GCK-MODY in the US Monogenic Diabetes Registry: description of 27 unpublished variants

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Abstract

We report on 134 unique GCK variants in 217 families, including 27 unpublished variants, identified in the US Monogenic Diabetes Registry in the last decade. Using ACMG guidelines, 26% were pathogenic, 56% likely pathogenic and 18% were of uncertain significance. Those with pathogenic variants had clinical features consistent with GCK-MODY.

Keywords

monogenic diabetes; MODY; glucokinase; pathogenic variants; next-generation sequencing

1. Introduction

Maturity-Onset Diabetes of the Young (MODY) refers to a clinically and genetically heterogeneous group of monogenic forms of diabetes. MODY is characterized by an autosomal dominant mode of inheritance, young onset typically before 35 years of age, and a non-insulin-dependent clinical presentation [1]. To date, 14 genetically distinct subtypes of MODY have been described and collectively account for about 2-5% of all diabetes cases [2-5]. Glucokinase (gene symbol-*GCK*), a key enzyme in glucose metabolism, regulates insulin secretion in response to variations in blood glucose levels. Loss of function

mutations in the heterozygous state in *GCK* cause GCK-MODY (formerly known as MODY-2), which is characterized by mild non-progressive fasting hyperglycemia which is present at birth but typically detected incidentally during routing medical screening [6]. Fasting blood glucose typically ranges from 5.5-8 mmol/L, and there is usually a small incremental rise in blood glucose during an oral glucose tolerance test (generally below 3 mmol/L). Glycated hemoglobin (HbA1c) levels are mildly elevated and usually range between 5.6-7.6% [7,8]. Despite lifelong mild hyperglycemia, microvascular and macrovascular complications are rare in patients with pathogenic *GCK* variants and pharmacological intervention is rarely required outside of pregnancy [9]. There are now over 800 reported variants in the Human Gene Mutation Database (HGMD) distributed throughout most of the coding regions and the exon—intron boundaries of the *GCK* gene [10]. Pathogenic *GCK* variants are present in 1 in 1000 individuals, yet most cases of GCK-MODY are undiagnosed or misdiagnosed as type 1 or type 2 diabetes [11, 12]. The Institutional Review Board (IRB)-approved US Monogenic Diabetes Registry, housed at the University of Chicago, was established to correctly diagnose and follow families with monogenic forms of diabetes (http://monogenicdiabetes.uchicago.edu/) [13]. To date, 1552 families with a known or suspected form of monogenic diabetes have been consented for participation in the Registry. A total of 371 families were found to have pathogenic mutations causative of MODY of which 189 were found to have a previously reported pathogenic variant causative of GCK-MODY. Here, we describe the molecular characteristics and associated clinical features of 27 unreported *GCK* variants identified in 28 families.

2. Materials and Methods

Within the Registry a clinical diagnosis of GCK-MODY was suspected in patients with persistent mild fasting hyperglycemia and/or an HbA1c level within the expected range for GCK-MODY [8]. A genetic diagnosis was performed using Sanger sequencing or a targeted next-generation sequencing (NGS) panel as described previously [14, 15]. Briefly, NGS data quality was assessed using fastQC and aligned to the hg19 reference using Burrow-Wheeler Aligner. Variants were called using the Genome analysis Toolkit based on the best practices for variant discovery and annotated using Alamut Batch (Interactive Biosoftware, Rouen, France). Variants with a global frequency >1% in EXAC were excluded [16]. Three different computational tools (PolyPhen-2, SIFT, and Human Splice Finder) were used to obtain pathogenicity predictions for each variant. All variants were classified based on degree of pathogenicity (pathogenic/likely pathogenic, variant of uncertain significance (VUS), and benign/likely benign) in accordance with the American College of Medical Genetics (ACMG) guidelines [17]. Pathogenic variants were confirmed by Sanger sequencing and co-segregation with the diabetic phenotype was evaluated by targeted sequencing analysis of enrolled family members. HGMD and Pubmed were used to determine whether the variant was previously reported. ClinVar was used as a reliable source supporting variant interpretation [18]. However, sequence changes identified in this study that were reported in ClinVar but not previously published were considered novel.

