

Targeted Panel Exome Sequencing in Suspected Monogenic Diabetes: Single-Center Pilot Study

Sangwoo Lee, Gi Min Lee, MiSeon Lee, Rosie Lee, Jung Eun Moon

Department of Pediatrics, School of Medicine, Kyungpook National University, Kyungpook National University Hospital, Daegu, Korea

Purpose: Maturity onset diabetes of the young (MODY) is the most common hereditary form of diabetes mellitus (DM), with similar clinical manifestations to type 1 or type 2 DM, leading to diagnostic ambiguity. Despite increased genetic research on monogenic DM, studies with Asian populations are limited. Therefore, we investigated mutation in possible monogenic DM and MODY in Korean children and adolescents. **Methods:** Targeted panel exome sequencing including 32 target genes was performed for 41 patients with suspected monogenic DM at Kyungpook National University Children's Hospital. **Results:** Variants were detected in 19 patients, including those in known MODY-associated genes (*HNF4A*, *GCK*, *HNF1A*, *CEL*, *PAX4*, *INS*, and *BLK*) and monogenic DM-associated genes (*WFS1*, *FRX6*, and *GLIS3*). **Conclusion:** MODY variants were detected more than expected. Targeted exon sequencing is helpful in diagnosing MODY or possible monogenic DM patients.

Key words: Diabetes mellitus, Genes, Koreans

ORIGINAL ARTICLE

Received: March 11, 2022

Revised: April 8, 2022

Accepted: April 11, 2022

Correspondence to: Jung Eun Moon
Division of Pediatric Endocrinology, Kyungpook National University Children's Hospital, 807 Hoguk-ro, Buk-gu, Daegu 41404, KOREA
Tel: +82-53-200-5704
Fax: +82-53-425-6683
E-mail: subuya@daum.net

ORCID

Jung Eun Moon:
<https://orcid.org/0000-0001-9786-7898>

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INTRODUCTION

Monogenic DM, which is associated with a single-gene disorder or hereditary diseases [1,2]. The most common form of monogenic diabetes is maturity-onset diabetes of the young (MODY), and this category also includes neonatal diabetes and deafness [3]. MODY has similar characteristics to those of type 1 and 2 DM, resulting in diagnostic ambiguity. In addition, disease treatment and progression differ between the two conditions, further emphasizing the importance of an accurate differential diagnosis [4,5]. Recently, there has been an explosion in research related to the genetic diagnosis of monogenic DM using targeted panel exome sequencing, leading to the identification of 14 MODY-specific genetic variants that change the function of beta cells to cause DM [6-8]. However, studies on the genotype of monogenic DM have mainly focused on Caucasian patients, with limited genetic screening performed for Asian patients to date. Therefore we performed targeted panel exome sequencing on pediatric patients suspected of having monogenic DM at Kyungpook National University Children's Hospital to determine how many Patients have MODY gene variation.

MATERIALS AND METHODS

Participants

Participants included pediatric patients from the Endocrinology Unit at Kyungpook National University Children's Hospital who were receiving insulin therapy by injection following a diagnosis of DM prior to the age of 25 years. A total of 41

patients who have a family history of two generation or more were selected for targeted panel exome sequencing. Patients who received a diagnosis of type 1 DM (C-peptide <0.6 ng/mL, or anti-pancreas antibody-positive) were excluded from the study [9].

Clinical parameters and Targeted exome sequencing

Patient clinical characteristics were reviewed with respect to age, sex, family history, body mass index (BMI), and C-peptide levels. Targeted exome sequencing in the patient was performed using the TruSight One Sequencing Panel, 32 genes, including 14 identified MODY genes (*HNF4A*, *GCK*, *HNF1A*, *PDX1*, *HNF1B*, *NEUROD1*, *KLF11*, *CEL*, *PAX4*, *INS*, *BLK*, *ABCC8*, *KCNJ11*, *APPL1*) and 18 monogenic DM-related genes (*EI-F2AK3*, *FOXP3*, *GATA4*, *GATA6*, *GLIS3*, *IER3IP1*, *INSR*, *MNX1*, *NEUROG3*, *NKX2-2*, *PAX6*, *PTF1A*, *RFX6*, *SLC19A2*, *PTPRD*, *SYT9*, *WFS1* and *PAX1*). The pathogenicity of the genetic variants was evaluated according to the American College of Medical Genetics and Genomics and Association for Molecular Pathology guidelines.

