JDI Journal of Diabetes Investigation Open access
Official Journal of the Asian Association for the Study of Diabetes
ORIGINAL ARTICLE

# Targeted gene panel analysis of Japanese patients with maturity-onset diabetes of the young-like diabetes mellitus: Roles of inactivating variants in the *ABCC8* and insulin resistance genes

Tohru Yorifuji[1,2,3,4]*, Yoh Watanabe[1], Kana Kitayama[1], Yuki Yamada[1], Shinji Higuchi[1], Jun Mori[1], Masaru Kato[2], Toru Takahashi[2], Tokuko Okuda[3], Takane Aoyama[3]

[1]Division of Pediatric Endocrinology and Metabolism, Children's Medical Center, Osaka City General Hospital, Osaka, Japan, [2]Department of Genetic Medicine, Osaka City General Hospital, Osaka, Japan, [3]Clinical Research Center, Osaka City General Hospital, Osaka, Japan, and [4]2nd Department of Internal Medicine, Date Red Cross Hospital, Date, Hokkaido, Japan

**Keywords**
*ABCC8*, *INSR*, Maturity-onset diabetes of the young

***Correspondence**
Tohru Yorifuji
Tel.: +81-142-23-2211
Fax: +81-142-23-5249
E-mail address:
yorifuji.tohru.l94@kyoto-u.jp

*J Diabetes Investig* 2023; 14: 387–403

doi: 10.1111/jdi.13957

## ABSTRACT

**Aims/Introduction:** To investigate the genetic background of Japanese patients with suspected maturity-onset diabetes of the young (MODY).

**Materials and Methods:** On 340 proband patients referred from across Japan, genomic variants were analyzed using a targeted multigene panel analysis combined with the multiplex ligation probe amplification (MLPA) analysis, mitochondrial m.3243A > G analysis and methylation-specific polymerase chain reaction of the imprinted 6q24 locus. Pathogenic/likely pathogenic variants were listed according to the 2015 American College of Medical Genetics and Genomics and the Association for Molecular Pathology criteria. Additionally, variants with a population frequency <0.001 and Combined Annotation Dependent Depletion score >20 (CS >20) were listed as rare variants of uncertain significance-CS >20.

**Results:** A total of 157 pathogenic/likely pathogenic variants and 44 rare variants of uncertain significance-CS >20 were identified. In the pathogenic/likely pathogenic variants, alterations in the *GCK* gene were the most common (82, 52.2%) followed by *HNF1A* (29, 18.5%), *HNF4A* (13, 8.3%) and *HNF1B* (13, 8.3%). One patient was a 29.5% mosaic with a truncating *INSR* variant. In the rare variants of uncertain significance-CS >20, 20 (45.5%) were in the genes coding for the adenosine triphosphate-sensitive potassium channel, *KCNJ11* or *ABCC8*, and four were in the genes of the insulin-signaling pathway, *INSR* and *PIK3R1*. Four variants in *ABCC8* were previously reported in patients with congenital hyperinsulinism, suggesting the inactivating nature of these variants, and at least two of our patients had a history of congenital hyperinsulinism evolving into diabetes. In two patients with *INSR* or *PIK3R1* variants, insulin resistance was evident at diagnosis.

**Conclusions:** Causative genomic variants could be identified in at least 46.2% of clinically suspected MODY patients. *ABCC8*-MODY with inactivating variants could represent a distinct category of MODY. Genes of insulin resistance should be included in the sequencing panel for MODY.

Received 14 July 2022; revised 23 October 2022; accepted 24 November 2022

## INTRODUCTION

Monogenic diabetes mellitus accounts for 1–4% of pediatric or young adult diabetes cases[1], and typical clinical presentation includes maturity-onset diabetes of the young (MODY), neonatal diabetes, insulin resistance syndromes, lipodystrophy or other syndromic diabetes.

Diagnosing and differentiating monogenic diabetes mellitus from the vast majority of type 1/type 2 diabetes is important both for the clinical management and genetic counseling of the patients[2–4]. Particularly, the diagnosis of MODY caused by pathogenic variants in the glucokinase (*GCK*), hepatic nuclear factor 1A (*HNF1A*), hepatic nuclear factor 4A (*HNF4A*) genes or in the *KCNJ11*, *ABCC8* genes coding for the adenosine triphosphate (ATP)-sensitive potassium channel ($K_{ATP}$ channel) could be critical for the management of these patients, with sulfonylureas often being effective for *HNF1A*-, *HNF4A*- or $K_{ATP}$ channel-MODY, whereas no medical intervention is generally required for *GCK*-MODY[5–8]. The diagnosis of other types of monogenic diabetes also helps properly manage these patients with the accumulated knowledge of comorbidities and the natural course specific for each causative gene[9].

Unfortunately, monogenic diabetes is currently extremely underdiagnosed[1,10–13], especially when the diabetes is not associated with specific clinical features, such as neonatal diabetes, lipodystrophy or syndromic diabetes. Multiple factors make a diagnosis of monogenic diabetes difficult. For example, MODY is generally accepted as diabetes characterized by the early-onset before 35 years, non-obesity, dominant inheritance and negative pancreatic autoantibodies. However, not rarely, MODY is diagnosed after middle age, in obese patients, or without any affected family members[14–16]. The diagnosis is particularly difficult for East Asians with inherently diminished insulin secretory capacities. They develop type 2 diabetes at lower body mass index (BMI; >23 for Japanese), as compared with white Europeans[17,18], and often, multiple family members are affected. Therefore, differentiating MODY from early-onset type 2 diabetes has always been challenging for this population, resulting in lower rates of mutation identification in previous studies[19–21]. Additionally, molecular diagnosis is generally not covered by insurance, making the correct diagnosis even more difficult.

In the present study, we report the results of our comprehensive, multigene mutational analysis on 340 proband patients with early-onset, MODY-like diabetes who were referred to us under the diagnosis of suspected MODY. To address the possibility of different monogenic diabetes misdiagnosed as MODY, a broader range of monogenic diabetes genes were sequenced, including those typically presenting with insulin resistance or those associated with progressive endoplasmic reticulum stress.

## MATERIALS AND METHODS

### Participants

The study participants were 340 proband patients with early-onset (0–42 years, median 11 years) diabetes who were referred to us between 2005 and 2022 from across Japan under the diagnosis of suspected MODY. All were Japanese, and the age distribution at diagnosis is shown in Figure 1. The diagnoses were made by pediatric or adult diabetologists, and the patients fulfilled at least two of the following criteria of MODY; (i) dominant inheritance of early-onset diabetes; (ii) non-obesity with persistently detectable C-peptide; and (iii) negative pancreatic autoantibodies. Patients with the onset of diabetes before 6 months of age; that is, neonatal diabetes, were excluded. The study protocol was approved by the institutional review board of Osaka City General Hospital (No. 742). Written informed consent was obtained either from the patient or their legal guardians. Molecular and clinical features of a fraction of these patients were previously reported separately[22–24].

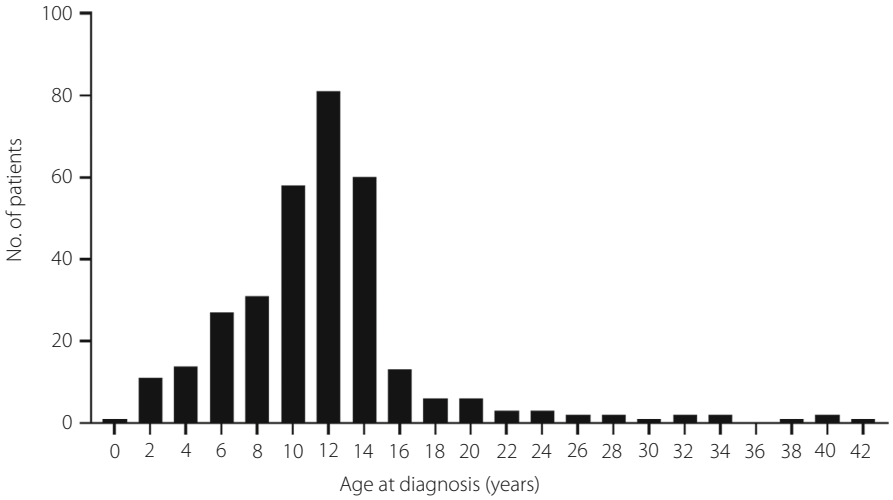


**Figure 1** | The distribution of age at diagnosis of the study participants.

## Methods

### Clinical data

For each patient, clinical data including sex, age at diagnosis, hemoglobin A1c at diagnosis, height and weight at diagnosis, and inheritance of diabetes were obtained from the questionnaire to the referral source. For children aged <18 years, the standard deviation score of BMI (BMI-SDS) was determined by using the Excel-based clinical tool for growth evaluation of children available from the Japanese Society for Pediatric Endocrinology (taikakushisu_v3.1, http://jspe.umin.jp/medical/chart_dl.html). For adults, the data from the 2018 National Health and Nutrition Survey by the Ministry of Health, Labor and Welfare were used for the calculation of BMI-SDS (https://www.e-stat.go.jp/dbview?sid=0003224178).

### Detection of genomic variants

All analyses were carried out using the DNA extracted from peripheral blood using the QIAmp DNA mini kit (QIAGEN, Hilden, Germany). For patients with maternal inheritance, mitochondrial m.3243A > G mutation was first excluded by the polymerase chain reaction restriction fragment length polymorphism analysis as described[25], then the remaining patients underwent the multiplex ligation-dependent probe amplification (MLPA) analysis covering the common MODY genes (SALSA MLPA P241 including the *GCK*, *HNF1A*, *HNF1B*, *HNF4A* genes, MRC Holland, Amsterdam) and targeted multigene panel sequencing using the Ion-PGM next-generation sequencer (Thermo Fisher, Waltham, MA, USA). To identify patients with monogenic diabetes other than MODYs, the target genes were set broadly to include all exons of the following genes; *ABCC8*, *APPL1*, *CISD2*, *EIF2AK3*, *FOXP3*, *GATA4*, *GCK*, *GLIS3*, *HNF1A*, *HNF1B*, *HNF4A*, *INS*, *INSR*, *KCNJ11*, *NEUROD1*, *NEUROG3*, *PAX6*, *PCBD1*, *PDX1*, *PIK3R1*, *RFX6*, *STAT3*, *WFS1* and *ZFP57*. The primer sets were generated using the Ion AmpliSeq Designer (Thermo Fisher), and sequencing was carried out using the Ion PGM next-generation sequencer (Thermo Fisher) as per the protocol of the manufacturer. The output data were analyzed using the Ion Reporter system (Thermo Fisher). Identified variants were also visualized using the Integrative Genomics Viewer tool[26] (https://software.broadinstitute.org/software/igv/, Broad Institute), and further confirmed by Sanger sequencing when necessary. Patients referred before 2021 mostly underwent the MLPA analysis and Sanger sequencing of exons in the *GCK*, *HNF1A*, *HNF4A* and *HNF1B* genes taking into consideration the clinical features. Only those without pathogenic (P)/likely pathogenic (LP) variants in these genes underwent the above-described targeted next-generation sequencing. Finally, patients without P/LP variants underwent the methylation-specific polymerase chain reaction of the chromosome 6q24 imprinted region, as described previously[23].

