

# Identification of candidate gene variants of monogenic diabetes using targeted panel sequencing in early onset diabetes patients

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**To cite:** Lee D-H, Kwak S-H, Park HS, *et al.* Identification of candidate gene variants of monogenic diabetes using targeted panel sequencing in early onset diabetes patients. *BMJ Open Diab Res Care* 2021;**9**:e002217. doi:10.1136/bmjdr-2021-002217

► Supplemental material is published online only. To view, please visit the journal online (<http://dx.doi.org/10.1136/bmjdr-2021-002217>).

Received 14 February 2021  
Accepted 1 June 2021



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## ABSTRACT

**Introduction** Monogenic diabetes is attributed to genetic variations in a single gene. Maturity-onset diabetes of the young (MODY) is the most common phenotype associated with monogenic diabetes, but is frequently misdiagnosed as either type 1 or type 2 diabetes. Increasing our basic understanding of genetic variations in MODY may help to improve the accuracy of providing the correct diagnosis and personalize subsequent treatment regimens in different racial populations. For this reason, this study was designed to identify nucleotide variants in early onset diabetes patients with clinically suspected MODY in a Korean population.

**Research design and methods** Among 2908 Korean patients diagnosed with diabetes, we selected 40 patients who were diagnosed before 30 years old and were clinically suspected of MODY. Genetic testing was performed using a targeted gene sequencing panel that included 30 known monogenic diabetes genes. The pathogenicity of the identified variants was assessed according to the American College of Medical Genetics and Genomics and Association for Molecular Pathology (ACMG-AMP) guidelines.

**Results** A total of six rare missense variants (p.Ala544Thr in HNF1A, p.Val601Ile and p.His103Tyr in ABCC8, p.Pro33Ala in PDX1, p.Gly18Glu in INS, and p.Arg164Gln in PAX4) in five distinct MODY genes were identified in five patients. In addition, a variant was identified in mitochondrial DNA at 3243A>G in one patient. The identified variants were either absent or detected at a rare frequency in the 1000 Genomes Project. These variants were classified as uncertain significance using the ACMG-AMP guidelines.

**Conclusion** Using a targeted gene sequencing panel, we identified seven variants in either MODY genes or mitochondrial DNA using a Korean patient population with early onset diabetes who were clinically suspected of MODY. This genetic approach provides the ability to compare distinct populations of racial and ethnic groups to determine whether specific gene is involved in their diagnosis of MODY.

## INTRODUCTION

Diabetes is a heterogeneous disease of metabolic disorders that share hyperglycemia as

## Significance of this study

### What is already known about this subject?

- Maturity-onset diabetes of the young (MODY) is caused by mutations in specific genes and is characterized by early onset.
- In patients with early onset diabetes, it is important to distinguish MODY from type 1 or type 2 diabetes.
- Genetic variants causing MODY have been identified using various methodologies.

### What are the new findings?

- Genetic testing for 30 known monogenic diabetes genes was performed using a targeted gene sequencing panel.
- A total of six rare missense variants in five distinct MODY genes and an additional mitochondrial variant were identified, which were either absent or detected at a rare frequency in the 1000 Genomes Project.

### How might these results change the focus of research or clinical practice?

- These results provide new information about genetic variants in a Korean population that could be used to help accurately predict the diagnosis of MODY.

a common clinical characteristic. Diabetes can be classified in the following three categories: (1) type 1 diabetes (T1D), (2) type 2 diabetes (T2D), (3) gestational diabetes mellitus. Although most diagnosed cases of diabetes are linked to either T1D or T2D, a considerable proportion of patients do not fit into these classifications and can fall into other causes of diabetes, which includes different forms of monogenic diabetes, including neonatal diabetes and maturity-onset diabetes of the young (MODY), diseases of the exocrine pancreas, and drug-induced or chemical-induced diabetes.<sup>1</sup>

