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Identification of Candidate Gene Variants in Korean MODY Families by Whole-Exome Sequencing

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Key Words
Maturity-onset diabetes of the young · Type 2 diabetes mellitus · Whole-exome sequencing · *PTPRD* · *SYT9* · *WFS1*

Abstract
Aims: To date, 13 genes causing maturity-onset diabetes of the young (MODY) have been identified. However, there is a big discrepancy in the genetic locus between Asian and Caucasian patients with MODY. Thus, we conducted whole-exome sequencing in Korean MODY families to identify causative gene variants. ***Methods:*** Six MODY probands and their family members were included. Variants in the dbSNP135 and TIARA databases for Koreans and the variants with minor allele frequencies >0.5% of the 1000 Genomes database were excluded. We selected only the functional variants (gain of stop codon, frameshifts and nonsynonymous single-nucleotide variants) and conducted a case-control comparison in the family members. The selected variants were scanned for the previously introduced gene set implicated in glucose metabolism. ***Results:*** Three variants c.620C>T:p.Thr207Ile in *PTPRD*, c.559C>G:p.Gln187Glu in *SYT9*, and c.1526T>G:p.Val509Gly in *WFS1* were respectively identified in 3 families. We could not find any disease-causative alleles of known MODY 1–13 genes. Based on the predictive program, Thr207Ile in *PTPRD* was considered pathogenic. ***Conclusions:*** Whole-exome sequencing is a valuable method for the genetic diagnosis of MODY. Further evaluation is necessary about the role of *PTPRD, SYT9* and *WFS1* in normal insulin release from pancreatic beta cells.

Introduction

Maturity-onset diabetes of the young (MODY) is one of monogenic diabetes mellitus caused by a single-gene defect [1–3]. MODY is characterized by a young age onset before 25 years and autosomal dominant inheritance and shows an impaired insulin secretion from pancreatic beta cell without autoimmune cause. To date, 13 MODY genes have been identified worldwide – *HNF4A*, *GCK*, *HNF1A*, *PDX1*, *HNF1B*, *NEUROD1*, *KLF11*, *CEL*, *PAX4*, *INS*, *BLK*, *ABCC8* and *KCNJ11* [1–5].

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In the Caucasian population, *HNF1A*, *GCK*, *HNF4A* and *HNF1B* mutations comprise more than 95% of causes of MODY, and the others are known to be very rare [1, 3]. There are close genotype-phenotype correlations between the genetic locus of mutation and the clinical manifestation in MODY patients. Thus, an accurate diagnosis is important for special implications in the individualized treatment for a generic counselling of the family and for the prognosis.

However, there is a big discrepancy in the disease-causing genetic locus between the Asian and Caucasians MODY patients. In Korea, only 10% of 40 MODY or early-onset type 2 diabetes patients showed a known MODY gene defect (*HNF1A* 5%, *GCK* 2.5% and *HNF1B* 2.5%) among MODY 1–9 genes [6, 7]. Also in Japan and China were only 10–20% of known MODY gene defects reported [8–11]. In other words, there are much more 'MODY X' patients in Asia, whose range is 80–90%.

Recently, next generation sequencing methods have been applied for MODY X patients to discover unknown genetic variants [12, 13]. Whole-exome sequencing is a useful method to discover unknown rare germline mutations of mendelian disorders [14]. Thus, in this study, we conducted exome sequencing in Korean MODY patients to identify new candidate gene variants and to compare the results with Caucasian patients. This research was intended for MODY probands and their family members with available blood samples.

Materials and Methods

Ethics Statement

This research was approved by the relevant Ethics Committees (The Institutional Review Board in Kyungpook National University Hospital, approval No. 2013-07-037). This study was performed in accordance with the Declaration of Helsinki. Informed consent was obtained from all study subjects before blood sampling.

Subjects

Six MODY probands and their family members with available blood samples were included for whole-exome sequencing. Five MODY subjects were selected using the following inclusion criteria: (1) fasting serum C-peptide >0.6 ng/ml, (2) negative islet cell antibody (1A-2) test, glutamic acid decarboxylase (GAD) antibody test and insulin autoantibody (IAA) test, (3) early age at diabetes onset before 25 years, (4) autosomal dominant inheritance and (5) family history of diabetes in at least 3 generations. The fasting serum C-peptide in one proband was 0.5 ng/ml; however, we included her family in our study because she satisfied all other inclusion criteria. Genetic testing for MODY is also recommended for patients classified as type 1 diabetes and with strong family history because those patients may have a progression to severe hyperglycemia and insulin dependence as time goes on in the case of *HNF1A* or *HNF4A* alteration [1]. The partial pedigrees for the participants are described in figure 1. The clinical characteristics of all 6 probands and their family members with diabetes are described in table 1. We followed the classification of body mass index (BMI) and weight status for the Asian population for adults [15] because Asian populations including Korean have higher diabetes prevalence at lower cutoff values of BMI [16]. In the case of children and adolescents (age from 2 to 20 years), we followed the criteria as follows: underweight (<5th percentile), normal weight (≥5 and <85 percentile), overweight (≥85 and <95 percentile), and obesity (≥95 percentile) [17, 18]. The growth curve of BMI percentile was based on Korean pediatric data.