### Variant assessment

The pathogenicity of identified variants was assessed according to the 2015 American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG/AMP) criteria[27] using the InterVar website (https://wintervar.wglab.org/)[28] and the ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/), then the P/LP variants were listed as the causative variants. For genes of recessively inherited disorders; that is *CISD2*, *EIF2AK3*, *GLIS3*, *PAX6*, *PCBD1* and *ZFP57*, the P/LP variants were not listed unless they were identified in both alleles. For *RFX6*, variants were not listed unless the identified variant was a truncating variant[29], and for *WFS1*, monoallelic variants were not listed unless the variant was previously reported to cause dominantly inherited diabetes[30].

In addition, variants classified as variants of uncertain significance (VUS) were separately listed as rare VUS with a Combined Annotation Dependent Depletion (CADD) score >20 (VUS-CS >20) if they were with a population frequency <0.001 in all ethnic groups in the gnomAD database (https://gnomad.broadinstitute.org/) and the Japanese 14KJPN genomic variants database (https://jmorp.megabank.tohoku.ac.jp/202112/variants), and also if they had a CADD score >20 (https://cadd.gs.washington.edu/)[31]. These cut-offs were arbitrarily set, because the incidence of pathogenic variants in the most common, known MODY gene, *GCK*, is approximately one in 1000[32], and a cut-off of the CADD score at 10–20 was recommended by the developer[31].

Finally, the Human Gene Mutation Database professional version (https://digitalinsights.qiagen.com/products-overview/clinical-insights-portfolio/human-gene-mutation-database/; QIAGEN) was used to find previous reports of identified variants in association with the disease phenotype.

## RESULTS

Of the 340 proband patients, we could identify 157 P/LP causative variants (46.2%; Figure 2a). In addition, we identified 44 variants in 42 patients with a population frequency <0.001 and with the CADD score >20 as rare VUS-CS >20 (12.4%; Figure 2b). Tables 1 and 2 show the details of the identified variants, and the demographic data of those with the P/LP variants and rare VUS-CS >20, respectively. The demographic features of patients without these variants are also shown in Table 3.

In the P/LP causative variants, alterations in the *GCK* gene were the most common (82, 52.2%) followed by *HNF1A* (29, 18.5%), *HNF4A* (13, 8.3%) and *HNF1B* (13, 8.3%). The mitochondrial m.3243A > G variant was found in eight (5.1%), all with maternal inheritance, and variants in the rare MODY genes, *INS*, *KCNJ11* and *ABCC8*, were found in four (2.5%), one (0.6%) and three (1.9%), respectively. As previously reported, abnormalities in the 6q24 imprinted locus were found in three patients (1.9%), and none of these had a history of transient neonatal diabetes, which was confirmed by chart review (details previously reported[23]). Additionally, a variant in the *INSR* gene (c.282_283TG > GT, p.TyrGly94_95*Trp), normally associated with type A insulin resistance, was found in a single patient as a mosaic with the wild type.

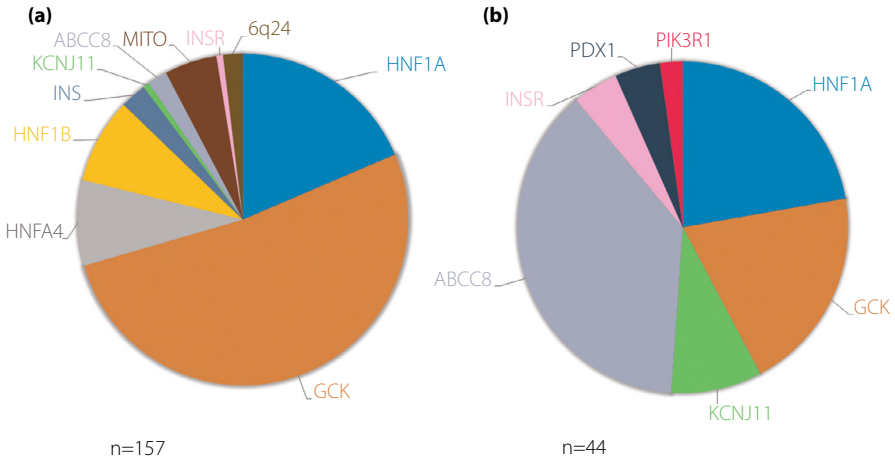


**Figure 2** | (a). Breakdown of genes with pathogenic/likely pathogenic (P/LP) variants. (b). Breakdown of genes with variants of uncertain significance of a population frequency <0.001 and the Combined Annotation Dependent Depletion score >20.

The 44 variants in the rare VUS-CS >20 category did not fulfill the ACMG/AMP criteria to reach the P/LP status. However, they are rare and possibly deleterious, with a population frequency of <0.001 and with the CADD score >20. The CADD is a tool for scoring the deleteriousness of single-nucleotide variants, which integrates multiple annotations into one metric, and a score of 20 represents the top 1% of the likelihood of pathogenicity[31]. In fact, 21 of these rare VUS-CS >20 were previously reported in association with the disease phenotype and included in the HGMD professional database. Of these, variants in the *ABCC8* gene were the most commonly found in 16 (36.4%), followed by *HNF1A* (10, 22.7%), *GCK* (8, 18.2%), *KCNJ11* (4, 9.1%) and *PDX1* (2, 4.5%) genes. Additionally, three variants in the *INSR* (p.Thr1181Asn, p.Pro269Leu, p.Lys294Arg) and one in the *PIK3RI* (p.Ser83Leu) genes, both normally associated with insulin resistance, were found in three patients (patients 191, 192, 193), one of them (patient 191) having variants in both genes.

Compared with the group of patients without these variants, patients with P/LP variants or rare VUS-CS >20 showed significantly lower BMI-SDS at diagnosis ($P < 0.0001$ by the Kruskal–Wallis test; Figure 3a), whereas there were no significant differences in the age at onset ($P = 0.16$, Figure 3b). These results suggest that those without P/LP variants or rare VUS-CS >20 are more similar to polygenic type 2 diabetes.

In total, there were four $K_{ATP}$ channel variants (3 *ABCC8*, 1 *KCNJ11*) in the P/LP group, and 21 (17 *ABCC8*, 4 *KCNJ11*) in the rare VUS-CS >20 group. Of these, 11 were listed in the HGMD database. Interestingly, one of the P/LP variants in the *ABCC8* gene was a frameshift, loss-of-function variant (patient 5), and thus, was expected to cause hyperinsulinism. This patient was not obese, with a BMI-SDS of 0.25 and developed diabetes at the age of 7 years. His fasting C-peptide was not diminished at 1.8 nmol/L, and the homeostatic model assessment for insulin resistance (HOMA-IR) was 7.1, suggesting the presence of insulin resistance. Additionally, of the *ABCC8* variants in the rare VUS-CS >20, four (p.Arg1420His, patient 161; p.Arg1486Lys, patient 162; p.Gly1378Ser, patient 163; p.Asp1030Asn, patient 170) were listed in the HGMD database in association with congenital hyperinsulinism. The unique clinical course of patient 161 was previously reported by Saito-Hakoda *et al.*[33] Briefly, the patient was born large-for-gestational-age (4,244 g after 36 weeks' gestation), and presented with hyperinsulinemic hypoglycemia requiring diazoxide treatment until the age of 6 years. Then, she gradually began to present with postprandial hyperglycemia and was diagnosed with diabetes at the age of 11 years. After the diagnosis of diabetes, she still experienced reactive postprandial hypoglycemia, which was successfully treated by a combination of glinide and alpha-glucosidase inhibitor. Patient 162 was also an 11-year-old girl who presented with fasting hypoglycemia associated with postprandial hyperglycemia. On the oral glucose tolerance test, her fasting plasma glucose was low at 3.9 mmol/L, with inappropriately elevated insulin at 29.2 pmol/L. After 2 h, however, her plasma glucose was elevated at 15.0 mmol/L. Patient 163 was a non-obese, 10-year-old girl. Her oral glucose tolerance test showed a sign of insulin resistance with the HOMA-IR index at 4.5. Her insulinogenic index was low at 0.08, and her plasma glucose after 2 h was 16.6 mmol/L. Patient 170 was born large-for-gestational-age, with a birthweight of 4,111 g. She reported a history of hospital admission for neonatal apneic episodes. She was not obese, but an oral glucose tolerance test at the age of 9 years showed a sign of insulin resistance with the HOMA-IR index at 4.5. Her insulinogenic index was low at 0.34 and her plasma glucose after 2 h was 16.2 mmol/L.

Also, interestingly, there was one *INSR* variant, p.TyrGly94_95*Trp, in the P/LP group (patient 148), which is expected to cause type A insulin resistance. This patient was a 29.5% mosaic with a truncating variant, and presented with symptoms resembling acute type 1 diabetes at the age of 14 years, with