Monogenic diabetes, which is caused by variants in a single gene, accounts for ~2% of all known cases of diabetes.<sup>2</sup> The most

common phenotype of monogenic diabetes is MODY,<sup>3</sup> which is considered as a heterogeneous group of disorders caused by genetic variants that play a fundamentally crucial role in  $\beta$ -cell development, function and regulation, glucose sensing, and the proper function of the insulin gene. The prevalence of MODY is relatively rare, with only 1%–5% of all cases of diabetes and 1%–6% of diabetes in pediatric cases.<sup>4</sup> In general, MODY is characterized by early onset (typically before 25 years of age), autosomal dominant mode of inheritance, and no dependence on insulin.<sup>5</sup>

Patients with MODY can be misdiagnosed as either T1D or T2D.<sup>6,7</sup> MODY can be relatively easy to distinguish from T1D because of its distinct pathogenesis, such as maintenance of  $\beta$ -cell function. On the other hand, the difference between MODY and T2D is more challenging due to their similar characteristics in terms of sustained insulin secretion and the existence of strong family history. However, one prominent difference between these two forms of diabetes is that MODY patients generally are not obese unlike patients with T2D. However, the degree of obesity in a specific population may confound the proper diagnosis of MODY versus T2D depending on their race or ethnicity.<sup>8</sup> As an example, a previous study reported that approximately 10%–15% of Japanese children with T2D are non-obese.<sup>9</sup> Therefore, alternate diagnostic methods were needed to complement the existing methods to properly distinguish the types of diabetes, particularly MODY, in a particular patient.

A newer method was to develop genetic testing to distinguish the various forms of diabetes in various patient populations. To date, the prevalence of specific variants in genes that cause MODY differs according to race or ethnic groups. Variants in the *GCK*, *HNFI1A*, *HNFI4A*, and *HNFI1B* genes are the most common causes of MODY, and they account for 32%, 52%, 10%, and 6% of cases in the UK, respectively.<sup>10</sup> In other parts of Europe, specifically France (56%) and Italy (41%), *GCK* variants were more prevalent as a suspected cause of MODY.<sup>11,12</sup> In Asians, there was a considerable difference in the frequencies of variants that normally cause MODY in Caucasians. In Korea, only 10% of 40 MODY or early onset T2D patients possessed known MODY gene defects, such as *HNFI1A* (5%), *GCK* (2.5%), and *HNFI1B* (2.5%) among MODY 1–6 genes.<sup>4,13</sup> Similar results were shown in Japan and China, only 10%–20% of MODY cases were caused by known MODY gene defect.<sup>14,15</sup> This would suggest that Asians may have other as yet to be identified genetic variations that are involved in the pathogenesis of MODY.

In the present study, we designed a clinical approach to genotype patients that have been previously diagnosed as T2D to determine whether any genetic variants could be identified that would help to reclassify under the category of monogenic diabetes (or MODY). To perform this analysis, we recruited a small population of Korean patients to isolate cells for genetic testing for a target gene panel for next-generation sequencing to identify either existing or novel variants that may be associated with MODY.

## MATERIALS AND METHODS

### Ethics statement

This research was approved by the relevant Ethics Committees (The Institutional Review Board at Chungbuk National University Hospital, approval No. 2019-12-010-001). This study was performed following the Declaration of Helsinki.<sup>16</sup> Informed consent was obtained from all study subjects before blood sampling.

### Study participants

The study participants were recruited from a hospital-based cohort with diabetes at Chungbuk National University Hospital from March 2011. Diabetes was diagnosed clinically using the American Diabetes Association criteria.<sup>1</sup> On the basis of onset age, especially less than 30 years old, 183 patients were enrolled in a total of 2908 patients with diabetes. Among them, patients with clinically suspected T1D ( $n=101$ ) who treated with insulin alone and with the presence of autoantibodies to glutamic acid decarboxylase or a fasting C-peptide level  $<0.6$  ng/mL were excluded. After calculation of MODY probability by the standard MODY probability calculator,<sup>17</sup> 42 patients who had less than a 50% probability of MODY were excluded. Finally, a total of 40 patients with suspected monogenic diabetes who had more than 50% of MODY probability were included in the final analysis. None of the patients had a familial relationship. A flowchart of the patient enrollment process of the study is presented in online supplemental figure 1. Clinical information, including demographics, family history, and treatment history of diabetes, physical examination, and laboratory test results for the 40 participants were obtained at the time of enrollment (table 1).

