# Identifying Pathogenic Variants of Monogenic Diabetes Using Targeted Panel Sequencing in an East Asian Population

Seung Shin Park,[1]* Se Song Jang,[2]* Chang Ho Ahn,[1] Jung Hee Kim,[1] Hye Seung Jung,[1] Young Min Cho,[1,3] Young Ah Lee,[4] Choong Ho Shin,[4,5] Jong Hee Chae,[4,5] Jae Hyun Kim,[6] Sung Hee Choi,[3,7] Hak C. Jang,[3,7] Jee Cheol Bae,[8] Jong Cheol Won,[9] Sung-Hoon Kim,[10,11] Jong-Il Kim,[2,12,13] Soo Heon Kwak,[1] and Kyong Soo Park[1,3,14]

[1]Department of Internal Medicine, Seoul National University Hospital, Seoul 03080, Republic of Korea; [2]Department of Biomedical Sciences, Seoul National University Graduate School, Seoul 03080, Republic of Korea; [3]Department of Internal Medicine, Seoul National University College of Medicine, 03080 Seoul, Republic of Korea; [4]Department of Pediatrics, Seoul National University Hospital, Seoul 03080, Republic of Korea; [5]Department of Pediatrics, Seoul National University College of Medicine, Seoul 03080, Republic of Korea; [6]Department of Pediatrics, Seoul National University Bundang Hospital, Seongnam 13620, Republic of Korea; [7]Department of Internal Medicine, Seoul National University Bundang Hospital, Seongnam 13620, Republic of Korea; [8]Department of Internal Medicine, Samsung Changwon Hospital, Changwon 51353, Republic of Korea; [9]Department of Internal Medicine, Sanggye Paik Hospital, Seoul 01757, Republic of Korea; [10]Department of Internal Medicine, Cheil General Hospital & Women's Healthcare Center, Seoul 04619, Republic of Korea; [11]Department of Internal Medicine, Dankook University College of Medicine, Seoul 301116, Republic of Korea; [12]Department of Biochemistry and Molecular Biology, Seoul National University College of Medicine, Seoul 03080, Republic of Korea; [13]Genomic Medicine Institute, Medical Research Center, Seoul National University, Seoul 03080, Republic of Korea; and [14]Department of Molecular Medicine and Biopharmaceutical Sciences, Graduate School of Convergence Science and Technology, Seoul National University, Seoul 03080, Republic of Korea

ORCiD numbers: 0000-0003-1230-0919 (S. H. Kwak).

**Purpose:** Monogenic diabetes is a specific type of diabetes in which precision medicine could be applied. In this study, we used targeted panel sequencing to investigate pathogenic variants in Korean patients with clinically suspected monogenic diabetes.

**Methods:** The eligibility criteria for inclusion were patients with nontype 1 diabetes with age at onset ≤30 years and body mass index (BMI) ≤30 kg/m$^2$. Among the 2090 patients with nontype 1 diabetes, 109 had suspected monogenic diabetes and underwent genetic testing. We analyzed 30 monogenic diabetes genes using targeted panel sequencing. The pathogenicity of the genetic variants was evaluated according to the American College of Medical Genetics and Genomics and Association for Molecular Pathology guidelines.

**Results:** Among the 109 patients with suspected monogenic diabetes, 23 patients (21.1%) harbored pathogenic/likely pathogenic variants. A total of 14 pathogenic/likely pathogenic variants of common maturity-onset diabetes of the young (MODY) genes were identified in *GCK*, *HNF1A*, *HNF4A*, and *HNF1B*. Other pathogenic/likely pathogenic variants were identified in *WFS1*, *INS*, *ABCC8*, and *FOXP3*. The mitochondrial DNA 3243A>G variant was identified in five participants. Patients with pathogenic/likely pathogenic variants had a significantly higher MODY probability, a lower BMI, and a lower C-peptide level than those without pathogenic/likely pathogenic variants (*P* = 0.007, *P* = 0.001, and *P* = 0.012, respectively).

**Conclusions:** Using targeted panel sequencing followed by pathogenicity evaluation, we were able to make molecular genetic diagnoses for 23 patients (21.1%) with suspected monogenic diabetes. Lower BMI, higher MODY probability, and lower C-peptide level were characteristics of these participants. (*J Clin Endocrinol Metab* 104: 4188–4198, 2019)

Diabetes truly comprises a heterogeneous group of metabolic disorders that share hyperglycemia as a common clinical characteristic. The relative contributions of genetic and environmental risk factors vary by individual and may determine optimal therapeutic strategies as well as clinical outcomes for the patient (1). However, the pathophysiological processes underlying diabetes are not fully understood, and there are unmet needs for determining the core pathophysiological process disrupted in each patient. Recently, precision medicine has been widely discussed, and substantial effort has been directed toward applying it in the field of diabetes, specifically regarding incorporation of genetic information (2).