Statistical analysis

Data were using a t-test with PAWS Statistics 20 (SPSS Inc., Chicago, IL, USA) after confirming the normality of the data distribution. Significance was defined as $P < 0.05$

RESULTS

The characteristics of participants (Table 1)

Of the 41 patients, 23 were male (56.1%) and 18 were female (43.9%). At the time of diagnosis, the average age of all patients was 11.80 ± 3.7 years, the HbA1c level was $12.14 \pm 2.42\%$, body weight was 59.95 ± 26.92 kg, BMI was 23.11 ± 4.72 kg/m², serum

insulin level before and after meals was 6.40 ± 4.01 uU/mL and 10.72 ± 9.83 uU/mL, respectively, and the serum level of C-peptide before and after meals was 2.50 ± 1.61 ng/mL and 3.84 ± 3.38 ng/mL, respectively.

Identification of gene variants (Table 2)

Variants were identified in 19 of the 41 patients (46.3%), including variants in seven MODY-identified genes (*HNF4A*, *GCK*, *HNF1A*, *CEL*, *PAX4*, *INS*, and *BLK*) and three monogenic DM-related genes (*WFS1*, *RFX6*, *GLIS3*).

DISCUSSION

In this study, targeted panel exome sequencing was performed on pediatric patients suspected of having monogenic DM. In a review of the literature, Kim et al. [10] confirmed that the MODY3 and MODY2 genotypes account for 20% and <5% of all MODY cases in Korea and China, respectively. In this study, monogenic DM variants were identified in 19 of 41 (46.3%) patients. Actually, the frequency of PV or LPV variations excluding the actual VUS is 9.75% (4 of 41), and the frequency of PV or LPV variations excluding the VUS variations is more reliable as the frequency of the actual Monogenic DM [11]. However, compared to studies where the previous VUS and PV/LPV ratios were 6% and 2.1%, respectively, we can see a high positive rate [12]. Instead, Yang et al. [13] reviewed studies of Korean patients with monogenic DM, highlighting that the prevalence of MODY, which has been studied mainly in Western populations to date, may be different for Asians. Similarly, we found a high prevalence of the MODY8 genotype (4/17, 21.0%), which has been reported at a low frequency in previous studies [14].

Based on a review of the state of monogenic DM in Korea, MODY exhibits various pathophysiological mechanisms and clinical manifestations [13]. Unlike type 1 DM, which is caused by the destruction of pancreatic beta cells, some MODY subtypes do not require insulin therapy and can be improved with oral antidiabetic medications, diet management, and treatment with sulfonylurea. However, we did not find any difference in disease progression according to the presence or absence of variants, with respect to a similar significant in HbA1c and BMI.

The limitation of this study were many patients with gene variants showed VUS. Further more, there are not many patient numbers. In order to secure these limitations in the future, it is necessary to conduct a family examination on patients with confirmed genetic variation, and increased targeted study numbers.

Table 1. The characteristics of participants

Variables	Patients
Number (M/F)	41 (23/18)
Age	11.8 ± 3.7
HbA1c	
At Diagnosis	12.1 ± 2.4
BMI (kg/m ²)	23.1 ± 4.7
Insulin (uU/mL)	
Pre-meal	6.4 ± 4.0
Post-meal	10.7 ± 9.8
C-peptide (ng/mL)	
Pre-meal	2.50 ± 1.61
Post-meal	3.84 ± 3.38

Table 2. Clinical and molecular characteristics of patients with MODY gene variant