### Protocol for targeted panel sequencing

In this study, DNA was extracted from peripheral blood leukocytes and was used in a targeted panel sequencing by MacroGen (Seoul, Republic of Korea). The custom-designed capture probes were previously published by Park *et al*<sup>18</sup> to include the exonic and untranslated regions of 30 genes (target region of approximately 93 kb) that were known to cause either MODY, lipodystrophy, or neonatal diabetes (online supplemental table 1).

### Variant selection and assessment

The sequenced reads were aligned to the human reference genome (GRCh37) using the Burrows-Wheeler Aligner (V.0.7.15).<sup>19</sup> Using PICARD software (V.2.9.0) (<http://broadinstitute.github.io/picard/>) and the Genome Analysis Toolkit (V.3.8),<sup>20</sup> PCR duplicates were removed and base quality recalibration and indel realignment were conducted. Using ANNOVAR<sup>21</sup> and InterVar,<sup>22</sup> annotation was conducted for all identified variants. Further annotation was performed using the Human Gene Mutation Database (HGMD) professional version release 2018.1.<sup>23</sup>

Variants selection was performed through a sequential process. At first, non-silent variants, such as

**Table 1** Clinical characteristics of study participants

Variables	Total (N=40)
Male, N (%)	21 (52.5)
Current age, years	25.6±5.5
Age at diagnosis, years	20.8±5.0
Family history of diabetes, N (%)	32 (80.0)
Height, cm	167.3±12.1
Weight, kg	77.8±22.1
BMI, kg/m <sup>2</sup>	27.3±5.2
SBP, mmHg	127.9±17.5
HbA1c, %	9.4±3.0
C-peptide, ng/mL	2.0±1.1
Insulin, mIU/mL	10.8±10.5
Oral antidiabetics use, N (%)	35 (87.5)
Insulin use, N(%)	8 (20.0)
MODY probability, %	59.9±19.1
Number of MODY criteria fulfilled	
1	2 (5.0)
2	4 (10.0)
3	18 (45.0)
4	13 (32.5)
5	3 (7.5)

Data are shown as mean ± SD or N (%).

BMI, body mass index; MODY, maturity-onset diabetes of the young; SBP, systolic blood pressure.

non-synonymous, stop gain, stop loss, start loss, frameshift, and splice site variants, were selected. Rare variants in general population with a frequency <0.5% reported were selected. We used several population databases (Genome Aggregation Database,<sup>24</sup> 1000 Genomes Project,<sup>25</sup> Exome Aggregation Consortium Project,<sup>24</sup> and National Heart, Lung, and Blood Institute Exome Sequencing Project<sup>26</sup>) to evaluate the frequency in general population. In addition, we selected variants previously reported as being likely pathogenic or pathogenic in ClinVar or with a high confidence that the variation would cause disease in HGMD. In addition, we selected variants to have a possibility to be clinically meaningful considering the known inheritance pattern in the final analysis. The representative example of the process of variant selection is presented in online supplemental figure 2. After selecting non-silent variants, we conducted a detailed evaluation to assess the pathogenicity according to the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG-AMP) guidelines.<sup>27</sup> The ACMG-AMP guideline is consisted of two sets of criteria: (1) pathogenic or likely pathogenic variants, and (2) benign or likely benign variants. Evidence for pathogenic or likely pathogenic variants is provided as follows: one very strong (PVS1), four strong (PS1 to PS4), six moderate (PM1 to PM6), and five supporting (PP1 to PP5) attributes served as evidence of pathogenicity

(online supplemental table 2). InterVar software was used to automatically determine 8 of 16 pathogenic criteria. In this study, two investigators independently analyzed the pathogenicity of each identified rare, non-silent variant according to the ACMG-AMP guidelines.

