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Case Report

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Congenital Hyperinsulinism Due to a Novel Activating Glucokinase Mutation: A Case Report and Literature Review

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Abstract

Background: *Congenital Hyperinsulinism (CHI) constitutes a major cause of persistent and recurrent hypoglycemia, especially in the neonatal period, showing notable phenotypical heterogeneity among affected subjects. Activating mutations of the Glucokinase gene (GCK) are responsible for mild forms of hypoglycemia, due to CHI, usually easily medically managed.*

Case report: *We present a patient at the age of 3.5 years old investigated for persistent hypoglycemia. Laboratory evaluation showed hyperinsulinism during the hypoglycemic episode with a required glucose infusion rate greater than 8-10 mg/kg/min to maintain normoglycemia. Targeted gene panel sequencing revealed an activating missense novel mutation p.Val71Ala in exon 3 of GCK gene, dominantly inherited by his mother. In silico, analysis of this novel missense variant assessed its pathogenicity as being of uncertain significance*

Conclusions: *GCK gene mutations result in varying phenotypic characteristics and responsiveness to diazoxide depending on the type of activating mutation.*

Keywords: Congenital Hyperinsulinism; Glucokinase Gene; Hypoglycemia; Diazoxide; Mutation

Background

Tight control of glucose levels is crucial for normal brain function and neurocognitive development. As though, in the context of hypoglycemia neurological impairment or even brain damage can be observed, specifically during the neonatal period and early infancy. Therefore, the early identification of the etiology of hypoglycemia along with the prompt management of the situation seems critical, due to the severe sequelae [1].

Congenital Hyperinsulinism (CHI) constitutes a major cause of persistent and recurrent hypoglycemia, especially during the neonatal period, with a frequency of 1 in 30.000 to 50 000 live births [1]. Mutations of genes implicated in insulin release represent 55% of the cases of CHI, while the etiology of the rest 45% remains unknown [2]. At present, mutations in 12 genes (*ABCC8, KCNJ11, GLUD1, GCK, HADH, SLC16A1, UCP2, HNF4A, HNF1A, HK1, PGM1*, and *PMM2*) have been identified to play a key role in the process of dysregulated insulin exocytosis by the pancreatic β-cell [3]. In the majority of situations there is an increase in ATP: ADP ratio, with anarchic Ca influx in the β-cell, inducing autonomous insulin release [3].

Among these genes, the *GCK* gene, encoding for glucokinase enzyme, shows great interest, due to its action as a glucose sensor determining the degree of insulin secretion, depending on the plasma glucose concentration [4]. As a consequence, mutations affecting *GCK* activity present with various phenotypic characteristics depending on the type of mutation [5]. As such, inactivating mutations result in Maturity-Onset Diabetes of the Young (GCK-MODY), due to *GCK* deficiency, without need for special treatment. On the contrary, activating *GCK* gene mutations cause persistent hypoglycemia, due to congenital hyperinsulinism (GCK-CHI), with treatment options based on pharmacological agents (diazoxide, diuretics, octreotide, etc.), depending on the severity of underlying mutation and the subsequent level of GCK activation [5, 6].

GCK gene gain-of-function variants constitute a rare cause of CHI, inherited in an autosomal dominant manner. They are characterized by inappropriate release of insulin (insulin levels >10 μU/mL), despite the low plasma glucose levels [7]. The glucose-stimulated insulin secretion threshold is lower, while insulin secretion remains tightly regulated [7]. Key biochemical features include high insulin levels (>10 μU/mL), with low levels of ketone bodies (β-hydroxybutyrate <15mg/dl) and free fatty acids (28-42mg/d). The insulin-to-glucose ratio may range from 0.4-2.7 (normal <0.3) [8].

To date, 20 different gain-of-function *GCK* variants responsible for hyperinsulinism (GCK-CHI) have been described, the majority of which are located in the allosteric activator site of the enzyme,

except for the p.Met197Ile and p.Val389Asn variants [9]. In the present study, we describe one patient with CHI due to a novel missense gain-of-function *GCK* variants, namely, p.Val71Ala (c.212T>C).