http://wileyonlinelibrary.com/journal/jdi

**Table 1** | Summary of the clinical features and the identified pathogenic/likely pathogenic variants for each patient

| Patient No. | Sex | At diagnosis | | | | | Inheritance | Gene | cDNA | Protein | ACMG/AMP classification | HGMD (phenotype) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Age | HbA1c | Height | Weight | BMI-SDS | | | | | | |
| 1 | F | 14 | 7.2 | 157.8 | 50.7 | 0.06 | NO | 6q24 | pat dup | | P | – |
| 2 | F | 9 | 7.8 | 133.2 | 27.7 | −0.43 | NO | 6q24 | pUPD | | P | – |
| 3 | M | 12 | 9.2 | 149 | 45 | 0.65 | NO | 6q24 | pUPD | | P | – |
| 4 | F | 14 | 6.7 | NA | NA | NA | M | ABCC8 | c.1819G > A | p.Val607Met | LP | DM |
| 5 | M | 7 | 9.5 | 163 | 50.5 | 0.25 | P | ABCC8 | c.716delC | p.Thr239Metfs*19 | LP | – |
| 6 | F | 6 | 7.3 | 108.7 | 16.3 | −1.17 | P | ABCC8 | c.3544C > T | p.Arg1182Trp | LP | DM |
| 7 | M | 5 | 6 | 104 | 16 | −0.44 | M | GCK | c.175C > T | p.Pro59Ser | LP | DM |
| 8 | M | 2 | 6.5 | NA | NA | NA | P | GCK | c.1016A > G | p.Glu339Gly | LP | DM |
| 9 | M | 9 | 6.4 | 126.8 | 24.5 | −0.66 | P | GCK | c.1019G > C | p.Ser340Thr | LP | DM |
| 10 | M | 9 | 6.5 | 125 | 24.7 | −0.32 | NO | GCK | c.1019G > C | p.Ser340Thr | LP | DM |
| 11 | F | 17 | 5.9 | NA | NA | NA | P | GCK | c.1055 T > G | p.Leu352Arg | LP | DM |
| 12 | M | 14 | 6.5 | 163 | 54 | 0.22 | P | GCK | c.1092C > A | p.Cys364* | P | DM |
| 13 | F | 6 | 7 | NA | NA | NA | P | GCK | c.1142 T > C | p.Met381Thr | LP | DM |
| 14 | M | 14 | 6.6 | 158.5 | 50.9 | 0.2 | P | GCK | c.1142 T > G | p.Met381Arg | LP | DM |
| 15 | F | 12 | 6.7 | 159 | 53 | 0.76 | P | GCK | c.1144_1144insTGCTCG | p.Cys382_Ser383dup | LP | DM |
| 16 | F | 7 | 6.6 | 117.5 | 19 | −1.25 | P | GCK | c.1144 T > C | p.Cys382Arg | LP | DM |
| 17 | M | 8 | 6.7 | 132 | 26.1 | −0.6 | P | GCK | c.1183_1209delGAGAG CCGCAGCGAGGACGTAATGCGC | p.Glu395_Arg403del | P | – |
| 18 | M | 8 | 7.5 | 118.6 | 19.2 | −1.67 | P | GCK | c.1183G > T | p.Glu395* | P | – |
| 19 | M | 4 | 6.1 | NA | NA | NA | M | GCK | c.118G > A | p.Glu40Lys | LP | DM |
| 20 | M | 5 | 7.1 | 108.7 | 16.8 | −0.93 | P | GCK | c.1249C > A | p.Pro417Thr | LP | – |
| 21 | M | 6 | 6.8 | 118.5 | 23.8 | 0.82 | M | GCK | c.1278_1286dupCGTGCGCAG | p.Ser426_Arg428dup | LP | DM |
| 22 | M | 5 | 6.6 | 107.2 | 19.3 | 0.93 | P | GCK | c.130G > A | p.Gly44Ser | LP | DM |
| 23 | M | 17 | 6.8 | 175 | 65 | 0.09 | BI | GCK | c.130G > A | p.Gly44Ser | LP | DM |
| 24 | F | 9 | 6.3 | NA | NA | NA | M | GCK | c.1324 delG | p.Glu442Argfs*613 | P | – |
| 25 | F | 3 | 6.4 | NA | NA | NA | P | GCK | c.1340_1368del29 | p.Arg447Leufs*2 | P | – |
| 26 | F | 10 | 6.4 | 144.6 | 40.4 | 0.8 | NO | GCK | c.1340G > A | p.Arg447Gln | LP | DM |
| 27 | F | 8 | 6.3 | NA | NA | NA | M | GCK | c.146C > T | p.Thr49Ile | LP | DM |
| 28 | F | 8 | 6 | 114.8 | 19.2 | −0.8 | M | GCK | c.182A > G | p.Tyr61Cys | LP | DM |
| 29 | M | 12 | 6.3 | 146.1 | 40.1 | −0.77 | M | GCK | c.234C > G | p.Asp78Glu | LP | DM |
| 30 | F | 1 | 6.4 | NA | NA | NA | M | GCK | c.214G > A | p.Gly72Arg | LP | DM |
| 31 | F | 0 | 6 | 39.5 | 1.542 | −5.43 | M | GCK | c.214G > A | p.Gly72Arg | LP | DM |
| 32 | F | 13 | 6.2 | 156.9 | 41.8 | −1.12 | NO | GCK | c.234C > G | p.Asp78Glu | LP | DM |
| 33 | F | 8 | 7 | 131.6 | 26 | −0.58 | M | GCK | c.264G > A | p.Ala188Thr | LP | DM |
| 34 | M | 5 | 6.6 | 100.9 | 14.5 | −0.91 | M | GCK | c.364C > T, | p.Leu122Phe | LP | DM |
| 35 | M | 12 | 6.4 | 146.1 | 36 | −0.67 | M | GCK | c.469G > T | p.Glu157* | P | DM |
| 36 | F | 5 | 6.1 | 98.1 | 11.9 | −2.58 | M | GCK | c.500G > A | p.Trp167* | P | DM |
| 37 | M | 1 | 5.7 | 69 | 7.2 | −1.34 | P | GCK | c.526G > C | p.Ala176Pro | LP | – |
| 38 | M | 9 | 6.8 | 134 | 27 | −0.8 | P | GCK | c.533G > C | p.Gly178Ala | LP | DM |
| 39 | M | 13 | 6.6 | 161.9 | 45.1 | −0.81 | NO | GCK | c.544G > A | p.Val182Met | LP | DM |
| 40 | F | 13 | 6.3 | 149.2 | 36.6 | −1.41 | NO | GCK | c.556C > T | p.Arg186* (CGA > TGA | P | DM |
| 41 | F | 6 | 7.1 | 120 | 22 | −0.11 | P | GCK | c.556C > T | p.Arg186* | P | DM |

**Table 1.** (*Continued*)

| Patient No. | Sex | At diagnosis | | | | | Inheritance | Gene | cDNA | Protein | ACMG/AMP classification | HGMD (phenotype) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Age | HbA1c | Height | Weight | BMI-SDS | | | | | | |
| 42 | F | 9 | 6.5 | 127 | 25 | −0.49 | P | GCK | c.556C > T | p.Arg186* | P | DM |
| 43 | F | 3 | 6.7 | 98 | 15 | 0.25 | M | GCK | c.563C > T | p.Ala188Val | LP | DM |
| 44 | M | 11 | 6.8 | 144.8 | 42.8 | 0.9 | M | GCK | c.571C > T | p.Arg191Trp | LP | DM |
| 45 | M | 9 | 6.4 | 133 | 32.7 | 0.8 | M | GCK | c.571C > T | p.Arg191Trp | LP | DM |
| 46 | M | 3 | 6.7 | 100.8 | 15.2 | −0.37 | M | GCK | c.571C > T | p.Arg191Trp | LP | DM |
| 47 | M | 9 | 6.5 | NA | NA | NA | P | GCK | c.571C > T | p.Arg191Trp | LP | DM |
| 48 | F | 12 | 6.5 | NA | NA | NA | M | GCK | c.571C > T | p.Arg191Trp | LP | DM |
| 49 | F | 3 | 6.8 | 89.2 | 13.9 | 1.52 | M | GCK | c.571C > T | p.Arg191Trp | LP | DM |
| 50 | F | 5 | 7 | 103.6 | 18.1 | 0.9 | M | GCK | c.571C > T | p.Arg191Trp | LP | DM |
| 51 | F | 6 | 6.4 | 119 | 18 | −2.21 | P | GCK | c.571C > T | p.Arg191Trp | LP | DM |
| 52 | M | 10 | 6.9 | 144.8 | 32 | −0.95 | P | GCK | c.571C > T | p.Arg191Trp | LP | DM |
| 53 | F | 6 | 6.7 | 113.7 | 16.9 | 0.74 | P | GCK | c.571C > T | p.Arg191Trp | LP | DM |
| 54 | F | 10 | 6.8 | 132.5 | 28.6 | −0.38 | P | GCK | c.571C > T | p.Arg191Trp | LP | DM |
| 55 | M | 7 | 6.8 | 118.6 | 20.1 | −0.98 | M | GCK | c.572G > A | p.Arg191Gln | LP | DM |
| 56 | M | 5 | 6.1 | 108.7 | 17 | −0.78 | P | GCK | c.572G > A | p.Arg191Gln | LP | DM |
| 57 | M | 12 | 6 | 123.9 | 22.4 | −0.66 | P | GCK | c.577G > T | p.Gly193Trp | LP | DM |
| 58 | F | 7 | 6.5 | NA | NA | NA | M | GCK | c.605 T > C | p.Met202Thr | LP | DM |
| 59 | F | 8 | 6.2 | 125.8 | 26 | 0.22 | P | GCK | c.617C > G | p.Thr206Arg | LP | DM |
| 60 | F | 3 | 6.3 | 90.5 | 14.5 | 1.67 | M | GCK | c.617C > T | p.Thr206Met | LP | DM |
| 61 | F | 6 | 6.8 | 111.4 | 18 | −0.62 | NO | GCK | c.617C > T | p.Thr206Met | LP | DM |
| 62 | F | 10 | 6.9 | 128.4 | 25.4 | −0.85 | P | GCK | c.617C > T | p.Thr206Met | LP | DM |
| 63 | F | 10 | 6.5 | 132 | 27 | −0.8 | NO | GCK | c.635_637delCCT | p.S212Cfs | P | DM |
| 64 | M | 9 | 6.2 | 136.4 | 26.6 | −1.34 | P | GCK | c.671 T > A | p.Met224Lys | LP | DM |
| 65 | M | 3 | 6 | 100.1 | 16.45 | 0.82 | UN | GCK | c.706G > A | p.Glu236Lys | LP | DM |
| 66 | M | 12 | 10.5 | 168 | 45.6 | −1.07 | NO | GCK | c.743delA | p.Asp248Alafs*47 | P | – |
| 67 | F | 9 | 6.5 | 142.8 | 32.7 | −0.21 | P | GCK | c.751A > G | p.Met251Val | LP | DM |
| 68 | F | 14 | 6.8 | 155.6 | 38.05 | −2.21 | M | GCK | c.764C > G | p.Thr255Ser | LP | DM |
| 69 | F | 19 | 6.4 | 152 | 43 | −1.04 | P | GCK | c.76C > T | p.Gln26* | P | DM |
| 70 | F | 9 | 6.6 | 129.6 | 26.2 | −0.43 | P | GCK | c.76C > T | p.Gln26* | P | DM |
| 71 | M | 11 | 6.1 | 143.3 | 31.2 | −1 | P | GCK | c.773G > T | p.Gly258Val | LP | – |
| 72 | M | 6 | 6 | 116 | 22.9 | 0.86 | M | GCK | c.775G > A | p.Ala259Thr | LP | DM |
| 73 | F | 4 | 6.5 | 89.6 | 11 | −1.29 | M | GCK | c.781G > A | p.Gly261Arg | LP | DM |
| 74 | M | 10 | 6.4 | 140 | 32 | −0.33 | M | GCK | c.781G > A | p.Gly261Arg | LP | DM |
| 75 | M | 18 | 7 | 165 | 53 | −0.61 | P | GCK | c.781G > A | p.Gly261Arg | LP | DM |
| 76 | F | 11 | 6.7 | 141 | 42 | 1.07 | M | GCK | c.838_839delAG | p.Ser280Leufs*10 | P | DM |
| 77 | F | 5 | 6.7 | 102 | 15.7 | −0.16 | UN | GCK | c.864-2A > G | | LP | DM |
| 78 | M | 12 | 6.2 | 144.6 | 36.5 | −0.38 | NO | GCK | c.873G > C | p.Lys291Asn | LP | – |
| 79 | M | 6 | 6.5 | NA | NA | NA | NO | GCK | c.895G > A | p.Gly299Ser | LP | DM |
| 80 | M | 9 | 6.5 | NA | NA | NA | M | GCK | c.895G > C | p.Gly299Arg | LP | DM |
| 81 | M | 10 | 6.2 | 133.5 | 28.4 | −0.54 | NO | GCK | c.898G > T | p.Glu300* | P | DM |
| 82 | M | 12 | 6.7 | 156 | 47 | 0.35 | P | GCK | c.908G > T | p.Arg303Leu | LP | DM |