DNA Extraction and Quality Estimation

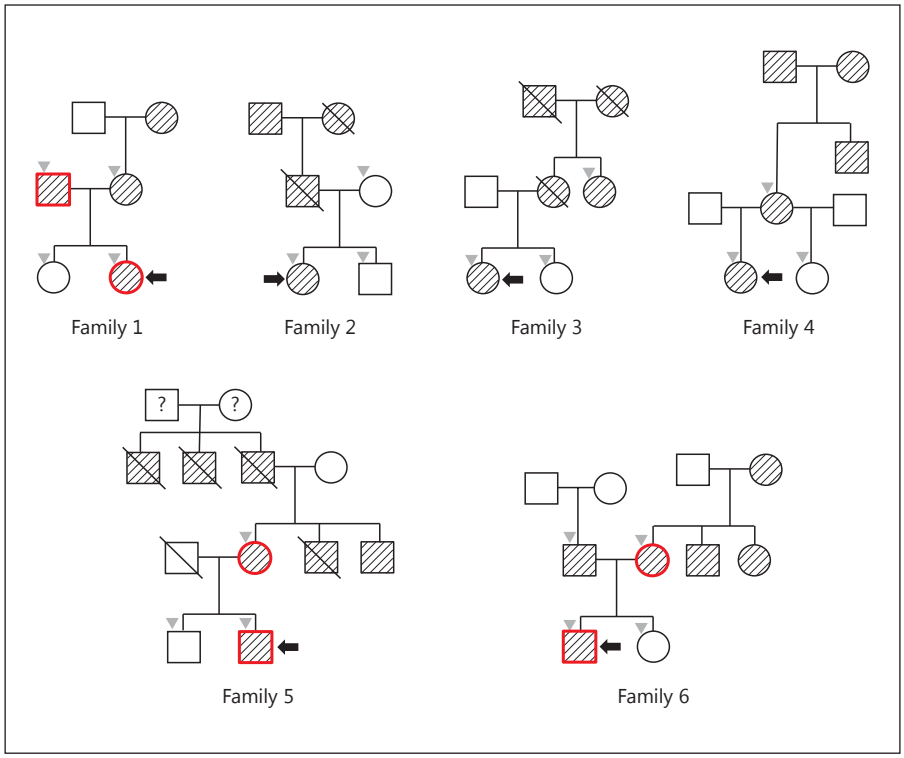
Genomic DNA was extracted from the subject's peripheral blood using the Wizard DNA purification Kit (Promega Corporation, Madison, Wisc., USA) based on the manufacturer's manual. The DNA quantity measurement was done by Picogreen (Invitrogen, Calif., USA) method using Victor 3 fluorometry. The measurement of DNA purity was done by NanoDrop instrument. DNA condition assessment was done by the gel electrophoresis method.

Whole-Exome Sequencing

Capturing of whole exome was performed from the subjects' genomic DNA using SureSelect Human All Exon V4 (Agilent Technologies, Calif., USA). Sequencing was undertaken by HiSeq2000 (Illumina, Calif., USA). The generated reads were mapped against UCSC hg19 (http://genome.ucsc.edu/) using a mapping program Burrow-Wheeler Alignment tool (http://biobwa.sourceforge.net/). The PCR duplicates were removed by the PICARD tool (http://picard.sourceforge.net/). The single nucleotide variants (SNVs) and insertions-deletions (Indels) were detected by SAMTOOLS (http://samtools.sourceforge.net/). The variants were filtered to include only those with >8 read depth and >30 mapping quality, and annotated by ANNOVAR (http://www.openbioinformatics.org/annovar/).

