Study of Thirteen Causal Genes in Turkish Patients with Clinically Suspected Maturity-Onset Diabetes of the Young (MODY) using a Targeted Next-Generation Sequencing Panel

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Abstract

Background: Maturity-onset diabetes of the young (MODY), which is the most common cause of monogenic diabetes, has an autosomal dominant pattern of inheritance and exhibits marked clinical and genetic heterogeneity. The aim of the current study was to investigate molecular defects in patients with clinically suspected MODY using a next-generation sequencing (NGS)-based targeted gene panel. Candidate patients with clinical suspicion of MODY and their parents were included in the study. Molecular genetic analyses were performed on genomic DNA by using NGS. A panel of thirteen MODY-related genes involving *ABCC8*, *BLK*, *CEL*, *GCK*, *HNF1A*, *HNF1B*, *HNF4A*, *INS*, *KCNJ11*, *KLF11*, *NEUROD1*, *PAX4*, *PDX1* was designed and subsequently implemented to screen 44 patients for genetic variants. Ten different pathogenic or likely pathogenic variants were identified in MODY-suspected patients, with a diagnostic rate of 22.7%. Eight variants of uncertain significance were also detected. Four novel pathogenic or likely pathogenic variants were detected in the genes *GCK* (c.1301G>T [p.Cys434Phe]), *HNF1A* (c.505_506delAA [p.Lys169AlafsTer18]), *ABCC8* (c.3584C>T [p.Thr1195Ile]), and *CEL* (c.679-1G>A). Intriguingly, we were able to detect variants associated with rare forms of MODY in our study population. Our results suggest that in heterogenous diseases such as MODY, NGS analysis enables accurate identification of underlying molecular defects in a timely and cost-effective manner. Although MODY accounts for 1–2% of all diabetic cases, molecular genetic diagnosis of MODY is necessary for optimal long-term treatment and prognosis as well as for effective genetic counseling.

Introduction

Monogenic diabetes phenotypes include neonatal diabetes, maturity-onset diabetes of the young (MODY), mitochondrial diabetes, and diabetes mellitus associated with syndromic diseases. MODY, which is the most frequent cause of monogenic diabetes, is inherited in an autosomal dominant fashion and shows both clinical and genetic heterogeneity [1].

The MODY type of diabetes occurs mostly under the age of 25, but it can also occur in the adulthood. MODY is usually considered in people with a history of diabetes in at least two generations (which is indicative of autosomal dominant transmission) and in those who do not have islet autoantibody positivity (which is suggestive of type 1 diabetes). However, in patients without evidence of insulin resistance and in those who do not require high-dose insulin therapy in their long-term follow-up, MODY should also be considered [2].

Molecular genetic diagnosis of MODY has a significant impact on precise treatment, prognosis, and genetic counseling for patients. With advancements in next-generation sequencing (NGS) technology, it is now possible to understand the genetic basis of phenotypically and genotypically heterogeneous diseases. In addition to whole-genome and -exome sequencing, many genes can be sequenced by using targeted NGS panels simultaneously [3]. In this study, we aimed to analyzing 13 different genes (*ABCC8*, *BLK*, *CEL*, *GCK*, *HNF1A*, *HNF1B*, *HNF4A*, *INS*, *KCNJ11*, *KLF11*, *NEUROD1*, *PAX4*, *PDX1*) using the NGS method in cases with clinically suspected MODY.

Subjects And Methods

44 unrelated patients with a clinical suspicion of MODY before 25 years of age were included in the study after informed consents were obtained from the patients and their parents for medical examination and genomic analysis. The procedures adhered to the Declaration of Helsinki, and the relevant studies were approved by the Ethics Committee of the Faculty of Medicine at Duzce University. Possible MODY in this study was defined as the presence of at least two of the following criteria: (i) a family history of diabetes consistent with autosomal dominant transmission in at least two or three generations; (ii) negative autoantibody test results (for two or more of the most commonly observed antibodies including anti-glutamic acid decarboxylase [anti-GAD] antibody, anti-islet cell antibody [ICA], and anti-insulin antibody [IAA]); (iii) C-peptide levels ≥ 0.59 ng/mL and no evidence of insulin resistance (obesity, acanthosis nigricans, etc.); (iv) a low insulin requirement (< 0.5 IU/kg/day) two years after the diagnosis; and (v) incidental identification of hyperglycemia.

Genomic DNA was extracted from peripheral blood, and NGS was performed by capture of the coding regions and splice sites of target genes (*GCK*, *HNF1A*, *HNF4A*, *HNF1B*, *PDX1*, *NEUROD1*, *KLF11*, *CEL*, *PAX4*, *INS*, *BLK*, *ABCC8*, and *KCNJ11*) using the Celeomics targeted capture kit for NGS (Seoul, South Korea). After library enrichment and quality control, the samples were sequenced on the Illumina MiSeq platform (San Diego, CA, USA) with 100-bp paired-end reads at an average sequencing depth of 100 $\times$. The entire list of target genes in the panels is provided in Table 1. The sequencing reads were aligned to the human reference genome assembly (GRCh37: Genome Reference Consortium Human Build 37) using BWA. Then, BAM files were sorted, indexed and de-duplicated using SAMtools and Picard. For the filtering process, exonic and splicing variants, including missense/nonsense variants, and indels were selected. Annotation of detected variants were performed using Illumina BaseSpace Variant Interpreter, InterVar, Franklin, VarSome, ClinVar, OMIM, and Pubmed. Variants with a frequency higher than 0.5% were filtered out. dbNSFP (contains SIFT, PolyPhen-2, LRT, Mutation Taster) was used to predict the pathogenicity (deleteriousness) of variants. Rare variants were classified according to the American College of Medical Genetics and Genomics (ACMG)/Association for Molecular Pathology (AMP) variant interpretation framework [4].

