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).

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in USA

Received 6 November 2018. Accepted 8 April 2019.
First Published Online 12 April 2019

*S.S.P. and S.S.J. contributed equally to this study.

Abbreviations: ACMG-AMP, American College of Medical Genetics and Genomics and the Association for Molecular Pathology; BMI, body mass index; CNV, copy number variation; HGMD, Human Gene Mutation Database; MIDD, maternally inherited diabetes with deafness; MODY, maturity-onset diabetes of the young.

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/m^2 (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/m^2) (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

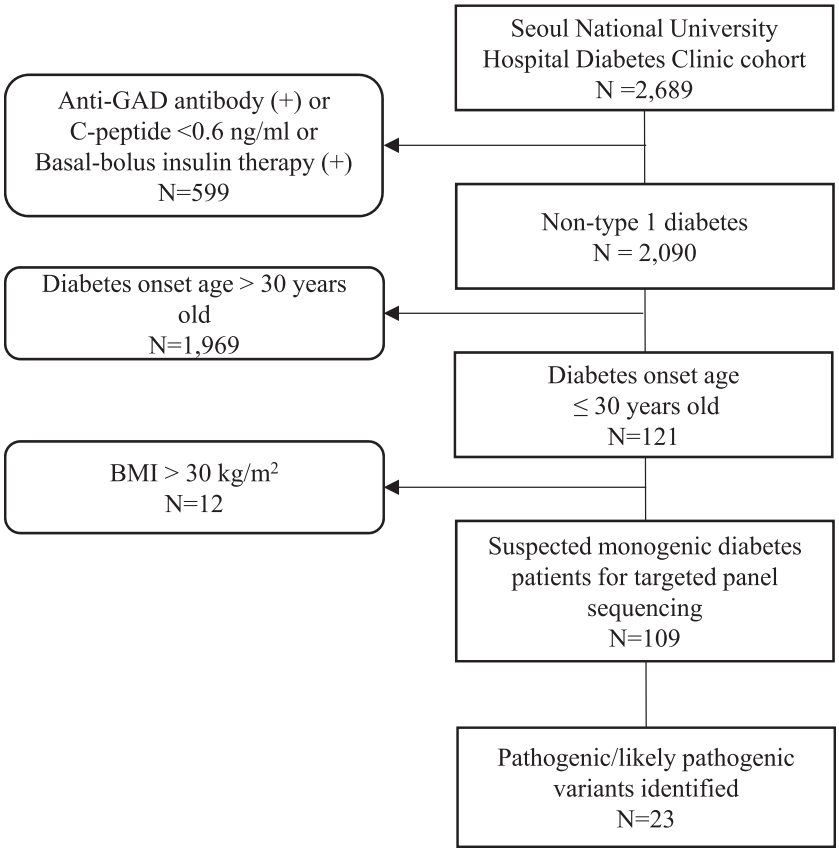

Figure 1. Flowchart of patients recruited for targeted panel sequencing of monogenic diabetes. Patients with nontype 1 diabetes with an age at onset ≤30 y and a BMI ≤30 kg/m^2 were selected from the Seoul National University Hospital Diabetes Clinic cohort. A total of 109 patients with suspected monogenic 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 χ^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/m^2)	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 ± 3.0 vs 23.6 ± 3.2 kg/m^2; $P = 0.001$). None of the participants with pathogenic/likely pathogenic variant had BMI ≥27.5 kg/m^2. The average MODY probability was also significantly higher in participants with pathogenic/likely pathogenic variants (64.5% ± 17.1% vs 51.8% ± 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 ± 1.2 vs 2.7 ± 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.

Acknowledgments

Computational analysis in this study was performed with support from the Seoul National University Hospital Next-Generation Sequencing Server.

Financial Support: This research was supported by a grant from the Korea Health Technology R&D Project through the Korea Health Industry Development Institute, funded by the Ministry of Health & Welfare, Republic of Korea [grant nos. HI15C3131 (to S.H.K.) and HI13C1468 to J.H.C.)].

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.

Disclosure Summary: The authors have nothing to disclose.