Identification of Disease-Causative Candidate Variants

Variants were filtered out in the dbSNP135 and TIARA database for Koreans (http://tiara.gmi.ac.kr/) to identify the disease-causing mutations. Variants with minor allele frequencies >0.5% of the 1000 Genomes database (http://www.1000genomes.org/) were also excluded. We selected only the functional variants (gain of stop codon, frameshifts and nonsynonymous SNVs) as pathogenic mutations. Then, we conducted a case-control comparison in the family members and selected those variants commonly found in both the MODY proband and the family member with diabetes which were not found in a healthy family member. Finally, the selected variants were scanned for the previously introduced gene set implicated in glucose metabolism [12]. These are the genes with an essential role in pancreatic beta cells, genes previously known to cause monogenic diabetes or associated syndromes and genes from the genome-wide association data of type 2 diabetes [19–26]. The list of genes of interest is shown in table 2. The finally selected candidate variants were verified by the in silico analysis database, PROVEAN (http://provean.jcvi.org/index.php/) [27], SIFT (http://sift.jcvi.org/) [28], and PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/) [29]. The final corresponding



Color version available online

Fig. 1. Partial pedigrees of the 6 clinical MODY families involved in this study. Shaded symbols with deviant crease lines indicate the individuals with diabetes. Crossed symbols denote the deceased individuals. Black arrows indicate respective probands in each family. Gray inverted triangles denote the family members whose blood sampling was available. Red-bordered symbols means the subjects who shared a common variant among the genes of interest for MODY and not in other family members (color refers to the online version only).

Table 1. Characteristics of the 6 clinical MODY probands and their family members with diabetes in this study

Family	1			2	3		4		5		6		
	proband	her father	her mother	proband	proband	her mother's sister	proband	her mother	proband	his mother	proband	his father	his mother
Sex/age, years	F/17	M/45	F/43	F/23	F/16	F/38	F/21	F/44	M/13	F/47	M/18	M/50	F/46
Age at diagnosis, years	15	35	33	14	14	26	12	30	11	33	16	34	30
BMI, kg/m^2	22.2[a]	20.6[b]	27.1[b]	18.4[a]	29.0[a]	26.7[a]	13.1[a]	18.8[b]	23.1[a]	23.7[b]	23.9[a]	23.4[b]	27.1[b]
BMI percentile	75th			25–50th	>95th		<5th		90–95th		75–85th		
Weight status	normal	normal	obesity	normal	obesity	overweight	underweight	normal	overweight	normal	normal	normal	obese
HbA1c, %	10.1	11.3	10.4	13.7	10.0	9.9	9.6	8.7	12.2	n.a.	7.9	n.a.	n.a.
Fasting C-peptide, ng/dl	1.73	1.86	2.02	3.20	4.41	0.80	0.50	0.80	2.39	n.a.	2.84	n.a.	n.a.
Diabetic ketoacidosis	no	n.a.	n.a.	no	no	no	no	no	no	n.a.	no	n.a.	n.a.
Total cholesterol, mg/dl	160	n.a.	n.a.	130	n.a.	143	202	186	140	n.a.	131	n.a.	n.a.
CRP, mg/dl	0.00	n.a.	n.a.	n.a.	0.23	0.55	0.06	0.00	1.05	n.a.	0.03	n.a.	n.a.
Treatment	insulin	insulin	No	No	OHA	insulin and OHA	insulin	insulin and OHA	insulin	OHA	OHA	OHA	insulin
Complication	no	no	no	no	no	yes	no	yes	no	n.a.	no	no	no
Last HbA1c, %	6.3	n.a.	n.a.	5.3	8.6	9.5	8.5	7.7	8.4	n.a.	8.9	n.a.	n.a.

n.a. = Not available; OHA = oral hypoglycemic agent. [a] Initial BMI at diagnosis of diabetes. [b] These values are the last BMI because initial BMI values at diagnosis were not available in family members.