http://wileyonlinelibrary.com/journal/jdi

**Table 1.** (*Continued*)

| Patient No. | Sex | At diagnosis | | | | | Inheritance | Gene | cDNA | Protein | ACMG/AMP classification | HGMD (phenotype) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Age | HbA1c | Height | Weight | BMI-SDS | | | | | | |
| 83 | M | 9 | 6.3 | 135.3 | 36 | 1.13 | NO | GCK | c.957_984delGGAGGCCT CCGAGCAGCTGCGCACACGC | p.Ala321Profs*24 | P | — |
| 84 | M | 10 | 6.3 | 127.6 | 24.4 | −1.14 | M | GCK | exon 6 del | | P | DM |
| 85 | M | 9 | 6.5 | 127.4 | 23.8 | −1.06 | M | GCK | exon 4–5 del | | P | DM |
| 86 | M | 4 | 6.4 | 97.4 | 13 | −1.48 | M | GCK | all exon del | | P | DM |
| 87 | M | 3 | 6 | NA | NA | NA | M | GCK | c.617C > T | p.Thr206Met | LP | DM |
| 88 | M | 14 | 6.5 | 146.2 | 32.2 | −2.52 | NO | GCK | c.898G > C | p.Glu300Gln | LP | DM |
| 89 | F | 11 | NA | 130.1 | 27.5 | −0.74 | M | HNF1A | c.1054delT | p.Ser352Profs*12 | P | DM |
| 90 | F | 15 | 10.5 | 137 | 40.9 | 0.38 | P | HNF1A | c.1136delC | p.Pro379Leufs*5 | P | DM |
| 91 | F | 13 | 6.5 | 157.9 | 44.6 | −0.68 | UN | HNF1A | c.1181delC | p.Pro394Glnfs*19 | P | DM |
| 92 | F | 13 | 7.4 | 141.4 | 32 | −1.66 | P | HNF1A | c.1340C > T | p.Pro447Leu | LP | DM |
| 93 | F | 12 | 6.7 | 156.7 | 47.6 | 0.25 | M | HNF1A | c.1340C > T | p.Pro447Leu | LP | DM |
| 94 | F | 14 | 7 | 150.2 | 65 | 2.08 | M | HNF1A | c.142delG | p.Glu48Serfs*107 | P | DM |
| 95 | F | 13 | 6.9 | 147.4 | 50.6 | 1.14 | M | HNF1A | c.1768 + 1G > T | | P | DM |
| 96 | M | 10 | 8.3 | NA | NA | NA | P | HNF1A | c.391C > T | p.Arg131Trp | LP | DM |
| 97 | M | 5 | 5.7 | 109.7 | 19.95 | 0.8 | M | HNF1A | c.391C > T | p.Arg131Trp | LP | DM |
| 98 | F | 11 | NA | NA | NA | NA | P | HNF1A | c.392G > A | p.Arg131Gln | LP | DM |
| 99 | M | 15 | 7.2 | 150.9 | 43.1 | −0.53 | NO | HNF1A | c.493delT | p.Trp165Glyfs*21 | P | – |
| 100 | M | 14 | 5.6 | 167.2 | 41.1 | −2.82 | M | HNF1A | c.598C > T | p.Arg200Trp | LP | DM |
| 101 | F | 12 | 11 | 142.3 | 41.2 | 0.57 | P | HNF1A | c.598C > T | p.Arg200Trp | LP | DM |
| 102 | F | 14 | 16.6 | NA | 40.8 | NA | M | HNF1A | c.618G > A | p.Trp206* | P | DM |
| 103 | F | 11 | 6.4 | 143.8 | 40.1 | 0.55 | NO | HNF1A | c.618G > A | p.Trp206* | P | DM |
| 104 | F | 14 | 13 | 154.2 | 42.4 | −1 | P | HNF1A | c.685C > T | p.Arg229* | P | DM |
| 105 | F | 11 | 8.8 | 152 | 52 | 1.4 | P | HNF1A | c.686G > A | p.Arg229Gln | LP | DM |
| 106 | F | 12 | 6.4 | 152.8 | 50.1 | 0.9 | P | HNF1A | c.779C > T | p.Thr260Met | LP | DM |
| 107 | M | 8 | 7.2 | 130 | 27.3 | 0.09 | M | HNF1A | c.788G > A | p.Arg263His | LP | DM |
| 108 | M | 10 | 7.6 | 145 | 35 | −0.17 | M | HNF1A | c.811C > T | p.Arg271Trp | LP | DM |
| 109 | M | 10 | 6 | 145.5 | 40.7 | 0.8 | P | HNF1A | c.827C > A | p.Ala276Asp | LP | DM |
| 110 | F | 12 | 8.5 | 160.2 | 43.7 | −0.73 | M | HNF1A | c.872dupC | p.Gly292Argfs*25 | P | DM |
| 111 | M | 6 | 11.6 | NA | NA | NA | P | HNF1A | c.872dupC | p.Gly292Argfs*25 | P | DM |
| 112 | F | 13 | 6.4 | 141 | 30 | −2.23 | P | HNF1A | c.872dupC | p.Gly292Argfs*25 | LP | DM |
| 113 | F | 7 | NA | NA | NA | NA | M | HNF1A | ex7,8,9 del | | P | DM |
| 114 | M | 10 | 7.8 | 136.8 | 41.8 | 1.49 | M | HNF1A | p.Arg229Gln | c.686G > A | LP | DM |
| 115 | F | 9 | 8.7 | 130.8 | 38 | 1.7 | M | HNF1A | c.872delC | p.Pro291Glnfs*51 | P | DM |
| 116 | F | 4 | 7.7 | 102.8 | 16.1 | −0.02 | NO | HNF1A | all exon del | | P | DM |
| 117 | M | 8 | 6.5 | 132.2 | 36.4 | 1.61 | M | HNF1A | c.872delC | p.Pro291Glnfs*51 | P | DM |
| 118 | M | 9 | NA | NA | NA | NA | P | HNF1B | c.286C > T | p.Gln96* | P | – |
| 119 | M | 7 | NA | NA | NA | NA | NO | HNF1B | c.395A > C | p.His132Pro | LP | DM |
| 120 | M | 13 | 12.7 | 158 | 36 | −2.69 | NO | HNF1B | c.494G > A | p.Arg165His | LP | DM |
| 121 | F | 12 | 12.3 | 155.6 | 47.3 | 0.3 | M | HNF1B | c.544 + 1G > A | | P | DM |
| 122 | M | 0y11m | 9.3 | 75 | 9.67 | 0.4 | NO | HNF1B | exon 1–4 del | | P | DM |
| 123 | M | 9 | 8 | 117.8 | 21.6 | −0.46 | NO | HNF1B | exon 3–4 del | | P | DM |

**Table 1.** (*Continued*)

| Patient No. | Sex | At diagnosis | | | | | Inheritance | Gene | cDNA | Protein | ACMG/AMP classification | HGMD (phenotype) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Age | HbA1c | Height | Weight | BMI-SDS | | | | | | |
| 124 | M | 37 | 10 | NA | NA | NA | NO | HNF1B | all exon del | | P | DM |
| 125 | F | 12 | 6.5 | 151 | 35.35 | −1.55 | P | HNF1B | all exon del | | P | DM |
| 126 | M | 21 | 16.7 | 176 | 38.9 | −2.72 | P | HNF1B | all exon del | | P | DM |
| 127 | F | 14 | 16.1 | 149.9 | 45.1 | −0.05 | NO | HNF1B | all exon del | | P | DM |
| 128 | F | 15 | 7.2 | 161.6 | 45.4 | −1.49 | NO | HNF1B | all exon del | | P | DM |
| 129 | F | 12 | 18.1 | NA | NA | NA | UN | HNF1B | exon 3–4 del | | P | DM |
| 130 | F | 13 | NA | NA | 47.2 | NA | NO | HNF1B | all exon del | | P | DM |
| 131 | F | 8 | NA | 126.2 | 26.8 | 0.39 | NO | HNF4A | c.1079C > T + c.1052 T > C (same allele) | p.Ala360Val + p.Met351Thr (same allele) | LP | – |
| 132 | F | 23 | 8.7 | 167.7 | 51.9 | −0.76 | P | HNF4A | c.146A > C | p.His49Pro | LP | DM |
| 133 | M | 40 | 7.1 | 169 | 54 | −1.51 | P | HNF4A | c.359 + 1G > A | | P | – |
| 134 | M | 18 | 6 | 165 | 64 | 0.97 | P | HNF4A | c.427-2A > G | | P | DM |
| 135 | F | 13 | 8.9 | 158 | 57.2 | 1.04 | NO | HNF4A | c.518 T > C | p.Leu173Pro | LP | – |
| 136 | F | 9 | 7.5 | 149 | 47.9 | 1.58 | M | HNF4A | c.582 + 2_582 + 10delTGAGGATGG | | LP | – |
| 137 | F | 9 | NA | 136.8 | 34.8 | 0.81 | NO | HNF4A | c.802C > T (*de novo*) | p.Gln268* | P | – |
| 138 | F | 11 | 6.5 | 147 | 39.4 | 0.14 | M | HNF4A | c.916insT | p.Tyr306Leufs*2 | P | – |
| 139 | F | 16 | 9.2 | 158.5 | 49.7 | −0.46 | M | HNF4A | c.857 T > A | p.Ile286Asn | LP | DM |
| 140 | M | 12 | 10.7 | 148.9 | 38.1 | −0.51 | NO | HNF4A | c.874C > T | p.Gln292* | P | DM |
| 141 | F | 12 | 7.9 | 151.2 | 40.7 | −0.37 | P | HNF4A | c.956_958dupTGC | p.Leu319dup | LP | DM |
| 142 | F | 13 | 10.5 | 147 | 41.4 | −0.15 | P | HNF4A | c.925C > T | p.Arg309Cys | LP | DM |
| 143 | F | 12 | 15.6 | 149.3 | 39.2 | −0.47 | M | HNF4A | c.713A > C | p.Glu238Ala | LP | – |
| 144 | M | 21 | 8.2 | 173 | 62 | −0.4 | P | INS | c.101A > T | p.His34Leu | LP | – |
| 145 | F | 8 | 5.8 | 127 | 24.4 | −0.45 | M | INS | c.163C > T | p.Arg55Cys | LP | DM |
| 146 | M | 7 | 6.5 | 130.1 | 29.3 | 0.8 | NO | INS | c.212delG | p.Gly71Alafs | P | – |
| 147 | F | 1 | 11.1 | 73 | 7.7 | −1.07 | P | INS | c.94G > A | p.Gly32Ser | LP | – |
| 148 | F | 13 | 14.9 | 149.8 | 46.2 | 0.36 | NO | INSR | c.282_283TG > GT | p.TyrGly94_95*Trp | P | – |
| 149 | M | 6 | 8.2 | NA | NA | NA | NO | KCNJ11 | c.685G > A | p.Glu229Lys | LP | DM |
| 150 | M | 42 | NA | NA | NA | NA | BI | MITO | m.3243A > G | | P | |
| 151 | F | 15 | NA | NA | NA | NA | M | MITO | m.3243A > G | | P | |
| 152 | F | 18 | 10.5 | 166.9 | 60 | 0.57 | M | MITO | m.3243A > G | | P | |
| 153 | F | 31 | 5.6 | NA | NA | NA | M | MITO | m.3243A > G | | P | |
| 154 | M | 14 | NA | NA | NA | NA | M | MITO | m.3243A > G | | P | |
| 155 | M | 25 | 11.2 | 165.9 | 59.8 | −0.11 | M | MITO | m.3243A > G | | P | |
| 156 | F | 26 | 8.3 | 143.5 | 44.7 | 0.16 | M | MITO | m.3243A > G | | P | |
| 157 | F | 28 | 10.1 | 149.4 | 53.6 | 0.92 | M | MITO | m.3243A > G | | P | |

The reference sequences for each gene: NM_000162.3 (*GCK*), NM_000545.5 (*HNF1A*), NM_175914.3 (*HNF4A*), NM_000458.2 (*HNF1B*), NM_000207.2(*INS*), NM_000525.3(*KCNJ11*), NM_000352.3 (*ABCC8*), NM_000208.2 (*INSR*).

ACMG/AMP classification, classification of pathogenicity by the criteria of American College of Medical Genetics and Genomics/Association for Molecular Pathology with P (pathogenic) and LP (likely pathogenic); BI (both parents affected); BMI-SDS, standard deviation score of body mass index; del, deletion; HGMD, listing in the Human Gene Mutation Database professional with "–(minus)" representing absence and diabetes representing diabetes phenotype; Inheritance, P (paternal), M (maternal), NO (no affected parents), MITO, mitochondrial gene; pat dup, duplication of the paternal allele; NA, not available; pUPD, paternal uniparental disomy.