Table 1
The pathogenicity assessments of the detected variants

<i>P No</i>	<i>Gene</i>	<i>V Name</i>	<i>AA change</i>	<i>Zyg</i>	<i>V Loc</i>	<i>V Type</i>	<i>ClinVar</i>	<i>ACMG Class</i>	<i>ACMG Pat Crit</i>	<i>Transcript n</i>
P1	<i>GCK</i>	c.449_451delTCT	p.150delF	Het	Exon 4	Del	NP	LP	PM1,PM2,PM4,PP3	NM_000162
P2	<i>GCK</i>	c.537delG	p.Asn180ThrfsTer25	Het	Exon 5	Frms	NP	Pat	PVS1,PM2,PP3	NM_033507
P3	<i>HNF1A</i>	c.862G > A	p.Gly288Arg	Het	Exon 4	Mis	NP	VUS	PP2,PP3,BS2	NM_000545
P4	<i>CEL</i>	c.268A > C	p.Thr90Pro	Het	Exon 3	Mis	NP	LB	BS2,BP1,BP4	NM_001807
P5	<i>CEL</i>	c.679-1G > A	-	Het	Intron 5	Splic	NP	Pat	PVS1,PM2,PP3	ENST00000
P6	<i>NEUROD1</i>	c.-81C > A	-	Het	5'UTR	Mis	NP	VUS	PM2,BP4	NM_002500
P7	<i>BLK</i>	c.211G > A	p.Ala71Thr	Het	Exon 4	Mis	Conf In	Bng	BS1,BS2,BP4	NM_001715
P8	<i>KLF11</i>	c.1382G > A	p.Arg461Gln	Het	Exon 4	Mis	NP	VUS	PM2,BS2,PP3	NM_003597
P9	<i>NEUROD1</i>	c.-71G > A	-	Het	5'UTR	Mis	NP	VUS	PM2,BP4	NM_002500
P10	<i>NEUROD1</i>	c.590C > A	p.Pro197His	Het	Exon 2	Mis	NP	Bng	BS1,BS2	NM_002500
P11	<i>NEUROD1</i>	c.590C > A	p.Pro197His	Het	Exon 2	Mis	NP	Bng	BS1,BS2	NM_002500
P12	<i>ABCC8</i>	c.1943G > A	p.Arg648His	Het	Exon 14	Mis	NP	VUS	PM2,PP2,PP3	NM_000352
P13	<i>HNF4A</i>	c.83C > T	p.Ala28Val	Het	Exon 2	Mis	NP	VUS	PM2,PP2	NM_175914
	<i>WFS1</i>	c.2168T > C	p.Leu723Pro	Hom	Exon 8	Mis	NP	VUS	PM2,PP3	NM_006005
P14	<i>GCK</i>	c.664G > A	p.Glu222Lys	Het	Exon 6	Mis	Pat	Pat	PM1,PM2,PP2,PP3, PP5	NM_033507
P15	<i>KLF11</i>	c.1447C > T	(p.Pro483Ser)	Het	Exon 4	Mis	NP	VUS	PM2	(NM_003597)
P16	<i>HNF1A</i>	c.505_506delAA	p.Lys169AlafsTer18	Het	Exon 2	Frms	NP	Pat	PVS1, PM2, PP3	(NM_000545)
P17	<i>ABCC8</i>	c.3584C > T	(p.Thr1195Ile)	Het	Exon 29	Mis	NP	LP	PM1, PM2,PP2,PP3	(NM_000352)
P18	<i>HNF1A</i>	c.376 C > T	p.His126Tyr	Het	Exon 2	Mis	NP	VUS	PM2, PP2,PP3	(NM_000545)
P19	<i>GCK</i>	c.1301G > T	p.Cys434Phe	Het	Exon 10	Mis	NP	LP	PM1,PM2, PP2,PP3	NM_000162
P20	<i>HNF1A</i>	c.476G > A	p.Arg159Gln	Het	Exon 2	Mis	Pat	LP	PM2,PM5, PP2,PP3,PP5	NM_000545
P21	<i>HNF1B</i>	c.704G > A	p.Arg235Gln	Het	Exon 3	Mis	LP	LP	PM1,PM2,PM5,PP2,PP3,PP5	NM_000458
P22	<i>GCK</i>	c.790G > A	p.Gly264Ser	Het	Exon 7	Mis	NP	LP	PM1, PM2, PP2,PP3,PP5	NM_000162

P: Patients **V Loc:** Variant Location **AA:** Aminoacid **Zyg:** Zygosity **ACMG Class:** The American College of Medical Genetics and Genomics Classification **ACMG** **ACMG** Pathogenicity Criteria **Het:** Heterozygous **Hom:** Homozygous **Del:** Deletion **Frms:** Frameshift **Mis:** Missense **Splic:** Splicing **NP:** Not Provided **Conf In:** Confirmed interpretation **Pat:** Pathogenic **LP:** Likely Pathogenic **VUS:** A variant of uncertain significance **Bng:** Benign **LB:** Likely Benign

All variants identified by NGS were confirmed by Sanger sequencing, and patients and their parents were tested in order to determine whether the identified variants were *de novo* or inherited. Sanger sequencing was performed using the Applied Biosystems (ABI) 3130 Genetic Analyzer (Foster City, CA, USA). Detected variants were classified as "pathogenic" "likely pathogenic" or "variant of uncertain significance (VUS)" according to the international guidelines of ACMG. They are listed in Table 1.