## RESULTS

### Characteristics of the study participants

A total of 40 patients with early onset diabetes were enrolled in this study, and their clinical data at the time of study enrollment are displayed in table 1. The average age of all patients at the time of their diagnosis was 20.8±5.0 years. A high percentage (80%; n=32) of the patients had a family history of diabetes, in which 10 of these 32 patients (31.3%) had a family history of diabetes for at least three generations. The mean body mass index (BMI) of participants was 27.3±5.2 kg/m<sup>2</sup>. The average HbA1c and C-peptide levels at the time of diagnosis was 9.4%±3.0% and 2.0±1.1 ng/mL, respectively. A total of 35 patients (87.5%) were using oral antidiabetic medications, but only 8 (20.0%) were using insulin. The average MODY probability was 59.9%±19.1%, but nearly half (47.5%; n=19) of the patients had a higher calculated MODY probability of >75%. A total of 34 patients (85.0%) fulfilled more than three clinical diagnostic criteria of MODY, and 3 subjects (7.5%) satisfied all five diagnostic criteria.

### Variant classification and prevalence of monogenic diabetes

The average depth of coverage for each gene and percentages of the targeted region were more than 30X and 100X, respectively, as shown in online supplemental table 3. The average depth of coverage for the entire target region was 730X. More than 98% of bases covered more than 30X in most of the genes, except for *GATA4*, *CEL*, *PTF1A*, *KCNJ11*, and *GATA6*. No copy number variants (CNVs) were detected in the 30 selected genes in our cohort. A total of 16 rare, non-silent variants were identified in nine distinct genes and were evaluated for pathogenicity according to the ACMG-AMP guideline. After the initial review by two study investigators who remained blinded to each other's analysis, the two investigators reached a consensus decision on the final results through a group discussion. A total of six uncertain significances and one pathogenic variant were identified (table 2). These variants were located closely in variants previously reported (figure 1). In addition to the 80 nuclear DNA variants, a pathogenic variant in mitochondrial DNA, 3243A>G, was identified in one patient.

### Identified rare non-silent genetic variants of MODY

Among the four most common MODY genes (*GCK*, *HNFA1A*, *HNF4A*, and *HNF1B*), only one of these variants was identified in the present study. Other rare monogenic diabetes genes, including *ABCC8*, *PDX1*, *INS-IGF2*, *PAX4*, and mitochondrial *MT-TL1*, were identified. The evidence for pathogenicity classification according to the ACMG-AMP guidelines is provided in table 3. The



**Table 2** List of identified rare non-silent genetic variants of monogenic diabetes

ID	Chr	Gene name	Position	cDNA change	Amino acid change	dbSNP ID	ASN AF (1000 genomes)	East Asian AF (gnomAD)	Effect	Pathogenicity
P07	11	ABCC8	17 452 377	c.1801G>A	p.Val601Ile	-	-	-	Missense	Uncertain significance
P10	11	ABCC8	17 491 753	c.307C>T	p.His103Tyr	rs751209734	-	0.0004	Missense	Uncertain significance
P10	13	PDX1	28 494 372	c.97C>G	p.Pro33Ala	-	-	0.003	Missense	Uncertain significance
P14	11	INS-IGF2	2 182 149	c.53G>A	p.Gly18Glu	-	-	-	Missense	Uncertain significance
P17	7	PAX4	127 253 857	c.491G>A	p.Arg164Gln	-	-	0.000	Missense	Uncertain significance
P28	12	HNF1A	121 437 271	c.1630G>A	p.Ala544Thr	rs193922585	0.001	0.0009	Missense	Uncertain significance
P35	M	MT-TL1	3243	m.3243A>G		rs199474657	-	-	tRNA variant	Pathogenic

ASN AF, alternative allele frequency in Asian subjects; Chr, chromosome; dbSNP ID, NCBI Short Genetic Variations database ID.

p.Ala544Thr variant of HNF1A was classified to have conflicting interpretations of pathogenicity in ClinVar. The p.Val601Ile variant of ABCC8 was classified to have uncertain significance, but it was absent from the 1000 Genomes Project. This variant was previously reported as a cause of congenital hyperinsulinism (CHI).<sup>28</sup> Another previously reported variant of ABCC8 gene, p.His103Tyr, which was initially categorized as likely pathogenic,<sup>29</sup> was currently classified as uncertain significance. In the present study, p.Pro33Ala of PDX1 was identified with no report in the 1000 Genomes Project. The p.Gly18Glu of INS-IGF2 and the p.Arg164Gln of PAX4 were identified with uncertain significance, and no report in the 1000 Genomes Project. The mitochondrial variant m.3243A>G, which is well known to be a causative mutation of Maternally Inherited Deafness and Diabetes (MIDD), was confirmed in five participants. Interestingly, one patient with the mitochondrial DNA 3243A>G mutation had a positive maternal history of diabetes, but hearing loss was not evident in both the patient and their mother.