Case Presentation

The proband was the first male child from a non-consanguineous Albanian family born at 37th weeks of gestation after an uneventful pregnancy. His birth weight was 3330 g (3rd-10th percentile), head circumference 34 cm, and length 53 cm (95th percentile). At the chronological age of 3.5 years, the child was admitted in the emergency department of the hospital with unconsciousness along with dizziness, facial cyanosis, sweating and transient loss of muscle tone. Hypoglycemia was suspected and immediately he was treated with intravenous infusion of glucose at a rate of 8-10 mg/kg/min, which corrected low levels of plasma glucose and maintained normoglycemia. The initial laboratory evaluation revealed low blood glucose levels at 35 mg/dl (1.9mmol/L) and plasma ketone levels at 0.4 mg/dl. During the episode of hypoglycemia [40 mg/dl (2.2 mmol/ L)] insulin levels were measured at 24.6 μU/ ml (170.8 pmol/ L) and c-peptide at 1.77 ng/ml.

As hypoglycemia was confirmed, the next step in differential diagnosis of hypoglycemia was a glucagon test, which confirmed hyperinsulinism: blood glucose value, prior to glucagon administration was 36mg/dl, while blood glucose post glucagon administration was 107 mg/dl. Further laboratory revealed suppressed levels of free-fatty acid levels, while urine organic acids, plasma acylcarnitines, ammonia, growth hormone and serum cortisol levels were within normal range. Clinical examination, electrocardiograph and imaging assessment with cardiac and abdomen ultrasound, were all proved to be normal.

Through investigation of the the type of pancreatic abnormality (focal or diffuse) of CHI in the index boy included 18F-fluoro-L-DOPA positron emission tomography (PET). The test revealed diffuse pancreatic uptake of 18F-DOPA, with normal homogeneous activity in the liver and gallbladder. His medical history revealed surgical repair of inguinal hernia and hypospadias. Family history was indicative of maternal mild hyperinsulinemia with high insulin levels after fasting hypoglycemia (insulin/glucose>0.3). She has been on dietary instructions without need for medical treatment. During pregnancy an oral glucose tolerance test (OGTT), revealed impaired glucose tolerance with normal insulin response.

Targeted gene panel sequencing was performed for the investigation of mutations in genes associated with CHI after informed consent of the parents. DNA was isolated from peripheral blood leucocytes. A Next Generation Sequencing Target Gene Panel of seven MODY genes (*GCK, HNF1A, HNF4A, HNF1B, INS, ABCC8* and *KCNJ11*) was employed and NGS was performed on an Ion Torrent™ Personal Genome Machine™ (PGM) platform (Thermo Fisher Scientific, Waltham, MA, USA) using the Ion PGM™ Hi-Q™ View Sequencing Kit and ion314™ chip v2. The analysis revealed a heterozygous missense activating mutation in exon 3 of the GCK gene, c.212T>C, p.Val71Ala (V71A) (Figure 1). This finding was verified by Sanger Sequencing of exon 3 of the *GCK* gene. Parents were tested by Sanger sequencing and it was found that the mother was also heterozygote for this variant. Father's genetic testing revealed no variant. To date, the nucleotide change c.212T>C in exon 3 of the *GCK* gene, p.Val71Ala constitutes a novel variant as it has not been reported in the literature.

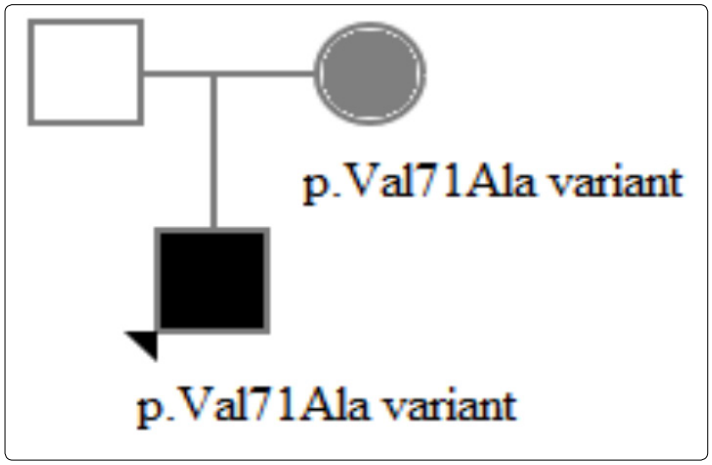

Figure 1A: Family pedigree

The arrow indicates the index case, black square: CHI male with p.Val71Ala variant detected, grey circle: female carrier of the variant with impaired glucose tolerance during gestation, white square: no CHI or diabetic male.