To potentially link genetic variants to MODY, we further investigated protein structures in a computational setting. The primary structures of the proteins of interest were retrieved from the UniProt Knowledgebase (available at <https://www.uniprot.org/>; UniProt Consortium, 2021), along with the information on sequence similarities to other proteins belonging to the same family and the domains present in each protein. The tertiary/quaternary structures of the

proteins of interest were retrieved from the RCSB Protein Data Bank (available at <https://www.rcsb.org/>; Berman *et al.*, 2000). The PyMOL Molecular Graphics System, Version 1.8 (Schrödinger LLC, Portland, OR, USA) was used to visualize and analyze protein structures and related data.

Results

A total of 44 patients were included in the study. There were 18 males (40.9%) and 26 females (59.1%), with a mean age of 13.307 ± 7.177 years (minimum: 3.5; maximum: 40) and a mean age at diagnosis of 10.773 ± 6.167 years (minimum: 1.5; maximum: 30). The average insulin requirement of patients and the mean level of C-peptide were 0.833 ± 0.358 U/kg/day (minimum: 0.19; maximum: 1.55) and 0.617 ± 0.433 ng/mL (minimum: 0.10; maximum: 2.41), respectively. The mean BMI, HbA_{1c} at diagnosis and level of fasting blood glucose were 19.568 ± 4.216 kg/m² (minimum: 13.80; maximum: 34.50), 9.362 ± 2.705 per cent (minimum: 5.80; maximum: 15) and 273.5 ± 123.941 mg/dL (minimum: 100; maximum: 543), respectively. Presenting complaints included incidental identification of hyperglycemia in 14 patients (31.8%), polyuria and polydipsia in 18 patients (40.9%), ketoacidosis in 7 patients (15.9%), polyuria in 3 patients (6.8%), polydipsia in 2 patients (4.5%). All these cases were negative for ICA, with one of them being positive for IAA and three of them being positive for anti-GAD.

Based on the molecular genetic analysis results, patients were divided into those with a variant classified as possibly pathogenic/pathogenic and others. The clinical and laboratory characteristics belonging to them are given in Tables 2 and 3. Thirteen different genes were analysed in 44 unrelated cases with a pre-diagnosis of MODY. Pathogenic/likely pathogenic variants in 10 (22.7%) cases, variants of uncertain significance (VUS) in 8 (18.2%) cases, likely benign/benign variants in 4 (9%) cases were detected in our study. The distribution of detected pathogenic/likely pathogenic variants were as follows: five variants in the *GCK* gene, two variants in the *HNF1A* gene, one variant in the *ABCC8* gene, one variant in *HNF1B* gene and one variant in the *CEL* gene. VUS were detected in the following numbers: two variants in the *HNF1A* gene, two variants in the *KLF11* gene, two variants in the *NEUROD1* gene and one variant in the *ABCC8* gene. The distribution of likely benign/benign variants were as follows: two variants in the *NEUROD1* gene, one variant in the *BLK* gene, one variant in the *CEL* gene (Table 1). Overall, a total of 21 different variants were identified in this study (Table 1). We were able to identify four novel pathogenic/likely pathogenic variants (one in *GCK*, one in *ABCC8*, one in *HNF1A*, one in *CEL*) and two novel VUS (both in *NEUROD1*). All variants detected in this study were heterozygous, and the pathogenicity assessments of them are provided in Table 1.

Table 2
Symptoms, clinical and genetic findings of the patients

<i>P No</i>	<i>Sex</i>	<i>Age</i>	<i>Age at diagnosis</i>	<i>first symptom</i>	<i>Treatment</i>	<i>Diabetic parents</i>	<i>Seg Anly Res of Par</i>	<i>Mother</i>	<i>Phenotype</i>
P1	F	4	1.5	Coincidental	diet	Mother	N	c.449_451delTCT	MT 2
P2	F	9	8	Coin	diet	Father	c.537delG	N	MT 2
P3	M	17	14	Puria, Pdips	Insulin 0.66 u/kg	-	N	N	MT 3
P4	F	13	10	Puria, Pdips	Insulin 0.76 u/kg	-	N	c.268A>C	DM
P5	F	12	9	Puria, Pdips	Insulin 1.05 u/kg	Father	c.679-1G>A	N	DM
P6	M	17	15	Puria, Pdips	Insulin 0.8 u/kg	Mother	N	N	DM
P7	M	10	9	Puria, Pdips	Insulin 0.57 u/kg	Father	N	N	DM
P8	F	12	7	Puria, Pdips	Insulin 0.58 u/kg	Father	c.1382G>A	N	MT 7
P9	F	10	8	Puria, Pdips	Insulin 0.62 u/kg	Father	c.-71G>A	N	DM
P10	F	9	7	Puria	Insulin 0.8 u/kg	-	N	N	DM
P11	M	14	11	Puria, Pdips	Insulin 0.44 u/kg	-	N	N	DM
P12	M	7	5	Puria, Pdips	Insulin 0.5 u/kg	Mother	N	c.1943G>A	MT 12
P13	M	28	20	Puria	Insulin 0.5 u/kg	Mother/Father	c.2168T>C, c.83C>T	c.2168T>C	MT 1 /WFS
P14	M	9	8	Coincidental	diet	Mother	N	c.664G>A	MT 2
P15	F	17	15	Ketoasidozis	Insulim 0.74u/kg	Mother	NA	NA	MT 7
P16	M	30	25	Coincidental	0.5 u/kg	-	N	N	MT 3
P17	M	40	30	Pdips	OAD	Father	NA	NA	MT 12
P18	M	25	24	Coincidental	OAD	-	N	N	MT 3
P19	M	6	3	Coincidental	diet	Father	c.1301G>T	N	MT 2
P20	M	16	14	Puria, Pdips	Insulin 0.48 u/kg	Mother	N	c.476G>A	MT 3
P21	M	21	19	Puria, Pdips	Insulin 0.81 u/kg	-	N	N	MT 5
P22	F	7	6	Coincidental	Diet	father	c.790G>A	N	MT 2
P: Patients Puria: Polyuria Pdips: Polydipsia OAD: Oral antidiabetic drugs Seg Anly Res of Par: Segregation Analaysis results of the parents N: No NA: Not applicable MT: Mody type DM: Diabetes Mellitus WFS: Wolfram syndrome									

