CLINICAL IMPLICATIONS OF THE GLUCOKINASE IMPAIRED FUNCTION – GCK-MODY TODAY

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SUMMARY

Heterozygous inactivating mutations of the glucokinase (*GCK)* gene are causing GCK-MODY, one of the most common forms of the Maturity Onset Diabetes of the Young (MODY). GCK-MODY is characterized by fasting hyperglycemia without apparent worsening with aging and low risk for chronic vascular complications. Despite the mild clinical course, GCK-MODY could be misdiagnosed as type 1 or type 2 diabetes. In the diagnostic process, the clinical suspicion is often based on the clinical diagnostic criteria for GCK-MODY and should be confirmed by DNA analysis. However, there are several issues in the clinical and also in genetic part that could complicate the diagnostic process. Most of the people with GCK-MODY do not require any pharmacotherapy. The exception are pregnant women with a fetus which did not inherit *GCK* mutation from the mother. Such a child has accelerated growth, and has increased risk for diabetic foetopathy. In this situation the mother should be treated with substitutional doses of insulin. Therefore, distinguishing GCK-MODY from gestational diabetes in pregnancy is very important. For this purpose, special clinical diagnostic criteria for clinical identification of GCK-MODY in pregnancy are used. This review updates information on GCK-MODY and discusses several currently not solved problems in the clinical diagnostic process, genetics, and treatment of this type of monogenic diabetes.

KEY WORDS:

GCK, MODY, diabetes, clinical diagnostic criteria, pregnancy

ABBREVIATIONS:

ADP - Adenosine Diphosphate, **ATP** - Adenosine Triphosphate, **CHI** - Congenital hyperinsulinism, **DM –** diabetes mellitus, **G6P** - glucose-6-phosphate, **GCK** – enzyme glucokinase, ***GCK*** – gene encoding enzyme glucokinase, **GCK-CHI** - Congenital hyperinsulinism due to a heterozygous activating mutation, **GCK-MODY -** Maturity Onset Diabetes of the Young due to a heterozygous inactivating mutation of the *GCK* gene, **GCK-PNDM -** Permanent Neonatal Diabetes Mellitus due to homozygous or compound heterozygous inactivating mutations of the *GCK* gene, **GDM** – Gestational Diabetes Mellitus, **GIP** - Gastric inhibitory polypeptide, **GKRP** – glucokinase regulatory protein, **GLP-1** - Glucagon-like peptide-1, **HbA1c –** glycated

hemoglobin, **HNF1A-MODY** - Maturity Onset Diabetes of the Young due to a heterozygous inactivating mutation of the *HNF1A* gene, **HNF4A-MODY** - Maturity Onset Diabetes of the Young due to a heterozygous inactivating mutation of the *HNF4A* gene, **MODY –** Maturity Onset Diabetes of the Young, **oGTT** - oral glucose tolerance test, **PFK2/FBPase-2** - 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase, **PNDM -** Permanent Neonatal Diabetes Mellitus, **T1D** – Type 1 Diabetes, **T2D** – Type 2 Diabetes

INTRODUCTION

Enzyme glucokinase (GCK) is involved into the insulin secretion and glucose homeostasis. Activating heterozygous mutations of the *GCK* gene cause congenital hyperinsulinism, whereas phenotype of the inactivating *GCK* mutations is diabetes mellitus (DM) (Hattersley *et al.* 2018). Homozygous or compound heterozygous inactivating *GCK* mutations cause permanent neonatal diabetes, and heterozygous inactivating *GCK* mutations are causing Maturity Onset Diabetes of the Young (MODY). MODY is the most common type of the monogenic DM, and is characterized by familial occurrence with dominant type of inheritance and an early onset (typically < 30 years of age) (Ellard *et al.* 2008, Hattersley et al. 2018).

Maturity Onset Diabetes of the Young caused by a *GCK* mutation (GCK-MODY) is a frequent type of MODY with mild course and low risk of chronic diabetes complications. GCK-MODY accounts for 10-60% of MODY and 1% of women with gestational diabetes mellitus (Chakera *et al.* 2014, Rudland *et al.* 2016). It is the most common type of MODY in the central and southern European countries, unlike the North Europe, where other type of MODY (HNF1A-MODY) prevails (Velho *et al.* 1997, Feigerlova *et al.* 2006, Sagen *et al.* 2008, Lorini *et al.* 2009, Shields *et al.* 2010). In this review, we focus on genetic and clinical aspects of the GCK-MODY caused by *GCK* mutations.

GCK GENE

The *GCK* gene (MIM 138079) encoding enzyme glucokinase is located on chromosome 7 (RefSeq NG_008847.2). Two main transcripts are transcribed from two organ-specific promoters (Iynedjian 1993, Iynedjian 2009). The constitutively active upstream neuroendocrine promoter (NE-GCK-promoter) gives rise to NM_000162.5 transcript (2745 bp, 10 exons, 465 amino acids) in the endocrine pancreas, brain, pituitary, adrenal, and entero-endocrine cells (Fig. 1). On the other hand, the downstream promoter that controls expression of the *GCK* gene in liver (HEP-GCK-promoter) is insulin-dependent and gives rise to transcript NM_033507.3 (2424 bp, 10 exons, 466 amino acids). Thus, the two transcripts differ in the first exon and the two GCK isoforms have 15/16 different N-terminal amino acids. However, they are functionally indistinguishable (Matschinsky and Wilson 2019).

GLUCOKINASE PROTEIN STRUCTURE AND FUNCTION

Enzyme glucokinase is one of the four members of hexokinase family (Iynedjian 1993), which mediate phosphorylation of their preferred substrate glucose to glucose-6-phosphate (G6P). It is the first step of glycolytic pathway in utilization of glucose, as well as glycogen synthesis (Iynedjian 2009).