Table 2. The list of genes of interest for MODY used in this study

MODY	*ABCC8, BLK, CEL, GCK, HNF1A, HNF1B, HNF4A, INS, KCNJ1, KLF11, NEUROD1, PAX4, PDX1*
Genome-wide association studies	*ADAM30, ADAMTS9, ADCY5, ADRA2A, ARAP1, BCL11A, C2CD4B, CAMK1D, CDC123, CDKAL1, CDKN2A, CDKN2B, CHCHD9, CRY2, DGKB, DUSP8, DUSP9, FADS1, FTO, G6PC2, GCKR, GIPR, GLIS3, HHEX, HMGA2, HNF1A, HNF1B, IDE, IGF1, IGF2BP2, IRS1, ITGB6, JAZF1, KCNJ11, KCNQ1, KLF14, LGR5, MADD, MTNR1B, NOTCH2, PPARG, PRC1, PROX1, PTPRD, RBMS1, SLC2A2, SLC30A8, SRR, TCF7L2, THADA, TMEM195, TP53INP1, TSPAN8, VEGFA, VPS13C, WFS1, ZBED3, ZFAND6*
Neonatal diabetes mellitus	*ABCC8, GCK, INS, INS, KCNJ11, NEUROG3, RFX6*
Congenital hyperinsulinism of infancy	*ABCC8, GCK, GLUD1, HADH, HNF4A, INSR, KCNJ11, SLC16A1*
Syndrome	*AGPAT2, AKT2, ALMS1, BSCL2, CAV1, CISD2, EIF2AK3, FXN, HFE, LMNA, LMNB2, WFS1, ZAC*
Candidate	*APPL1, FOXA1, FOXA2, FOXA3, GATA4, GATA6, INSM1, ISL1, LMX1A, MAFA, MAFB, MNX1, MYT1, NKX2-2, NKX6-1, ONECUT1, PAX6, PBX1, PTF1A, SOX2, SOX4, SOX9, SREBF1, SYT9, UCP2*

This is the previously introduced gene set implicated in glucose metabolism with an essential role in pancreatic beta cells, genes previously known to cause monogenic diabetes or associated syndromes and genes from the genome-wide association data of type 2 diabetes. Adapted from references [1–5, 12, 19–26].

genomic regions were amplified using an automatic genetic analyzer Dr. MAX DNA Polymerase (MG Taq-HF DNA Polymerase) kit. The PCR products were confirmed by Sanger sequencing in both forward and reverse directions.

Results

Whole-Exome Sequencing and Filtering

We performed whole-exome sequencing in 6 MODY probands and 14 family members. The number of total reads [mean (range)] was 91,756,007 (75,996,864~148,983,998) and the observed variants were 68,801 (67,791~70,073). After filtering out the variants with a frequency greater than 0.5% in the 1000 Genomes database and in the dbSNP135 and TIARA databases, residual exonic mutations were 464 (428~481) in each MODY proband, and 236 (224~250) were functional variants of them. After a case-control comparison between probands and family members, the number of variants with disease-causing possibility was decreased to 50 (31~63) in each MODY proband in one-side pedigree. After scanning regarding the specific gene list implicated in the glucose metabolism, 3 variants in *PTPRD*, *SYT9* and *WFS1* were identified in families 1, 5 and 6, respectively. Three variants were verified by PROVEAN, SIFT, and PolyPhen-2. Because there was no disease-causing variant in family 4 and the proband had insulin dependent manifestations, we checked the respective function of residual 52 genes in family 4. Finally, one variant in *PTPRN2*, the well-known type 1 diabetes gene, was identified in family 4. We were not able to detect the disease-causing mutation in families 2 and 3. A summary of the results of exome sequencing and the process of variant reduction are described in table 3 for all MODY probands.

Identification of Potential Disease-Causative Variants of MODY

PTPRD in Family 6

In family 6, one nonsynonymous mutation, exon6: c.620C>T:p.Thr207Ile, in *PTPRD* was found in both the proband and his mother. The proband visited our hospital because of a fasting hyperglycemia (168 mg/dl) during the regular medical checkup. He had no diabetes-specific symptoms. His HbA1c was 7.9% and fasting C-peptide 2.84 ng/ml. IA-2, GAD antibody and IAA were negative. His parents were both diabetics and he had a strong maternal family history (mother, mother's brother, mother's sister and maternal grandmother). He was treated with an oral hypoglycemic agent, and his last follow-up HbA1c was 8.9%. His mother was diagnosed with diabetes at 30 years of age. She has been treated with insulin by her physician. In the predictive program, Thr207Ile in *PTPRD* was deleterious/damaging/possibly damaging (table 4).

PTPRD (protein tyrosine phosphatase receptor type delta) is a member of a receptor type IIA subfamily, which also includes receptor type F (PTPRF) and sigma (PTPRS). A genetic alteration of *PTPRD* is associated with multifactorial forms of diabetes according to a genome-wide association study [25, 30]. Especially in Han Chinese, *PTPRD* alteration seems to cause a progression to diabetes through insulin resistance [31]. In addition, an enhanced phosphatase activity can cause defective insulin secretion by a reduced ATP/ADP ratio or the phosphorylation of

Table 3. Results of whole-exome sequencing of 6 MODY probands and the process of variant reduction