**Table 2** | Summary of the clinical features and the identified rare variants of uncertain significance of a population frequency <0.001 and the Combined Annotation Dependent Depletion score >20 for each patient

| Patient No. | | At diagnosis | | | | | Inheritance | Gene | cDNA | protein | ACMG/AMP classification | CADD | TMMo | MAF in gnomAD ALL | HGMD (phenotype) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sex | Age | HbA1c | Height | Weight | BMI-SDS | | | | | | | | | |
| 158 | M | 11 | NA | NA | 52 | NA | P | ABCC8 | c.1513G > A | p.Gly505Ser | VUS | 31 | – | – | DM |
| 159 | M | 14 | 10 | NA | NA | NA | NO | ABCC8 | c.1596C > G | p.Ser532Arg | VUS | 23.5 | – | – | – |
| 160 | M | 11 | 13.5 | 144.3 | 31.7 | −1.32 | NO | ABCC8 | c.3533A > G | p.Gln1178Arg | VUS | 23.9 | – | – | DM |
| 161 | F | 11 | 6 | 153.2 | 50.8 | 1.20 | NO | ABCC8 | c.4259G > A | p.Arg1420His | VUS | 31 | 0.00014 | – | HI/DM |
| 162 | F | 11 | 5.1 | NA | 42 | NA | M | ABCC8 | c.4457G > A | p.Arg1486Lys | VUS | 28.3 | – | – | HI |
| 163 | F | 10 | 7.4 | 133.7 | 34.9 | 0.86 | NO | ABCC8 | c.4132G > A | p.Gly1378Ser | VUS | 24.7 | – | – | HI |
| 164 | M | 11 | 6.7 | NA | NA | NA | BI | ABCC8 | c.1432G > T | p.Ala478Ser | VUS | 24 | – | – | – |
| 165 | F | 10 | 9.5 | 150 | 49 | 1.44 | P | ABCC8 | c.1673C > T | p.Thr558Ile | VUS | 32 | – | – | – |
| 166 | F | 22 | NA | NA | NA | NA | P | ABCC8 | c.2957C > T | p.Ser986Leu | VUS | 24.8 | – | 0.00003 | – |
| 167 | M | 28 | NA | 170 | 91.4 | 2.72 | NA | ABCC8 | c.2405 T > C | p.Ile802Thr | VUS | 20.3 | – | – | – |
| 168 | F | 9 | 8.5 | 148.4 | 52.5 | 1.98 | M | ABCC8 | c.1337 T > C | p.Ile446Thr | VUS | 23 | — | – | DM |
| 169 | F | 15 | 13.8 | 172 | 56 | −0.71 | M | ABCC8 | c.359C > G | p.Ser120Cys | VUS | 25 | – | – | – |
| 170 | F | 9 | 6.7 | 133.9 | 44.45 | 2.12 | BI | ABCC8 | c.3088G > A | p.Asp1030Asn | VUS | 25.1 | – | – | HI |
| 171 | M | 11 | 5.6 | 129.7 | 26.8 | −0.86 | M | ABCC8 | c.3875A > C | p.Asn1292Thr | VUS | 24.2 | – | – | – |
| 172 | F | 13 | 6.5 | 162.4 | 46.1 | −0.87 | M | ABCC8 | c.2300G > A | p.Gly767Asp | VUS | 23.1 | 0.00053 | – | – |
| | | | | | | | | ABCC8 | c.1753A > G | p.Ile585Val | VUS | 23.4 | – | – | – |
| 173 | M | 12 | 6.8 | 151 | 50 | 1.07 | P | GCK | c.1151C > T | p.Ala384Val | VUS | 29.9 | 0.00004 | – | DM |
| 174 | F | 14 | 6.7 | 152 | 40.2 | −1.22 | M | GCK | c.1174C > G | p.Arg392Gly | VUS | 31 | – | – | DM |
| 175 | F | 8 | 5.7 | 127.2 | 26.1 | 0.08 | NO | GCK | c.1232C > T | p.Ser411Phe | VUS | 31 | – | – | DM |
| 176 | M | 8 | 6.9 | 118.5 | 19.1 | −1.71 | NO | GCK | c.437 T > G | p.Leu146Arg | VUS | 28.7 | – | – | DM |
| 177 | M | 7 | 6.3 | 120.9 | 22.3 | −0.27 | P | GCK | c.538A > C | p.Asn180His | VUS | 25.8 | – | – | DM |
| 178 | F | 11 | 6.6 | 149.7 | 35.3 | −1.01 | M | GCK | c.707A > C | p.Glu236Ala | VUS | 25.1 | – | – | DM |
| 179 | F | 10 | 6.5 | 134.3 | 27.6 | −0.91 | M | GCK | c.1144 T > C | p.Cys382Arg | VUS | 32 | – | – | – |
| 180 | M | 14 | 6.8 | 159.8 | 44 | −1.1 | M | GCK | c.164 T > G | p.Val55Gly | VUS | 26 | – | – | – |
| 181 | M | 5 | 6.1 | 118 | 19 | −1.5 | NA | HNF1A | c.505A > G | p.Lys169Glu | VUS | 23 | – | – | — |
| 182 | F | 12 | 6.5 | 149 | 37 | −0.19 | NO | HNF1A | c.1544C > A | p.Thr515Lys | VUS | 26.8 | 0.00011 | – | DM |
| 183 | F | 12 | 8.8 | NA | NA | NA | M | HNF1A | c.1043 T > C | p.Leu348Pro | VUS | 26.3 | – | – | DM |
| 184 | M | 13 | 6.2 | 154 | 44 | −0.19 | UNK | HNF1A | c.397G > T | p.Val133Leu | VUS | 23.7 | – | – | DM |
| 185 | M | 11 | 7.3 | NA | NA | NA | M | HNF1A | c.485 T > C | p.Leu162Pro | VUS | 26.3 | – | – | DM |
| 186 | F | 12 | 6.4 | 140.1 | 30.7 | −1.47 | P | HNF1A | c.493 T > C | p.Trp165Arg | VUS | 27.2 | – | – | DM |
| 187 | M | 10 | 10.3 | NA | NA | NA | NA | HNF1A | c.494G > C | p.Trp165Ser | VUS | 27.9 | – | – | – |
| 188 | F | 10 | 8.7 | 144.9 | 45.9 | 1.46 | M | HNF1A | c.778A > T | p.Thr260Ser | VUS | 23.6 | – | – | DM |
| 189 | F | 11 | 9.7 | 153.3 | 44 | 0.32 | P | HNF1A | c.791 T > C | p.Val264Ala | VUS | 26 | – | – | DM |
| 190 | M | 34 | 6.2 | NA | NA | NA | M | HNF1A | c.794A > T | p.Tyr265Phe | VUS | 25.4 | – | – | – |
| 191 | F | 12 | 10.6 | 154.2 | 42.1 | −0.42 | NO | INSR | c.3542C > A | p.Thr1181Asn | VUS | 26.8 | – | – | – |
| | | | | | | | | PIK3R1 | c.248C > T | p.Ser83Leu | VUS | 24.9 | 0.00004 | – | – |
| 192 | M | 33 | NA | NA | NA | NA | NO | INSR | c.806C > T | p.Pro269Leu | VUS | 28.4 | 0.00014 | – | – |
| 193 | M | 8 | NA | 126.2 | 26.6 | 0.34 | NM | INSR | c.881A > G | p.Lys294Arg | VUS | 23.1 | 0.00074 | 0.0002 | – |
| 194 | F | 8 | 8.9 | 136.3 | 37.4 | 1.44 | M | KCNJ11 | c.139A > G | p.Lys47Glu | VUS | 23.8 | – | – | – |

**Table 2.** (*Continued*)

| Patient No. | Sex | At diagnosis | | | | Inheritance | Gene | cDNA | protein | ACMG/AMP classification | CADD | TMMo | MAF in gnomAD ALL | HGMD (phenotype) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Age | HbA1c | Height | Weight | BMI-SDS | | | | | | | | |
| 195 | F | 9 | 5.6 | 138.8 | 30 | −0.45 | M | KCNJ11 | c.1105C > T | p.Arg369Cys | VUS | 26.5 | 0.00002 | – | – |
| 196 | M | 20 | 8 | NA | NA | NA | P | KCNJ11 | c.10C > T | p.Arg4Cys | VUS | 28.7 | – | 0.00003 | DM |
| 197 | F | 2 | 10.1 | NA | NA | NA | P | KCNJ11 | c.968A > G | p.Asp323Gly | VUS | 24.4 | – | – | – |
| 198 | M | 14 | NA | NA | NA | NA | M | PDX1 | c.239C > T | p.Ala80Val | VUS | 23.5 | – | – | – |
| 199 | F | 8 | NA | 122 | 28 | 1.09 | M | PDX1 | c.119G > A | p.Arg40His | VUS | 29.9 | 0.00021 | – | – |

Variants classified as variants of unknown significance (VUS) by the 2015 American College of Medical Genetics and Genomics and the Association for Molecular Pathology criteria, but with a population frequency <0.001 and with a scaled C-score by the Combined Annotation Dependent Depletion (CADD score) >20 are listed. The reference sequences for each gene: NM_000209.3 (*PDX1*), NM_181523.2 (*PIK3R1*), NM_000162.3 (*GCK*), NM_000545.5 (*HNF1A*), NM_175914.3 (*HNF4A*), NM_000458.2 (*HNF1B*), NM_000207.2 (*INS*), NM_000525.3 (*KCNJ11*), NM_000352.3 (*ABCC8*), NM_000208.2 (*INSR*). ACMG/AMP classification, classification of pathogenicity by the criteria of American College of Medical Genetics and Genomics/Association for Molecular Pathology with P (pathogenic) and LP (likely pathogenic); BI (both parents affected); BMI-SDS, standard deviation score of body mass index; del, deletion; DM, diabetes; HGMD, listing in the Human Gene Mutation Database professional with "–(minus)" representing absence and diabetes representing diabetes phenotype; HI, hyperinsulinemic hypoglycemia; Inheritance, P (paternal), M (maternal), NO (no affected parents), MITO, mitochondrial gene; pat dup, duplication of the paternal allele; MAF in gnomAD ALL, minor allele frequency in the gnomAD database for all ethnic groups with "–(minus)" representing absence; NA, not available; pUPD, paternal uniparental disomy; TMMo, allele frequency in the Japanese 14 K database (jMorp) with "–(minus)" representing absence.

highly elevated plasma glucose at 24.8 mmol/L, hemoglobin A1c at 14.9% in association with diminished serum C-peptide at 0.2 nmol/L. Pancreatic autoantibodies were negative. Her BMI at presentation was 20.6 (64th centile), and she had a paternal history of diabetes. Three variants in the *INSR* gene (p.Thr1181Asn, p.Pro269Leu, p.Lys294Arg) were also identified in the rare VUS-CS >20 group (patients 191, 192, 193). In addition to the *INSR* variant, patient 191 had an additional variant, p.Ser83Leu, in the *PIK3R1* gene, which is responsible for SHORT syndrome characterized by low birthweight and insulin resistance after puberty. This patient was born small-for-gestational-age, with a birthweight of 2,416 g after 39 weeks of pregnancy. She presented with incidentally identified hyperglycemia at the age of 12 years. She had a maternal history of diabetes, and was lean with a BMI-SDS of −0.42. Retrospectively, her C-peptide and insulin at presentation were elevated at 3.0 nmol/L and 2413.4 pmol/L in the presence of plasma glucose at 16.1 mmol/L. Patient 192 was diagnosed with diabetes at the age of 33 years. He had a three-generation paternal inheritance of diabetes. Clinical data at diagnosis was unavailable, but after 30 years of diabetes, his insulin secretion was diminished (increment of C-peptide after arginine loading at 0.5 nmol/L). The patient was not obese and was negative for pancreatic autoantibodies. Patient 193 developed diabetes at the age of 8 years. His BMI-SDS at diagnosis was 0.34, and his fasting insulin was not diminished at 241.7 pmol/L when plasma glucose was 5.9 mmol/L.