GCK has a large and a small domain interconnected with a flexible hinge. The glucose binding site is located in a cleft space between the domains and is composed of residues of the large domain (Glu 256 and Glu 290), the small domain (Thr 168 and Lys 169), and the interconnecting region (Asn 204 and Asp 205) (Kamata *et al.* 2004). Glucokinase can make three conformations - the "super open" conformation corresponding to the inactive form of the enzyme in the absence of glucose; - glucose binding promotes the formation of the intermediate "open" structure with high affinity to glucose; - and the catalytically active "closed conformation" formed upon binding of glucose, that binds ATP and performs the phosphorylation of glucose (Sternisha and Miller 2019)

Due to specific characteristics, glucokinase serves a different purpose as the other hexokinases. Glucokinase has a lower affinity for glucose ($S_{0.5}$ 8 mmol/l), moderate cooperativeness with glucose (Hill number of 1.7) (Matschinsky 1996, Osbak *et al.* 2009), and lower reverse inhibition by G6P (Iynedjian 1993). These kinetic characteristics allow glucokinase to phosphorylate the substrate above the physiological levels of plasma glucose (4–15 mmol/l) (Matschinsky 2002). Hence, the glucokinase has the glucose-sensing capability crucial for insulin secretion and glucose homeostasis. It is present in substantial amounts only in cells and tissues that serve as glucose sensors. Other cells express hexokinase instead of glucokinase, with a higher affinity for glucose, which makes it insensitive to changes in glucose concentration in the physiological range (Matschinsky and Wilson 2019).

Glucose homeostasis involves a complex regulatory system that includes, in addition to pancreatic β-cells, a number of other cells/tissues, notably α- and δ-cells of pancreatic islets, entero-endocrine cells, the adrenal glands, glucose sensing neurons in the CNS, the pituitary gland, and the liver.
The **pancreatic β-cell**–specific *GCK* promoter is only slightly regulated by insulin or glucagon and the main pancreatic *GCK* regulator is postprandial glucose level (Matschinsky 1996). In β-

cells, an increase of glucose concentration activates GCK and promotes glycolysis. The subsequent increase of [ATP]/[ADP] ratio closes the K^+_{ATP} channel and membrane depolarization opens voltage-gated Ca^{2+} channels. Increased intracellular Ca^{2+} triggers the exocytosis of insulin granules (Ashcroft 2005). The metabolism of β-cells is characterized by high glycerol phosphate shuttle, high pyruvate dehydrogenase and pyruvate carboxylase activities, and low lactate dehydrogenase activity that stream the glycolysis through pyruvate to the oxidative phosphorylation. Simultaneously, β-cells have low plasma membrane monocarboxylate transport that minimizes pyruvate loss and prevent increased blood lactate levels from interfering with glucose sensing. Low pentose-phosphate shunt, low glucose-6-P phosphatase activity, as well as low glycogen synthase activity limit utilization of glucose by other pathways in β-cell and allow their glucose sensing function (Matschinsky and Wilson 2019).

Glucokinase serves as a sensor also in glucagon releasing α-cells, where the downstream signaling is similar to that of β-cells. Increased glucose increases the [ATP]/[ADP] ratio leading to the closure of K^+_{ATP} and membrane depolarization. In α-cells, however, depolarization leads to voltage-dependent inactivation of voltage-gated Na+ channels involved in action potential firing. Diminution of action potential height decreases activation of the P/Q Ca2+ channels that mediate Ca2+ entry, and this decreases glucagon release (Basco *et al.* 2018).

Glucokinase in the **brain** is thought to play a role as a glucose sensor in hypothalamus triggering cell responses that affect glucose homeostasis in the whole body (Osundiji *et al.* 2012, Rosario *et al.* 2016, Ma *et al.* 2018). The mechanism of glucose sensing is similar to that of a β-cell with closure of $K^+_{ATP,}$ membrane depolarization and Ca2+ influx, but it leads to neurotransmitter secretion (De Backer *et al.* 2016).

In the **liver**, glucokinase plays a role in intermediary metabolism and the *GCK* gene transcription is driven from the liver-specific promoter by insulin and glucagon related to the current nutritional state Due to the higher activity level of the hepatic *GCK* promoter the glucose phosphorylation activities in liver are 10- to 20-fold higher than in pancreatic β-cells. Thus, the liver GCK represents a high-capacity glucose-phosphorylating system in comparison to the low capacity system in β-cells and neuroendocrine cells (Matschinsky *et al.* 2006). GCK in the liver is regulated

by the GCK regulatory protein (GKRP). It is a ligand dependent competitive inhibitor of GCK. GKRP regulates a translocation of the GCK protein between the cytoplasm and the nucleus of the hepatocytes (Toyoda *et al.* 1995, Brown *et al.* 1997, Niculescu *et al.* 1997, Shiota *et al.* 1999). Ligands for GCKR are fructose-6-phosphate and fructose-1-phosphate, which are mutual competitors. Binding of fructose 6-phosphate to GCKR favors the GCKR-GCK interaction causing GCK inactivation, while binding of fructose-1-phosphate releases active GCK. Intrahepatic fructose-1-phosphate rises postprandially after intestinal absorption of fructose and its conversion to fructose-1-phosphate by liver fructokinase. Conversely, fructose 6- phosphate is in equilibrium with glucose 6-phosphate (the product of the GCK reaction) through the phosphohexose isomerase step of glycolysis and would provide indirect negative feedback (Van Schaftingen *et al.* 1994). Also glucose releases GCK from the GCK-GKRP complex through conformational changes of the enzyme protein (Agius and Peak 1993, Agius and Peak 1997). Therefore, at low glucose concentrations or in the presence of fructose-6-phosphate, GCK is concentrated in the nucleus, while high glucose concentrations or fructose-1-phosphate trigger a rapid release of GCK from GKRP and the translocation to the cytoplasm.