References and Notes

1. Kwak SH, Park KS. Pathophysiology of type 2 diabetes in Koreans. *Endocrinol Metab (Seoul)*. 2018;**33**(1):9–16.
2. McCarthy MI. Painting a new picture of personalised medicine for diabetes[published correction appears in *Diabetologia*. 2017;**60**(5):940]. *Diabetologia*. 2017;**60**(5):793–799.
3. Ellard S, Lango Allen H, De Franco E, Flanagan SE, Hysenaj G, Colclough K, Houghton JA, Shepherd M, Hattersley AT, Weedon MN, Caswell R. Improved genetic testing for monogenic diabetes using targeted next-generation sequencing. *Diabetologia*. 2013;**56**(9):1958–1963.
4. Johansson S, Irgens H, Chudasama KK, Molnes J, Aerts J, Roque FS, Jonassen I, Levy S, Lima K, Knappskog PM, Bell GI, Molven A, Njølstad PR. Exome sequencing and genetic testing for MODY. *PLoS One*. 2012;**7**(5):e38050.
5. Kim MK, Kwak SH, Kang S, Jung HS, Cho YM, Kim SY, Park KS. Identification of two cases of ciliopathy-associated diabetes and their mutation analysis using whole exome sequencing. *Diabetes Metab J*. 2015;**39**(5):439–443.
6. Permutt MA, Wasson J, Cox N. Genetic epidemiology of diabetes. *J Clin Invest*. 2005;**115**(6):1431–1439.
7. Irgens HU, Molnes J, Johansson BB, Ringdal M, Skrivarhaug T, Undlien DE, Søvik O, Joner G, Molven A, Njølstad PR. Prevalence of monogenic diabetes in the population-based Norwegian Childhood Diabetes Registry. *Diabetologia*. 2013;**56**(7):1512–1519.
8. Bansal V, Gassenhuber J, Phillips T, Oliveira G, Harbaugh R, Villarasa N, Topol EJ, Seufferlein T, Boehm BO. Spectrum of mutations in monogenic diabetes genes identified from high-throughput DNA sequencing of 6888 individuals. *BMC Med*. 2017;**15**(1):213.
9. Hattersley AT, Patel KA. Precision diabetes: learning from monogenic diabetes. *Diabetologia*. 2017;**60**(5):769–777.
10. Ellard S, Bellanné-Chantelot C, Hattersley AT; European Molecular Genetics Quality Network (EMQN) MODY group. Best practice guidelines for the molecular genetic diagnosis of maturity-onset diabetes of the young. *Diabetologia*. 2008;**51**(4):546–553.
11. Shepherd M, Shields B, Ellard S, Rubio-Cabezas O, Hattersley AT. A genetic diagnosis of *HNF1A* diabetes alters treatment and improves glycaemic control in the majority of insulin-treated patients. *Diabet Med*. 2009;**26**(4):437–441.
12. Shields BM, Hicks S, Shepherd MH, Colclough K, Hattersley AT, Ellard S. Maturity-onset diabetes of the young (MODY): how many cases are we missing? *Diabetologia*. 2010;**53**(12):2504–2508.
13. Pihoker C, Gilliam LK, Ellard S, Dabelea D, Davis C, Dolan LM, Greenbaum CJ, Imperatore G, Lawrence JM, Marcovina SM, Mayer-Davis E, Rodriguez BL, Steck AK, Williams DE, Hattersley AT; SEARCH for Diabetes in Youth Study Group. 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**(10):4055–4062.
14. Porter JR, Rangasami JJ, Ellard S, Gloyn AL, Shields BM, Edwards J, Anderson JM, Shaw NJ, Hattersley AT, Frayling TM, Plunkett M, Barrett TG. Asian MODY: are we missing an important diagnosis? *Diabet Med*. 2006;**23**(11):1257–1260.
