Case Report

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A rare, likely pathogenic *GCK* variant related to maturity-onset diabetes of the young type 2: A case report

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Maturity-onset diabetes of the young (MODY) is caused by autosomal dominant pathogenic variants in one of 14 currently known monogenic genes. Characteristics of patients with MODY include early-onset clinical disease with a family history of diabetes and negative autoantibodies and may present with heterogeneous phenotypes according to the different subtypes. Here, we report a patient with early-onset diabetes who presented asymptomatic mild fasting hyperglycemia with the absence of autoantibodies. She was diagnosed with glucokinase (GCK)-MODY caused by a *GCK* variant, c.1289T>C (p.L430P), identified by targeted gene-panel testing, and the affected father had the same variant. We interpreted this rare missense variant as a likely pathogenic variant and then she stopped taking oral medication. This case highlights the usefulness of gene-panel testing for accurate diagnosis and appropriate management of MODY. We also note the importance of familial genetic testing and genetic counseling for the proper interpretation of MODY variants.

Key words: Maturity-onset diabetes of the young, Glucokinase, Genetic testin.

Introduction

Maturity-onset diabetes of the young (MODY) is a rare disease that accounts for only 1% to 6% of all pediatric diabetes cases; however, it is one of the most common forms of monogenic hereditary diabetes mellitus caused by autosomal dominant pathogenic variants [1-5]. Accurate and timely diagnoses can be difficult as MODY may be misdiagnosed due to overlap of its clinical manifestations with type 2 and type 1 diabetes mellitus [6]. To date, 14 candidate genes have been identified to be associated with MODY: *HNF4A, GCK, HNF1A, PDX1, HNF1B, NEUROD1, KLF11, CEL, PAX4, INS, BLK, ABCC8, KCNJ11*, and *APPL1*. Clinical characteristics, frequency of microvascular complications, and preferred treatments vary according to the subtype of MODY based on the gene involved. To accurately understand the clinical situation, it is necessary to determine the genetic cause of the disease.

To determine which gene is involved in any particular case of MODY, molecular genetic testing is needed. Direct Sanger sequencing has been traditionally used for molecular genetic testing. However, this technique is labor-intensive and only a few genes can be tested at a time. Therefore, it is limited in its application for genetically heterogeneous diseases, including MODY. As gene-panel testing using next-generation sequencing (NGS)

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allows for the simultaneous analysis of several candidate genes, it is currently the preferred approach for the diagnosis of MODY in which several subtypes are known. According to the literature, the molecular diagnosis rate of monogenic diabetes through a gene-panel test is approximately 20% in clinically suspected patients [7,8].

Here, we report a patient with MODY type 2 associated with a rare "likely pathogenic" glucokinase (GCK) variant (NM_000162.5:c.1289T>C) that was identified by gene-panel testing using targeted NGS. This study includes our experience regarding the challenge of the missense variant interpretation process according to the American College of Medical Genetics and Genomics/Association for Molecular Pathology (ACMG/AMP) 2015 guidelines [9]. To our knowledge, only two cases with this variant have been reported.

Case

1. Clinical manifestation

An eight-year-old Korean female was transferred to our hospital from a local clinic with a complaint of fasting hyperglycemia lasting three months. She had no symptoms of diabetes. The patient was born small for her gestational age of 38 weeks, weighing 2.38 kg. When transferred to our hospital, her height was 131.6 cm (75th-90th percentile), weight was 31.1 kg (75th-90th percentile), and body mass index was 17.9 kg/m^2 (75th-85th percentile).

Laboratory test results were as follows: fasting blood glucose, 134 mg/dL; 2-hour postprandial glucose, 179 mg/dL; glycated hemoglobin (HbA1c), 7.0%; insulin, 4.8 μIU/mL (normal range: 2.5-25.0 μIU/mL), C-peptide 1.17 ng/mL (normal range: 1.1-4.4 ng/mL); and negative for insulin, glutamic acid decarboxylase, and islet cell antibodies. She was initially diagnosed with type 2 diabetes and treated with glimepiride. Meanwhile, family history revealed the father was diagnosed with diabetes in his 40s. The mother of the patient was healthy. Diabetes had also been diagnosed in her father's twin brother at a health checkup when he was in his 40s and in the patient's paternal grandmother.