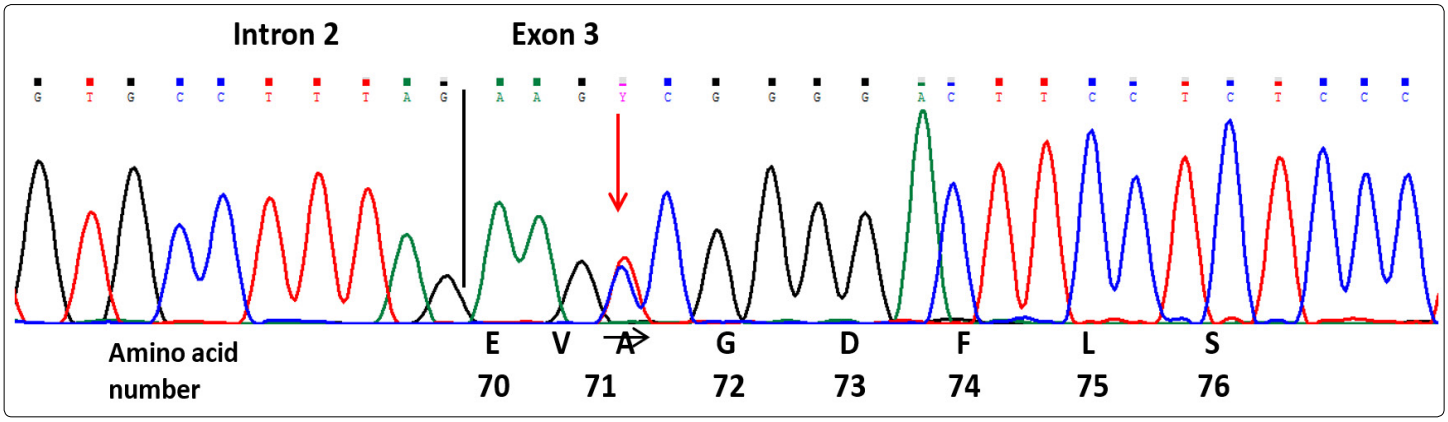

Figure 1B: Part of the GCK gene exon 3 sequence electropherogram, showing the heterozygous c.212T> C, p.Val71Ala variant identified. Black straight line depicts the intron 2-exon 3 boundaries and the red arrow points the variant.

In silico analysis of this novel missense variant using various bioinformatics tools was performed to assess its pathogenicity (Table 1), however the results of the various tools differed (from benign to pathogenic) and according to the American College of Medical Genetics (ACMG) classification, it is a variant of uncertain significance. After diagnosing GCK-HI, a treatment with diazoxide was started at an initial dose of 15mg/ kg/day in combination with frequent glucose-enriched oral feedings to maintain normoglycemia. However, as the boy was partially responsive to medical treatment, with frequent episodes of hypoglycemia, diazoxide was increased at a dose of 20mg/kg/d with significant reduction of hypoglycemic episodes. Currently, the boy at 5 years of age receives diazoxide at a dose of 15mg/kg/d to maintain glucose levels at 60-80mg/dl with rare episodes of hypoglycemia, while it is able to participate in school sports. His growth profile is a weight 16kg (-1.20SD) and a height of 106cm (-0.68SD). During treatment, the child developed hypertrichosis, while the parents reported loss of appetite, with amelioration of symptoms after dose tapering. The boy, also, was advised to avoid simple carbohydrates and be fed with frequent meals that help to maintain adequate glucose levels, such as complex carbohydrates, unsaturated fats, and high-protein foods. Finally, the importance of adherence to the treatment and regular follow-up by a pediatric endocrinologist and dietician was underlined.