15. Kawakita R, Hosokawa Y, Fujimaru R, Tamagawa N, Urakami T, Takasawa K, Moriya K, Mizuno H, Maruo Y, Takuwa M, Nagasaka H, Nishi Y, Yamamoto Y, Aizu K, Yorifuji T. Molecular and clinical characterization of glucokinase maturity-onset diabetes of the young (GCK-MODY) in Japanese patients. *Diabet Med*. 2014;**31**(11):1357–1362.
16. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E, Voelkerding K, Rehm HL. 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**(5):405–424.
17. Santana LS, Caetano LA, Costa-Riquetto AD, Quedas EPS, Nery M, Collett-Solberg P, Boguszewski MCS, Vendramini MF, Crisostomo LG, Floh FO, Zarabia ZI, Kohara SK, Guastapaglia L, Passone CGB, Sewaybricker LE, Jorge AAL, Teles MG. Clinical application of ACMG-AMP guidelines in *HNF1A* and *GCK* variants in a cohort of MODY families. *Clin Genet*. 2017;**92**(4):388–396.
18. Park SS, Jang SS, Ahn CH, Kim JH, Jung HS, Cho YM, Lee YA, Shin CH, Chae JH, Kim JH, Choi SH, Jang HC, Bae JC, Won JC, Kim S-H, Kim J-I, Kwak SH, Park KS. Data from: Identifying pathogenic variants of monogenic diabetes using targeted panel sequencing in an East Asian population. Figshare 2019. Deposited 13 February 2019. https://dx.doi.org/10.6084/m9.figshare.7296536.
19. Vaxillaire M, Froguel P. Monogenic diabetes in the young, pharmacogenetics and relevance to multifactorial forms of type 2 diabetes. *Endocr Rev*. 2008;**29**(3):254–264.
20. Shields BM, McDonald TJ, Ellard S, Campbell MJ, Hyde C, Hattersley AT. The development and validation of a clinical prediction model to determine the probability of MODY in patients with young-onset diabetes. *Diabetologia*. 2012;**55**(5):1265–1272.
21. World Medical Association. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. *JAMA*. 2013;**310**(20):2191–2194.
22. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics*. 2009;**25**(14):1754–1760.
23. McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky A, Garimella K, Altshuler D, Gabriel S, Daly M, DePristo MA. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Res*. 2010;**20**(9):1297–1303.
24. Wang K, Li M, Hakonarson H. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. *Nucleic Acids Res*. 2010;**38**(16):e164.
25. Li Q, Wang K. InterVar: clinical interpretation of genetic variants by the 2015 ACMG-AMP Guidelines. *Am J Hum Genet*. 2017;**100**(2):267–280.
26. The 1000 Genomes Project Consortium, Auton A, Abecasis GR, Altshuler DM, Durbin RM, Abecasis GR, Bentley DR, Chakravarti A, Clark AG, Donnelly P, Eichler EE, Flicek P, Gabriel SB, Gibbs RA, Green ED, Hurles ME, Knoppers BM, Korbel JO, Lander ES, Lee C, Lehrach H, Mardis ER, Marth GT, McVean GA, Nickerson DA, Schmidt JP, Sherry ST, Wang J, Wilson RK, Gibbs RA, Boerwinkle E, Doddapaneni H, Han Y, Korchina V, Kovar C, Lee S, Muzny D, Reid JG, Zhu Y, Wang J, Chang Y, Feng Q, Fang X, Guo X, Jian M, Jiang H, Jin X, Lan T, Li G, Li J, Li Y, Liu S, Liu X, Lu Y, Ma X, Tang M, Wang B, Wang G, Wu H, Wu R, Xu X, Yin Y, Zhang D, Zhang W, Zhao J, Zhao M, Zheng X, Lander ES, Altshuler DM, Gabriel SB, Gupta N, Gharani N, Toji LH, Gerry NP, Resch AM, Flicek P, Barker J, Clarke L, Gil L, Hunt SE, Kelman G, Kulesha E, Leinonen R, McLaren WM, Radhakrishnan R, Roa A, Smirnov D, Smith RE, Streeter I, Thormann A, Toneva I,