The possibility of monogenic diabetes was explored based on the evidence of the patient being diagnosed with diabetes under 25 years of age, autosomal dominant inheritance over three generations, fasting insulin levels within the normal range (insulin ≥2.0 μIU/mL or plasma C-peptide ≥0.6 ng/mL), and not being associated with obesity [8]. Accordingly, the patient underwent gene-panel testing for suspected MODY. The gene-panel testing used NGS (Illumina, San Diego, CA, USA) and included 38 genes associated with different MODY subtypes or syndrome with diabetes phenotype: *HNF4A* (MODY1), *GCK* (MODY2), *HNF1A* (MODY3), *PDX1* (MODY4), *HNF1B* (MODY5), *NEUROD1* (MODY6), *KLF11* (MODY7), *CEL* (MODY8), *PAX4* (MODY9), *INS* (MODY10), *BLK* (MODY11), *ABCC8* (MODY12), *KCNJ11* (MODY13), *APPL1* (MODY14), *EIF2AK3, FOXP3, GATA4, GATA6, GLIS3, GLUD1, HADH, IER3IP1, INSR, MNX1, NEUROG3, NKX2-2, PAX6, PPARG, PTF1A, PTPRD, RFX6, SLC16A1, SLC19A2, SLC2A2, SYT9, UCP2, WFS1,* and *ZFP57*.

The gene-panel analysis revealed the heterozygous missense variant NM_000162.5(*GCK*):c.1289T>C in the coding region of the large hexokinase subdomain of *GCK*, which was verified by Sanger sequencing (Fig. 1). For further evaluation, the patient's father and mother underwent familial genetic testing, which revealed that the father had the same missense variant. The mother had no detected mutations. The patient's uncle and grandmother refused to undergo familial genetic testing. Through the following variant interpretation, we classified the identified variant as likely pathogenic and the cause of the MODY.

The patient was ultimately diagnosed with MODY type 2 based on the gene-panel testing and stopped taking oral medication. Her diabetes was controlled with exercise and diet, and her HbA1c level was maintained at 6.7% to 7.0%.

2. Variant interpretation

Information regarding the *GCK* missense variant NM_000162.5(*GCK*):c.1289T>C, p.L430P is shown in Table 1. According to computational prediction tools, including Polyphen-2,

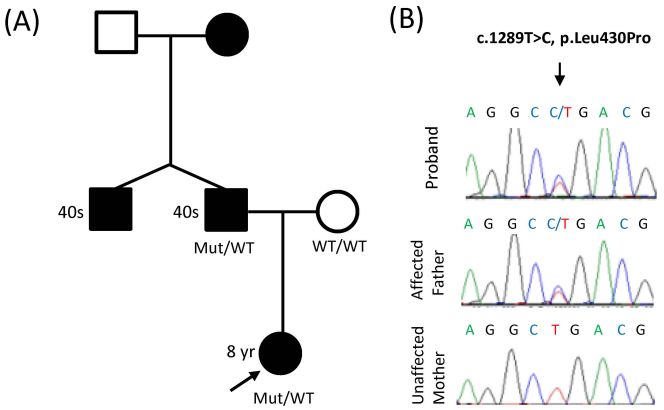

Fig. 1. Pedigree and Sanger sequencing. (A) Pedigree of the case family with the GCK p.Leu430Pro variant, which is likely pathogenic. (B) Variant segregation confirmed by Sanger sequencing. Square, male; circle, female; arrow, proband; open white symbol, no diabetes mellitus (DM) phenotype; solid black symbol, DM affected; WT, wild type; Mut, pathogenic variant type.