Table 3
Clinical and laboratory characteristics between patients who were carrying a LP/P variant and the other patients

Pathogenicity			
	The others(n = 34)	LP/P(n = 10)	p
Age(Years)	12.735 ± 5.265	15.250 ± 11.793	0.855
Age at diagnosis(Years)	10.308 ± 4.914	12.350 ± 9.464	0.877
c-peptide level (ng/ml)	0.621 ± 0.475	0.607 ± 0.287	0.453
Insulin requirement (U/kg)	0.852 ± 0.370	0.710 ± 0.272	0.479
BMI in Diagnosis (kg/m ²)	18.535 ± 4.547	22.150 ± 1.334	0.002*
HbA1c (%) in Diagnosis	9.966 ± 2.659	7.430 ± 1.880	0.004*
Fasting Blood Glucose in Diagnosis (mg/dl)	299.353 ± 125.388	185.600 ± 68.084	0.011*
Last Observed HbA1c Value (%)	9.885 ± 10.391	6.720 ± 0.814	0.003*
History of diabetes in at least two generations	Y:26(76.5%)/ N:8(23.5%)	Y:8(80%)/ N:2(20%)	0.815
BMI:Body mass index Y:Yes N:No LP/P : likely pathogenic/pathogenic			

Clinical and molecular analysis results of the variant (+) patients

GCK-MODY (MODY 2)

We detected two pathogenic and three likely pathogenic variants in the *GCK* gene, all of them being previously described in the literature except for the c.1301G > T (p.Cys434Phe) variant. The corresponding patients are shown in Tables 1 and 2 as P1, P2, P14, P19 and P22, along with the clinical and molecular findings in them.

Fasting blood glucose levels in P1 were between 120 and 180 mg/dL and monitored by diet. In the family history, the fasting blood glucose of the mother of P1 did not exceed 200 mg/dL and she did not use any medication. Through the targeted mutational analysis, it was shown that the mother carries the c.449_451delTCT (p.150delF) variant. The results of the segregation analysis demonstrated that P2 inherited the c.537delG (p.Asn180ThrfsTer25) variant from her father. The father's fasting blood sugar levels were around 150–160 mg/dL, and he was first diagnosed when he was being examined for other reasons at 25 years old. Currently, he is using oral metformin. Fasting blood glucose levels in P14 were between 100 and 150 mg/dL and monitored by diet. DNA sequence analysis revealed that the patient inherited the variant from his mother. It is worth noting that the patient's mother and grandmother also have a history of diabetes.

Fasting blood glucose levels in P19 were 90–160 mg/dL and monitored by diet. The same variant (c.1301G > T [p.Cys434Phe]) was found in the patient's father by Sanger sequencing. In the last routine follow-up visit of P19 who had high fasting sugar for three years, HbA_{1c} was measured as 6.3%, body mass index as 22 kg/m² and C-peptide as 0.8 ng/mL.

The *de novo* heterozygous c.790G > A (p.Gly264Ser) variant was detected in P22. The patient was first noticed that he had a high fasting blood glucose level coincidentally. Today, the patient does not use any medication and fasting blood sugar levels vary between 80 and 160 mg/dL. The clinical and molecular findings of the above-mentioned patients were consistent with GCK-MODY.

GCK encodes glucokinase, a 465-residue-long monomeric enzyme that is responsible for glucose phosphorylation in hepatocytes and pancreatic β-cells. Asn180 is located in an α-helical region (helix 5; residues 180–193), while Phe150 is located in a β-stranded region (strand 5; residues 145–150) (Fig. 1A). Although Phe150 lies in the close vicinity of the enzyme's active site, an analysis of polar interactions occurring between the enzyme and substrate reveals that it does not interact with glucose (Fig. 1B). Similarly, Asn180 itself is not engaged in favorable contacts with glucose. The majority of glucose-binding residues, however, are situated after position 180, meaning that the frameshift variant affecting Asn180 is expected to result in a severely truncated protein (if translated) with no catalytic activity.