Table 1: In silico analysis

***In silico* tool**	**SCORE**	**RANGE**	**PREDICTION**
SIFT	0.143	0-1 (Damaging-Tolerated)	**Tolerated**
POLYPHEN2	0.995	0-1 (Benign-Damaging)	**Damaging**
MUTATION TASTER	0.999	0-1 (Polymorphism-Disease Causing)	**Damaging**
PANTHER	456	>450	**Probably damaging**
GRANTHAM	64	5 to 215 (Tolerated – Deleterious)	
UMD	63		**Probable polymorphism**
LRT	0	0-1	**Damaging**
MUTATION ASSESSOR	0.935	-5.135 to +6.49	**Low**
DANN	0.997	0-1 (benign-Damaging)	**Damaging**
GERP (Genomic Evolutionary Rate Profiling)	4.67	-12.3 to 6.17 (6.17=more conserved)	**Conserved**
FATHMM	-4.72	-16.13 to +10.64.	**Damaging**
FATHMM-MKL	0.912	0-1	**Damaging**
MetaSVM	0.976	-2.0058 to +3.0399	**Damaging**
MetaLR	0.925	0 to 1	**Damaging**
Provean	0.64	-14 to +14	**Neutral**
VEST3	0.576	0-1 (neutral –damaging)	**Neutral**
CADD	23.5	-7 to 20 (benign-deleterious)	**Deleterious**
M-CAP	0.223		**Damaging**
ACMG			**Uncertain Significance**

Discussion

To date, more than 800 different *GCK* gene variants have been described in the literature with the majority of them being responsible for hyperglycemia [9]. However, according to the latest data, of the aforementioned variants only 21 different gain-of-function mutations of the *GCK* gene have been reported, including the variant identified in the present study (Table 2), causing mild forms of CHI. The majority of them are missense variants, while one insertion has been described (Table 2). As far as the inheritance pattern is concerned, the GCK-CHI variants can either be autosomal dominantly (AD) inherited or *de novo* [9]. In our case, the variant was dominantly inherited from his mother; however, she was not reported to be diabetic. Kinetic analysis of the reported GCK gain-of-function variants confirms the increased enzymatic relative activity index (RAI), ranging from 1.6-130, which is responsible for hyperinsulinism. However, various degrees of RAI are not well correlated with the severity of phenotypic characteristics of GCK-CHI [9]. Many factors should be considered, including the incidence of hypoglycemia, the dose of diazoxide, the glucose infusion rate, and so on. As such, p.Met197Ile with RAI 3.1 resulted in hypoglycemia soon after birth, whereas p.Thr103Ser with RAI 8.4 caused hypoglycemia at 15 years of age. By that, age of onset of GCK-HI symptoms ranged from birth to 44 years, with the average age of confirmatory diagnosis being at the age of 16 years [10, 11]. Another factor with large variability is diazoxide responsiveness of the patient, depending on the severity of the variant effect [9]. Of the 20 so far reported variants, a large proportion of the patients have been diazoxide responsive, whereas the remaining being partial responsive or unresponsive to diazoxide. Further investigation revealed that among the diazoxide non-responder patients, the majority harbored *de novo* variants [9]. It is noteworthy that in two cases of children carrying the p.Tyr214Cys and the 454insA variants, near total pancreatectomy had to be performed and even so, hypoglycemia was not totally controlled [12]. Indeed, the variants p.Tyr214Cys and ins454A may cause severe and possibly fatal hypoglycemia, in contrast with the majority of the *GCK* variants described to date, which result in a phenotype of mild hypoglycemia, often asymptomatic and responsive to pharmacological treatment [13].

Table 2: Data of published patients with GCK-HI

	Mutant	Inheritance patterns	Proband: Gender/ Age of diagnosis	Diazoxide response	RAI
1.	p.S64Y (9)	De novo	M/17 years	Yes	22
2.	p.T65I (9)	AD	M/neonate	Yes	9.8
3.	p.G68V (9)	AD	F/7 months	Yes	16
4.	p.V71A (9)	AD	M/3.5 years	Partial	-
5.	p.K90R (9)	AD	F/20 years	NA	1.6
6.	p.V91L (9)	AD	F/2 years	Yes	24
7.	p.W99R (9)	AD	M/10 months	Partial	4.1
8.	p.W99L (9)	De novo	M/6 y	Partial	8.9
9.	p.W99C (9)	NA	F/25 years	Yes	11.6
10.	p.T103S (9)	AD	F/15 years	Yes	8.4
11.	p.N180D (9)	AD	F/childhood	Partial	NA
12.	p.M197V (9)	De novo	M/9 years	Partial	4.7
13.	p.M197I (9)	De novo	M/16 years	Yes	3.1
14.	p.M197T (9)	AD	F/44 years	NA	2.9
15.	p.Y214C (9)	De novo	F/29years	No	130
16.	p.V389L (9)	AD	M/2years	Partial	6.0
17.	p.E442K (9)	AD	F/6years	Yes	3.3
18.	p.V452L (9)	De novo	M/3years	Yes	10.8
19.	Pins454A (9)	De novo	M/17 years	No	26
20.	p.V455M (9)	AD	M/31 years	Yes	5.2
21.	p.A456V (9)	AD	M/14 years	Yes	37.9