Interestingly, endocrine gut cells, K and L-cells, which secrete incretins GLP-1, GIP, that in turn stimulate insulin secretion by β-cells have not been proven to utilize glucokinase as main glucose sensor in intestines (Murphy *et al.* 2009).

THE *GCK* GENE MUTATIONS

Inactivating mutations of the glucokinase gene cause mild stable hyperglycemia in the heterozygous condition (GCK-MODY) or permanent neonatal diabetes mellitus (PNDM) with severely diminished insulin secretion in the homozygous or compound heterozygous states. *GCK* mutations leading to MODY decrease functional reserve of glucokinase in B-cells, tolerating only a less than 50% decrease of enzyme activity (Gloyn *et al.* 2003). Homozygous *GCK* knockout mice experiment led to death due to hyperglycemia and showed the GCK expression as crucial for insulin secretion stimulation in β-cells (Grupe *et al.* 1995, Terauchi *et al.* 1995). Conversely, heterozygous activating *GCK* mutations are responsible for a hypoglycemic condition caused by excessive insulin secretion (GCK- congenital hyperinsulinism) (Davis *et al.* 1999, Galcheva *et al.* 2019). Most of the mutations (59%) in GCK-MODY are reported to be private (reported within

one family) or novel (Osbak et al. 2009). *GCK* mutations are the most often point mutations where 65% are missense, followed by nonsense, splice site, and frameshift mutations (Chakera *et al.* 2015). Partial or whole gene deletions occur rarely (3.5%) (Ellard *et al.* 2007).

Routine genetic testing of the pancreatic *GCK* gene transcript is usually aimed to the scanning of the coding regions (exon 1a, 2-10) and exon-intron boundaries. Earlier DNA analyses did not include analysis of regulatory sequences facilitating transcription of the gene. However, the study in 2009 by Gasperikova et al. (Gasperikova *et al.* 2009) reported so far the only variant in the promoter that is proved as pathogenic c.-557G>C (published as c.-71G>C, also reported as c.-87G>C). It is a founder mutation responsible for ~30% of known cases of GCK-MODY in Slovakia (D.G., M.S., and J.S. unpublished data). Functional study of the c.-557G>C promoter variant demonstrated dramatic reduction in promoter activity due to loss of Sp1 transcription factor binding. Results of this study strongly support inclusion of the *GCK* promoter region in routine testing for GCK-MODY (Gasperikova et al. 2009).

Since GCK-MODY is an autosomal dominant condition, 50% of offspring are at risk of inheriting the mutation. Although vast majority of the people with GCK-MODY have inherited the *GCK* mutation from one of their parents, several people with *de novo GCK* mutations have been reported. Prevalence of *de novo* mutations in *GCK* gene has not been established, since not all newly diagnosed GCK-MODY patients have had their parents tested for GCK-MODY as well. Nevertheless, at least 16 cases of *de novo GCK* inactivating mutations have been reported (Hager *et al.* 1994, Velho et al. 1997, Massa *et al.* 2001, Birkebaek *et al.* 2011, Cappelli *et al.* 2011, Stanik *et al.* 2014, Chakera et al. 2015, Haliloglu *et al.* 2016, Alvelos *et al.* 2020). Our previous study observed, that 7.3% (11/150) of referrals that fulfill clinical criteria for MODY, except for family history of diabetes or hyperglycemia, have been diagnosed with *de novo* mutations in most common forms of MODY (*GCK, HNF1A,* and *HNF4A*) (Stanik et al. 2014).

PATHOPHYSIOLOGY OF *GCK* MUTATIONS

GCK mutations most frequently alter the kinetics of glucokinase. Inactivating mutations (MODY, PNDM) usually decrease the glucokinase affinity to glucose (increased $S_{0.5}$). The glucokinase catalytic efficiency for ATP (ATP $K_{m)}$ and Hill coefficient are either increased or decreased and

the maximal specific activity (K_{cat}) is decreased (Davis et al. 1999). In some GCK-MODY mutations, the kinetics of glucokinase is not impaired; rather the stability of the molecule is decreased. Binding with regulatory molecules such as GKRP, bi-functional enzyme PFK2/FBPase-2 (6-phosphofructo-2-kinase/fructose-2,6-biphosphatase) may be altered, too (Osbak et al. 2009, Valentínová *et al.* 2012).

By contrast, some activating *GCK* mutations (e.g. T65I and A456V) in individuals with congenital hyperinsulinism tend to have the opposite GCK enzyme kinetic properties. GCK glucose affinity is increased (lower $S_{0.5}$) and it is frequently catalytically more active (higher K_{cat}), whereas other mutations (W99R, Y214C, V455M) mediate activity of GCK enzyme by isomerization (Heredia *et al.* 2006).

CLINICAL COURSE, DIAGNOSTICS AND DIFFERENTIAL DIAGNOSIS OF GCK-MODY

GCK-MODY is the most common cause of persistent fasting hyperglycemia in pediatric population (Feigerlova et al. 2006). The clinical phenotype of GCK-MODY is remarkably homogenous in many patients (Stride *et al.* 2002, Hattersley et al. 2018). However, GCK-MODY has no defining features that may be pathognomonic. Therefore, we need to assess a broad spectrum of clinical criteria before stating the clinical suspicion on GCK-MODY and decision for genetic testing.