Vaughan B, Zheng-Bradley X, Bentley DR, Grocock R, Humphray S, James T, Kingsbury Z, Lehrach H, Sudbrak R, Albrecht MW, Amstislavskiy VS, Borodina TA, Lienhard M, Mertes F, Sultan M, Timmermann B, Yaspo M-L, Mardis ER, Wilson RK, Fulton L, Fulton R, Sherry ST, Ananiev V, Belaia Z, Beloslyudtsev D, Bouk N, Chen C, Church D, Cohen R, Cook C, Garner J, Hefferon T, Kimelman M, Liu C, Lopez J, Meric P, O'Sullivan C, Ostapchuk Y, Phan L, Ponomarov S, Schneider V, Shekhtman E, Sirotkin K, Slotta D, Zhang H, McVean GA, Durbin RM, Balasubramaniam S, Burton J, Danecek P, Keane TM, Kolb-Kokocinski A, McCarthy S, Stalker J, Quail M, Schmidt JP, Davies CJ, Gollub J, Webster T, Wong B, Zhan Y, Auton A, Campbell CL, Kong Y, Marcketta A, Gibbs RA, Yu F, Antunes L, Bainbridge M, Muzny D, Sabo A, Huang Z, Wang J, Coin LJM, Fang L, Guo X, Jin X, Li G, Li Q, Li Y, Li Z, Lin H, Liu B, Luo R, Shao H, Xie Y, Ye C, Yu C, Zhang F, Zheng H, Zhu H, Alkan C, Dal E, Kahveci F, Marth GT, Garrison EP, Kural D, Lee W-P, Fung Leong W, Stromberg M, Ward AN, Wu J, Zhang M, Daly MJ, DePristo MA, Handsaker RE, Altshuler DM, Banks E, Bhatia G, del Angel G, Gabriel SB, Genovese G, Gupta N, Li H, Kashin S, Lander ES, McCarroll SA, Nemesh JC, Poplin RE, Yoon SC, Lihm J, Makarov V, Clark AG, Gottipati S, Keinan A, Rodriguez-Flores JL, Korbel JO, Rausch T, Fritz MH, Stütz AM, Flicek P, Beal K, Clarke L, Datta A, Herrero J, McLaren WM, Ritchie GRS, Smith RE, Zerbino D, Zheng-Bradley X, Sabeti PC, Shlyakhter I, Schaffner SF, Vitti J, Cooper DN, Ball EV, Stenson PD, Bentley DR, Barnes B, Bauer M, Keira Cheetham R, Cox A, Eberle M, Humphray S, Kahn S, Murray L, Peden J, Shaw R, Kenny EE, Batzer MA, Konkel MK, Walker JA, MacArthur DG, Lek M, Sudbrak R, Amstislavskiy VS, Herwig R, Mardis ER, Ding L, Koboldt DC, Larson D, Ye K, Gravel S, Swaroop A, Chew E, Lappalainen T, Erlich Y, Gymrek M, Frederick Willems T, Simpson JT, Shriver MD, Rosenfeld JA, Bustamante CD, Montgomery SB, De La Vega FM, Byrnes JK, Carroll AW, DeGorter MK, Lacroute P, Maples BK, Martin AR, Moreno-Estrada A, Shringarpure SS, Zakharia F, Halperin E, Baran Y, Lee C, Cerveira E, Hwang J, Malhotra A, Plewczynski D, Radew K, Romanovitch M, Zhang C, Hyland FCL, Craig DW, Christoforides A, Homer N, Izatt T, Kurdoglu AA, Sinari SA, Squire K, Sherry ST, Xiao C, Sebat J, Antaki D, Gujral M, Noor A, Ye K, Burchard EG, Hernandez RD, Gignoux CR, Haussler D, Katzman SJ, James Kent W, Howie B, Ruiz-Linares A, Dermitzakis ET, Devine SE, Abecasis GR, Min