	Family of the proband					
	1	2	3	4	5	6
Total reads	75,996,864	81,447,800	91,148,066	148,983,998	101,749,122	84,199,586
Mappable reads	75,264,066	81,154,224	90,840,274	147,621,080	100,805,966	83,962,588
On-target reads	55,621,495	59,864,707	66,864,766	106,053,901	74,527,885	60,389,279
Coverage of target region (>10×)	97.7%	98.0%	98.1%	98.6%	98.3%	98.0%
Mean read depth of target regions	93.5	100.6	112.7	179.7	125.4	101.6
Total SNPs	69,063	68,637	69,341	68,858	69,200	67,791
Exonic regions	20,178	19,891	20,044	18,981	19,859	19,971
After filtering[a]	468	470	428	481	476	463
Functional variants[b]	250	224	224	235	238	243
After case-control comparison	98 (43 paternal and 55 maternal)	51	31	52	63	120 (60 paternal and 60 maternal)
In genes of interest	1 (*WFS1*, paternal side)	0	0	0	1 *(SYT9)*	1 (*PTPRD*, maternal side)

[a] The variants with a frequency of ≤0.5% in the 1000 Genomes database and not in the dbSNP135 or the TIARA database. [b] Gain of stop codon, frameshifts, and nonsynonymous SNVs.

Table 4. Finally identified variants in MODY families and the results of the predictive program

Family	Gene	Chr:position	Variant	Frequency in 1000 Genomes/dbSNP135/ TIARA	PROVEAN	SIFT	Polyphen-2
1	*WFS1*	4:6303048	exon8:c.T1526G:p.Val509Gly	0/0/0	deleterious (–3.35)	tolerated (0.086)	benign (0.002)
5	*SYT9*	11:7334687	exon3:c.C559G:p.Gln187Glu	0/0/0	neutral (0.02)	tolerated (0.715)	benign (0.059)
6	*PTPRD*	9:8524975	exon6:c.C620T:p.Thr207Ile	0/0/0	deleterious (–3.80)	damaging (0.003)	possibly damaging (0.796)

Figures in parentheses indicate scores.

proteins regulating the insulin release in pancreatic beta cells [32]. Considering the structural similarity of receptor type IIA subfamily members, we kept in mind the possibility that *PTPRD* mutation should concern not only insulin resistance but also insulin release from pancreatic beta cells.

SYT9 in Family 5

In family 5, another nonsynonymous SNV, exon3: c.559C>G:p.Gln187Glu, in *SYT9* was found in both the proband and his mother. The proband visited our hospital at the age of 11 years because of a random hyperglycemia (295 mg/dl) noted at a regular medical checkup. He had persistent polyphagia, polydipsia, polyuria and weight loss for 1 year. His initial HbA1c was 12.1% and fasting C-peptide 4.67 ng/ml. IA-2, GAD antibody and IAA were all negative. He had a strong maternal family history of diabetes (mother, 2 mother's brothers, maternal grandfather and 2 maternal grandfather's brothers). His last follow-up HbA1c was 8.4% during insulin infusion. His mother was diagnosed with diabetes at 33 years. Although his mother has been treated with an oral hypoglycemic agent, additional insulin was recommended by her physician. Because Gln187Glu in *SYT9* was neutral/tolerated/benign in the predictive program (table 4), the role of *SYT9* in MODY needs more study.

Synaptotagmin 9 (SYT9) is one of the synaptotagmin family proteins known to play a role as an important Ca^{2+} sensor in exocytosis. The glucose-induced hormone release was decreased by the reduction of the expression

of both SYT5 and 9 isoforms, indicating that they are directly involved in the Ca^{2+}-dependent stimulation of exocytosis [33]. In addition, the insulin release induced by glucose was decreased in the pancreas islets of rats after an adenovirus-mediated silencing of SYT9 [34].

WFS1 in Family 1

In family 1, another nonsynonymous SNV, exon 8: c.1526T>G:p.Val509Gly, in *WFS1* was identified in both the proband and her father. The proband visited the clinic at the age of 15 years due to glucosuria noticed at a regular checkup in her school. She had a general weakness and polydipsia. Her initial HbA1c was 10.1%, and fasting C-peptide was 1.73 ng/ml. IA-2, GAD antibody and IAA were all negative. Her last follow-up HbA1c was 6.3% following an intermittent low-dose insulin treatment (0.5 IU/kg, once a day, 3 times per week). She did not need insulin injections after physical activity. Her father, with the same variant in *WFS1*, was diagnosed as having diabetes at the age of 35 years. His HbA1c was 11.3% and fasting C-peptide 1.86 ng/ml. Although he initially had been treated with insulin by his physician, he was not on any medication at the time of the study. In the predictive program PROVEAN, Val509Gly in *WFS1* was deleterious (table 4).