### Clinical characteristics of patients with variants of monogenic diabetes

The variants of monogenic diabetes were identified in a total of six patients, of which two were female and four were male. The clinical characteristics of these patients are presented in table 4. The age of the patients at the time of their diabetic diagnosis tended to be on the younger side compared with the whole population (range 18–21 years). Four out of the six patients (67%) had a family history of diabetes, of which three out of the four patients had prolonged family history through at least three generations. The HbA1c level at diagnosis was diverse among patients (range 6.4%–14.7%). However, there were no patients who were treated with insulin, and  $\beta$ -cell function assessed by C-peptide was preserved in all patients. Eighty-three per cent of the patients (five out of six) were calculated to exhibit a MODY probability score >75%. All six patients fulfilled more than three clinical diagnostic criteria of MODY, with two of the subjects satisfied all five diagnostic criteria. There were no patients who showed other clinical phenotypes, including extra-pancreatic features related to the variants.

### DISCUSSION

In this study, we have investigated the genetic variants of monogenic diabetes in 40 South Korean patients with early onset diabetes using targeted panel sequencing. Among these patients, six patients had one mitochondrial and six non-silent variants in five distinct candidate genes that may be involved in monogenic diabetes, specifically MODY. All of the non-silent variants were classified to have uncertain significance using the criteria in the ACMG-AMP guidelines, but from our limited population pool, they may participate in driving the diabetic phenotype associated in MODY.

**A. ABCC8**

**B. PDX1**

**C. INS-IGF2**

**D. PAX4**

**E. HNF1A**


**Figure 1** The spectrum of genetic variants in five MODY genes identified. Each note above represents a variant in this study. Each colored asterisk below represents a genetic mutation reported in ClinVar (red—putative loss of function, orange—missense, black—other). (A) *ABCC8* gene. (B) *PDX1* gene. (C) *INS-IGF2* gene. (D) *PAX4* gene. (E) *HNF1A* gene. Adapted from gnomAD (<https://gnomad.broadinstitute.org/>).

Although MODY accounts for a small percentage (1%–2%) of all cases of diabetes, the accurate diagnosis of MODY is very important for patients and their families to ensure that the proper treatment regimen is initiated to treat their malady. Once we accumulate sufficient genetic data in patients with diabetes, genetic testing and counseling will likely have a major impact in a positive way to

provide more accurate and earlier diagnosis of diabetes, which will inevitably improve the long-term outcome of the affected patient through optimized treatment protocols. In fact, there is emerging evidence that genetic testing has shown success in distinguishing monogenic diabetes subtypes, MODY, and neonatal diabetes.<sup>30</sup> In particular, MODY can be diagnosed by direct sequencing

**Table 3** Evidence attributes of the identified variants of monogenic diabetes

Gene	Variant	PVS1	PS1-5	PM1	PM2	PM3	PM4	PM5	PM6	PP1	PP2	PP3	PP4	PP5	Final
<i>ABCC8</i>	c.1801G>A			+	+								+		US
<i>ABCC8</i>	c.307C>T				+								+		US
<i>PDX1</i>	c.97C>G				+			+					+		US
<i>INS-IGF2</i>	c.53G>A			+	+						+				US
<i>PAX4</i>	c.491G>A			+	+								+		US
<i>HNF1A</i>	c.1630G>A				+									+	US

US, uncertain significance.