Finally, there were two patients, patients 198 and 199, with rare VUS-CS >20 in the *PDX1* gene. Detailed clinical data are missing for patient 198, except that the patient had a three-generation family history of early-onset diabetes, was not obese, and could be treated with metformin and sulfonylurea for at least 10 years. Patient 199 had a typical history of MODY. She was not obese and had a three-generation family history of non-obese diabetes, and with homeostatic model assessment for β-cell function at 32.0.

## DISCUSSION

To the best of our knowledge, this is the most comprehensive analysis of monogenic diabetes in East Asians. Responsible P/LP variants were identified in 46.2% of the patients, and the identification rate could be higher, as at least some of the rare VUS-CS >20 are apparently pathogenic. These figures are higher than those previously reported for East Asians[19–21]. Possible explanations include: (i) a higher fraction of pediatric patients in the present study; (ii) most referral sources being diabetologists; and (iii) the study design with a broader range of target genes. As the incidence of type 2 diabetes increases with age, in the pediatric age group, the chances of identifying monogenic diabetes would be higher, especially by diabetologists. Also, with a broader range of target genes, the variant identification rate would be higher compared with studies focusing only on common MODY genes.

Limiting to the P/LP variants, *GCK*-MODY was the most common, followed by *HNF1A*-, *HNF4A*- and *HNF1B*-MODY

**Table 3** | Summary of the clinical features of patients without pathogenic/likely pathogenic or rare variants of uncertain significance of a population frequency <0.001 and the Combined Annotation Dependent Depletion score >20

| Patient No. | At diagnosis | | | | | | Inheritance |
|---|---|---|---|---|---|---|---|
| | Sex | Age | HbA1c | Height | Weight | BMI-SDS | |
| 200 | M | 6 | 8.2 | NA | NA | NA | NO |
| 201 | M | 12 | NA | 153.8 | 64.6 | 1.93 | NA |
| 202 | M | 5 | 5.8 | 108.1 | 18.1 | 0.11 | P |
| 203 | M | 13 | 10.9 | 161.4 | 57.05 | 0.88 | M |
| 204 | M | 1 | 5.8 | 89 | 11.6 | −1.58 | NO |
| 205 | F | 10 | 7.5 | NA | NA | NA | M |
| 206 | F | 15 | 8.9 | 144.8 | NA | NA | P |
| 207 | M | 10 | 12.7 | 147 | 34 | −0.65 | NO |
| 208 | F | 10 | 7.4 | 151.2 | 28.2 | −3.24 | M |
| 209 | M | 6 | 9.3 | NA | NA | NA | NO |
| 210 | M | 14 | 7.5 | 165.7 | 59.4 | 0.63 | NO |
| 211 | F | 14 | 9.6 | 148.2 | 52.5 | 1.11 | M |
| 212 | F | 32 | NA | 160.6 | 60 | 0.295 | NO |
| 213 | F | 13 | 7.4 | 145.7 | 54.4 | 1.66 | M |
| 214 | F | 10 | 6.4 | 139 | 35 | 0.4 | M |
| 215 | M | 14 | 5.1 | 165.5 | 54 | 0.01 | NO |
| 216 | M | 15 | NA | 155 | 46.2 | −0.4 | M |
| 217 | M | 15 | 15.6 | NA | 58 | NA | P |
| 218 | F | 6 | NA | 109.1 | 16.8 | −0.92 | M |
| 219 | F | 14 | 15.8 | 143.7 | 33 | −2.04 | NO |
| 220 | M | 9 | 7.14 | 137.2 | 31.8 | 0.21 | M |
| 221 | F | 10 | 8.7 | NA | NA | NA | P |
| 222 | F | NA | NA | NA | NA | NA | NA |
| 223 | F | 5 | 5.8 | 105.1 | 18 | 0.59 | M |
| 224 | F | 10 | 11.5 | 145.1 | 53.3 | 2.08 | NO |
| 225 | F | 10 | 10.5 | 144.7 | 43.2 | 1.17 | NO |
| 226 | F | 11 | 5.3 | 151.5 | 38.3 | −0.52 | M |
| 227 | F | 8 | 10.7 | 134 | 23.5 | −1.96 | NO |
| 228 | F | 1 | NA | 80 | 11 | 1.12 | NO |
| 229 | F | 9 | NA | NA | NA | NA | M |
| 230 | F | 14 | 14.1 | 159 | 70 | 1.9 | NO |
| 231 | F | NA | NA | NA | NA | NA | NA |
| 232 | M | 8 | 5.1 | 126.2 | 26.6 | 0.35 | M |
| 233 | M | 3 | 5.4 | 93.5 | 13.9 | 0.43 | NO |
| 234 | M | 1 | 8.5 | 72 | 11.8 | 3.98 | M |
| 235 | F | 13 | 11 | NA | NA | NA | NO |
| 236 | F | 2 | 9.9 | NA | NA | NA | P |
| 237 | M | 14 | 10.9 | 171 | 52 | −0.81 | M |
| 238 | F | 9 | NA | NA | NA | NA | M |
| 239 | M | 9 | 10.1 | 140 | 44.3 | 1.69 | NO |
| 240 | F | 11 | 10.7 | 143.3 | 36 | −0.14 | M |
| 241 | F | 10 | 8.7 | 142 | 42 | 1.22 | BI |
| 242 | F | 23 | 5.9 | 156.2 | 44.9 | −0.76 | BI |
| 243 | F | 20 | 6 | 154.8 | 45 | −0.65 | NO |
| 244 | F | 11 | 6.8 | 138.5 | 34.7 | 0.09 | P |
| 245 | M | 12 | 9.1 | 158.7 | 51.3 | 0.68 | NO |
| 246 | F | 20 | 8 | NA | NA | NA | P |
| 247 | F | 11 | 10.7 | 144 | 41.1 | 0.69 | NO |
| 248 | F | 10 | 6 | 149 | 58.5 | 2.22 | M |
| 249 | M | NA | NA | NA | NA | NA | NA |
| 250 | F | 12 | NA | NA | NA | NA | BI |

**Table 3.** (*Continued*)

| Patient No. | At diagnosis | | | | | | Inheritance |
|---|---|---|---|---|---|---|---|
| | Sex | Age | HbA1c | Height | Weight | BMI-SDS | |
| 251 | F | 14 | NA | 148.1 | 69.2 | 2.47 | M |
| 252 | F | 11 | 12.7 | 143.6 | 39.95 | 0.55 | M |
| 253 | M | 12 | 5.5 | NA | NA | NA | M |
| 254 | M | 23 | 9.9 | NA | NA | NA | P |
| 255 | NA | 13 | 8.3 | NA | NA | NA | P |
| 256 | NA | 19 | 6.8 | NA | NA | NA | NA |
| 257 | NA | 12 | 8.1 | NA | NA | NA | P |
| 258 | NA | 11 | 7.3 | NA | NA | NA | M |
| 259 | M | 12 | 6 | 155 | 58 | 1.5 | M |
| 260 | F | 14 | 5.4 | 148.8 | 41.3 | −0.62 | NO |
| 261 | F | 40 | NA | NA | NA | NA | M |
| 262 | F | 13 | 6.4 | 153 | 60.2 | 1.68 | NA |
| 263 | F | 12 | 8.2 | 155.5 | 70.9 | 2.42 | M |
| 264 | F | 11 | 11.4 | 159.8 | 81.9 | 2.8 | M |
| 265 | F | 12 | 9.3 | 147.2 | 42 | 0.25 | M |
| 266 | F | 10 | NA | 136.9 | 32.8 | 0.16 | M |
| 267 | F | 6 | 6.7 | 104 | 17.7 | 0.5 | P |
| 268 | M | 13 | 10.1 | 171.8 | 85 | 2 | NO |
| 269 | F | 11 | 7.4 | 149.4 | 58.2 | 2.1 | M |
| 270 | F | NA | NA | NA | NA | NA | NA |
| 271 | M | 12 | 11.2 | 169 | 70.6 | 1.59 | P |
| 272 | F | 13 | 8.8 | 145.6 | 52.5 | 1.48 | M |
| 273 | F | 8 | 5.4 | 134.1 | 43.5 | 2.17 | M |
| 274 | M | 12 | 14.8 | 171 | 86 | 2.13 | NO |
| 275 | F | 11 | 11.3 | 157 | 54 | 1.26 | BI |
| 276 | M | 20 | 6.6 | 169 | 79.5 | 1.64 | M |
| 277 | F | 13 | 8.7 | 151.7 | 47.1 | 0.32 | BI |
| 278 | M | 11 | NA | NA | NA | NA | M |
| 279 | F | 7 | 9.5 | 132.6 | 33.2 | 1.3 | M |
| 280 | M | 11 | 13.5 | 151 | 42.4 | 0.36 | M |
| 281 | F | 12 | 13.2 | 149.3 | 47.9 | 0.91 | NA |
| 282 | F | NA | NA | NA | NA | NA | NA |
| 283 | F | NA | NA | 144.5 | 28.2 | NA | M |
| 284 | F | 4 | 13 | 102.1 | 14.7 | −0.93 | P |
| 285 | F | 10 | NA | 135.2 | 42.7 | 1.76 | BI |
| 286 | M | 14 | 6.2 | 157.5 | 53.1 | 0.56 | NO |
| 287 | M | 11 | 9.2 | 140.5 | 39.8 | 0.84 | P |
| 288 | M | 11 | 12 | 161 | 86.5 | 2.4 | P |
| 289 | F | 11 | 6.6 | 125.6 | 23.1 | −1.68 | M |
| 290 | F | 12 | 10 | 146.8 | 39 | −0.25 | M |
| 291 | M | 11 | 12 | 148.7 | 45 | 0.89 | M |
| 292 | M | 11 | 10.9 | 142.4 | 40.8 | 0.83 | P |
| 293 | M | 11 | 8 | NA | NA | NA | P |
| 294 | M | 8 | 5.5 | 131.6 | 32.8 | 1.15 | M |
| 295 | F | 11 | 9 | 142 | 32 | −0.94 | M |
| 296 | M | 12 | 12.4 | 168.2 | 113.3 | 2.71 | P |
| 297 | F | 10 | 9.8 | 145.8 | 42.4 | 0.98 | M |
| 298 | M | 14 | 6 | NA | NA | NA | M |
| 299 | M | 14 | 11.9 | 157.6 | 49.1 | 0.03 | M |
| 300 | M | 12 | 13.1 | 152 | 60 | 1.77 | P |
| 301 | M | 12 | 12.8 | 170 | 65.4 | 1.22 | NO |
| 302 | F | 4 | 13.7 | 105.1 | 16.7 | −0.11 | NO |