Table 1. The causative variant characteristics of *GCK*-MODY found in this case

Information	Variant	
Chromosome	7	
Position	44184844	
Reference	A	
Alteration	G	
Gene	GCK	
Reference sequence	NM_000162.5	
Nucleotide change	c.1289T>C	
Protein change	p.Leu430Pro	
Variant type	Missense variant	
Zygosity	Heterozygous	
GnomAD/KRGDB	Not found	
In silico database	PROVEAN	−6.21 (deleterious)
	PolyPhen-2 HVAR	0.999 (probably damaging)
	SIFT	0 (damaging)
	REVEL score	0.986 (pathogenic)
Final classification	Likely pathogenic variant (evidence: PM2, PP3, PS4_supporting [ref 1], PP2, PP4)[a]	

KRGDB, Korean Reference Genome Database.
[a]See the context of the variant interpretation part.

SIFT, PROVEAN, and REVEL, the variant was predicted to adversely affect protein stability. However, no functional study demonstrating its impact on proteins in vivo has been reported. Furthermore, this variant was not been found in the large population databases Genome Aggregation Database (gnomAD; https://gnomad.broadinstitute.org/) or Korean Reference Genome Database (KRGDB; http://coda.nih.go.kr/coda/KRGDB/index.jsp).

According to ACMG/AMP 2015 guidelines [9], the following evidence codes could be applied to the variant identified in the current case study: PM2 (absent from control population databases), PP3 (a deleterious effect proven by multiple lines of computational evidence), and PS4_supporting (increased odds ratios with case control study data, downgraded as supporting level of evidence if previously identified in one unrelated affected individual) (Table 1). Another possible evidence code that could be applied to the variant was PP2, which is used for missense variants in genes with a low rate of benign variation. According to the 2020 Association for Clinical Genetics Science (ACGS) practice guidelines [10], PP2 evidence can be considered significant when the missense Z-score of a gene according to gnomAD constraint scores is more than 3.09. The Z-score from gnomAD for the *GCK* gene in the current study was 3.07, which nearly met the recommended cut-off value. Additionally, among 229 pathogenic/likely pathogenic variants (PV/LPV) of *GCK* genes in the ClinVar database, which included 34 frameshifts, 5 in-frame deletions or insertions, 147 missense mutations, 24 nonsense mutations, and 19 splice-site mutations, missense-type mutations were the main causes of PV/LPV. In exon 10 of the *GCK* gene, where the variant was found, there were 34 PV/LPV but only 2 benign/likely benign variants; therefore, we included the PP2 evidence code.

The evidence code PP1 (proven co-segregation, four or more segregations in an autosomal dominant gene) could be applied based on the familial genetic test results; however, the evidence code was not applied to the current variant due to the lack of four or more segregations. Had co-segregation been confirmed through the genetic testing of the other affected family members (uncle and grandmother), supporting evidence could have been added. However, this additional evidence could not be gathered as the extended family members refused testing.

An additional consideration was the evidence code PP4. This code is the evidence criterion applied when a "patient's phenotype or family history is highly specific for a disease with a single genetic etiology". In our case, the patient's phenotype was young-onset diabetes with mild elevation of fasting blood glucose levels and no positive beta-cell autoantibodies. Additionally, we calculated the chance of testing positive using MODY Probability Calculator (https://www.diabetesgenes.org/exeter-diabetes-app/ModyCalculator) resulting in 75.5%. Accordingly, we applied the PP4 code. Finally, the variant identified in our case was classified as "likely pathogenic" according to the 2015 ACMG [9] and ACGS best practice guidelines [10].