Advances in next-generation sequencing technology have allowed us to investigate—at lower cost and with improved efficiency—sequence variants that cause monogenic diabetes (3–5). Monogenic diabetes includes maturity-onset diabetes of the young (MODY), neonatal diabetes, maternally inherited diabetes with deafness (MIDD), and genetic syndromes such as Wolfram syndrome, Bardet-Biedl syndrome, and lipodystrophies. Collectively, these diseases account for approximately 1% to 5% of all diabetes cases (6–8). Monogenic diabetes is a specific type of diabetes in which precision medicine could be readily applied for accurate diagnosis, individualized therapy, and prediction of clinical outcomes (9). In addition, precision medicine can help identify family members at risk and provide a basis for genetic counseling.

Despite previous efforts in identifying and characterizing monogenic diabetes, several areas require further investigation. There are certain clinical criteria for using genetic screening to diagnose MODY (10). However, not all patients with monogenic diabetes fulfill these criteria, and they are often undiagnosed or misdiagnosed as having type 1 or type 2 diabetes (11–13). It is not known which clinical criteria are sufficient for identifying patients who should undergo genetic testing. Most genetic studies on monogenic diabetes were conducted in Europe. The clinical characteristics of monogenic diabetes and the spectrum of mutations require further investigation in other populations, including East Asians (14, 15). An increasing number of genomic sequences are being generated using either targeted panel, whole exome, or genome sequencing. However, it is often difficult to interpret the pathogenicity of the identified genetic variant, especially when only the proband is available for investigation. Recently, the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG-AMP) published standards and guidelines for interpreting the pathogenicity of genetic variants (16). These guidelines are expected to improve the interpretation and classification of genetic variants of monogenic diabetes (17).

In this study, we used targeted panel sequencing to identify the genetic variants of 30 genes (including mitochondrial DNA) in 109 Korean patients with suspected monogenic diabetes from our cohort of 2090 patients with nontype 1 diabetes. The pathogenicity of the identified variants was analyzed according to the ACMG-AMP guidelines. In addition, the prevalence of monogenic diabetes in Koreans was estimated, and the clinical characteristics of these patients were analyzed.

## Methods

### Participants

This study was conducted as part of the ongoing Seoul National University Hospital Diabetes Clinic cohort, which was initiated in January 2001 and has enrolled 2689 patients with diabetes. We first excluded those with type 1 diabetes, defined as the presence of antiglutamic acid decarboxylase antibody or a C-peptide level <200 pmol/L or treated with basal-bolus insulin therapy. Among the remaining 2090 participants with nontype 1 diabetes (18), we selected 109 with suspected monogenic diabetes according to the clinical criteria of early-onset with an age at diagnosis ≤30 years and a body mass index (BMI) ≤30 kg/m² (Fig. 1). Clinical information, including demographics, initial presentation, family history and treatment history of diabetes, physical examination results, and laboratory test results for the 109 participants, were obtained at the time of enrollment (Table 1). The age at diagnosis was estimated by the patient's recall or review of the medical record. Five generally accepted criteria were used for the clinical diagnosis of MODY: (i) age at diagnosis <25 years; (ii) autosomal dominant inheritance across three generations; (iii) absence of insulin therapy within 5 years of diagnosis; (iv) insulin level within the normal range (plasma insulin ≥2.0 μIU/mL or plasma C-peptide ≥0.6 ng/mL); and (v) not obese (BMI <25 kg/m²) (19). The MODY probability of each participant was calculated using the MODY probability calculator, which incorporates clinical and biochemical information (20). Each participant provided written informed consent, which indicated whether he or she would receive the analysis results. The Institutional Review Board of the Biomedical Research Institute at Seoul National University Hospital approved the
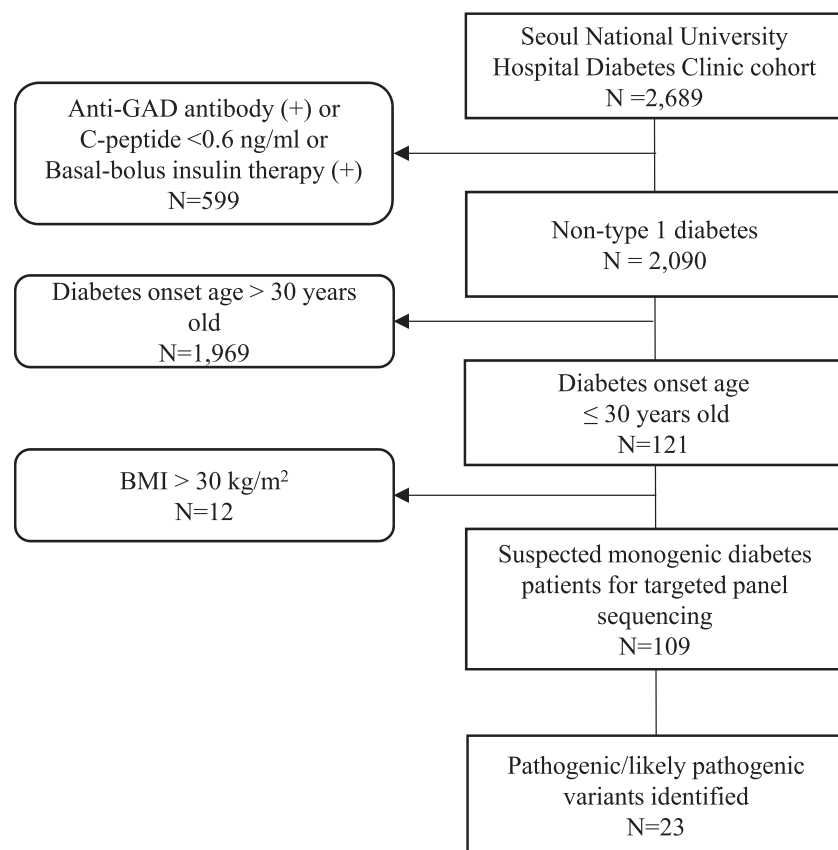
**Figure 1.** Flowchart of patients recruited for targeted panel sequencing of monogenic diabetes. Patients with nontype 1 diabetes with an age at onset ≤30 y and a BMI ≤30 kg/m² were selected from the Seoul National University Hospital Diabetes Clinic cohort. A total of 109 patients with suspected monogenic diabetes were selected for targeted panel sequencing. GAD, glutamic acd decarboxylase.