ABCC8-MODY (MODY 12)

We detected the c.1943G > A (p.Arg648His) variant as "VUS" in the *ABCC8* gene of our 7-year-old male patient, P12, with elevated sugar levels for two years. The allele frequency of this variant is estimated to be 0.000012 in the gnomAD exomes database. The same variant was detected in his mother. In the last routine follow-up visit of P12, fasting blood glucose levels ranged from 100 to 160 mg/dL, insulin requirement was 0.19 U/kg/day, body mass index was 17.7 kg/m² and C-peptide was 0.87 ng/mL. In the family history, it was noted that the patient's mother had high blood sugar levels in the past, with the exact value being 124 mg/dL at the time of the follow-up visit. It was also noted that the patient's grandmother had a history of diabetes, which is being controlled by oral antidiabetics since the age of 30. Clinical and laboratory findings of this family were consistent with MODY.

The heterozygous c.3584C > T (p.Thr1195Ile) variant, which was predicted to be “likely pathogenic”, was detected in the *ABCC8* gene of P17. The high levels of fasting sugar in the patient were first noticed 10 years ago, and the patient was on oral antidiabetic agents. He had no insulin requirement, and his fasting blood glucose levels never exceeded 250 mg/dL. The patient’s father had a history of diabetes, and it was noted that he was being treated with insulin. We did not have the opportunity to perform segregation analysis involving the patient’s parents.

ABCC8 codes for the regulatory subunit sulfonylurea receptor 1 (SUR1) which, together with the pore-forming subunit Kir6.2, constitutes the ATP-sensitive potassium (K_{ATP}) channels in pancreatic β -cells. Arg648 is located in a cytosolic loop structure between the eleventh transmembrane domain (TM11) and the first nucleotide-binding domain (NBD1) of SUR1 (Fig. 2). Both SUR1 and Kir6.2 contain a tripeptide endoplasmic reticulum retention motif termed RKR. In SUR1, this motif is composed of Arg648-Lys649-Arg650. The occurrence of the RKR motif simultaneously in SUR1 and Kir6.2 ensures that only fully functional β -cell K_{ATP} channels are trafficked to the plasma membrane, since each motif is only shielded when the two subunits are properly assembled or folded [5]. Therefore, it is plausible to assume that the Arg648His substitution may lead to dysregulation of intracellular SUR1 trafficking, interfering with the critical role of β -cell K_{ATP} channels in the glucose-stimulated insulin secretion pathway.

HNF1A-MODY (MODY 3)

The heterozygous c.505_506delAA (p.Lys169AlafsTer18) variant was observed in P16 in a *de novo* case of MODY. The presence of high fasting blood glucose levels in the patient was first noticed five years ago by coincidence. Today, the patient’s condition is controlled by low-dose insulin injections, and his fasting blood sugar levels vary between 90 and 200 mg/dL.

The heterozygous c.376 C > T (p.His126Tyr) variant, which was predicted to be “VUS”, was detected as a *de novo* mutation in P18. The patient’s parents had no previous history of diabetes. This variant is not present in the gnomAD exomes database and has not been reported in the ClinVar archive before. Sequence- or structure-based prediction tools further support the pathogenicity of this variant. The patient has been diagnosed with diabetes for one year, and his blood glucose levels are controlled by oral antidiabetics.

We identified the *de novo* c.862G > A (p.Gly288Arg) variant in exon 4 of the *HNF1A* gene. The allele frequency of this variant is calculated to be 0.0000461 in the gnomAD exomes database and has not previously been registered in ClinVar. Sequence- or structure-based prediction tools further support the pathogenicity of this variant and considering familial co-segregation analysis results, we think that this variant may be a disease-causing variant.

We identified the c.476G > A (p.Arg159Gln) variant in exon 2 of the *HNF1A* gene, which has been classified as pathogenic in ClinVar. In the last follow-up visit of P20, fasting blood glucose values were measured to vary from 100 to 244 mg/dL, insulin requirement to be 0.48 U/kg/day, body mass index to be 23 kg/m² and C-peptide concentrations to be 0.77 ng/mL. The same variant was detected in his mother and she was diagnosed with diabetes at the age of 25 and it was noticed that she had insulin requirement.

HNF1A encodes hepatocyte nuclear factor1 alpha, a transcription factor that has been shown to regulate the tissue-specific expression of multiple genes including the gene for insulin in pancreatic β -cells and the gene for GLUT2 in glucose-sensing cells [6, 7]. Gly288 appears to be situated in a proline-rich, low-complexity region (residues 287–315) adjacent to the homeobox (HOX) domain of the protein. This region belongs to the C-terminal transactivation domain of the protein, indicating that the Gly288Arg substitution may impair the interactions of HNF1 α with coactivators. This, however, remains to be investigated by biochemical and structural approaches. To date, no human HNF1 α structures containing the C-terminal transactivation domain have been deposited in the PDB.

HNF1B-MODY (MODY 5)

The heterozygous c.704G > A (p.Arg235Gln) variant was detected as a *de novo* mutation in P21. The patient was diagnosed with diabetes two years ago, and his blood glucose levels fall in the range of 100–310 mg/dL. In his last follow-up visit, C-peptide was found to be 0.45 ng/mL and HbA_{1c} to be 7.1%.

Sequence- or structure-based prediction tools further support the pathogenicity of this variant and considering the segregation analysis results we propose this variant as “likely pathogenic”.