Kang H, Kidd JM, Blackwell T, Caron S, Chen W, Emery S, Fritsche L, Fuchsberger C, Jun G, Li B, Lyons R, Scheller C, Sidore C, Song S, Sliwerska E, Taliun D, Tan A, Welch R, Kate Wing M, Zhan X, Awadalla P, Hodgkinson A, Li Y, Shi X, Quitadamo A, Lunter G, McVean GA, Marchini JL, Myers S, Churchhouse C, Delaneau O, Gupta-Hinch A, Kretzschmar W, Iqbal Z, Mathieson I, Menelaou A, Rimmer A, Xifara DK, Oleksyk TK, Fu Y, Liu X, Xiong M, Jorde L, Witherspoon D, Xing J, Eichler EE, Browning BL, Browning SR, Hormozdiari F, Sudmant PH, Khurana E, Durbin RM, Hurles ME, Tyler-Smith C, Albers CA, Ayub Q, Balasubramaniam S, Chen Y, Colonna V, Danecek P, Jostins L, Keane TM, McCarthy S, Walter K, Xue Y, Gerstein MB, Abyzov A, Balasubramanian S, Chen J, Clarke D, Fu Y, Harmanci AO, Jin M, Lee D, Liu J, Jasmine Mu X, Zhang J, Zhang Y, Li Y, Luo R, Zhu H, Alkan C, Dal E, Kahveci F, Marth GT, Garrison EP, Kural D, Lee W-P, Ward AN, Wu J, Zhang M, McCarroll SA, Handsaker RE, Altshuler DM, Banks E, del Angel G, Genovese G, Hartl C, Li H, Kashin S, Nemesh JC, Shakir K, Yoon SC, Lihm J, Makarov V, Degenhardt J, Korbel JO, Fritz MH, Meiers S, Raeder B, Rausch T, Stütz AM, Flicek P, Paolo Casale F, Clarke L, Smith RE, Stegle O, Zheng-Bradley X, Bentley DR, Barnes B, Keira Cheetham R, Eberle M, Humphray S, Kahn S, Murray L, Shaw R, Lameijer E-W, Batzer MA, Konkel MK, Walker JA, Ding L, Hall I, Ye K, Lacroute P, Lee C, Cerveira E, Malhotra A, Hwang J, Plewczynski D, Radew K, Romanovitch M, Zhang C, Craig DW, Homer N, Church D, Xiao C, Sebat J, Antaki D, Bafna V, Michaelson J, Ye K, Devine SE, Gardner EJ, Abecasis GR, Kidd JM, Mills RE, Dayama G, Emery S, Jun G, Shi X, Quitadamo A, Lunter G, McVean GA, Chen K, Fan X, Chong Z, Chen T, Witherspoon D, Xing J, Eichler EE, Chaisson MJ, Hormozdiari F, Huddleston J, Malig M, Nelson BJ, Sudmant PH, Parrish NF, Khurana E, Hurles ME, Blackburne B, Lindsay SJ, Ning Z, Walter K, Zhang Y, Gerstein MB, Abyzov A, Chen J, Clarke D, Lam H, Jasmine Mu X, Sisu C, Zhang J, Zhang Y, Gibbs RA, Yu F, Bainbridge M, Challis D, Evani US, Kovar C, Lu J, Muzny D, Nagaswamy U, Reid JG, Sabo A, Yu J, Guo X, Li W, Li Y, Wu R, Marth GT, Garrison EP, Fung Leong W, Ward AN, del Angel G, DePristo MA, Gabriel SB, Gupta N, Hartl C, Poplin RE, Clark AG, Rodriguez-Flores JL, Flicek P, Clarke L, Smith RE, Zheng-Bradley X, MacArthur DG, Mardis ER, Fulton R, Koboldt DC, Gravel S, Bustamante CD, Craig DW, Christoforides A, Homer N, Izatt T, Sherry ST, Xiao C, Dermitzakis