study protocol (IRB no. 1612-068-813). This study was conducted according to the principles of the Declaration of Helsinki [21].

## Protocol for targeted panel sequencing

Clinical-grade targeted panel sequencing was performed using genomic DNA extracted from peripheral blood leukocytes. The sequencing experiments were performed at Macrogen, Inc. (Seoul, Republic of Korea). The custom-designed capture probes included the exonic and untranslated regions of 30 genes (target region of approximately 93 kb) known to cause monogenic diabetes [18]. The probe was designed using Agilent SureDesign (Agilent Technology, Santa Clara, CA) software and was captured using the SureSelectXT Custom Kit (Agilent Technology). Captured DNA fragments were paired-end sequenced with a clinical diagnostic purpose using the Illumina HiSeq 2500 Sequencing System (Illumina, San Diego, CA) according to the manufacturer's instructions.

## Variant calling and annotation

The sequenced reads were aligned to the human reference genome (GRCh37) using the Burrows-Wheeler Aligner (v.0.7.15) [22]. Picard software (v.2.9.0) (http://broadinstitute.github.io/picard/) and the Genome Analysis Toolkit (v.3.8) [23] were used for the elimination of PCR duplicates, realignment around insertions or deletions, and base recalibration. The Genome

Analysis Toolkit HaplotypeCaller (genomic variant call format mode) was used for calling variants, such as single nucleotide variants, small insertions, and deletions. All variants were annotated using ANNOVAR [24] and InterVar [25]. Both annotation tools integrate a number of population databases such as the 1000 Genomes Project [26] and the Exome Aggregation Consortium Project [27], disease phenotype databases such as ClinVar [28], and the National Center for Biotechnology Information Reference Sequence Database. Further annotation was achieved using the Human Gene Mutation Database (HGMD) professional version release 2018.1 [29]. For copy number variation (CNV) calling, the outlier-based approach using reads per kilobase per million mapped reads for each capture probe was used; these values were calculated with Copy Number Inference From Exome Reads software [30], including only the reads with mapping quality above 15.

## Variant selection

We selected variants according to the following procedures. First, variants in highly repetitive sequences were removed using the Repeat Masker program (http://repeatmasker.org). Second, nonsilent variants (nonsynonymous, stop gain, stop loss, start loss, frameshift, splice site variants) were selected. Third, variants with a frequency <1% in all population databases [Genome Aggregation Database [27], 1000 Genomes Project [26], Exome Aggregation Consortium Project [27], and National Heart, Lung, and Blood Institute Exome Sequencing Project [31]] were selected. In addition, variants previously reported as being likely pathogenic or pathogenic in ClinVar or as high-confidence disease-causing mutations in HGMD were selected. A total of 80 rare and nonsilent variants were selected for a further detailed evaluation of pathogenicity according to the ACMG-AMP guidelines.