Clinical picture of people with GCK-MODY is characterized by asymptomatic fasting hyperglycemia (FPG 5.5-8.5 mmol/l). They have only mild increase in HbA1c, that amounts up to 0.018 % (0.2 mmol/mol) increase per year (similar increase as in the general population) (Steele *et al.* 2013). Glycated hemoglobin is usually within range of 5.6-7.6% (38–60 mmol/mol). A study by Steele et al. (Steele et al. 2013) found, that people with GCK-MODY aged ≤ 40 years had an HbA1c reference range of 5.6–7.3% (38–56 mmol/mol) and people aged ≥ 40 years had a reference range 5.9–7.6% (41–60 mmol/mol).

People with GCK-MODY also maintain their increased glucose levels by altered counter regulating mechanisms reacting at higher glycemic levels. Evidence from euglycemic hyperinsulinemic clamps in GCK-MODY patients suggests that suppression of hepatic glucose production is decreased by physiological insulin levels (Clement *et al.* 1996). As level of hyperglycemia in GCK-MODY is not high enough to transcend renal threshold, the osmotic symptoms (polyuria, polydipsia), and weight loss are rare. Therefore, most patients are diagnosed incidentally, at any age via mild hyperglycemia found at health screening in an asymptomatic individual, or at admission to a hospital for different conditions. Pregnant women can be identified during the glycemic screening for gestational diabetes mellitus (GDM) within 24^{th} to 28^{th} gestational week.

Clinical diagnosis. Identifying monogenic form of diabetes and assumption of particular subtype nowadays still depends on recognition of the pattern of clinical phenotype and later confirmation by molecular-genetic testing. Additional biochemical tests can lead to more precise discrimination and prioritization for molecular-genetic testing between diabetes subtypes (Hattersley et al. 2018).

One of the clinical features is the familial appearance of diabetes, as GCK-MODY has high prevalence in the first degree family members. One of the parents is usually a carrier of a *GCK* mutation and is most likely not diagnosed as diabetic or misdiagnosed as young-onset type T2D with no complications (Ellard et al. 2008). When suspecting a diagnosis of GCK-MODY in a child, testing HbA_1c and/or fasting glucose in parents can be useful (Hattersley et al. 2018).

Age at diagnosis does not play crucial role as many GCK-MODY patients go unnoticed or misdiagnosed for a long time. However, presence of mild stable hyperglycemia found at younger age (<25 years) or in a childhood is uncharacteristic for developing T2D, as prevalence of T2D in Caucasian population is rather small in children and adolescents. Presence of mild unprogressive hyperglycemia is thus more likely to be caused by *GCK* mutations in children (prevalence of 40%) (Feigerlova et al. 2006) than adults (~1% in adults diagnosed over 50 years of age) (Gloyn *et al.* 2009).

Fasting glucose and glycosylated hemoglobin can reliably distinguish GCK-MODY from young-onset T1D or T2D, as patients with T1D/T2D usually have higher fasting glucose (>7 mmol/l) and HbA_{1c} levels surpassing the upper limit for GCK-MODY in 73% (438/597) of patients (Steele et al. 2013). Exceeding the upper age-related limit can as well reflect possibility of concurrent T1D/T2D developed in a known GCK-MODY patient (Stanik *et al.* 2012). People with GCK-MODY are at the same risk of developing T1D or T2D as general population. Management of such individual with hypoglycemics may only decrease glucose levels to those found in untreated GCK-MODY individuals (Chakera et al. 2015).

C-peptide is usually present in serum of GCK-MODY patients even beyond 5 years after diagnosis of diabetes, suggesting active insulin secretion. However, diagnostic power of measurable C-peptide levels is limited: T1D patients who usually do not have measurable levels of C-peptide due to total destruction of β-cells, have some active β-cells secreting insulin during the "honeymoon" period, hence having measurable levels of C-peptide. T2D patients are usually hyperinsulinemic, compensating for lowered insulin sensitivity and have measurable C-peptide levels as well.

In contrast to other subtypes of monogenic diabetes, people with GCK-MODY have adequate insulin secretion at slightly higher glucose set-point than unaffected people. Fasting plasma glucose levels in GCK-MODY are mildly increased, but the insulin response to glucose load is appropriate and the first phase of insulin response maintained (Velho *et al.* 1992). In an oral glucose tolerance test (oGTT), 71% of individuals with GCK-MODY have 2h-blood glucose increment <3 mmol/l (Stride et al. 2002), and 90% of GCK-MODY less than 4.6 mmol/l (Ellard et al. 2008). OGTT can also be used as a distinguishing factor, as other types of diabetes such as T1D/T2D tend to have higher glycemic increment >3mmol/l. Majority (67%) of the people with monogenic types of diabetes HNF1A-MODY and HNF4A-MODY have also >3 mmol/l 2h-blood glucose increment in an oGTT (Ellard et al. 2008).

Unlike the T1D, the GCK-MODY individuals mostly do not have positive pancreatic auto-antibodies. Only 1% of people with GCK-MODY have positive auto-antibodies (particularly against glutamic acid decarboxylase) (McDonald *et al.* 2011a).

Unlike the T2D, inactivating *GCK* mutations are not linked to obesity. GCK-MODY individuals are affected by obesity at the same rate as the rest of the population. Therefore, increasing prevalence of obesity in population makes it impossible to exclude patients from *GCK* DNA analysis based on obesity (Njolstad and Molven 2012).

Clinical diagnostic criteria summarize the clinical signs in the diagnostic process. Current clinical diagnostic criteria suggesting *GCK* mutation, provided by International Society for Pediatric and Adolescent Diabetes (ISPAD) in 2018 (Hattersley et al. 2018) are displayed in Table 1.