**Table 4** Clinical characteristics of patients with variants of monogenic diabetes

ID	Current age	Age at Dx	FHx of DM	Three-generation FHx	Insulin Tx	BMI (kg/m <sup>2</sup> )	HbA1c (%)	Fasting C-peptide (ng/mL)	Fasting insulin (mIU/mL)	MODY criteria	Probability of MODY (%)
P07	20s	20s	Yes	No	0	22.2	10.9	1.14	4.7	4/5	75.5
P10	20s	10s	Yes	No	0	24.9	12.5	1.16	8.1	4/5	75.5
P14	20s	10s	No	No	0	25.2	14.7	1.64	4.7	3/5	75.5
P17	10s	10s	Yes	Yes	0	23.7	8.4	1.20	4.9	5/5	75.5
P28	20s	20s	Yes	No	0	26.8	6.4	2.22	9.1	3/5	62.4
P35	10s	10s	Yes	Yes	0	17.7	8.7	0.91	2.8	5/5	75.5

BMI, body mass index; DM, diabetes mellitus; Dx, diagnosis; FHx, family history; MODY, maturity-onset diabetes of the young; Tx, therapy.

with up to 100% sensitivity.<sup>10</sup> The major drawback with genetic testing is the relatively high cost due in part to its availability in select specialized laboratories. Therefore, it is important to use the patient history and clinical values to select patients with clinically suspected monogenic diabetes. In our study using a small pilot group of preselected Korean patients with suspected probability of MODY, we performed a genetic analysis using a targeted gene panel.

Overall, variations in HNF1A are considered to be the most common cause of MODY in Europe, North America and Asia,<sup>31</sup> with a current total of 414 different HNF1A variants out of 1247 families.<sup>32</sup> In Asians, the prevalence of HNF1A variations was initially detected in 5%–8% of patients diagnosed with MODY. More specifically, in Japanese and Chinese, the prevalence of HNF1A variants was 8% and 5%, respectively.<sup>33 34</sup> In Korea, one patient (6.3%) with an HNF1A R267L mutation was detected among 16 unrelated patients diagnosed with T2D before the age of 35 years and a family history of autosomal dominant inheritance of T2D for at least two generations.<sup>35</sup> In this current study, the prevalence in the detection of an HNF1A variant was lower (2.5%) compared with the other studies, but this was consistent with another recent study by our group using a similar selection criteria and sequencing protocol.<sup>18</sup> In our other study, we found 3 out of 109 patients having one of three distinct HNF1A variants (p.Tyr166Asn, p.Leu26Gln, p.Val567Ile), which are classified as likely pathogenic.<sup>18</sup> The present study detected a distinct HNF1A variant (p.Ala544Thr) that was not identified in our other study, and this particular variant is listed in the 1000 Genomes Project with an as yet to be determined biological role due to conflicting interpretations of its pathogenicity in ClinVar.

In our study, the patient with the HNF1A variant did not have a family history, and his BMI was >25 kg/m<sup>2</sup>. The patient exhibited good glycemic control using his treatment with metformin and DPP4 inhibitors, where his latest HbA1c level was 6.3%. Generally, patients with HNF1A mutations are sensitive to sulfonylurea. Considering the clinical characteristics of this patient and other previous reports, it still remains unclear whether this distinct HNF1A variant influenced the onset of MODY. No other variants in other common MODY genes, including *GCK*, *HNF4A*, *HNF1B*, were identified in this patient. Overall, the relatively lower prevalence of the HNF1A variants within Asian countries, particularly our recent findings in Korea, demonstrates the importance of genetic testing and analysis between distinct racial and ethnic groups, but also may be attributed to the criteria used to select patients.<sup>36</sup>

Novel variants in other genes, including *ABCC8*, *PDX1*, *INS*, and *PAX4*, believed to be involved in the pathogenesis of MODY, although on a rarer basis were identified in our study. The p.Val601Ile and p.His103Tyr in *ABCC* were discovered in two patients. In a previous study conducted in the UK, p.Val601Ile was identified in patients with CHI.<sup>28</sup> Furthermore, it was reported that dominant

inheritance of *ABCC8* mutations can cause CHI with predisposition to insulin deficiency and diabetes later in life.<sup>37</sup> The p.His103Tyr variant was reported as likely pathogenic in a Korean patient with diabetes.<sup>29</sup> The clinical manifestations of MODY caused by *ABCC8* gene variations are similar to those with *HNF4A/1A* mutations.<sup>4</sup> The two patients in this study did not show insulin deficiency or a history that would suggest CHI. In addition, they were treated with three classes of oral glycemic agents. Their clinical symptoms showed inconsistencies with known features associated with MODY due to the *ABCC8* mutation.