## Application of ACMG-AMP guidelines

ACMG-AMP standards and guidelines were used to evaluate the pathogenicity of the selected sequence variants [16]. In brief, the ACMG-AMP guideline classifies variants as pathogenic, likely pathogenic, of uncertain significance, likely benign, and benign according to the combination of 28 evidence attributes for pathogenicity and benign impact [18]. 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. As evidence of benign impact, one stand-alone, four strong, and seven supporting attributes exist. Two study investigators independently analyzed the pathogenicity of each identified rare, nonsilent variant by strictly following the ACMG-AMP guidelines. InterVar software was used to automatically determine eight of 16 pathogenic criteria

**Table 1.    Clinical Characteristics of Study Participants With or Without Pathogenic/Likely Pathogenic Variants**

| Variables | With Pathogenic/Likely Pathogenic Variants (N = 23) | Without Pathogenic/Likely Pathogenic Variants (N = 86) | Total (N = 109) | P |
|---|---|---|---|---|
| Male, N (%) | 10 (43.5) | 40 (46.5) | 50 (45.9) | 0.981 |
| Age at diagnosis, y | 18.9 ± 7.7 | 20.6 ± 7.0 | 20.2 ± 7.1 | 0.301 |
| SBP, mmHg | 116.7 ± 12.0 | 123.6 ± 14.9 | 122.2 ± 14.5 | **0.046** |
| BMI, kg/m$^2$ | 21.2 ± 3.0 | 23.6 ± 3.2 | 23.1 ± 3.3 | **0.001** |
| HbA1c, mmol/mol | 65.0 ± 22.0 | 72.6 ± 25.4 | 71.0 ± 24.8 | 0.194 |
| HbA1c, % | 8.1 ± 2.0 | 8.8 ± 2.3 | 8.6 ± 2.3 | 0.194 |
| C-peptide, ng/mL | 1.7 ± 1.2 | 2.7 ± 2.5 | 2.5 ± 2.3 | **0.012** |
| Fasting glucose, mg/dL | 145.4 ± 62.8 | 179.0 ± 84.3 | 171.9 ± 81.1 | 0.078 |
| Oral antidiabetics use, N (%) | 16 (69.6) | 62 (72.1) | 78 (71.6) | 1.000 |
| Insulin use, N (%) | 12 (52.2) | 50 (58.1) | 62 (56.9) | 0.782 |
| MODY probability, % | 64.5 ± 17.1 | 51.8 ± 25.4 | 54.5 ± 24.4 | **0.007** |
| Number of MODY criteria fulfilled | | | | 0.054 |
| 2 | 5 (21.7) | 7 (8.1%) | 12 (11.0) | |
| 3 | 4 (17.4) | 36 (41.9) | 40 (36.7) | |
| 4 | 8 (34.8) | 31 (36.0) | 39 (35.8) | |
| 5 | 6 (26.1) | 12 (14.0) | 18 (16.5) | |

Data are shown as mean ± SD or N (%). Boldface type denotes $P < 0.05$.

Abbreviations: HbA1c, hemoglobin A1c; SBP, systolic blood pressure.

(PVS1, PS3, PM1, PM2, PM4, PM5, PP2, PP3) (25). PS1 was coded positive when the same amino acid change was identified as pathogenic (two or more gold star reviews) in ClinVar or as high-confidence disease-causing mutations in the HGMD database. When DNA samples were available for the family members, Sanger sequencing results were used to determine PS2 and PP1. In the absence of familial sequencing data, PM6 was coded positive only when there was no familial history of early-onset diabetes and *de novo* mutation was strongly suspected. In the autosomal recessive disorder, PM3 was determined to be positive when *trans*-mutations were identified in one gene. PP4 status was activated when subjects fulfilled more than three clinical diagnostic criteria described previously or had specific characteristics of monogenic diabetes, such as renal cysts in the case of *HNF1B*.

### Statistical analysis

The characteristics of patients with or without pathogenic/likely pathogenic variants were compared. The Student *t* test was used to compare continuous variables. For categorical variables, the $\chi^2$ test was used to analyze differences between two groups. Data are shown as the means ± SD or N (%). $P < 0.05$ was considered statistically significant.