MODY probability calculator (https://www.diabetesgenes.org/mody-probability-calculator/) is another tool for distinguishing MODY (including GCK-MODY) from type 1 and type 2 diabetes mellitus. MODY calculator was developed by Shields and colleagues (Shields *et al.* 2012) and the model was validated in a set of 350 patients with these three types of diabetes. This tool discriminates MODY and type 1 diabetes by lower HbA1c, parent with diabetes, female sex and older age at diagnosis. Discrimination from type 2 diabetes is based on lower BMI, younger age at diagnosis, female sex, lower HbA1c, parent with diabetes, and not being treated with oral hypoglycemic agents or insulin. Both model tools showed excellent discrimination, low rates of cross-validated misclassification. Using the optimal cut-offs, the probability models improved the sensitivity (91% vs 72%) and specificity (94% vs 91%) for identifying MODY. Nevertheless, the MODY calculator has some limitations. It can be used only for patients with diabetes onset before the age of 35 years. In addition, MODY calculator was established for Europeans and the research for other ethnics should be done. It is also good to remind that this tool discriminates only HNF1A/GCK/HNF4A-MODY from T1D or T2D and it will not pick up other forms of monogenic diabetes (Njolstad and Molven 2012). The probability calculator works best for patients who are not treated with insulin. Additional non-genetic tests (e.g. islet autoantibody testing and C-peptide analysis) should be considered as 'rule-out tests' in patients treated by the insulin as the presence of islet autoantibodies and/or C-peptide < 200 pmol/l effectively rules out MODY (Besser *et al.* 2011, McDonald et al. 2011a). As the name indicates, MODY calculator is able to calculate the clinical probability for an individual to have MODY. Therefore biomarkers, other clinical data and

the costs of the tests could be considered before the decision of DNA analysis in a patient (Njolstad and Molven 2012).

Biomarkers. High sensitivity C-reactive protein (hs-CRP) is a useful distinguishing factor between HNF1A-MODY and GCK-MODY (McDonald *et al.* 2011b). Although hs-CRP is particularly helpful and in clinical practice limited to discriminating HNF1A-MODY from T2D (Owen *et al.* 2010, McDonald et al. 2011b), it cannot be used for discrimination of GCK-MODY from T1D or T2D. However, another biomarker 1,5-anhydroglucitol (1,5AG) may be used as a discriminant for GCK-MODY from other subtypes of diabetes. 1,5AG is a metabolically inactive monosaccharide, which reaches steady state between ingestion and urinary excretion, and is usually nearly fully reabsorbed in kidneys (Pal *et al.* 2010). Thanks to similarity, glucose inhibits competitively this kidney reabsorption of 1,5AG especially in people with significant glycosuria. In such situation 1,5AG does not get reabsorbed and is excreted in urine, thus its serum levels drop. In GCK-MODY is the post glucose load glycemic increment modest and does rarely exceed renal threshold for glucose, resulting in close to normal levels of 1,5AG (13.06 (5.74–29.74) μg/ml) (Pal et al. 2010). All these characteristics predispose GCK-MODY to have significantly higher serum levels of 1,5AG than any other diabetes subtypes including T2D (5.43 (2.12–13.23) μg/ml), HNF1A-MODY (4.23(2.12–8.44) μg/ml) (Pal et al. 2010). ROC curve analysis has proven that 1,5AG has the biggest discriminative accuracy between GCK-MODY and HNF1A (ROC 0.86), and lower for discrimination of GCK-MODY from T2D (ROC 0.79). When adjusted for HbA_{1C}, ROC values reached 0.96 and 0.94, respectively. To distinguish GCK-MODY and HNF1A-MODY, an oGTT could be carried out. However, 1,5AG similarly reflects postprandial glucose increase in non-fasting blood, thus could prove to be more efficient than oGTT (Pal et al. 2010).

DNA ANALYSIS IN THE GCK-MODY

After assessment of phenotype via clinical diagnostic criteria and/or by MODY calculator (Njolstad and Molven 2012), individuals with a suspicion on GCK-MODY should be tested for a causal mutation in the *GCK* gene. Routine screening of *GCK* gene should include all pancreatic glucokinase exons 1a-10, as mutations are dispersed throughout the whole gene and are not clustered in particular segment of DNA. Based on the study by Gasperikova et al. (Gasperikova et

al. 2009), the *GCK* promoter region should be included in routine diagnostic testing for GCK-MODY because of the promoter mutation (c.-557G>C) which is highly prevalent in Central Europe (Gasperikova et al. 2009). Nowadays, next-generation sequencing (NGS) enabled analysis of multiple genes at lower cost per gene and has replaced other methods such as single gene Sanger sequencing (Stanik *et al.* 2018). However, it still remains a rather expensive method. Therefore, many diagnostic centers analyze the *GCK* gene by Sanger sequencing prior NGS.

Identification of a causal *GCK* mutation has implications for the therapy in a proband (see below), and also enables genetic counseling and extended genetic testing of other diabetic family members, whose diabetes may be reclassified (Rubio-Cabezas *et al.* 2014). Screening of diabetic family members for a known mutation carried by their relative is cheaper than analyzing the whole *GCK* gene and is inexpensive compared to life-long improper treatment due to incorrect diagnosis. As diabetic family members are usually thought to have T1D/T2D, reclassification to GCK-MODY constitutes a major change in quality of their life as treatment by insulin or oral hypoglycemic agents can be discontinued (Chakera et al. 2015).