WFS1 is the gene which encodes Wolframin, the transmembrane protein of the endoplasmic reticulum [23]. A homozygous mutation of *WFS1* is associated with an autosomal recessive inheritance of Wolfram syndrome, which means the complex of diabetes mellitus, diabetes insipidus, hearing impairment and optic atrophy [35], while the heterozygous mutation of *WFS1* causes an early onset of autosomal dominant diabetes without other syndromic appearances [36]. The proband and her father in family 1 had no specific symptoms of Wolfram syndrome except diabetes mellitus. Although family 1 shows a strong maternal history of diabetes (mother and maternal grandmother), we suppose that p.Val509Gly in *WFS1* as a potential variant caused MODY because the proband and her father had very similar clinical manifestations. An offspring study will be necessary in the future.

Validation by Sanger Sequencing

The final 3 candidate gene variants for MODY, p.Thr207Ile in *PTPRD*, p.Gln187Glu in *SYT9* and p.Val509Gly in *WFS1* were confirmed by Sanger sequencing and are shown in figure 2. The primers used are as follows: ATTGAATCGACGTTGAGTGG (forward) and CAAACTCCAAGCCTCAGGAC (reverse) in *PTPRD*, AAAGGCCCACAGATCTAAGC (forward) and GCC AAGTCTAGGAAGTGATCC (reverse) in *SYT9*, CTT TACCGTGACCAGCTACC (forward) and TCTTGG TGAGCTCCAGAGAC (reverse) in *WFS1*.

Exclusion from MODY

PTPRN2 in Family 4

In family 4, another nonsynonymous SNV, exon 1:c.85A>C:p.Arg29Ser, in *PTPRN2* was found in both the proband and her mother. The proband was referred to our hospital at the age of 12 years because of a fasting hyperglycemia (259 mg/dl) that was noticed in the clinic. She had polydipsia, polyuria, polyphagia and general weakness for 2 weeks. Her initial HbA1c was 9.6%, and the fasting C-peptide was 0.05 ng/ml. IA-2, GAD antibody, and IAA were all negative. She had a strong maternal family history of diabetes (mother, maternal grandfather and maternal grandmother). Her last follow-up HbA1c was 8.5% during insulin treatment. Her mother was diagnosed with diabetes at the age of 30 years and has been treated with insulin and oral hypoglycemic agent. She had an early diabetic peripheral neuropathy. The clinical manifestations of the proband and her mother are very similar. Both of them had Hashimoto's thyroiditis and took oral thyroid hormone.

PTPRN2 is a well-verified gene causing type 1 diabetes and is also known as *IA-2beta* or *phogrin*. Both, IA-2beta and IA-2 are precursors for autoantigens of pancreatic islet cells [37], and their intracellular domains are very similar (73% identical) [38]. An overexpression of IA-2beta reduced the insulin secretion stimulated by glucose in insulinoma cells [39]. The clinical manifestations in family 4 were in accordance with a *PTPRN2* alteration considering their relatively low C-peptide level and accompanying autoimmune thyroid disease with negative laboratory tests for IA-2, GAD antibody and IAA. Thus, family 4 was ruled out to have MODY by genetic testing. Family 4 seems to belong to the rare group of autosomal dominantly inherited type 1 diabetes, which is known to occur in <10% of diabetes patients [3].

Discussion

We conducted whole-exome sequencing in 6 clinical MODY families to identify MODY genetic variants in Korea for the first time. Surprisingly, we could not find any disease-causative alleles among known MODY 1–13 genes. One synonymous exonic mutation, exon 2:c.294G>A:p.Gln98Gln, in *PAX4* (MODY9) was identified in both the proband and her mother in family 1.