## Results

### Characteristics of the study participants

The clinical characteristics of the study participants are shown in Table 1. Among the 109 participants with clinically suspected monogenic diabetes who met the eligibility criteria, 50 (45.9%) were men, and the average age at diagnosis was 20.2 ± 7.1 years. There were 45 participants (41.3%) who were diagnosed with diabetes before the age of 20 years. The average hemoglobin A1c of study participants was 71.0 mmol/mol (8.6%). A total

of 78 participants (71.6%) were using oral antidiabetic medications, and 62 (56.9%) were using insulin. The average MODY probability, calculated using the MODY probability calculator, was 54.5%. For 42 participants (38.5%), there was a positive family history of diabetes in three generations, and for 53 participants (48.6%), there was a family history of diabetes in two generations. A total of 97 participants (89.0%) fulfilled more than three clinical diagnostic criteria of MODY, and 18 subjects (16.5%) 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 that covered more than 30× and 100× are shown in an online repository (18). The average depth of coverage for the entire target region was 730×. More than 98% of bases covered more than 30× in most of the genes except for *GATA4*, *CEL*, *PTF1A*, *KCNJ11*, and *GATA6*. No CNVs were detected in the 30 selected genes in our cohort. A total of 80 rare, nonsilent variants were identified in 22 genes and were evaluated for pathogenicity according to the ACMG-AMP guideline (18). There were nine variants (11.3%) with discrepant interpretations between the two investigators after initial review, and the two investigators reached a consensus after discussion (18). Among the 80 variants, one variant was identified as benign, 15 variants were identified as likely benign, and 46 variants were classified as having uncertain significance. A total of 14 likely pathogenic and four pathogenic variants were identified (Table 2). In addition to the 80 nuclear DNA

**Table 2. List of Identified Pathogenic/Likely Pathogenic Variants of Monogenic Diabetes**

| ID | Sex | Age at Dx (y) | BMI (kg/m²) | Three-Generation FHx | Extra-Pancreatic Features | Diagnostic Criteria | MODY PPV (%) | Chr | Gene Name | Position (hg19) | HGVS | Effect | Pathogenicity |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| MDT054 | F | 24 | 25.2 | X | — | 5/5 | 75.5 | Chr20 | HNF4A | 43,042,354 | p.Arg136Trp | Missense | Likely pathogenic |
| MDT034 | F | 10 | 21.9 | O | — | 3/5 | 75.5 | Chr20 | HNF4A | 42,984,447 | p.Met1Ile | Start lost | Pathogenic |
| MDT071 | M | 17 | 23.1 | O | — | 5/5 | 75.5 | Chr20 | HNF4A | 43,034,848 | p.Arg89Gln | Missense | Likely pathogenic |
| MDT011 | M | 28 | 22.2 | O | — | 5/5 | 58.0 | Chr7 | GCK | 44,187,420 | p.Asn231Ser | Missense | Likely pathogenic |
| MDT090 | M | 16 | 19.6 | X | — | 3/5 | 32.8 | Chr7 | GCK | 44,190,569 | p.His156fs | Frameshift | Pathogenic |
| MDT092 | F | 30 | 19.6 | X | — | 4/5 | 62.4 | Chr7 | GCK | 44,185,121 | p.Gly410Ser | Missense | Likely pathogenic |
| MDTC003 | F | 12 | 19.6 | O | — | 5/5 | 75.5 | Chr7 | GCK | 44,189,657 | p.Leu164Phe | Missense | Pathogenic |
| MDT116 | F | 30 | 26.1 | X | — | 2/5 | 32.9 | Chr7 | GCK | 44,186,189 | p.Met298Val | Missense | Likely pathogenic |
| MDTC007 | F | 11 | 14.3 | O | — | 4/5 | 12.6 | Chr7 | GCK | 44,184,791 | p.Gly448Ser | Missense | Likely pathogenic |
| MDT129 | F | 30 | 20.8 | X | — | 2/5 | 62.4 | Chr7 | GCK | 44,191,868 | c.363+2T>C | Splice site | Pathogenic |
| MDTB002 | F | 27 | 20.5 | O | — | 4/5 | 75.5 | Chr12 | HNF1A | 121,426,805 | p.Tyr166Asn | Missense | Likely pathogenic |
| MDTC001 | F | 9 | 17.1 | O | — | 5/5 | 75.7 | Chr12 | HNF1A | 121,416,648 | p.Leu26Gln | Missense | Likely Pathogenic |
| MDT114 | F | 27 | 21.1 | O | — | 4/5 | 62.4 | Chr12 | HNF1A | 121,437,361 | p.Val567Ile | Missense | Likely pathogenic |
| MDTC002 | M | 11 | 22.4 | X | Multiple renal cysts | 4/5 | 58.0 | Chr17 | HNF1B | 36,099,472 | p.Leu168Pro | Missense | Likely pathogenic |
| MDT019 | M | 17 | 24.3 | O | — | 5/5 | 75.5 | Chr4 | WFS1 | 6,303,407 | p.Arg629Trp | Missense | Likely pathogenic |
| MDT033 | F | 10 | 25.6 | O | — | 2/5 | 75.5 | Chr11 | INS | 2,182,533 | c.−60C>G | 5' UTR | Likely pathogenic |
| MDT113 | M | 14 | 22.9 | X | — | 4/5 | 75.5 | Chr11 | ABCC8 | 17,415,926 | p.Gly1478Arg | Missense | Likely pathogenic |
| MDT006 | M | 18 | 22.1 | X | — | 5/5 | 75.5 | ChrX | FOXP3 | 49,112,207 | p.Gln200Arg | Missense | Likely pathogenic |
| MDT041 | M | 24 | 18.8 | X | MHx | 4/5 | 75.5 | Mitochondria | MT-TL1 | 3243 | m.3243A>G | tRNA variant | Pathogenic |
| MDT097 | M | 27 | 18.3 | X | Hearing loss, MHx | 2/5 | 45.5 | Mitochondria | MT-TL1 | 3243 | m.3243A>G | tRNA variant | Pathogenic |
| MDT118 | M | 19 | 25.0 | X | MHx | 3/5 | 62.4 | Mitochondria | MT-TL1 | 3243 | m.3243A>G | tRNA variant | Pathogenic |
| MDT121 | F | 30 | 18.3 | X | MHx | 2/5 | 62.4 | Mitochondria | MT-TL1 | 3243 | m.3243A>G | tRNA variant | Pathogenic |
| MDT126 | F | 13 | 17.8 | X | Hearing loss, MHx | 3/5 | 8.2 | Mitochondria | MT-TL1 | 3243 | m.3243A>G | tRNA variant | Pathogenic |