Discussion

Gene-panel testing data are routinely generated in clinical practice and various rare variants have been identified using gene-panel testing [11,12]. In the past, clinical laboratories had interpreted the variants using their own criteria. Their interpretation was often subjective and therefore resulted in inconsistent classifications among different clinical laboratories [13]. Since the 2015 ACMG guidelines were published, most clinical laboratories have interpreted the germline variants according to the guidelines. The guidelines introduced a scoring system to classify a variant into five tiers using specific rules and evidence, and help to interpret variants in a consistent manner among clinical laboratories. Since the guidelines, several recommendations have been published to specifically apply each piece of evidence, which can reduce the rate of inconsistent classification [10,14-16]. Despite the systemic interpretation approach, deter-

mining the pathogenicity of rare variants remains challenging, especially for rare missense variant types. Evidence of variant pathogenicity strength depends on the type of the variant and effects of its genetic mechanism. For the interpretation of novel or rare missense variants, the strength of available evidence is usually weaker than that of evidence applied to insertion-deletion (INDEL)-type variants [10,14]. Therefore, assessing the pathogenicity of novel missense variants compared to INDEL variants is a challenge and many missense variants remain classified as a "variant of uncertain significance.

The *GCK* missense variant identified in our current case was ultimately classified as "likely pathogenic" based on the 2015 ACMG and ACGS best practice guidelines, but the interpretation process was challenged in meeting the "likely pathogenic" criteria. In this case, segregation evidence (PP1), which provides evidence of co-segregation with the disease, could not be applied. For this evidence, at least three affected individuals who share the same variant of the dominant inheritance gene are needed [16]. Only genetic data of the patient and her parents were obtained, which revealed the variant affected the patient and her father, but the variant was absent in her unaffected mother. The missense variant of the case study would have more easily met the "likely pathogenic" criteria, if there had been segregation evidence from a familial genetic study of affected and unaffected extended members been available. Considering this point, obtaining co-segregation data of family members through pedigree analysis and in-depth genetic counseling may be essential for confirming pathogenic classification. This is especially the case for understanding missense variants that are strongly suspected to be pathogenic, but do not meet the pathogenic criteria.

We report here a LPV *GCK* missense variant that was identified through the variant interpretation process described above. To our knowledge, only two previous cases with this *GCK* variant have been reported. The first case was an individual with suspected MODY2 harboring the *GCK* variant [1]. In this case, the variant was considered a "variant of uncertain significance" and no detailed information was presented. The second case was found in the ClinVar database (variation ID: 36194). This variant was reported to be likely pathogenic, but no evidence was presented regarding the detailed variant interpretation. Our current case is the third reported case. There has been no other literature or reputable source assessing the clinical significance of this variant.

GCK plays a critical regulatory role in glucose metabolism and the *GCK* gene, according to race, is the most or second-most frequent gene associated with MODY [17-19]. While hyperglycemia associated with *GCK*-MODY is present at birth and persists throughout life, its clinical course may be mild and nonprogressive, and micro- and macro-vascular complications rarely develop [17,20]. Furthermore, the *GCK*-MODY subtype does not usually require medication. Due to its mild clinical symptoms and the limited availability and high-cost of diagnostic genetic testing, *GCK*-MODY is often undiagnosed.

In our current case, the patient exhibited mild fasting hyperglycemia (134 mg/dL), relatively steady 2-hour glucose levels (179 mg/dL), and 7% HbA1c, which was consistent with the clinical features of *GCK*-MODY. Fortunately, the hyperglycemia was detected relatively quickly through routine blood tests and the easy access to gene-panel testing allowed for diagnosis at an early age. Simultaneously using several MODY-related gene-panel testing and NGS techniques, not only led to a diagnosis of MODY, but also provided for the disease subtype to be determined. Through subtype classification, a clinician is able to make a treatment decision based on subtype-specific treatment guidelines. In the current case, the patient was initially prescribed oral medication. However, after a specific subtype diagnosis of MODY type 2 was made through identification of the "likely pathogenic" *GCK* variant, her clinician decided to stop the oral medication and to continue following-up through the outpatient clinic.

In conclusion, we have reported a patient with MODY type 2 having a rare and likely pathogenic *GCK* missense variant. Additional cases are needed to assist with variant interpretation of rare and novel variants through genomic data sharing. Genetic counseling and family genetic testing may also be critical.

Authors' Contributions

Conception and design: JH and HSK. Acquisition of data: MKS. Analysis and interpretation of data: MKS. Drafting the article: MKS. Critical revision of the article: HSK and JH. Final approval of the version to be published: all authors.

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