3. Results

Through December 2018, 359 people were referred for suspected or confirmed GCK-MODY, with 217 probands testing positive for a *GCK* variant either by Sanger sequencing or MODY gene panel. Of these, 72% were tested either on a research or clinical basis through the University of Chicago. Seventy-four probands have yet to be tested. Clinical information was available for a subset of probands with *GCK* variants (Table 1). Overall, clinical findings were similar to those with previously described pathogenic *GCK* variants in the [12]. Sequence analysis identified 134 *GCK* variants in 217 families from the year 2008 to 2018. Of these, 107 variants were previously reported in the literature as causal. The remaining 27 variants, present in 28 probands, were to the best of our knowledge not previously described or reported (Table 2). These included 17 missense, 1 nonsense, 2 splice sites, 1 in-frame deletion, 1 in-frame duplication, and 5 insertions/deletions. After interpretation using the ACMG guidelines, 26% (7/27) of the novel *GCK* variants were found to be pathogenic, 56% (15/27) were likely pathogenic and 18% (5/27) were of uncertain significance. All novel variants except for Met210dup were identified in a single family. Seven variants (p.Lys13X, p.Leu185Pro, p.Val207Leu, p.Glu227Hisfs*47, p.Cys252Trp, p.Arg275Leu, and p.Ser281Pro) were previously reported by our group [12, Supplementary Table] but are not included in the professional version of HGMD and are therefore further described in Table 2. Parental or extended family DNA was available for 10 out of 28 probands and co-segregation with the diabetes phenotype was established in those cases. Four of the five VUS (p.Ser127Pro, p.His141Pro, c.579+4delA, and p.Leu307Phe) were predicted to be damaging by *in-silico* tools. However, due to the lack of segregation data and/or phenotypic data on family members, these variants did not satisfy classification requirements for pathogenic or likely pathogenic. The p.Leu307Phe sequence change was identified in one proband with no family history of diabetes. Targeted sequencing of the parents' DNA did not detect this sequence change and was thus presumed to be *de novo* in the proband. Paternity testing was not performed. The p.His317Gln moderately conserved sequence change was predicted to be tolerated by *in-silico* tools. This variant was detected in one proband and her mother, both of whom had stable, mild fasting hyperglycemia levels of 5.8-6.1mmol/l and 6-6.3mmol/l respectively. However, the proband reported an HbA1c level of 5.2% which is below the expected range for GCK-MODY.

4. Discussion

Pathogenic variants in *GCK* represent the most common cause of MODY in the Registry, accounting for 57% of all MODY cases. This is consistent with other studies reporting a high prevalence of GCK-MODY as a percentage of all MODY patients (France, 56%; Italy, 41–61%; Spain, 25–80%; Czech Republic, 31%; Norway 12%, Chile, 50%) [19-23]. In this study, we identified 27 previously unreported *GCK* variants distributed throughout the gene with no particular variant associated with a more severe phenotype (Table 1). Their phenotype was similar to probands with pathogenic variants previously reported as causal for GCK-MODY. The main limitations of this study include incomplete clinical information, family history, and segregation studies which may have provided further evidence of pathogenicity.

Given the mild hyperglycemia and lack of associated complications, GCK-MODY can be considered a discrete genetic cause of mild hyperglycemia rather than a subtype of diabetes. Yet, there remains a significant clinical utility in making a correct diagnosis as patients with GCK-MODY do not require treatment except possibly in pregnancy [24]. Our report of 22 pathogenic and likely pathogenic variants will help to correctly diagnose other patients with these same variants. Moreover, our report of the 5 VUS will assist in future studies to determine the pathogenicity of these variants.

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Table 1:

Clinical characteristics of probands with novel *GCK* variants

	All variants	**P variants**	**LP variants**	**VUS**
Total Number of Probands (*n*)	**28**			
Gender				
• Female	18 (64%)			
• Male	10 (36%)			
Ethnicity				
• Caucasian	19 (68%)			
• Asian	2 (7%)			
• Hispanic	2 (7%)			
• Unreported	5 (18%)			
Previous antibody testing (n=11)				
• Negative	10 (91%)			
Positive*	1 (9%)			
Family history with hyperglycemia (reported parent with diabetes or hyperglycemia) (n=20)				
• Yes	17 (85%)			
• No**	3 (15%)			
Age at Clinical Diagnosis (n=27)	16 years (7-29 years)	13 years (*n*=7)	15 years (*n*=15)	23 years (*n*=5)
• Females (n=18)	24 years (11-33 years)	7 years (*n*=5)	26 years (*n*=9)	24 years (*n*=4)
• Males (n=9)	10 years (6-19 years)	24.5 (*n*=2)	8 years (*n*=6)	5 years (*n*=1)
Fasting plasma glucose (n=14)	6.96mmol/L (6.55-7.21mmol/L)	6.85mmol/L (*n*=2)	6.99mmol/L (*n*=9)	6.27mmol/L (*n*=3)
HbA1c at clinical diagnosis (n=19)	6.3% (6-6.5%)	6.25% (*n*=4)	6.2% (*n*=11)	6.35% (*n*=4)
BMI at clinical diagnosis (m/kg²) for adult patients (*n*=14) ***	20.74	18.55 (*n*=4)	20.65 (*n*=8)	20.9 (*n*=2)

n: number of probands with available clinical data; P: pathogenic; LP: likely pathogenic; VUS: variant of unknown significance Data are presented as median values (interquartile range)

*
One proband carrying the p.Asn180Lys mutation tested positive for Znt8 autoantibody however oral glucose tolerance test and HbA1c levels were consistent with GCK-MODY. Furthermore, the proband's type 1 diabetes risk was below the 5th percentile [25].