Abbreviations: Chr, chromosome; Dx, diagnosis; F, female; FHx, family history of diabetes; hg19, genome reference consortium human build 37; HGVS, Human Genome Variation Society; M, male; MHx, maternal history of diabetes; PPV, positive predictive value.

variants, a pathogenic variant in mitochondrial DNA, 3243A>G, was identified in five participants. Overall, pathogenic/likely pathogenic variants were identified in 23 of 109 participants with suspected monogenic diabetes (21.1%; 95% CI: 14.5% to 29.7%) and constituted approximately 1.1% (95% CI: 0.7% to 1.7%) of the 2090 participants with nontype 1 diabetes in the Seoul National University Hospital Diabetes Clinic cohort. The pedigrees of these participants are shown in an online repository (18).

## Pathogenic variants of monogenic diabetes genes

Among four relatively common MODY genes (*GCK*, *HNF1A*, *HNF4A*, and *HNF1B*), a total of 14 pathogenic/likely pathogenic variants (12.8%) were identified in the 109 patients with suspected monogenic diabetes. *GCK* MODY was most common (N = 7; 50.0%), followed by *HNF1A* MODY (N = 3; 21.4%), *HNF4A* MODY (N = 3; 21.4%), and *HNF1B* MODY (N = 1; 7.1%). The pathogenic evidence attributes according to the ACMG-AMP guidelines for these variants are shown in Table 3. All pathogenic or likely pathogenic variants were absent in the 1000 Genomes Project database. Clinical characteristics of participants with pathogenic/likely pathogenic variants are shown in Table 2. The patient with an *HNF1B* pathogenic variant was confirmed to have multiple small cortical cysts in both kidneys. Pathogenic or likely pathogenic variants were identified in other rare monogenic diabetes genes, including *WFS1*, *INS*, *ABCC8*, *FOXP3*, and mitochondrial *MT-TL1*. Although variants in *WFS1* are usually known to cause Wolfram syndrome in recessive mode of inheritance, we considered one variant (p.Arg629Trp) to be likely pathogenic on the basis of previously noted studies, family history, and extremely low population frequency (32, 33). Mitochondria variant m.3243A>G, which is well known to be a causative mutation of MIDD, was confirmed in five participants. Among five patients with a mitochondrial DNA 3243A>G mutation, all had positive maternal history of diabetes and two had bilateral hearing loss.

## Characteristics of patients with pathogenic/likely pathogenic variants

The age at diabetes onset in subjects with pathogenic/likely pathogenic variants was not significantly different from that in subjects without pathogenic/likely pathogenic variants. The average BMI of participants with pathogenic/likely pathogenic variants was significantly lower than that of participants without pathogenic/likely pathogenic variants ($21.2 \pm 3.0$ vs $23.6 \pm 3.2$ kg/m$^2$; $P = 0.001$). None of the participants with pathogenic/likely pathogenic variant had BMI $\geq 27.5$ kg/m$^2$. The

average MODY probability was also significantly higher in participants with pathogenic/likely pathogenic variants ($64.5\% \pm 17.1\%$ vs $51.8\% \pm 25.4\%$; $P = 0.007$). The C-peptide levels of participants with pathogenic/likely pathogenic variants were significantly lower than those of participants without pathogenic/likely pathogenic variants ($1.7 \pm 1.2$ vs $2.7 \pm 2.5$ ng/mL; $P = 0.012$). The number of clinical diagnostic criteria fulfilled was not significantly different between the two groups ($P = 0.054$).