GCK gene gain-of-function variants can activate the enzyme via two distinct mechanisms, namely α-type and β-type, as described by latest findings [14]. Molecular investigation for each type of activation process indicates that α-type activation results from a shift in the conformational ensemble of unligated GCK toward a state resembling the glucose-bound conformation, whereas β-type activation is due to an accelerated rate of product release [14]. Variants near the allosteric activator (e.g. p.Val91Leu, p.Lys90Arg) represent a-type activation, while variants away from the GCK activator site (e.g. p.M197Val) lead to β-type activation [9, 14].

Since both types of activated *GCK* gene variants result in disease, uncovering the underlying molecular origins of activation could facilitate the development of targeted therapeutic approaches to treat hyperinsulinemia unique to each type of activation mechanism [14].

Overexpression of GCK in extra-pancreatic tissues has been also reported and may cause hepatic steatosis and metabolic dyslipidemia in both rodents and humans [15]. However, the majority of patients with *GCK* gain-of-function variants, including the child in our report, exhibit normal lipid profile, not supporting the strong association between such mutations and abnormal lipid profile [16]. Furthermore, the proband did not develop hepatic steatosis at the time of the diagnosis or any time up to today, as proven by hepatic ultrasound.

To a certain extent, it remains unclear whether patients with gain-of-function *CGK* gene variants are at increased risk of developing type 2 diabetes in adulthood [17]. Occasionally, patients with *HNF4A* and *ABCC8* gene variants have been reported to present with hypoglycemia in the neonatal period or infancy and diabetes in adulthood [10]. Patients harboring *GCK* gain-of-function mutations, where the defect lies in glucose sensing and not in excess insulin secretion, are not expected to develop β-cell exhaustion and consequently type 2 diabetes. However, there have been reports of families with *GCK* gain-of-function variants initially classified as having type 2 diabetes [10, 17]. Also, there is a case of a proband's father carrying the p.Val455Met variant and a history of hypoglycemia, who developed diabetes at the age of 48 years and a patient harboring the p.Val62Met variant, which has been associated with MODY2, raising the possibility of a possible association between *GCK* gain-of-function variants and diabetes later in life. Further longitudinal studies of such families are necessary to evaluate this possibility [17, 18]. In the case presented herein the mother, who also carried the variant, has not developed diabetes so far, however, a slightly impaired glucose tolerance detected during her pregnancy.

As for our case, the age of symptoms onset coincided with the age of diagnosis, in contrast to the mother's age of diagnosis, which occurred later in adult life, due to the lack of symptoms. The child was initially unresponsive to diazoxide with frequent episodes of hypoglycemia, but after dose adjustment, hypoglycemic episodes were significantly decreased. On the contrary, the mother has never shown any signs or symptoms, despite harboring the same variant as the proband. However, frequent laboratory evaluation is mandatory as she may develop diabetes later in life, as a result of β-cell exhaustion.

In summary, we have identified a novel gain-of-function variant, p.Val71Ala of *GCK* gene, with heterogeneous clinical manifestation among affected members of the same family. Although *GCK* gene variants are not as common as variants affecting the K_{ATP} channel genes (*ABCC8* and *KCNJ11*), they need careful evaluation and management due to persistent hypoglycemia.

Conclusion

Congenital hyperinsulinism is an important underlying disorder of severe persistent hypoglycemia, which should be emphasized in the

differential diagnosis of every case of hypoglycemia, necessitating high glucose supply, regardless of the age of symptoms onset. With the advancement of molecular genetics and the imaging techniques, a prompt diagnosis is nowadays attainable, with a variety of treating modalities for the resultant hypoglycemic episodes.