ET, Abecasis GR, Min Kang H, McVean GA, Gerstein MB, Balasubramanian S, Habegger L, Yu H, Flicek P, Clarke L, Cunningham F, Dunham I, Zerbino D, Zheng-Bradley X, Lage K, Berg Jespersen J, Horn H, Montgomery SB, DeGorter MK, Khurana E, Tyler-Smith C, Chen Y, Colonna V, Xue Y, Gerstein MB, Balasubramanian S, Fu Y, Kim D, Auton A, Marcketta A, Desalle R, Narechania A, Wilson Sayres MA, Garrison EP, Handsaker RE, Kashin S, McCarroll SA, Rodriguez-Flores JL, Flicek P, Clarke L, Zheng-Bradley X, Erlich Y, Gymrek M, Frederick Willems T, Bustamante CD, Mendez FL, David Poznik G, Underhill PA, Lee C, Cerveira E, Malhotra A, Romanovitch M, Zhang C, Abecasis GR, Coin L, Shao H, Mittelman D, Tyler-Smith C, Ayub Q, Banerjee R, Cerezo M, Chen Y, Fitzgerald TW, Louzada S, Massaia A, McCarthy S, Ritchie GR, Xue Y, Yang F, Gibbs RA, Kovar C, Kalra D, Hale W, Muzny D, Reid JG, Wang J, Dan X, Guo X, Li G, Li Y, Ye C, Zheng X, Altshuler DM, Flicek P, Clarke L, Zheng-Bradley X, Bentley DR, Cox A, Humphray S, Kahn S, Sudbrak R, Albrecht MW, Lienhard M, Larson D, Craig DW, Izatt T, Kurdoglu AA, Sherry ST, Xiao C, Haussler D, Abecasis GR, McVean GA, Durbin RM, Balasubramaniam S, Keane TM, McCarthy S, Stalker J, Chakravarti A, Knoppers BM, Abecasis GR, Barnes KC, Beiswanger C, Burchard EG, Bustamante CD, Cai H, Cao H, Durbin RM, Gerry NP, Gharani N, Gibbs RA, Gignoux CR, Gravel S, Henn B, Jones D, Jorde L, Kaye JS, Keinan A, Kent A, Kerasidou A, Li Y, Mathias R, McVean GA, Moreno-Estrada A, Ossorio PN, Parker M, Resch AM, Rotimi CN, Royal CD, Sandoval K, Su Y, Sudbrak R, Tian Z, Tishkoff S, Toji LH, Tyler-Smith C, Via M, Wang Y, Yang H, Yang L, Zhu J, Bodmer W, Bedoya G, Ruiz-Linares A, Cai Z, Gao Y, Chu J, Peltonen L, Garcia-Montero A, Orfao A, Dutil J, Martinez-Cruzado JC, Oleksyk TK, Barnes KC, Mathias RA, Hennis A, Watson H, McKenzie C, Qadri F, LaRocque R, Sabeti PC, Zhu J, Deng X, Sabeti PC, Asogun D, Folarin O, Happi C, Omoniwa O, Stremlau M, Tariyal R, Jallow M, Sisay Joof F, Corrah T, Rockett K, Kwiatkowski D, Kooner J, Tịnh Hiê`n Tn, Dunstan SJ, Thuy Hang N, Fonnie R, Garry R, Kanneh L, Moses L, Sabeti PC, Schieffelin J, Grant DS, Gallo C, Poletti G, Saleheen D, Rasheed A, Brooks LD, Felsenfeld AL, McEwen JE, Vaydylevich Y, Green ED, Duncanson A, Dunn M, Schloss JA, Wang J, Yang H, Auton A, Brooks LD, Durbin RM, Garrison EP, Min Kang H, Korbel JO, Marchini JL, McCarthy S, McVean GA, Abecasis GR. A global reference for human genetic variation. *Nature*. 2015;**526**(7571):68–74.