**
One proband reported an affected daughter with the same variant (c.680-2A>C), another was presumed to have a *de novo* variant (p.Leu307Phe), and the third reported no parental history (p.Gly410Asp).

***
BMI Normal weight: 18.5-24.8kg/m². BMI percentiles were calculated for pediatric patients (n=5). All pediatric patients had BMI percentiles in the normal range (5th-85th percentile).

Table 2:

Molecular characteristics of novel *GCK* variants

Nucleotide	Amino Acid[a]	Exon	GnomAD MAF[b]	Polyphen Score[c]	SIFT Score[d]	Human splice Finder	Variant Classification[e]	Number of Families (Number of members)
c.37A>T	Lys13*	1	0	-	-		P	1 (4)
c.98T>A	Val33Glu	2	0	0.99	0		LP	1 (1)
c.215G>A	Gly72Glu	3	0	1	0		LP	1 (3)
c.295del	Trp99Glyfs*3	3	0	-	-		P	1 (1)
c.317_333del	Gln106Argfs*9	3	0	-	-		P	1 (1)
c.344dup	Met115Ilefs*6	3	0	-	-		P	1 (2)
c.364C>A	Leu122Ile	4	0	0.985	0		LP	1 (3)
c.379T>C	Ser127Pro	4	0	0.832	0		VUS	1 (1)
c.422A>C	His141Pro	4	0	0.99	0		VUS	1 (1)[†]
c.503C>A	Thr168Asn	5	0	1	0		LP	1 (7)
c.511T>G	Phe171val	5	0	1	0		LP	1 (1)
c.540T>A	Asn180Lys	5	0	1	0		LP	1 (1)
c.554T>C	Leu185Pro	5	0	1	0		LP	1 (3)
c.554T>G	Leu185Arg	5	0	1	0		LP	1 (1)
c.579+4delA		Intron 5		-	-	Alteration of the WT donor site	VUS	1 (1)
c.619G>T	Val207leu	6	0	0.998	0		LP	1 (3)
c.630_632dup	Met210dup	6	0				LP	2 (2,3)
c.632T>A	Ile211Asn	6	0	1	0		LP	1 (1)
c.678_679+2del	Gly227Hisfs*47	6	0	-	-		P	2 (3,1)
c.680-2A>C		Intron 6		-	-	Alteration of the WT acceptor site	P	1 (2)
c.680G>A	Gly227Asp	7	0	1	0		LP	1 (2)
c.756C>G	Cys252Trp	7	0	1	0		LP	1 (2)
c.824G>T	Arg275Leu	7	0	0.616	0		LP	1 (1)
c.841T>C	Ser281Pro	7	0	1	0		LP	1 (1)

Nucleotide	Amino Acid[a]	Exon	GnomAD MAF[b]	Polyphen Score[c]	SIFT Score[d]	Human splice Finder	Variant Classification[e]	Number of Families (Number of members)
c.848del	Asn283Thrfs*11	7	0	-	-		P	1 (1)
c.908G>A	Arg303Gln	8	0.0004081%	1	0		LP	1 (1)
c.919C>T	Leu307Phe	8	0	0.718	0		VUS	1 (1)*
c.951 C>G	His317Gln	8	0	0.013	0.17		VUS	1 (2)
c.1082dupC	T362Hisfs*97	9	0	-	-		P	1 (1)
c.1113C>A	Cys371*	9	0	-	-		P	1 (1)
c.1130_1138del	Arg377_Ala379del	9	0	-	-		LP	1 (3)
c.1173C>A	Asn391Lys	9	0	1	0		LP	1 (1)
c.1181G>C	Arg394Pro	9	0	0.99	0		LP	1 (1)
c.1229G>A	Gly410Asp	9	0	1	0		LP	1 (1)

Abbreviations: gnomAD, Genome Aggregation Database; WT, Wild Type; MAF, Minor Allele Frequency; N/A, not available

[a] RefSeq reference transcript: NM_000162.3 (GCK)

[b] MAF from gnomAD was calculated using allele count / allele number.

[c] Polyphen-2 scores range from 0.0 to 1.0 and can be interpreted as follows: 0.0 to 0.15 –benign, 0.15 to 0.85 --possibly damaging, 0.85 to 1.0 -- probably damaging.

[d] SIFT scores range from 0.0 to 1.0 and can be interpreted as follows: 0.0 to 0.05 -deleterious, 0.05 to 1.0 - tolerated (benign)

[e] Variants were classified according to the ACMG 5-tier system: P, pathogenic; LP, likely pathogenic; VUS, variant of uncertain significance.

[†] Gestational diabetes

[*] Presumed to be *de novo*