Genetic counseling could be mediated also to all asymptomatic family members requesting predictive testing. Ellard et al. (Ellard et al. 2008) recommend that unaffected relatives should undergo a biochemical test first (fasting blood glucose for *GCK*). If the biochemical test is consistent with diagnosis of diabetes or hyperglycemia, proceeding with genetic testing will be conclusive, not predictive (Ellard et al. 2008). In contrary, fasting blood glucose < 5.4 mmol/l will in majority of cases rule out the possibility of GCK-MODY (Stride et al. 2002). If asymptomatic family members decide not to be screened for GCK-MODY, as their daily routine would not change even with the knowledge of being affected by GCK-MODY, they should at least be informed of the probability being affected by the mutation. Should they be picked up for hyperglycemia or diabetes during other medical checkups, knowledge of a relative having GCK-MODY can prevent them from being labeled as T1D or T2D. Women related to a GCK-MODY individual should have their fasting blood glucose tested prior to pregnancy, and if their fasting blood glucose is suspicious of GCK-MODY, genetic testing should be performed (Chakera et al. 2015).

THERAPEUTIC CONSEQUENCES OF THE GENETICALLY CONFIRMED GCK-MODY

Genetic confirmation of pathogenic *GCK* mutation is a necessary step toward diagnostic conclusion and therapeutic consequences. Individuals previously classified as T1D/T2D and treated by oral hypoglycemics or insulin can be successfully switched to no treatment, or just rational diet without any change in their glycemic levels. A study by Stride et al. (Stride *et al.* 2014) found no significant difference in HbA_{1C} levels between individuals without any treatment with medicaments, and treated by oral hypoglycemics or insulin. Furthermore, discontinuance of the treatment did not result in a change of HbA_{1C} in the same study (Stride et al. 2014). Due to kinetic difference of mutated glucokinase, glycemia is regulated at slightly higher setpoint, which is steady and does not pose a long-term danger of microvascular complications (Steele *et al.* 2014). Glucokinase has a role in counter regulatory mechanisms, therefore GCK-MODY individuals have higher glycemic threshold for counter regulatory response to hypoglycemia (5.0 ± 0.4 mmol.l^{-1}) (Guenat *et al.* 2000). As such, they can contribute to steady higher glycemic levels. Thus, therapy by low insulin doses is not justified nor rational, since no apparent change towards euglycemia will be observed (Stride et al. 2014). Increasing the treatment doses of insulin beyond replacement levels will cause major slump in pancreatic endocrine response along with decrease towards euglycemia which may be in some cases felt as hypoglycemic state (Chakera et al. 2015). Treatment by such doses is not recommended outside pregnancy, as it has more risks than benefits.

DIAGNOSTICS OF GCK-MODY IN PREGNANCY AND TREATMENT APPROACH

Distinguishing GCK-MODY in pregnancy from gestational diabetes mellitus (GDM) (Vejrazkova *et al.* 2015, Anderlova *et al.* 2019) is important, as GCK-MODY has different management approach. Most pregnant women with the GCK-MODY fulfill the International Association of the Diabetes and Pregnancy Study Groups (IADPSG) diagnostic criteria for GDM, having a fasting hyperglycemia ≥ 5.1 mmol/l (International Association of *et al.* 2010). Distinguishing factors between GDM and GCK-MODY can select individuals suitable for genetic testing. Recently, new pregnancy-specific screening criteria for GCK-MODY have been successfully tested in a sample of pregnant women from GDM database, selecting all GCK-MODY subjects with less candidates referred to genetic testing (in Anglo-Celtic women) (Rudland et al. 2016). The criteria include pre-pregnancy BMI < 25 kg/m^2 and fasting plasma glucose > 5.5 mmol/l. Specificity of this guideline

for GCK-MODY was 98%, and sensitivity 68% (Chakera et al. 2014). However, this study showed that HbA1c is not a suitable marker, as no significant differences in HbA1c levels between GCK-MODY and GDM were observed (Rudland et al. 2016).

In a pregnant woman with GCK-MODY the fetus is exposed to higher levels of glucose. However, the fetal growth will largely depend on genotype of the fetus, rather than on the treatment of the mother (Spyer *et al.* 2009). Probability that the child inherits the *GCK* mutation from the mother is 50%. If so, the glucose set-point of the fetus will be the same as that of the mother and the birth weight will not be altered (Spyer et al. 2009). If the fetus does not inherit the mutation, the glucose levels sensed by the fetus will be supra-physiological, causing increased secretion of insulin. These newborns suffer from diabetic fetopathy and typical macrosomic growth that increases birth weight on average by 700 g (Singh *et al.* 2007, Spyer et al. 2009). Macrosomic growth in such fetus can cause a serious obstetric difficulty. In order to predict these complications fetal genetic testing could be carried out. Invasive procedures (amniocentesis, chorionic villus sampling) are mostly not indicated. Therefore, non-invasive prenatal testing using cell-free fetal DNA is a promising way how to determine the fetal genotype without prenatal risk complications (De Franco *et al.* 2017). Nearby, several trials tried to determine presence of diabetes pregnancies by using fetal abdominal circumference. Fetus with abdominal circumference below 75th centile was not at risk of any adverse effect including macrosomia (*GCK* mutation is present in fetus). On the contrary, fetus exhibiting abdominal fetal circumference higher than 75th centile has high likelihood of not inheriting mother's mutation. In such a case, therapy by insulin is advised as a prevention of risks associated with macrosomia (Buchanan *et al.* 1994, Kjos *et al.* 2001). Low dosage insulin did not reduce fetal growth in a retrospective study of 82 newborns of GCK-MODY mothers. Insulin in median dose of 0.4 U/kg in the third trimester did not deliver significant difference in birth weight, centile birth weight, and corrected birth weight between diet- and insulin-treated groups (Spyer et al. 2009). This can be ascribed to the persistent active endogenous regulation of glycemia with slightly higher glucose-set point level. Lower doses of exogenous insulin tend to decrease endogenous production of insulin, thus do bring euglycemic state, unless the dose of insulin > 0.5-1.0 U/kg/day is administered (Spyer *et al.* 2001). To avoid risks of delivering macrosomic baby, fetal growth scans should be carried out fortnightly since 26th week of gestation and labor should be induced at 38 weeks (Osbak et al. 2009, Chakera et al. 2015). The

