneity with regard to age and severity of presentation, response to treatment and awareness of hypoglycaemia.[2,3,5,16,17] The family we describe highlights the challenges in timely diagnosis of genetic hyperinsulinism in older adults: those with the autosomal dominant activating GCK mutation, p.Val389Leu, either presented for the first time in adulthood or

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were unaware of their hypoglycaemia. Of three patients presenting with symptomatic hypoglycaemia, all met diagnostic biochemical criteria for endogenous hyperinsulinism prompting, in each case, unsuccessful and sometimes prolonged searches for an insulin-secreting tumour.[17] A suggestive feature of GCK-HH in these cases was the stability of hypoglycaemia during fasting, although negative 72-h fasts have been described for some cases of GCK-HH, likely reflecting GSIS threshold variability induced by different activating GCK mutations.[16] Exacerbation of hypoglycaemia by an oral glucose load was a striking feature of the patients studied; however, both a minority of insulinomas and some cases of severe insulin resistance also present with postprandial hypoglycaemia.[17,18] Moreover, a clear family history of hypoglycaemia was not available at the time of our proband's presentation, and indeed, even *bona fide* absence of family history does not exclude a diagnosis of GCK-HH given both the highly variable clinical phenotype and potential for *de novo* mutations.[19]

Glucokinase p.Val389Leu was previously identified in a boy who presented with hypoglycaemic seizures at 2 years of age and subsequently in his father with a much milder phenotype.[4] Biochemical characterization of GCK p.Val389Leu demonstrated increased affinity for glucose which potentiates the relative activity of the enzyme and lowers the threshold for glucose-stimulated insulin secretion by the pancreas.[4] The mutation is predicted also to lead to upregulation of GCK in liver and other tissues; however, teasing out tissue-specific contributions to disease in humans in whom all tissues are affected is intractable and may require future study of mice with tissue-specific overexpression of the mutant. How such a fixed perturbation of glucose sensing by GCK translates into such a wide spectrum of inter- and intrafamily clinical variation also remains unclear, although phenotypic modification by environmental and/or genetic factors seems likely.

Recent murine studies have suggested that chronic GCK activation increases β-cell activity and promotes β-cell death through p53-mediated apoptosis.[20] Moreover, pancreata from patients with GCK-HH showed increased DNA fragmentation and β-cell apoptosis[20,21] and carriers of the activating p.Val455-Met GCK mutation were reported either to develop diabetes or to show remission from hypoglycaemia over time, raising the possibility that eventual β-cell failure is a feature of GCK-HH.[2,20] Our data suggest, however, that β-cell failure is not an obligate outcome for those with GCK-HH: p.Val389Leu and p.Val455-Met exhibit similar activities *in vitro*, yet the proband in this report has persisting hypoglycaemia in her sixth decade, while her father has persisting hypoglycaemia (Hb_A1c 24 mmol/mol despite diazoxide treatment) in his ninth decade.

Glucokinase is expressed in extra-pancreatic tissues including the liver, enteroendocrine L cells and glucose-sensing neurons of the brainstem and hypothalamus, suggesting the existence of a network of GCK-containing cells that collectively may mediate a co-ordinated physiological response to maintain glucose homoeostasis.[1] L cells directly sense nutrients in the intestinal lumen facilitating release of the incretin hormone GLP-1, which, in turn, potentiates glucose-stimulated insulin secretion. Our findings that peak GLP-1 levels are not increased in subjects with GCK p.Val389Leu is in agreement with previously published data and do not support GCK-mediated glucose metabolism as an important regulator of GLP-1 secretion in humans.[15]