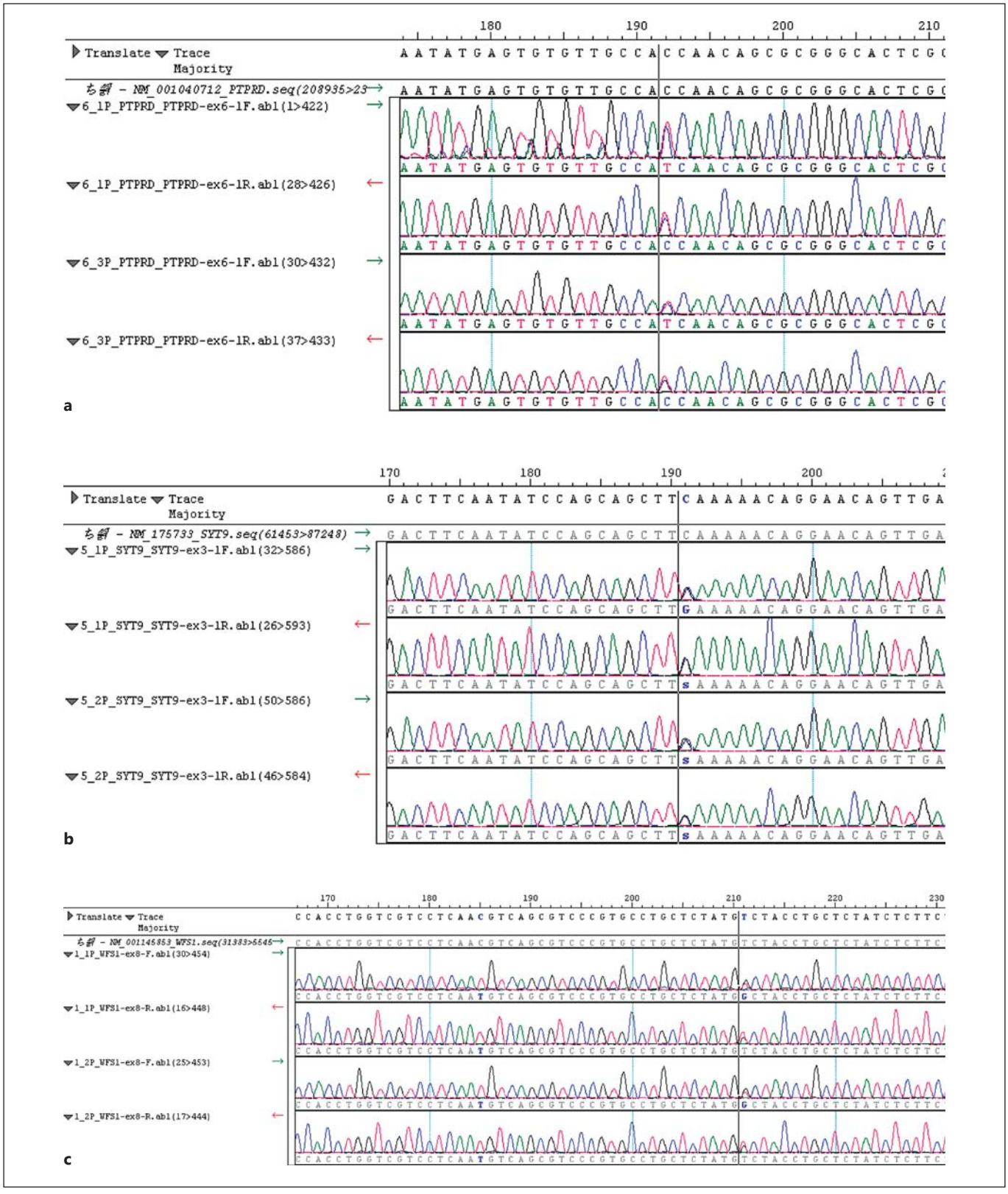


Fig. 2. Validation by Sanger sequencing for final potential disease-causative gene variants for MODY. **a** c.620C>T: p.Thr207Ile in *PTPRD*. **b** c.559C>G:p.Gln187Glu in *SYT9*. **c** c.1526T>G:p.Val509Gly in *WFS1*.

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However, it was excluded through the filtering step considering its prioritization because it is nonfunctional (silent).

Exome sequencing is a potential, economical method to identify novel genetic variants of rare monogenic disorders. However, it cannot be a panacea for all the rare monogenic disorders. The application of exome sequencing has both an advantage and disadvantage for MODY cases. Because MODY cases have an autosomal dominant inheritance, we can filter many meaningless variants which exist in healthy controls. On the other hand, it is a disadvantage that MODY is a heterogeneous group of monogenic diabetes. It is difficult to narrow the range of variants by filtering across only multiple unrelated, affected probands. We may overcome this problem by acquiring the blood of multiple affected family members from within a pedigree and by the application of a known interested gene list.

In this study, we mostly conducted the case-control method for family members of two generations due to practical issues like death of grandparents or failure to obtain consent from distant relations. We could reduce the number of functional variants from 220–250 to 30–60 in one side pedigree. In the case of two-generation families, we could reduce the variants from 1/4–1/5. In the case of family 3, it was possible to obtain the blood of the sister of the proband's mother, and so we could reduce the functional variants to 1/7. However, it was not enough to identify novel variants which do not exist in the genes of interest list. We closely follow them with the idea of further genetic evaluation like linkage analysis with other distant relations.