27. Lek M, Karczewski KJ, Minikel EV, Samocha KE, Banks E, Fennell T, O'Donnell-Luria AH, Ware JS, Hill AJ, Cummings BB, Tukiainen T, Birnbaum DP, Kosmicki JA, Duncan LE, Estrada K, Zhao F, Zou J, Pierce-Hoffman E, Berghout J, Cooper DN, Deflaux N, DePristo M, Do R, Flannick J, Fromer M, Gauthier L, Goldstein J, Gupta N, Howrigan D, Kiezun A, Kurki MI, Moonshine AL, Natarajan P, Orozco L, Peloso GM, Poplin R, Rivas MA, Ruano-Rubio V, Rose SA, Ruderfer DM, Shakir K, Stenson PD, Stevens C, Thomas BP, Tiao G, Tusie-Luna MT, Weisburd B, Won HH, Yu D, Altshuler DM, Ardissino D, Boehnke M, Danesh J, Donnelly S, Elosua R, Florez JC, Gabriel SB, Getz G, Glatt SJ, Hultman CM, Kathiresan S, Laakso M, McCarroll S, McCarthy MI, McGovern D, McPherson R, Neale BM, Palotie A, Purcell SM, Saleheen D,

Scharf JM, Sklar P, Sullivan PF, Tuomilehto J, Tsuang MT, Watkins HC, Wilson JG, Daly MJ, MacArthur DG; Exome Aggregation Consortium. Analysis of protein-coding genetic variation in 60,706 humans. *Nature*. 2016;**536**(7616):285–291.
28. Landrum MJ, Lee JM, Riley GR, Jang W, Rubinstein WS, Church DM, Maglott DR. ClinVar: public archive of relationships among sequence variation and human phenotype. *Nucleic Acids Res*. 2014;**42**(D1):D980–D985.
29. Stenson PD, Ball EV, Mort M, Phillips AD, Shaw K, Cooper DN. The Human Gene Mutation Database (HGMD) and its exploitation in the fields of personalized genomics and molecular evolution. Curr Protoc Bioinformatics. 2012;Chapter 1:Unit1.13.
30. Krumm N, Sudmant PH, Ko A, O'Roak BJ, Malig M, Coe BP, Quinlan AR, Nickerson DA, Eichler EE; NHLBI Exome Sequencing Project. Copy number variation detection and genotyping from exome sequence data. *Genome Res*. 2012;**22**(8):1525–1532.
31. Fu W, O'Connor TD, Jun G, Kang HM, Abecasis G, Leal SM, Gabriel S, Rieder MJ, Altshuler D, Shendure J, Nickerson DA, Bamshad MJ, Akey JM; NHLBI Exome Sequencing Project. Analysis of 6,515 exomes reveals the recent origin of most human protein-coding variants [published correction appears in Nature. 2013;495(7440):270]. *Nature*. 2012;**493**(7431):216–220.
32. Kadayifci A, Kepekci Y, Coskun Y, Huang Y. Wolfram syndrome in a family with variable expression. *Acta Med (Hradec Kralove)*. 2001;**44**(3):115–118.
33. Hofmann S, Philbrook C, Gerbitz KD, Bauer MF. Wolfram syndrome: structural and functional analyses of mutant and wild-type wolframin, the WFS1 gene product. *Hum Mol Genet*. 2003;**12**(16):2003–2012.
34. Xu JY, Dan QH, Chan V, Wat NM, Tam S, Tiu SC, Lee KF, Siu SC, Tsang MW, Fung LM, Chan KW, Lam KS. Genetic and clinical characteristics of maturity-onset diabetes of the young in Chinese patients. *Eur J Hum Genet*. 2005;**13**(4):422–427.
35. Misra S, Shields B, Colclough K, Johnston DG, Oliver NS, Ellard S, Hattersley AT. South Asian individuals with diabetes who are referred for MODY testing in the UK have a lower mutation pick-up rate than white European people. *Diabetologia*. 2016;**59**(10):2262–2265.
36. Kanthimathi S, Jahnavi S, Balamurugan K, Ranjani H, Sonya J, Goswami S, Chowdhury S, Mohan V, Radha V. Glucokinase gene mutations (MODY 2) in Asian Indians. *Diabetes Technol Ther*. 2014;**16**(3):180–185.
37. Yorifuji T, Fujimaru R, Hosokawa Y, Tamagawa N, Shiozaki M, Aizu K, Jinno K, Maruo Y, Nagasaka H, Tajima T, Kobayashi K, Urakami T. Comprehensive molecular analysis of Japanese patients with pediatric-onset MODY-type diabetes mellitus. *Pediatr Diabetes*. 2012;**13**(1):26–32.
38. Bonnycastle LL, Chines PS, Hara T, Huyghe JR, Swift AJ, Heikinheimo P, Mahadevan J, Peltonen S, Huopio H, Nuutila P, Narisu N, Goldfeder RL, Stitzel ML, Lu S, Boehnke M, Urano F, Collins FS, Laakso M. Autosomal dominant diabetes arising from a Wolfram syndrome 1 mutation. *Diabetes*. 2013;**62**(11):3943–3950.
39. Kwak SH, Park KS. Role of mitochondrial DNA variation in the pathogenesis of diabetes mellitus. *Front Biosci*. 2016;**21**(6):1151–1167.
40. Ng MC, Yeung VT, Chow CC, Li JK, Smith PR, Mijovic CH, Critchley JA, Barnett AH, Cockram CS, Chan JC. Mitochondrial DNA A3243G mutation in patients with early- or late-onset type 2 diabetes mellitus in Hong Kong Chinese. *Clin Endocrinol (Oxf)*. 2000;**52**(5):557–564.
41. Saker PJ, Hattersley AT, Barrow B, Hammersley MS, Horton V, Gillmer MD, Turner RC. UKPDS 21: low prevalence of the mitochondrial transfer RNA gene (tRNA $^{(Leu(UUR))}$) mutation at position 3243bp in UK Caucasian type 2 diabetic patients. *Diabet Med*. 1997;**14**(1):42–45.