## Discussion

In this study, we selected 109 patients with clinically suspected monogenic diabetes in our cohort of 2090 patients with nontype 1 diabetes and performed targeted panel sequencing. Among these participants, we confirmed a molecular genetic diagnosis in 23 (21.1%). A total of 80 rare, nonsilent nuclear DNA variants in 22 genes were identified. After stringent application of the ACMG-AMP guidelines, we classified 14 variants to be likely pathogenic and four variants to be pathogenic. In addition, we identified five participants with a mitochondrial variant that resulted in MIDD. To the best of our knowledge, this study is one of the first to systematically apply targeted panel sequencing and the guidelines of ACMG-AMP for genetic diagnosis of monogenic diabetes in an East Asian population.

The molecular genetic diagnosis rate was 21.1% for patients with clinically suspected monogenic diabetes and 1.1% for participants with overall nontype 1 diabetes. This finding was similar to that of the largest and most comprehensive study on monogenic diabetes, conducted in the United Kingdom, that involved 2072 referred probands and showed a genetic diagnosis rate of 27% (12). However, in a Chinese study, the prevalence rates of *HNF1A* MODY and *GCK* MODY among those with suspected MODY were only 9% and 1%, respectively, (34). Similarly, the diagnosis rate was 12.6% for South Asians residing in the United Kingdom, which was lower than that of Europeans (35). This discrepancy could be attributed to the inability of clinical criteria to differentiate between MODY and early-onset type 2 diabetes in Asians (35). The genetic diagnosis rate could vary according to the clinical criteria used to select patients for genetic testing (35). Both the genes included in genetic testing and the sequencing methods may also result in different diagnosis rates. Although it has been suggested that ethnic differences may exist in the prevalence of monogenic diabetes, further investigation is required (36).

Among the patients with genetically confirmed MODY, *GCK* MODY (50.0%) was the most common, followed

**Table 3. Evidence Attributes of the Pathogenic/Likely Pathogenic Variants of Monogenic Diabetes**

| Gene | Variant | PVS1 | PS1 | PS2 | PS3 | PS4 | PM1 | PM2 | PM3 | PM4 | PM5 | PM6 | PP1 | PP2 | PP3 | PP4 | PP5 | Final |
|------|---------|------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-------|
| HNF4A | p.Arg136Trp | | | | | | + | + | | | | | + | | + | + | + | Likely pathogenic |
| HNF4A | p.Met1Ile | + | | | | | | + | | | | | | | + | + | | Pathogenic |
| HNF4A | p.Arg89Gln | | + | | | | + | + | | | | | | | + | + | + | Likely pathogenic |
| GCK | p.Asn231Ser | | | | | | + | + | | | | | | | + | + | + | Likely pathogenic |
| GCK | p.His156fs | + | | | | | | + | | | | | | | + | + | | Pathogenic |
| GCK | p.Gly410Ser | | | | | | + | + | | | | | + | | + | + | | Likely pathogenic |
| GCK | p.Leu164Phe | | | | | | + | + | | | | | | | + | + | + | Pathogenic |
| GCK | p.Met298 Val | | | | | | + | + | | | | | | | + | + | | Likely pathogenic |
| GCK | p.Gly448Ser | | | | | | + | + | | | | | | | + | + | + | Likely pathogenic |
| GCK | c.363+2T>C | + | | | | | | + | | | | | | | + | + | | Pathogenic |
| HNF1A | p.Tyr166Asn | | | | | | + | + | | | | | + | | + | + | | Likely pathogenic |
| HNF1A | p.Leu26Gln | | | | | | + | + | | | | | | | + | + | | Likely pathogenic |
| HNF1A | p.Val567Ile | | | | + | | + | + | | | | | | | | + | | Likely pathogenic |
| HNF1B | p.Leu168Pro | | | | | | + | + | | | | + | | | + | + | | Likely pathogenic |
| WFS1 | p.Arg629Trp | | | | + | | | + | | | | | | | + | + | | Likely pathogenic |
| INS | c.-60C>G | | | | + | | + | + | | | | | | | | + | | Likely pathogenic |
| ABCC8 | p.Gly1478Arg | | | | | | + | + | | | | | | | + | + | | Likely pathogenic |
| FOXP3 | p.Gln200Arg | | | | | | + | + | | | | | | | | + | | Likely pathogenic |

Evidence attributes for the pathogenic/likely pathogenic variants of monogenic diabetes genes. Individual attributes for pathogenic evidence are shown. Interpretation and classification were based on the ACMG-AMP guidelines.