References

1. Palladino AA, Bennett MJ, Stanley CA (2008) Hyperinsulinism in infancy and childhood: when an insulin level is not always enough. Clin Chem 54: 256-263.
2. De Leon DD, Stanley CA (2017) Congenital Hypoglycemia Disorders: New Aspects of Etiology, Diagnosis, Treatment and Outcomes: Highlights of the Proceedings of the Congenital Hypoglycemia Disorders Symposium, Philadelphia April 2016. Pediatr Diabetes 18: 3-9.
3. Demirbilek H, Hussain K (2017) Congenital Hyperinsulinism: Diagnosis and Treatment Update. J Clin Res Pediatr Endocrinol 9: 69-87.
4. Gloyn AL, Noordam K, Willemsen MA, Ellard S, Lam WW, et al. (2003) Insights into the biochemical and genetic basis of glucokinase activation from naturally occurring hypoglycemia mutations. Diabetes 52: 2433-2440.
5. Lu B, Tonne JM, Munoz-Gomez M, Ikeda Y (2019) Hyperinsulinemic hypoglycemia subtype glucokinase V91L mutant induces necrosis in beta-cells via ATP depletion. Biochem Biophys Rep 17: 108-113.
6. Osbak KK, Colclough K, Saint-Martin C, Beer NL, Bellanne-Chantelot C, et al. (2009) Update on mutations in glucokinase (GCK), which cause maturity-onset diabetes of the young, permanent neonatal diabetes, and hyperinsulinemic hypoglycemia. Hum Mutat 30: 1512-1526.
7. Matschinsky FM (2002) Regulation of pancreatic beta-cell glucokinase: from basics to therapeutics. Diabetes 51: S394-404.
8. Mathur NB, Sharma M, Agarwal A, Kumar A (2011) Positron emission tomography in congenital hyperinsulinism. Indian Pediatr 48: 139-141.
9. Ping F, Wang Z, Xiao X (2019) Clinical and enzymatic phenotypes in congenital hyperinsulinemic hypoglycemia due to glucokinase-activating mutations: A report of two cases and a brief overview of the literature. J Diabetes Investig 10: 1454-1462.
10. Sayed S, Langdon DR, Odili S, Chen P, Buettger C, et al. (2009) Extremes of clinical and enzymatic phenotypes in children with hyperinsulinism caused by glucokinase activating mutations. Diabetes 58: 1419-1427.
11. Beer NL, van de Bunt M, Colclough K, Lukacs C, Arundel P, et al. (2011) Discovery of a novel site regulating glucokinase activity following characterization of a new mutation causing hyperinsulinemic hypoglycemia in humans. J Biol Chem 286: 19118-19126.
12. Saint-Martin C, Arnoux JB, de Lonlay P, Bellanne-Chantelot C (2011) KATP channel mutations in congenital hyperinsulinism. Semin Pediatr Surg 20: 18-22.
13. Kizu R, Nishimura K, Sato R, Kosaki K, Tanaka T, et al. (2017) Population Pharmacokinetics of Diazoxide in Children with Hyperinsulinemic Hypoglycemia. Horm Res Paediatr 88: 316-323.
14. Sternisha SM, Liu P, Marshall AG, Miller BG (2018) Mechanistic Origins of Enzyme Activation in Human Glucokinase Variants Associated with Congenital Hyperinsulinism. Biochemistry 57: 1632-1639.
15. Wabitsch M, Lahr G, Van de Bunt M, Marchant C, Lindner M, et al. (2007) Heterogeneity in disease severity in a family with a novel G68V GCK activating mutation causing persistent hyperinsulinaemic hypoglycaemia of infancy. Diabet Med 24: 1393-1399.
16. Challis BG, Harris J, Sleigh A, Isaac I, Orme SM, et al. (2014) Familial adult onset hyperinsulinism due to an activating glucokinase mutation: implications for pharmacological glucokinase activation. Clin Endocrinol (Oxf) 81: 855-861.
17. Meissner T, Marquard J, Cobo-Vuilleumier N, Maringa M, Rodriguez-Bada P, et al. (2009) Diagnostic difficulties in glucokinase hyperinsulinism. Horm Metab Res. 41: 320-326.
18. Gloyn AL, Odili S, Zelent D, Buettger C, Castleden HA, et al. (2005) Insights into the structure and regulation of glucokinase from a novel mutation (V62M), which causes maturity-onset diabetes of the young. J Biol Chem 280: 14105-14113.