incidence of labor induction was observed more frequently in insulin treated GCK-MODY women than diet-treated women (32% vs 15%) as well as Caesarean section (44% vs 15%) (Spyer et al. 2009). If a pregnant woman with GCK-MODY is misdiagnosed for GDM, the insulin therapy will not respect presence of *GCK* mutation in fetus, causing decreased fetal growth in *GCK* positive fetus (Spyer et al. 2001, Spyer et al. 2009). Newborns with GCK-MODY born to mothers without a *GCK* mutation (paternally inherited or *de novo* mutations) are usually small for their gestational age (<10th centile), with birth weight reduced by 400 g due to higher glucose set-point of the fetus that does not allow higher insulin secretion and its anabolic effects (Hattersley *et al.* 1998, Spyer et al. 2009). Influence of the GCK-MODY inheritance on the birth weight is summarized in Table 2.

The offspring of mothers with GDM are at increased risk of diabetes, obesity during childhood, inattention and hyperactivity, and impaired motor function (Gilmartin *et al.* 2008). The unaffected offspring of GCK-MODY mothers, that were prenatally exposed to moderate hyperglycemia are neither at risk of deterioration of glucose tolerance and reduction of β-cell function (Singh et al. 2007), nor abnormalities in BMI, lipid profile and blood pressure (Velho *et al.* 2000, Singh et al. 2007). Affected offspring of GCK-MODY mothers do not show any significant difference in the same modalities, similarly to GCK-MODY offspring of the healthy mothers (Velho et al. 2000). Hence, even though diabetic maternal environment can affect birth weight of offspring by increasing or decreasing fetal insulin secretion, it does not cause long-term consequences in adult life.

OTHER PHENOTYPES OF THE *GCK* MUTATIONS

Permanent neonatal diabetes mellitus (PNDM) can be caused by homozygous or compound heterozygous inactivating *GCK* mutations. PNDM is responsible for up to 50% of the children with neonatal diabetes mellitus and occurs with incidence of 1 in 215,417 live births (Stanik *et al.* 2007). Patients with PNDM have diabetes manifestation within the first 6 months of life (Flanagan *et al.* 2006) and do not experience remission period. Homozygous or compound heterozygous inactivating *GCK* mutations are a rare cause of PNDM. Only 8 individuals with GCK-PNDM have been reported so far. Homozygous *GCK* mutations suggest consanguinity between parents, as probability of both parents being GCK-MODY positive is rather low outside the family (Gloyn *et*

al. 2002, Rubio-Cabezas *et al.* 2008, Rubio-Cabezas *et al.* 2011). Possibility of second *de novo* mutation cannot be ruled out as well, although the probability is also low. 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, particularly when consanguinity is suspected (Vaxillaire *et al.* 2002). Individuals with PNDM caused by *GCK* mutations tend to not suffer from acute complications (ketoacidosis), unlike the other causes of PNDM (mutations in the genes *KCNJ11*, *ABCC8* or *INS*). It has also been suggested that homozygous absence of glucokinase, which is known to result in markedly low birth weight, could increase risk for intrauterine or neonatal death (Gloyn et al. 2002). People with GCK-PNDM are usually treated with insulin, as use of oral hypoglycemics has not proven to fully substitute the need of insulin (Turkkahraman *et al.* 2008).

Congenital hyperinsulinism (CHI) can be caused by heterozygous activating *GCK* mutations. CHI is labeled as persistent hyperinsulinemic hypoglycemia of infancy, and is characterized by increased insulin secretion despite of hypoglycemia. Mutations responsible for CHI can mostly be found in the β-cell potassium ATP channel genes (*ABCC8* and *KCNJ11*), followed by *HNF4A* and *HNF1A* mutations (Stanik *et al.* 2017). Heterozygous activating mutations of the *GCK* are one of the rare conditions, causing approximately 1.2% of all CHI cases (Christesen *et al.* 2008). Activating mutations in *GCK* result in an increased affinity of glucokinase for glucose and increased insulin secretion despite low blood glucose levels (Matschinsky 2002). The severity of hypoglycemia is variable and depends on the type of *GCK* mutation. Some mutations can cause severe and possibly fatal hypoglycemia (Sayed *et al.* 2009), whereas majority of mutations result in mild asymptomatic hypoglycemia, responsive to pharmacological treatment. Most of GCK-CHI patients respond well to diazoxide, the potassium channel opener (Gloyn et al. 2003, Wabitsch *et al.* 2007, Meissner *et al.* 2009), unlike CHI caused by recessive *KCNJ11*, *ABCC8* mutations, that are refractory to diazoxide (Touati *et al.* 1998) and often require surgical intervention (Gloyn et al. 2003). There is also evidence suggesting, that some GCK-CHI patients do not need pharmacological treatment, and may control their glycemia by regular eating (Gloyn et al. 2003, Wabitsch et al. 2007).

CONCLUSIONS

Mutations of the *GCK* gene are causing several types of monogenic diabetes. The most common of them is GCK-MODY. It has a mild course with low risk for chronic diabetes complications, and pharmacotherapy is mostly not needed. The exception are pregnant women with a macrosomic fetus, who should be treated with substitutional doses of insulin. Therefore, making the diagnosis of GCK-MODY is important not only because of consequences for the diabetes therapy, but it often improves also the quality of life for the affected individuals. However, still many people with GCK-MODY remain undiagnosed. Therefore, improving clinical identification of GCK-MODY and routine noninvasive prenatal testing in GCK-MODY pregnancies could contribute to better diagnostics in the future.