CEL-MODY (MODY 8)

We identified two patients carrying a variant in the *CEL* gene. P4 was found to bear the c.268A > C variant which was classified as “likely benign” according to the ACMG guidelines. In the last control visit of the patient, who has been followed up with a diagnosis of diabetes for three years, it was noted that fasting blood sugar levels ranged between 100 and 320 mg/dL and postprandial blood glucose levels between 100 and 240 mg/dL, and the patient’s insulin requirement, HbA_{1c} levels, C-peptide concentrations and body mass index were 0.72 U/kg/day, 7.3%, 0.38 ng/mL and 23 kg/m², respectively. The mother of P4 had no history of diabetes, her fasting blood glucose levels were measured as 110 mg/dL, and it was demonstrated that she also carried the same variant. Because of the low penetrance and variable expression of the *CEL* gene [8], additional studies are required to better evaluate the pathogenic potential of this variant which has a low allele frequency (0.000112) in the gnomAD exomes database.

CEL codes for bile salt-activated lipase (BAL), a secreted pancreatic enzyme that hydrolyzes a broad spectrum of lipids. Thr90 is located in a conserved 17-residue-long surface loop structure termed the Cys-loop (residues 84–100), which is adjacent to the catalytic site (Fig. 3). The Cys-loop is anchored by a disulfide bridge between Cys84 and Cys100 and forms a cover lid normally responsible for the water–oil interfacial activation of the related triacylglycerol lipases [9, 10]. It could be that simply introducing Pro90 into the Cys-loop or further having a prolyl *cis/trans* isomerization reaction at position 90 may affect the conformation of the Cys-loop, altering the biological function of BAL. This may eventually lead to pancreatic exocrine dysfunction which could be linked to MODY.

In P5, a heterozygous IVS5-1G > A variant in intron 5 of the *CEL* gene was detected. We showed that this variant, which has not been reported in the literature before, was also carried by the father of the patient. The effect of the variant on splicing mechanisms was predicted to be “deleterious” by the Human Splicing Finder. Biochemical parameters obtained in the last control visit of P5, who has been followed up with a diagnosis of diabetes for three years, were as follows: fasting blood glucose levels were 122–340 mg/dL, postprandial blood glucose levels were 130–210 mg/dL, C-peptide levels were 0.23 ng/mL and insulin requirement was 1.05 U/kg/day. Autoantibodies that were re-examined at the last examination were negative and BMI was 20 kg/m². Her father’s blood test revealed fasting blood glucose levels of 128 mg/dL and HbA_{1c} levels of 6.5%. Based on these results, it is plausible to assume that the variant detected in P5 may lead to the MODY phenotype. This, however, needs further studies to be substantiated.

NEUROD1-MODY (MODY 6)

We detected genetic alterations in *NEUROD1* in four patients. In the first patient (P6), we identified the heterozygous *de novo* c.-81C > A variant in the 5' UTR region of the gene. This variant is not present in the gnomAD exomes and genomes databases and has not been reported in ClinVar before. In the last control visit of the patient, who has been followed up with a diagnosis of diabetes for two years, BMI, HbA_{1c} levels and C-peptide concentrations were found to be 27 kg/m², 8.1%, 1.79 ng/mL, respectively, with negative assay results for autoantibodies. Collectively, these findings suggest that the variant may be a rare polymorphism contributing to type 2 diabetes.

In another patient (P9), the heterozygous *de novo* c.-71G > A variant in the 5' UTR region of the gene was detected. This variant has an estimated allele frequency of 0.0000637 in the gnomAD genomes database and has not been reported in ClinVar before. The father of the patient had a history of diabetes beginning at about the age of 35 and was on oral antidiabetics. In the last visit of the patient, C-peptide concentrations were 0.2 ng/mL, HbA_{1c} levels were 8.7%, BMI was 19 kg/m², and autoantibodies were negative except for anti-GAD.

Finally, two patients (P10 and P11) were found to bear the c.590C > A (p.Pro197His) variant in the *NEUROD1* gene. The allele frequency of this variant was calculated to be 0.0193 in gnomAD exomes and 0.0129 in gnomAD genomes. The variant has previously been reported as “benign” in Clinvar. According to some *in silico* tools, such as Mutation Taster, Provean and SIFT, this variant is damaging in nature. By increasing the number of cases available for analysis and having more detailed data regarding the population allele frequencies at this locus, we should be able to draw empirically well-based inferences.

NEUROD1 encodes neurogenic differentiation factor 1, a transcriptional activator that regulates insulin gene expression in pancreatic β-cells through binding to its E-box motif-containing promoter consensus sequence [11]. Pro197 is situated in the protein’s neuronal basic helix-loop-helix (bHLH) transcription factor domain (residues 160–284) which is involved in DNA binding. Therefore, it is tempting to think that Pro197His may lead to dysregulated insulin expression in β-cells, adversely affecting pancreatic cell-fate specification. Further biochemical and structural studies are required to delineate the pathobiology of this substitution. Currently, there are no human NeuroD1 structures deposited in the PDB.

BLK-MODY (MODY 11)

The heterozygous c.211G > A (p.Ala71Thr) variant was identified as *de novo* mutation in P7. The allele frequency of the variant is estimated to be 0.0116 in gnomAD exomes, while conflicting interpretations of pathogenicity have been reported in ClinVar. Bonnefond *et al.* stated in their study that the p.Ala71Thr variant, which causes a loss of function of the gene product, is highly unlikely to cause penetrating MODY and may weakly affect the risk of type 2 diabetes, although they also added that this awaits further clarification [12]. The patient has been diagnosed with diabetes mellitus for 2.5 years and tested negative for autoantibodies in the past. The test results from the last follow-up visit revealed that the patient’s fasting blood glucose levels were 90–160 mg/dL, postprandial blood glucose levels were 170–190 mg/dL, C-peptide concentrations were 0.25 ng/mL, BMI was 24 kg/m² and insulin requirement was 0.57 U/kg/day. Familial co-segregation analysis results and a number of sequence- or structure-based prediction tools support the pathogenicity of c.211G > A (p.Ala71Thr), but additional studies are needed in order to evaluate the causal role of this variant in MODY.