by *HNF1A* MODY (21.4%) and *HNF4* MODY (21.4%). Our results are similar to those of previous reports showing that *GCK* MODY is one of the most commonly identified MODY subtypes (8, 10). However, the frequency of *HNF1A* MODY was lower and the frequency of *HNF4A* MODY was higher than the frequencies in Europeans (8, 10). This finding could be related to the small number of patients with genetically confirmed MODY in our study, and the clinical criteria for genetic screening may have affected the frequency of each MODY subtype (37). Although we have systematically investigated CNVs, targeted sequencing may have limited sensitivity for detecting CNVs, and this could be an issue for *HNF1B* MODY. The interpretation regarding *WFS1* variants requires caution. Most Wolfram syndromes are inherited recessively, and only a few cases of monoallelic mutations are reported to cause diabetes (38). One of the interesting findings of this study is the number of patients with the m.3243A>G mutation resulting in MIDD. Ethnic differences in the frequency of this variant have been suggested. The prevalence of this variant was reported to be 1% to 3% among Asian patients with diabetes and much lower in Europeans (39–41).

Clinical criteria to screen patients with MODY for genetic testing are important. However, a universal clinical criterion does not exist. Screening should be based on various clinical characteristics, including family history, onset age, insulin dependency, BMI, and extrapancreatic features. In addition, there is no discrete threshold for the degree of family history, age at diagnosis, or BMI. To be as inclusive as possible, we used the broad criteria of patients with nontype 1 diabetes, age at diagnosis ≤30 years, and BMI ≤30 kg/m$^2$. None of our participants with a pathogenic/likely pathogenic variant had BMI ≥27.5 kg/m$^2$. This suggests that in East Asians or at least in our population, BMI ≥27.5 kg/m$^2$ could be an exclusion criterion for testing monogenic diabetes. Among patients with clinically suspected monogenic diabetes, subjects with pathogenic/likely pathogenic variants still had a lower BMI and lower C-peptide levels than those without these variants. This result is in accordance with previous reports showing that individuals with genetically confirmed MODY have fewer metabolic features common to type 2 diabetes (13).

One option for estimating the likelihood of finding a pathogenic variant is to use the MODY probability calculator, which takes eight clinical factors into account (20). This indicator, developed with clinical information derived mostly from Europeans, has not been validated in East Asians. However, it was interesting to find that those who had pathogenic/likely pathogenic variants had a significantly elevated MODY probability. This result suggests that the MODY probability calculator could be used as a screening tool in this population. Nevertheless, further

validation and refinement are necessary to determine the optimal cutoff values in non-European populations.

One of the strengths of this study is that we strictly applied ACMG-AMP guidelines for the interpretation of pathogenicity. We expected that applying the ACMG-AMP guidelines would result in a more objective and reproducible interpretation of variant pathogenicity. Two investigators reviewed the evidence attributes for 80 rare, nonsilent variants and made a consensus interpretation. However, it should be acknowledged that the initial discordance rate between the two investigators was 11.25%. In addition, as many as 46 variants were classified as having uncertain significance. Another strength of this study is that we screened participants from a relatively large patient cohort and performed targeted panel sequencing for 109 clinically selected participants. The high-quality sequencing was intended to cover more than 98% of the bases with more than 30× coverage for most genes.

Our study had certain limitations. First, the number of participants who underwent sequencing was not large. We may have missed participants with monogenic diabetes who had an onset after 30 years of age, and the overall prevalence may have been underestimated. Nevertheless, the molecular genetic diagnosis rate in individuals with an onset age >40 years was reported to be only 0.6% (8). Second, we had limited access to proband family members for genetic testing. Performing genetic testing on family members is still a critical step in confirming the diagnosis of monogenic diabetes. Third, as many as 78% of patients with suspected monogenic diabetes did not have a molecular genetic diagnosis. It is unclear whether these participants had early-onset type 2 diabetes or a not yet identified monogenic cause of diabetes, such as MODYX.

In conclusion, using targeted panel sequencing, we identified 23 patients (21.1%) among 109 participants with a clinically suspected monogenic cause of diabetes. *GCK* MODY was the most common MODY subtype, and participants with a molecular genetic diagnosis had a higher MODY probability, a lower BMI, and a lower C-peptide level. Additional large-scale studies are needed to confirm our findings and for a more detailed characterization of monogenic diabetes in Korea. We hope that our findings serve as a basis for precision medicine in terms of the diagnosis and treatment of monogenic diabetes in this East Asian population.

***Correspondence and Reprint Requests:*** Soo Heon Kwak, MD, Department of Internal Medicine, Seoul National University Hospital, 101 Daehak-ro, Jongno-gu, Seoul 03080, Republic of Korea. E-mail: shkwak@snu.ac.kr.

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