**Table 3.** (*Continued*)

| Patient No. | At diagnosis | | | | | | Inheritance |
|---|---|---|---|---|---|---|---|
| | Sex | Age | HbA1c | Height | Weight | BMI-SDS | |
| 303 | M | 14 | 10.8 | 164 | 89 | 2.41 | P |
| 304 | F | 11 | 9.6 | 149.2 | 43.8 | 0.65 | M |
| 305 | F | NA | NA | NA | NA | NA | NA |
| 306 | M | 12 | 15.1 | 149 | 35 | −1.31 | P |
| 307 | F | 7 | 12 | 127 | 24.8 | −0.15 | P |
| 308 | F | NA | NA | NA | NA | NA | NA |
| 309 | F | 14 | 16.1 | 167.5 | 42 | −2.73 | NO |
| 310 | M | 13 | 14.1 | 163.5 | 57.2 | 0.75 | NO |
| 311 | F | 12 | 7.6 | 146.9 | 44.1 | 0.6 | NO |
| 312 | M | 14 | 7.7 | 168.7 | 57.8 | 0.22 | P |
| 313 | M | 12 | 9 | 164.2 | 61.6 | 1.27 | P |
| 314 | F | NA | NA | NA | NA | NA | NA |
| 315 | F | NA | NA | NA | NA | NA | NA |
| 316 | M | 9 | 6.3 | 135.3 | 36 | 1.13 | NO |
| 317 | M | NA | NA | NA | NA | NA | NA |
| 318 | F | NA | NA | NA | NA | NA | NA |
| 319 | F | 10 | 9.6 | 147.9 | 55.8 | 2.11 | M |
| 320 | F | 15 | 12 | 171.6 | 55.4 | −0.76 | P |
| 321 | F | 14 | 8.7 | 160 | 50.2 | −0.22 | P |
| 322 | F | 15 | 5 | 158.7 | 50.6 | −0.22 | P |
| 323 | F | 15 | 10.4 | 151 | 55.2 | 1.07 | NP |
| 324 | F | 1 | 11.1 | 77 | 7.3 | −3.03 | P |
| 325 | F | 13 | NA | NA | NA | NA | NA |
| 326 | M | 13 | 6.1 | 143 | 32 | −1.74 | P |
| 327 | F | 13 | NA | 159.7 | 59.6 | 1.16 | M |
| 328 | M | NA | NA | NA | NA | NA | NA |
| 329 | F | 10 | 10 | NA | NA | NA | NO |
| 330 | F | 14 | 12.3 | 158 | 52 | 0.22 | M |
| 331 | F | 12 | 7.6 | 157.1 | 55.3 | 1.15 | M |
| 332 | F | 11 | 6.8 | 142.9 | 72.2 | 3.07 | M |
| 333 | M | 8 | 7.5 | 118.6 | 19.2 | −1.67 | P |
| 334 | M | 8 | 9.8 | 127 | 27.8 | 0.58 | P |
| 335 | F | 29 | 9.4 | 170 | NA | NA | BI |
| 336 | F | 12 | 9.5 | 143.6 | 36.7 | −0.38 | M |
| 337 | F | 13 | 16.6 | 156.4 | 43.5 | −0.73 | M |
| 338 | F | 17 | NA | NA | NA | NA | M |
| 339 | F | NA | NA | NA | NA | NA | NA |
| 340 | NA | 15 | 11.4 | NA | NA | NA | M |

These patients were characterized by female dominance with the female (F)/male (M) ratio at 1.70, the mean age of diagnosis at 11.8 years, the mean standard deviation score of body mass index (BMI-SDS) at 0.55 and the mean hemoglobin A1c (HbA1c) at 9.34%. M, maternal; NA, not available; NO, no affected parents; P, pathogenic.

(Figure 2). This is in line with the recent large-scale studies[21,34–36], and is not surprising considering the high population prevalence (estimated at 1.1 in 1000) of deleterious *GCK* variants in the general population[32]. When mildly hyperglycemic patients are included as in pediatric studies, *GCK* variants are likely to be the most common, whereas *HNF1A* variants tend to be more common in studies leaning toward symptomatic patients[37]. As few pathogenic variants with a strong founder effect have been identified in MODY genes, the true incidence of P/LP variants in MODY genes should be similar across different ethnicities if very large, population-based studies linking the genotypes and the serial measurements of blood glucose are obtained.

In addition to the P/LP variants, we tried to identify VUS variants that might be pathogenic as rare VUS-CS >20 by using the population frequency and the CADD score of the variants. For missense variants of rare MODY genes, it is often difficult to reach the P/LP status of the ACMG/AMP guidelines[27],

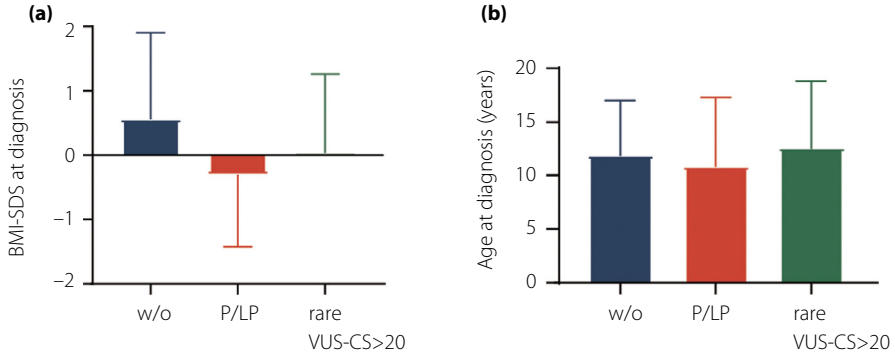


**Figure 3** | (a). Comparison of the standard deviation scores of body mass indices (BMI-SDS) at diagnosis of patients with pathogenic/likely pathogenic (P/LP) variants, with VUS variants of a population frequency <0.001 and A Combined Annotation Dependent Depletion score >20 (rare VUS-CS > 20), and those without these variants (w/o). For each category, data on BMI-SDS at diagnosis were available for 127, 30 and 100 patients, respectively. The mean and the standard deviations are shown. (b). Comparison of the ages of patients at diagnosis with pathogenic/likely pathogenic (P/LP) variants, VUS variants of a population frequency <0.001 and the CADD score > 20 (rare VUS-CS > 20), and those without these variants (w/o). For each category, data on age at diagnosis were available for 156, 43 and 127 patients, respectively. The mean and the standard deviations are shown.

unless extensive segregation studies or *in vitro* functional studies are additionally carried out. In this group of variants, nearly half of them were $K_{ATP}$ channel genes, *ABCC8* or *KCNJ11*. Combined with the P/LP patients, variants in the $K_{ATP}$ channel genes were identified in 25 patients, and 11 of them were listed in the HGMD database and seven were variants of the amino acids for which different alterations have already been reported (patients 159, 164, 172, 179, 186, 190, 195).

Interestingly, of the previously reported $K_{ATP}$ channel variants, four are listed in association with hyperinsulinemic hypoglycemia in the HGMD database. Described clinical phenotypes of patients with these variants (p.Arg1486Lys[38], p.Gly1378Ser[39], p.Asp1030Asn[40], p.Arg1420His[33]) are compatible with the diagnosis of congenital hyperinsulinism, and for p.Gly1378Ser and p.Arg1420His, *in vitro* evidence of the loss of function has been reported[41,42]. These findings could simply be the incidental identification of asymptomatic carriers of loss-of-function, hyperinsulinemic variants not related to diabetes. However, at least two of our patients (patients 161, 162) presented with a history of hyperinsulinemic hypoglycemia evolving into diabetes. Neonatal macrosomia with apneic spells found in patient 170 is also typical of congenital hyperinsulinism, although hypoglycemia was not documented for this patient.

Cases of $K_{ATP}$ channel congenital hyperinsulinism evolving into diabetes have been reported repeatedly[43–46]. This is likely to be a different category of $K_{ATP}$ channel MODY distinct from the patients with activating variants. As the treatment strategy for this group of patients could be different from that for the patients with activating mutations, recognition of this group of patients might be important for the management of diabetes. For example, Ovsyannikova *et al.*[47] reported a patient with a p.Ala1457Thr variant in the *ABCC8* gene with diabetes. This variant is known to be causative of hyperinsulinemic hypoglycemia[48,49]. Unlike neonatal diabetes caused by *ABCC8* variants, switching from insulin to sulfonylurea did not work well, with the extensive glucose excursion requiring an add-on treatment by an sodium–glucose cotransporter 2 inhibitor.

Another interesting finding in the present study was the identification of variants of the *INSR* gene, typically causing type A insulin resistance, in patients with suspected MODY; one in the P/LP group (patient 148) and three in the patients with rare VUS-CS >20 group (patients 191–193). Patient 148 was a 29.5% mosaic and presented with acute diabetes with diminished insulin secretion. The role of her insulin variant in the development of diabetes thus remains unknown. On the contrary, two patients with rare VUS-CS >20 *INSR* variants showed evidence of insulin resistance. Patient 191 presented with diabetes associated with elevated endogenous insulin and had an additional variant of the *PIK3R1* gene. As variants in the *INSR* and *PIK3R1* genes both cause insulin-resistant diabetes, it is unclear which of these variants is more responsible for her diabetes, although the low birthweight for gestational age is more consistent with the presentation of *PIK3R1* abnormality. Patient 193 also presented with mildly elevated fasting serum insulin (241.7 pmol/L) at diagnosis. The fact that he was not obese and had dominantly inherited diabetes made him a candidate for MODY. In our national survey in Japan, fasting serum insulin of genetically confirmed type A resistance could be as low as 243.1 pmol/L[50]. Insulin resistance syndrome, thus, needs to be included in the gene panel.

Finally, we identified two patients (patients 198; 199) with variants in the *PDX1* gene. As the pathogenicity of missense variants in this gene is difficult to interpret because of a large number of benign rare variants[51], the pathogenicity of these variants was not clear at the moment.

We believe the high diagnostic yield and its possible clinical implications support the cost-effectiveness of including multigene analysis of monogenic diabetes in the national health insurance system. The strength of the present study was in the comprehensiveness of target genes and a large number of patients with

suspected MODY. There were, however, several weaknesses in the present study. First, because of a lack of confirmatory studies, many variants remained in the category of VUS. To identify true pathogenic variants more efficiently, we generated a category of rare VUS-CS >20 in this study, but still, only a fraction of these appears responsible for the patients' diabetes. Second, the number of genes covered in this study might not be large enough. For example, many genes for syndromic diabetes were not included in this panel in the hope that those patients might be clinically diagnosed otherwise. Third, even for the genes included in this study, the detection of variants might not be complete. The MLPA analysis was carried out only for common MODY genes, and deletions/duplications in other genes were not examined. Detection of mitochondrial m.3243A > G might also be incomplete, given the heteroplasmic nature of mitochondrial variants. Larger-scale panel sequencing, whole-exome sequencing or even whole-genome sequencing combined with the more sensitive detection of copy number variation might be required to address these sensitivity problems, although the chances of capturing benign variants would be increased by scaling up the number of target genes.

## CONCLUSIONS

By using the comprehensive targeted gene panel analysis, causative variants could be identified in 157 (46.2%) of 340 real-world Japanese patients with suspected MODY. The identification rate could be higher, as at least some of the 44 rare VUS-CS >20 appear to be truly causative. In addition to common MODY genes, variants in the $K_{ATP}$-channel genes were frequently identified, and a proportion of them was with inactivating variants, probably representing a different category of $K_{ATP}$-channel MODY. An expanded multigene panel including genes of insulin resistance should be used for this population.

## ACKNOWLEDGMENTS

This work was supported by a grant-in-aid for scientific research from the Ministry of Education, Culture, Sports, Science and Technology of Japan (18 K07895).

## DISCLOSURE

The authors declare no conflict of interest.
Approval of the research protocol: The study protocol was approved by the institutional review board of Osaka City General Hospital (No. 742).
Informed consent: Written informed consent was obtained either from the patient or their legal guardians.
Registry and the registration no. of the study/trial: N/A.
Animal studies: N/A.