In a normal pancreatic beta cell, the glucose transportation by a GLUT-2 transporter allows glucokinase to phosphorylate glucose. ATP generated via glycolysis and Kreb cycle in mitochondria closes the potassium channel resulting in membrane depolarization and opening of the calcium channel. Ca^{2+} entry results in an exocytosis of insulin from the endoplasmic reticulum through secretory granules [1, 3]. The known MODY genes are involved in each step of exocytosis of insulin from the pancreatic beta cell. For example, the relatively frequent MODY genes *HNF4A*, *HNF1A* and *HNF1B* encode transcriptions factors. A *GCK* mutation reduces the glucokinase activity and glucose phosphorylation. The latest MODY genes, *ABCC8* and *KCNJ11*, are the genes coding the protein subunit of the beta cell potassium channel [1, 3, 23].

In this study, 3 potential candidate gene variants for MODY were identified in *PTPRD*, *SYT9* and *WFS1*. Considering the established roles of these genes or their family genes, we suppose the respective roles of them in the pancreatic beta cell as follows: (1) *PTPRD* should involve the generation of ATP or phosphorylation of proteins regulating the insulin release, (2) *SYT9* alteration should cause an impairment of the a Ca^{2+} channel resulting in decreased exocytosis of insulin stimulated by glucose, (3) *WFS1* mutation causes stress to the endoplasmic reticulum of the pancreatic beta cell resulting in a reduced insulin secretion.

PTPRD is a member of a receptor type IIA subfamily, which also includes PTPRF and PTPRS. They are known to be implicated in neural growth and regeneration, metabolic regulation and cancer [40]. *PTPRF* knockout mice showed both lower fasting insulin and glucose, suggesting a heightened level of insulin sensitivity [41]. Moreover, an increased expression of PTPRF in the muscle causes whole-body insulin resistance in mice most likely due to the dephosphorylation of specific regulatory phosphotyrosines on insulin receptor substrate proteins [42]. In PTPRS knockout mice, pancreatic islets were hypoplastic, and the immunoreactivity of insulin was decreased [43]. The glucose homeostasis is altered in mice lacking PTPRS [44]. An overexpression of PTPRS in beta cells suggests an increased consumption or degradation of ATP resulting in an impaired glucose-induced insulin secretion in hereditary diabetic rats [32]. Considering the structural similarity of receptor type IIA subfamily members, we also suggest that PTPRD is thought to have a role in a decreased insulin secretion and beta cell failure [30].

SYT is involved in a Ca^{2+}-regulated secretion and has been suggested to perform a general Ca^{2+} sensor on the membrane of secretory vesicles in neuronal cells [33, 45, 46]. The inhibition of SYT9 by direct antibodies decreased the calcium-induced norepinephrine release from PC12 cells in rats [45]. An acute deletion of SYT9 in striatal neurons severely impaired a fast synchronous release [46]. The pancreatic beta cell is another example of Ca^{2+} channel-mediated exocytosis. Iezzi et al. [34] reported that the glucose- or tolbutamide-induced insulin release was decreased in pancreas islets of rats after the adenovirus-mediated silencing of SYT9. On the other hand, there was no decline of a glucose-induced insulin secretion in genetic knockout *SYT9* mice [47]. Because there are differences in the glucose metabolism between rats, mice and human, more studies are needed about the role of *SYT9* in diabetes mellitus.

WFS1 null mice and genetic association studies suggest a role for the *WFS1* gene in insulin secretion [48–50].

In a previous study, another novel variant, c.2017T>C: p.Arg703Cys, in *WFS1* was also found in a Norwegian MODY family; however, the authors suggested further evaluation of the role of *WFS1* in MODY considering an inheritance pattern [12]. The localization of the WFS1 protein at the endoplasmic reticulum suggests that it has physiological functions in membrane trafficking, secretion, processing or regulation of calcium homeostasis. Disturbances or overloading of these functions induce stress of the endoplasmic reticulum [51]. This hypothesis was demonstrated by a functional study [36].

In conclusion, whole-exome sequencing is a valuable method for the genetic diagnosis of MODY. We suggest further evaluation of *PTPRD*, *SYT9* and *WFS1* in glucose metabolism and normal insulin release from pancreatic beta cell in other Asian countries. A large-scale control study in a local population cohort was not performed in this study. However, this research is valuable despite this limitation because all the 3 variants have a 0% frequency in the 1000 Genomes, dbSNP135 and TIARA databases for Koreans, and they were not found in diabetes-negative family members.

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Disclosure Statement

The authors declare that they have no conflict of interest.

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