BLK codes for B-lymphocyte kinase, a non-receptor tyrosine kinase that is involved in cellular development, differentiation and signaling. Ala71Thr, which lies in the SH3 domain (residues 58–118) of the protein, has previously been shown to cause MODY by attenuating the biological effects associated with BLK, especially the up-regulation of the transcription factors Pdx1 and Nkx6.1 which promote insulin synthesis and secretion in response to glucose in pancreatic β-cells [13]. At present, there are no human BLK structures deposited in the PDB.

KLF11-MODY (MODY 7)

We found a variant (c.1382G > A [p.Arg461Gln]) that we classified as “VUS” in the *KLF11* gene of P8. While the pathogenicity of this variant has not been clearly elucidated previously, using Mutation Taster and SIFT algorithms we predict that the variant is “damaging”. The variant has an allele frequency of 0.000497 in gnomAD exomes and appears to have been conserved in the evolutionary process. The patient’s father and grandfather had a history of diabetes, and it was noted that the father’s fasting blood glucose levels did not exceed 300 mg/dL and that he did not use insulin at any time. We confirmed that the father is a carrier for the same genetic alteration.

We detected the c.1447C > T (p.Pro483Ser) variant, which we classified as “VUS”, in the *KLF11* gene of our 17-year-old female patient (P15) present with elevated sugar levels for two years. The allele frequency of this variant is estimated to be 0.0000637 in the gnomAD exomes database. The last follow-up history of P15 revealed that her fasting blood glucose levels ranged from 100 to 250 mg/dL, insulin requirement was 0.74 U/kg/day, BMI was 21 kg/m² and C-peptide concentrations were 0.66 ng/mL. Although the patient’s mother and grandmother were known to have a history of diabetes starting after the age of 30, we did not have the opportunity to subject them to molecular genetic analysis.

KLF11 encodes Kruppel-like factor 11, a pancreas-enriched zinc finger transcription factor that regulates the expression of genes related to cellular differentiation and inflammation in early embryonic development. Arg461Gln is situated in the C2H2-type zinc finger domain (residues 454–476) of the protein, suggesting that this substitution may impair the interactions of KLF11 with coactivators, thereby leading to pancreatic β-cell dysfunction. Indeed,

KLF11 has previously been reported to activate Pdx1 [14], a transcription factor critical to pancreatic organogenesis and the maintenance of mature β -cell function. The precise mechanism of action of Arg461Gln is yet to be determined experimentally. Currently, there are no human KLF11 structures deposited in the PDB.

Wolfram syndrome

P13, who had a family history of diabetes for three generations and whose diabetes-related autoantibodies were negative, was first noticed eight years ago when he presented with high fasting sugar levels. The fasting blood glucose levels of the patient, who currently requires 0.5 U/kg/day insulin, vary between 90 and 240 mg/dL, and his HbA_{1c} at the last control was 7.6%. By running the MODY panel described in the present study for this patient, we identified the heterozygous c.83C > T (p.Ala28Val) variant in the *HNF4A* gene. The parents of the patient had a history of diabetes and were on oral antidiabetic drugs. The patient's father was also shown to carry the same variant. In addition, the *WFS1* gene was sequenced in order to determine the potential variants that cause Wolfram syndrome since the patient was followed up from an early age due to the presence of bilateral optic atrophy and mild hearing loss and there was a history of consanguineous marriage between his parents. The homozygous c.2168T > C (p.Leu723Pro) variant, which we classify as "VUS", was detected in the patient. This change has previously been described in the literature and is known to be associated with Wolfram syndrome (HGMD: CM112219).

Discussion

Frequently, it is clinically challenging to distinguish MODY from type 1 and type 2 diabetes. Therefore, it is safe to assume that several-to-many patients with this monogenic form of diabetes are misclassified or misdiagnosed. Although MODY accounts for 1–5% of all diabetic cases, molecular genetic diagnosis of MODY is necessary for optimal long-term treatment, prognosis, and especially genetic counseling. Studies have shown that pathogenic variants are mostly detected in the MODY-related genes *GCK*, *HNF1A* and *HNF4A* and that such variants in *GCK* and *HNF1A* are responsible for approximately 70% of all MODY cases [15]. It is believed that many genes associated with MODY have not been identified yet [16]. Through advancing molecular testing solutions and identification of new genes, the number of patients with monogenic diabetes can be expected to rise rapidly. Moreover, the frequency of disease-causing variants in MODY is likely to show differences among societies [16–18]. To date, 14 genes have been defined in the OMIM database, all of which have been proven to cause MODY.

In the current study, we detected a likely pathogenic/pathogenic MODY-related variant in approximately 23% of our patients and made a comprehensive description of the clinical and molecular characteristics of these patients. We believe that our cases will contribute to the development of the relevant scientific literature on MODY. According to our findings, a statistically significant difference ($p < 0.05$) was found between the patients who were carrying a likely pathogenic/pathogenic variant (i.e. the risk group) and the other patients with respect to BMI, HbA_{1c} values, and fasting blood glucose levels (Table 3). However, when the clinical data of patients such as gender, age at diagnosis, presence of diabetes in at least two generations, autoantibody positivity and C-peptide concentrations were compared, no statistically significant difference was found between the two groups ($p > 0.05$).