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FIGURE LEGENDS

Figure 1. Schematic representation of GCK isoform 1. The scheme is based on the data from the NCBI database. NG_008847.2 represents genomic sequence of the *GCK* gene. Boxes represent exons and blue line represents intronic regions. Coding region (CDS), marked by thick blue line, is 1 397 bp long (starts from 487bp of transcript and ends at the position of 1884bp). Thin green line indicates translated protein NP_000153.1. Amino-acid (aa) positions correspond to exon positions of the transcript. Upper amino-acid positions indicate the small and large hexokinase

subdomain. Brown amino-acid positions mark the protein sites which were described as substrate binding sites.

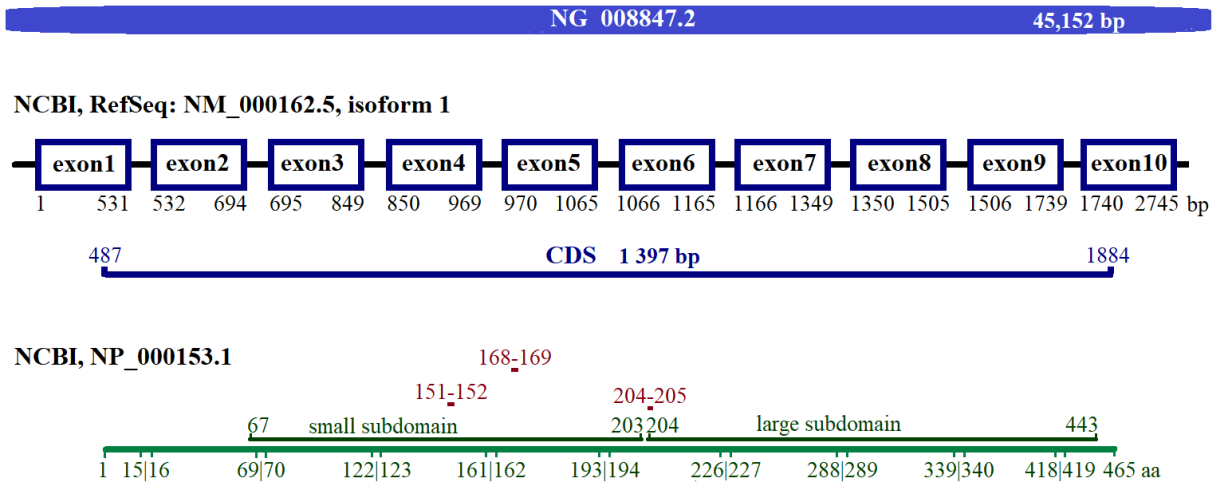

Table 1. Guidelines for the clinical identification of people with GCK-MODY (Ellard et al. 2008, Hattersley et al. 2018)

International Society for Pediatric and Adolescent Diabetes (ISPAD) 2018 guidelines	Best practise guidelines for molecular genetic diagnoses of MODY (Ellard, 2007)
1. Stable mild fasting hyperglycemia in range (FPG 5.5-8.0 mmol/l or 100-145 mg/dl).	1. The fasting hyperglycaemia is ≥5.5 mmol/l (98% of patients), persistent (at least 3 separate occasions) and stable over a period of months or years
2. HbA1c mildly elevated, but usually under 7.5%.	2. HbA1c is typically just above the upper limit of normal and rarely exceeds 7.5%
3. Small 2h-increment of blood glucose in oral glucose tolerance test oGTT (<60mg/dl or <3.5 mmol/l), but should not be considered an absolute criterion because of variability in oGTT test.	3. In an oGTT, the increment [(2h glucose)-(fasting glucose)] is small (71% of patients, in the large European study reported by Stride et al. 2002, had an increment <3 mmol/l). An increment of 4.6 mmol/l is often used to prioritize testing and corresponds to the 90th centile
4. Parent remains undiagnosed or misdiagnosed as early-onset type 2 diabetes, unless *de novo* mutation is present. On testing one parent will have a mildly raised fasting blood glucose, in the range of 5.5-8.5 mmol/l, as this is an autosomal dominant condition. Measuring fasting glucose of apparently unaffected parents is important when considering a diagnosis of a glucokinase mutation.	4. Parents may have 'type 2 diabetes' with no complications or may not be diabetic. On testing, one parent will usually have a mildly raised fasting blood glucose (range of 5.5 - 8 mmol/l), unless the mutation has arisen *de novo.* Testing af apparently unaffected parents´ fasting glucose is important when considering a diagnosis of glucokinase mutation.
5. Absence of obesity, acanthosis nigricans and/or other markers of metabolic syndrome.	
6. Asymptomatic at diagnosis, having no osmotic symptoms (polyuria, polydipsia).	
7. No concomitant pancreatic autoimmunity.	

Table 2. Impact of the inheritance of a GCK mutation on the birth weight of the child.

Genetic abnormalities:		
• GCK-MODY mother bearing child not inheriting *GCK* mutation.	• GCK-MODY positive mothers bearing child who possess one maternally/paternally/*de novo* mutated allele. • GCK-MODY negative mothers bearing child without *GCK* mutation (i.e. normal situation).	• GCK-MODY negative mother bearing child with *GCK* mutation inherited paternally or as *de novo* mutation. • GCK-MODY positive mother bearing child with *GCK* mutation, but falsely diagnosed with GDM and over-treated by insulin.
Macrosomia	**Normal weight**	**Reduced weight**