Hepatic GCK regulates glucose uptake and influences a multitude of anabolic pathways. Re-feeding and insulin induce hepatic GCK expression, whereas GCK activity is acutely regulated by endogenous inhibitory proteins such as GKRP.[22] At low glucose concentrations, GCK is inactivated and sequestered in the nucleus by the GKRP. Glucose-induced dissociation of this complex allows GCK to translocate rapidly to the cytoplasm where it is able to catalyse ATP-dependent phosphorylation of glucose to glucose 6-phosphate, thereby promoting further glucose uptake and flux through downstream metabolic pathways including those involved in hepatic lipogenesis. This suggests that increased hepatic GCK activity may promote hepatic steatosis and metabolic dyslipidaemia through increased rates of *de novo* lipogenesis. Indeed, transgenic overexpression of hepatic GCK in mice is associated with elevated circulating triglyceride levels and hepatic steatosis, and in humans, a GKRP polymorphism (p.Pro446Leu) that increases GCK activity is associated with hypertriglyceridaemia and fatty liver.[6,7,10,11]

Given these observations, pharmacological approaches that induce GCK activation for the treatment of type 2 diabetes have been subject to intense scrutiny. While activation of GCK is effective at reducing hyperglycaemia in some human and animal models of type 2 diabetes,[1] De Ceuninck *et al.*[8] found that administration of GKAs induced hepatic steatosis and increased hepatic triglycerides in both normal and diabetic rodents. Furthermore, in subjects with type 2 diabetes, chronic administration of a GKA was associated with an increased incidence of hypoglycaemia, hypertriglyceridaemia and increased systolic blood pressure.[12]

We demonstrate that untreated subjects with genetic, lifelong activation of GCK have normal rates of *de novo* lipogenesis and hepatic triglyceride content that is commensurate with their degree of adiposity. While rates of *de novo* lipogenesis or hepatic TG content have not been reported in patients treated with GKAs, hypertriglyceridaemia has been observed in subjects with type 2 diabetes mellitus treated for 54 weeks with MK-0941, an oral, allosteric GKA.[12] It is important to recognize, however, that unlike subjects with the p.Val389Leu GCK mutation, these individuals had suboptimal glycaemic control, suggesting that GKA treatment induced increased hepatic glucose uptake and flux through downstream metabolic pathways in the face of hyperglycaemia. In the context of activating GCK mutations, relative hypoglycaemia may be protective against development of fatty liver and dyslipidaemia presumably through reduced substrate provision for hepatic triglyceride and cholesterol synthesis and by increasing the fraction of hepatic GCK that is bound and inhibited by GKRP. This suggests the unfortunate paradox that a therapeutic strategy of GCK activation to lower blood glucose may be safe in patients with normoglycaemia at baseline, but not in those with hyperglycaemia, who are the target population. That this might be mitigated by concomitant use of an SGLT inhibitor to provide a renal conduit for excess glucose is an interesting possibility.

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Collectively, these findings present a management challenge and question whether pharmacological attempt to raise blood glucose levels in patients with activating GCK mutations is appropriate. Numerous reports describe successful treatment of GCK-related hypoglycaemia with diazoxide and octreotide; however, these findings are not consistent and there is a paucity of data describing metabolic consequences following alleviation of hypoglycaemia.[16] GCK hyperinsulinism was unresponsive to both diazoxide and subcutaneous octreotide therapy in the proband we describe, while although postprandial hypoglycaemia during OGTT supported treatment with an α-glucosidase inhibitor, gastrointestinal side effects proved intolerable, preventing assessment of efficacy. To date, management has focused on education around strict adherence to a low-glycaemic-index diet, exercise and reduced capillary glucose monitoring in an attempt to break a cycle of anxiety and overeating in response to asymptomatic hypoglycaemia. While collectively these efforts have not improved glycylated haemoglobin levels, symptomatic improvement and weight loss have been achieved.

In conclusion, activating GCK mutations are a rare cause of hyperinsulinaemic hypoglycaemia in adults and present with marked phenotypic heterogeneity. Our findings demonstrate that genetic GCK activation does not lead to the metabolic sequelae reported with pharmacological GCK activation. Further study of patients with naturally occurring GCK activating mutations will provide opportunities to study concepts relating to glucose sensing and therapeutic manipulation of GCK in man.

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