A careful diagnosis of HNF1B-MODY in an individual who is being followed up for diabetes provides clues about the extra pancreatic findings that accompany or co-occur with MODY and enables a multidisciplinary planning of the patient's follow-up [19]. However, in the abdominal MRI examination of our patient with the MODY phenotype (P21), in whom we detected a likely pathogenic variant in the *HNF1B* gene, no additional features, such as the commonly encountered renal cysts, were observed.

The results of this study clearly indicate that mutations in the *GCK* gene continue to rank first among the leading causes of MODY in a population of Turkish patients. Here, we found two pathogenic and three likely pathogenic variants in the *GCK* gene. If translated, c.537delG (p.Asn180ThrfsTer25) truncates ~ 60% of glucokinase, resulting in a loss of numerous hydrophilic, substrate-binding residues (such as Asn204, Asp205, Asn231, Glu256, Gln287, Glu290). Given the fact that glucokinase in pancreatic β -cells serves as a glucose sensor for amplifying insulin secretion as blood glucose concentration rises, there is no surprise as to the pathogenic nature of this frameshift variant in MODY. On the other hand, c.448_450delTTC (p.150delPhe) is likely to have a profound impact on the "molecular phenotype" of glucokinase, with no useful clues as to the exact mechanism of pathogenicity. Missense variants at position 150 (Phe150Leu, Phe150Ser, Phe150Tyr) as well as at the neighboring positions (Phe148Ile, Thr149Ile, Thr149Pro, Ser151Pro, Ser151Thr, Phe152Ile, Phe152Leu, Phe152Ser) have been reported to be associated with MODY in the Human Gene Mutation Database [20], indicating to the functional significance of this specific region of glucokinase. Indeed, the polar side chain of the adjoining residue Ser151 is involved in the hexahedral coordination of the Mg²⁺ cofactor through a mediating water molecule [21]. Therefore, it is tempting to speculate that Δ Phe150 may lead to a loss of Mg²⁺ coordination, affecting the catalytic properties of glucokinase. In four separate studies conducted recently by Turkish researchers about MODY, GCK-MODY has been proposed to be the most frequent type of MODY in Turkey [22–25].

The findings of the present study should be considered in the light of various limitations. First, the *APPL1* gene, which is associated with MODY 14, was not integrated into said NGS-based targeted gene panel. It may be worth mentioning here, however, that *APPL1* causes less than 1% of all MODY types [26]. Also, we did not attempt to measure the patients' enzyme activities or perform any other functional analyses of the newly identified variants. Last, we did not investigate the entire range of mutational events, including large deletions and duplications.

Conclusion

In conclusion, the practical yield of this study is considerable because it reflects professional experience gained in a single center and represents one of the first studies in Turkish children including molecular analysis of 13 MODY-related genes. We were able to analyze ABCC8 and KCNJ11 gene mutations in patients with clinically suspected MODY in our country and determine a patient whose test results were consistent with ABCC8-MODY. And besides, we identified two novel variants: One in GCK (c.537delG) and one in *CEL* (c.679-1G > A). In studies conducted with NGS method as in our study, it was observed that some patients have rare types of MODY. We believe that the frequency of these rare MODY alleles will be assessed more accurately through further

studies to be conducted with larger series and that family members under risk will be screened more rapidly via a detailed review of the genetic etiology of MODY.

Declarations

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Declarations Conflict of interest

The authors declare that they have no conflict of interest.

Ethics approval

It was approved by the Duzce University Faculty of Medicine Non-Invasive Clinical Research Ethics Committee with the approval number Decision No: 2016/24. Informed consents were obtained from the patients and their parents to participate in this study and publish their datas. All authors give consent for the article to be published in Molecular Biology Reports.

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Figures

A **B**

Figure 1

(A) A ribbon diagram showing the three-dimensional structure of human glucokinase (PDB ID: 3IDH). The bound glucose substrate is shown in brown sticks. The relative positions of the affected Phe150 and Asn180 residues are also indicated. (B) The polar interaction network that anchors glucose in the active-site pocket of glucokinase. Yellow dashed lines represent hydrogen bonds. Red spheres represent structurally relevant water molecules. These images were rendered using the PyMOL Molecular Graphics System, Version 1.8 (Schrödinger LLC, Portland, OR, USA).

Figure 2

A ribbon diagram showing the three-dimensional structure of human sulfonylurea receptor 1 (PDB ID: 6C3O). The affected Arg648 residue is located in a cytosolic loop structure (magenta; mostly missing in the crystal structure due to high flexibility) between the eleventh transmembrane domain (green) and the first nucleotide-binding domain (cyan) of the receptor. This image was rendered using the PyMOL Molecular Graphics System, Version 1.8 (Schrödinger LLC, Portland, OR, USA).

Figure 3

A close-up view of the catalytic site of human bile salt-activated lipase (PDB ID: 1JMY). The catalytic triad with Ser214–His455–Asp340 forms a charge relay network for substrate hydrolysis. The Cys-loop, which is anchored by a disulfide bridge between Cys84 and Cys100, forms a cover lid for lipase activation at a water–oil interface. The relative position of the affected Thr90 residue is also indicated. This image was rendered using the PyMOL Molecular Graphics System, Version 1.8 (Schrödinger LLC, Portland, OR, USA).