In the *PDX1* gene, a novel variant p.Pro33Ala was identified in this study, which was not listed in the 1000 Genomes Project. A previous study demonstrated that a missense variant changing the amino acid from proline to threonine (*PDX1*<sup>P33T/P33T</sup>) led to a deterioration of  $\beta$ -cell development and function.<sup>38</sup> The p.Gly18Glu in *INS* gene and the p.Arg164Gln in *PAX4* gene were also novel variants that were not previously reported. The patients with these variants did not have typical clinical features, such as ketosis, or requirement of insulin. Little is known about these variants due to their apparent rarity, so further research is needed to better understand whether these variants play a role in the pathogenesis of MODY.

In one patient with the A>G transition at position 3243 in the mitochondrial tRNA<sup>Leu</sup>-encoding (UUR) gene (m.3243A>G, MT-TL1), this variant is believed to be the most common mutation causing MIDD.<sup>39</sup> This syndrome usually affects metabolically active organs (such as endocrine pancreas and cochlea) and is accompanied by the severe childhood neurological phenotype mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS).<sup>40</sup> This one patient presented a strong maternal inheritance feature where the patient's maternal grandmother and mother were diagnosed with diabetes. However, the patient was not deaf, and there were no other features associated with MELAS. It is known that the clinical manifestation associated with MIDD and MELAS displays heterogeneous phenotypes,<sup>40</sup> so it is possible that our patient might have only a mild form of MIDD.

As with most association studies, there are limitations to the interpretation of our findings. First, there are several useful tools that have been developed to screen for MODY, including the use of clinical criteria and MODY probability calculators. In general, the criteria include clinical characteristics, such as age of onset for diabetes, BMI, family history, pancreatic function, and insulin use. However, no consensus clinical criteria have been used in all published studies. Moreover, the MODY probability calculator consists of eight clinical factors,<sup>17</sup> which has been developed using clinical information acquired mostly from Europeans and has not been validated in other races. In our results, our patient population with the identified variants fulfilled more than three of five clinical criteria and exhibited a high MODY probability

score. Therefore, these tools may not be limited in our study and could be properly applied to select the patients suspected of MODY before genetic testing. Second, the number of patients was relatively small. Because the inclusion criteria of the patients with age at diagnosis was limited under 30 years, there is a possibility of missing patients with monogenic diabetes who were diagnosed after 30 years old. However, the molecular genetic diagnosis rate in patients with an onset age >40 years was reported to be only 0.6%.<sup>41</sup> Third, the variants identified in this study were classified as uncertain significance except for one variant found in the mitochondria. These might be attributed to the lack of functional studies to identify variants and the absence of sequencing within family members. Lastly, many of the patients did not have variants for MODY, even though there were clinical data that suspected their genetic predisposition. It is unclear whether the unidentified variants for MODY, so-called MODYX, might exist or not in these patients. In addition, the present analysis did not include promoter variants so it may be possible that variants existed outside of the coding region.

In conclusion, our present study used targeted panel sequencing to identify seven variants, of which five were novel, in six distinct patients among a population of 40 patients with early onset diabetes and were clinically suspected of MODY. The results from this study continue to accumulate more data demonstrating the potential benefit of target panel sequencing to identify existing and new variants in genes associated with MODY for future development of genetic biomarkers for gene-related diabetes, especially in distinct racial and ethnic populations.

**Contributors** TKO and D-HL designed the study. D-HL researched the literature, interpreted the data, and wrote the manuscript and figures. EJK and HJJ contributed to data collection. HJJ and S-HK reviewed the manuscript and contributed to the discussion. All authors critically reviewed the manuscript and approved its submission. TKO is the guarantor of this work and, as such, had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

**Funding** This study was supported by a grant from the Korean Endocrine Society in 2020. The funding sources were not involved in oversight or design of the study, in the analysis or interpretation of the data, or in the decision to submit the manuscript for publication.

**Competing interests** None declared.

**Patient consent for publication** Not required.

**Provenance and peer review** Not commissioned; externally peer reviewed.

**Data availability statement** Data are available on reasonable request. All data relevant to the study are included in the article or uploaded as supplemental information. If there are requests for data sharing, contact to corresponding author by email (tgohkjs@chungbuk.ac.kr).

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