## REFERENCES

1. Pang L, Colclough KC, Shepherd MH, *et al*. Improvements in awareness and testing have led to a threefold increase over 10 years in the identification of monogenic diabetes in the U.K. *Diabetes Care* 2022; 45: 642–649.
2. Hattersley AT, Greeley SAW, Polak M, *et al*. ISPAD clinical practice consensus guidelines 2018: the diagnosis and management of monogenic diabetes in children and adolescents. *Pediatr Diabetes* 2018; 10(Suppl 27): 47–63.
3. Zhang H, Colclough K, Gloyn AL, *et al*. Monogenic diabetes: a gateway to precision medicine in diabetes. *J Clin Invest* 2021; 131: e142244.
4. Broome DT, Pantalone KM, Kashyap SR, *et al*. Approach to the patient with MODY-monogenic diabetes. *J Clin Endocrinol Metab* 2021; 106: 237–250.
5. Pearson ER, Starkey BJ, Powell RJ, *et al*. Genetic cause of hyperglycaemia and response to treatment in diabetes. *Lancet* 2003; 362: 1275–1281.
6. Pearson ER, Pruhova S, Tack CJ, *et al*. Molecular genetics and phenotypic characteristics of MODY caused by hepatocyte nuclear factor 4alpha mutations in a large European collection. *Diabetologia* 2005; 48: 878–885.
7. Pearson ER, Flechtner I, Njølstad PR, *et al*. Switching from insulin to oral sulfonylureas in patients with diabetes due to Kir6.2 mutations. *N Engl J Med* 2006; 355: 467–477.
8. Steele AM, Shields BM, Wensley KJ, *et al*. Prevalence of vascular complications among patients with glucokinase mutations and prolonged, mild hyperglycemia. *JAMA* 2014; 311: 279–286.
9. Bonnefond A, Semple RK. Achievements, prospects and challenges in precision care for monogenic insulin-deficient and insulin-resistant diabetes. *Diabetologia* 2022; 65: 1782–1795.
10. Shields BM, Hicks S, Shepherd MH, *et al*. Maturity-onset diabetes of the young (MODY): how many cases are we missing? *Diabetologia* 2010; 53: 2504–2508.
11. Kropff J, Selwood MP, McCarthy MI, *et al*. Prevalence of monogenic diabetes in young adults: a community-based, cross-sectional study in Oxfordshire. *UK Diabetologia* 2011; 54: 1261–1263.
12. Pihoker C, Gilliam LK, Ellard S, *et al*. Prevalence, characteristics and clinical diagnosis of maturity onset diabetes of the young due to mutations in HNF1A, HNF4A, and glucokinase: results from the SEARCH for diabetes in youth. *J Clin Endocrinol Metab* 2013; 98: 4055–4062.
13. Kleinberger JW, Pollin TI. Undiagnosed MODY: time for action. *Curr Diab Rep* 2015; 15: 110.
14. Bansal V, Gassenhuber J, Phillips T, *et al*. Spectrum of mutations in monogenic diabetes genes identified from high-throughput DNA sequencing of 6888 individuals. *BMC Med* 2017; 15: 213.
15. Stanik J, Dusatkova P, Cinek O, *et al*. De novo mutations of GCK, HNF1A and HNF4A may be more frequent in MODY than previously assumed. *Diabetologia* 2014; 57: 480–484.
16. Kleinberger JW, Copeland KC, Gandica RG, *et al*. Monogenic diabetes in overweight and obese youth diagnosed with type 2 diabetes: the TODAY clinical trial. *Genet Med* 2018; 20: 583–590.

17. Ma RC, Chan JC. Type 2 diabetes in east Asians: similarities and differences with populations in Europe and the United States. *Ann N Y Acad Sci* 2013; 1281: 64–91.
18. Okura T, Nakamura R, Fujioka Y, *et al*. Body mass index ≥23 is a risk factor for insulin resistance and diabetes in Japanese people: a brief report. *PLoS One* 2018; 13: e0201052.
19. Yang YS, Kwak SH, Park KS. Update on monogenic diabetes in Korea. *Diabetes Metab J* 2020; 44: 627–639.
20. Liang H, Zhang Y, Li M, *et al*. Recognition of maturity-onset diabetes of the young in China. *J Diabetes Investig* 2021; 12: 501–509.
21. Park SS, Jang SS, Ahn CH, *et al*. Identifying pathogenic variants of monogenic diabetes using targeted panel sequencing in an east Asian population. *J Clin Endocrinol Metab* 2019; 104: 4188–4198.
22. Yorifuji T, Fujimaru R, Hosokawa Y, *et al*. Comprehensive molecular analysis of Japanese patients with pediatric-onset MODY-type diabetes mellitus. *Pediatr Diabetes* 2012; 13: 26–32.
23. Yorifuji T, Matsubara K, Sakakibara A, *et al*. Abnormalities in chromosome 6q24 as a cause of early-onset, non-obese, non-autoimmune diabetes mellitus without history of neonatal diabetes. *Diabet Med* 2015; 32: 963–967.
24. Kawakita R, Hosokawa Y, Fujimaru R, *et al*. Molecular and clinical characterization of glucokinase maturity-onset diabetes of the young (GCK-MODY) in Japanese patients. *Diabet Med* 2014; 31: 1357–1362.
25. Yorifuji T, Kawai M, Momoi T, *et al*. Nephropathy and growth hormone deficiency in a patient with mitochondrial tRNA(Leu(UUR)) mutation. *J Med Genet* 1996; 33: 621–622.
26. Robinson JT, Thorvaldsdóttir H, Winckler W, *et al*. Integrative genomics viewer. *Nat Biotechnol* 2011; 29: 24–26.
27. Richards S, Aziz N, Bale S, *et al*. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med* 2015; 17: 405–424.
28. Li Q, Wang K. InterVar: Clinical interpretation of genetic variants by the 2015 ACMG-AMP guidelines. *Am J Hum Genet* 2017; 100: 267–280.
29. Patel KA, Kettunen J, Laakso M, *et al*. Heterozygous RFX6 protein truncating variants are associated with MODY with reduced penetrance. *Nat Commun* 2017; 8: 888.
30. Bonnycastle LL, Chines PS, Hara T, *et al*. Autosomal dominant diabetes arising from a Wolfram syndrome 1 mutation. *Diabetes* 2013; 62: 3943–3950.
31. Kircher M, Witten DM, Jain P, *et al*. A general framework for estimating the relative pathogenicity of human genetic variants. *Nat Genet* 2014; 46: 310–315.
32. Chakera AJ, Spyer G, Vincent N, *et al*. The 0.1% of the population with glucokinase monogenic diabetes can be recognized by clinical characteristics in pregnancy: the Atlantic diabetes in pregnancy cohort. *Diabetes Care* 2014; 37: 1230–1236.
33. Saito-Hakoda A, Yorifuji T, Kanno J, *et al*. Nateglinide is effective for diabetes mellitus with reactive hypoglycemia in a child with a compound heterozygous ABCC8 mutation. *Clin Ped Endocrinol* 2012; 21: 45–52.
34. Breidbart E, Deng L, Lanzano P, *et al*. Frequency and characterization of mutations in genes in a large cohort of patients referred to MODY registry. *J Pediatr Endocrinol Metab* 2021; 34: 633–638.
35. Zmysłowska A, Jakiel P, Gadzalska K, *et al*. Next- generation sequencing is an effective method for diagnosing patients with different forms of monogenic diabetes. *Diabetes Res Clin Pract* 2022; 183: 109154.
36. Rafique I, Mir A, Saqib MAN, *et al*. Causal variants in maturity onset diabetes of the young (MODY) - a systematic review. *BMC Endocr Disord* 2021; 21: 223.
37. Horikawa Y. Maturity-onset diabetes of the young as a model for elucidating the multifactorial origin of type 2 diabetes mellitus. *J Diabetes Investig* 2018; 9: 704–712.
38. Ohkubo K, Nagashima M, Naito Y, *et al*. Genotypes of the pancreatic beta-cell K-ATP channel and clinical phenotypes of Japanese patients with persistent hyperinsulinaemic hypoglycaemia of infancy. *Clin Endocrinol (Oxf)* 2005; 62: 458–465.
39. Dung VC, Liem NT, Thao BP, *et al*. Molecular genetics and phenotype of 26 Vietnamese patients with congenital hyperinsulinism. *Int J Pediatr Endocrinol* 2013; 2013(Suppl 1): P179.
40. Arya VB, Guemes M, Nessa A, *et al*. Clinical and histological heterogeneity of congenital hyperinsulinism due to paternally inherited heterozygous ABCC8/KCNJ11 mutations. *Eur J Endocrinol* 2014; 171: 685–695.
41. Saint-Martin C, Zhou Q, Martin GM, *et al*. Monoallelic ABCC8 mutations are a common cause of diazoxide-unresponsive diffuse form of congenital hyperinsulinism. *Clin Genet* 2015; 87: 448–454.
42. Baier LJ, Muller YL, Remedi MS, *et al*. ABCC8 R1420H loss-of-function variant in a southwest American Indian community: association with increased birth weight and doubled risk of type 2 diabetes. *Diabetes* 2015; 64: 4322–4332.
43. Huopio H, Reimann F, Ashfield R, *et al*. Dominantly inherited hyperinsulinism caused by a mutation in the sulfonylurea receptor type 1. *J Clin Invest* 2000; 106: 897–906.
44. Işık E, Demirbilek H, Houghton JA, *et al*. Congenital Hyperinsulinism and evolution to sulfonylurea responsive diabetes later in life due to a novel homozygous p.L171F ABCC8 mutation. *J Clin Res Pediatr Endocrinol* 2019; 11: 82–87.
45. Matsutani N, Furuta H, Matsuno S, *et al*. Identification of a compound heterozygous inactivating ABCC8 gene mutation responsible for young-onset diabetes with exome sequencing. *J Diabetes Investig* 2020; 11: 333–336.

46. Karatojima M, Furuta H, Matsutani N, *et al*. A family in which people with a heterozygous ABCC8 gene mutation (p.Lys1385Gln) have progressed from hyperinsulinemic hypoglycemia to hyperglycemia. *J Diabetes* 2020; 12: 21–24.
47. Ovsyannikova AK, Rymar OD, Shakhtshneider EV, *et al*. Diabetes mellitus associated with the mutation of the ABCC8 gene (MODY 12): features of clinical course and therapy. *Diabetes Mellitus* 2019; 22: 88–94.
48. Huopio H, Jääskeläinen J, Komulainen J, *et al*. Acute insulin response tests for the differential diagnosis of congenital hyperinsulinism. *J Clin Endocrinol Metab* 2002; 87: 4502–4507.
49. Macmullen CM, Zhou Q, Snider KE, *et al*. Diazoxide-unresponsive congenital hyperinsulinism in children with dominant mutations of the β-cell sulfonylurea receptor SUR1. *Diabetes* 2011; 60: 1797–1804.
50. Takeuchi T, Ishigaki Y, Hirota Y, *et al*. Clinical characteristics of insulin resistance syndromes: a nationwide survey in Japan. *J Diabetes Investig* 2020; 11: 603–616.
51. Edghill EL, Khamis A, Weedon MN, *et al*. Sequencing PDX1 (insulin promoter factor 1) in 1788 UK individuals found 5% had a low-frequency coding variant, but these variants are not associated with type 2 diabetes. *Diabet Med* 2011; 28: 681–684.