Pt	Gen	Age (yr)	BMI	HbA1c	c pep	Gene	DNA	AA Change	Zyg	Disease	Class	Clin	Nov- elity	In silico analysis score
1	F	9.9	1.0	9.0	5.19	<i>HNF4A</i>	c.778G>A	p.(Asp260Asn)	Het	MODY1	VUS	none		S/P/M Deleterous
2	M	3.2	-0.43	6.7	0.42	<i>GCK</i>	del (chr.44184714-44186245)	exon 9-11	Het	MODY2	PV	none		/
3	F	3.2	1.29	6.7	1.99	<i>GCK</i>	c.775G>A	p.(Ala259Thr)	Het	MODY2	PV	none		S/P/M Deleterous
4	M	6.9	1.83	6.4	1.70	<i>GCK</i>	c.579+1G>T	p.(?)	Het	MODY2	PV	none		M Tolerate
5	F	12.2	0.67	9.0	2.1	<i>HNF1A</i>	c.773T>C	p.(leu258pro)	Het	MODY3	VUS	none	Y	S/P/M Deleterous
6	M	15.5	1.47	6.1	2.6	<i>CEL</i>	c.1421C>T	p.(Thr474Met)	Het	MODY8	VUS	none		S Deleterous P/M Tolerate
7	F	13.3	1.35	13.2	1.61	<i>CEL</i>	c.2106_2171del	p.(Thr7004_Val725del)	Het	MODY8	VUS	none		/
8	M	10.1	1.75	14.7	1.47	<i>CEL</i>	c.1154C>T	P.(Thr385Met)	Het	MODY8	VUS	none		S/P Deleterous M Tolerate
9	M	14.1	1.49	14.3	1.97	<i>CEL</i>	c.1627C>T	p.(Arg543Cys)	Het	MODY8	VUS	none		S/P Deleterous M Tolerate
10	M	14.1	2.12	12.3	3.8	<i>PAX4</i>	p.(Arg121Trp)	p.(Arg 121 Trp)	Het	MODY9	VUS	none		S/P/M Deleterous
11	M	14.2	1.54	12.8	0.2	<i>PAX4</i>	c.374_412+1del	p.(Arg192His)	Het	MODY9	LPV	none	Y	S/P Deleterous M polymorphism automatic
12	M	10.2	1.36	17.3	1.0	<i>INS</i>	c.67G>A	P.(Ala23Thr)	Het	MODY10	VUS	deaf- ness		S/P/M Tolerate
13	M	14.2	1.55	12.4	4.0	<i>BLK</i>	c.337del	p.(val113TrpfsTer52)	Het	MODY11	VUS	none		/
14	F	11.2	1.71	13.0	3.3	<i>WFS1</i>	c.1846G>T	p.(Ala616Sat)	Het	WFS1	VUS	none		S/P/M Tolerate
15	F	14.2	0.35	11.6	1.1	<i>WFS1</i>	c.76C>T	P.(Arg26Ter)	Het	WFS1	LPV	none		M Deleterous
16	M	16.2	0.23	12.4	0.8	<i>WFS1</i>	c.2417C>G	p.(Ala806Gly)	Het	WFS1	VUS	none	Y	S Tolerate P/M Deleterous
17	F	16.2	1.74	12.6	2.0	<i>WFS1</i>	c.1264G>T	p.(Ala422Ser)	Het	WFS1	VUS	none		S/M Deleterous P Tolerate
18	F	17.2	-0.51	10.3	1.2	<i>RFX6</i>	c.280G>C	p.(Asp94His)	Het	MTCHRS	VUS	none		S/M Deleterous P Tolerate
19	M	17.2	-0.01	11.8	1.3	<i>GLIS3</i>	c.838G>C	p.(Glu280Gln)	Het	Diabetes Mellitus	VUS	none	Y	S/P/M Tolerate

Pt, patient; Gen, Gender; Age, Age when DM diagnosis/exon sequencing did; BMI, Body mass index, Z score; C.pep, C-peptide; Zyg, zygomatic; Ds, disease; C.pep, C-peptide; Path, pathophysiology; Het, heterozygosity; Clin, Clinical Pathology; VUS, variants of unknown significance; PV, pathogenic variants; LPV, likely pathogenic variants; WFS, wolfram-like syndrome; Y, novel variation; Zyg, zygosity; AA, aminoacid; in silico analysis S, SIFT; P, Polyphen2; M, MutationTaster.

In conclusion, using targeted panel sequencing, we were able to make molecular genetic diagnoses for 17/39 patients (44%) with MODY. MODY variants were detected more than expected in Korean children and adolescents with suspected monogenic DM.

ACKNOWLEDGMENTS

None

DISCLOSURE

All authors have no potential conflicts of interest.

ETHICAL STATEMENT

This study was approved by the Institutional Review Board (approval number: 2017-05-006) of Kyungpook National University Children's Hospital. This was a retrospective study; hence, it was exempted from the requirement of obtaining informed consent from patients.

AUTHOR CONTRIBUTIONS

Conceptualization: Moon JE. Data curation: Lee JM, and Lee SW. Formal analysis: Lee JM, Lee R, Lee MS, Lee SW and Moon JE. Methodology: Moon JE. Writing - original draft: Lee SW, Lee JM and Moon JE. Writing - review & editing: Lee JM and Moon JE.

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