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ORIGINAL ARTICLE

Study of ten 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.

Methods 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 ten MODY-causal genes involving *GCK, HNF1A, HNF1B, HNF4A, ABCC8, CEL, INS, KCNJ11, NEUROD1, PDX1* was designed and subsequently implemented to screen 40 patients for genetic variants.

Results Ten different pathogenic or likely pathogenic variants were identified in MODY-suspected patients, with a diagnostic rate of 25%. Three variants of uncertain significance were also detected in the same screen. A novel pathogenic variant in the gene *HNF1A* (c.505_506delAA [p.Lys169AlafsTer18]) was described for the first time in this report. Intriguingly, we were able to detect variants associated with rare forms of MODY in our study population.

Conclusions 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 2–5% 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.

Keywords Monogenic diabetes · Maturity-onset diabetes of the young · MODY · Next-generation sequencing · Targeted gene panel

Introduction

Maturity-onset diabetes of the young (MODY), which is the most frequent cause of monogenic diabetes, is inherited in an autosomal dominant manner and shows both clinical and genetic heterogeneity [1]. Other monogenic diabetes phenotypes include neonatal diabetes, mitochondrial diabetes, and diabetes mellitus associated with syndromic diseases. The MODY type of diabetes occurs mostly under the age of 25 years, but it can also occur later. 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).

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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]. At least 11 genes responsible for MODY have been identified to date, and these genes correspond to the following subtypes: *GCK*-MODY, *HNF1A*-MODY, *HNF4A*-MODY, *HNF1B*-MODY, *ABCC8*-MODY, *KCNJ1*-MODY, *INS*-MODY, *PDX1*-MODY, *NEUROD1*-MODY, *CEL*-MODY, and *APPL1*-MODY [3, 4]. Previous studies have shown that the most common MODY subtypes are *GCK*-MODY (30–70% of all MODY cases) [5], *HNF1A*-MODY (30–70% of all MODY cases)[6], *HNF4A*-MODY (5–10% of all MODY cases) [7], and *HNF1B*-MODY (5–10% of all MODY cases) [8]. MODY subtypes originating from variants in other MODY-related genes appear to be very rare [3].

Although variants detected in the *INS*, *ABCC8* and *KCNJ11* genes were initially linked to phenotypes such as neonatal diabetes and congenital hyperinsulinism, variants in these genes have also been described in the literature for MODY subjects [9–11]. Different MODY phenotypes require different therapeutic approaches. For instance, while the *GCK*-MODY subtype is characterized by mild non-progressive hyperglycemia and can be regulated by diet, the *HNF4A*-MODY and *HNF1A*-MODY subtypes are often misdiagnosed as type 1 or 2 diabetes and treated with insulin as opposed to sulfonylureas [12]. Molecular confirmation of the diagnosis prevents unnecessary insulin treatment in MODY patients and improves both metabolic control and quality of life. 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 [13]. In this study, we aimed at analyzing 10 different genes (*ABCC8, CEL, GCK, HNF1A, HNF1B, HNF4A, INS, KCNJ11, NEUROD1, PDX1*) using the NGS method in cases with clinically suspected MODY.

Subjects and methods

40 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. All the families met the generally accepted clinical diagnostic criteria for MODY as follows: a family history of diabetes consistent with autosomal dominant transmission in at least two or three generations; early-onset hyperglycemia (diagnosed aged < 25 years); no islet autoantibodies; non-insulin dependence (lack of a need for insulin treatment or a serum C-peptide level of > 0.60 ng/mL, even after 3 years of insulin treatment) [14]; lack the characteristics of type 2 diabetes (marked obesity, acanthosis nigricans). In addition, three more families, who did not meet the family history diagnostic criterion but met other diagnostic criteria, were also included in the study.

Genomic DNA was extracted from peripheral blood and NGS was performed by capture of the coding regions and

Table 1 Clinical presentation, treatment, and genetic findings of MODY in patients

P Number	*Sex*	*Age at Diagnosis (Years)*	*Clinical Presentation*	*Treatment*	*Inheritance*	*Gene*	*Seg Anly Res Par*	*Age of Onset of Hyperglycemia in the Parent (Years)*
P1	F	8	Incidental	Diet	Father	*GCK*	p.Asn179ThrfsTer25	25
P2	M	8	Incidental	Diet	Mother	*GCK*	p.Glu221Lys	23
P3	F	1.5	Incidental	Diet	Mother	*GCK*	p.Phe150del	20
P4	M	3	Incidental	Diet	Father	*GCK*	p.Cys434Phe	15
P5	F	6	Incidental	Diet	Father	*GCK*	p.Gly264Ser	30
P6	M	25	Incidental	Insulin at 0.5 U/kg	Father	*HNF1A*	p.Lys169AlafsTer18	30s
P7	M	14	Puria, Pdips	Insulin at 0.48 U/kg	Mother	*HNF1A*	p.Arg159Gln	25
P8	M	14	Puria, Pdips	Insulin at 0.66 U/kg	-	*HNF1A*	N	-
P9	M	24	Incidental	OAD	-	*HNF1A*	N	-
P10	M	19	Puria, Pdips	Insulin at 0.81 U/kg	-	*HNF1B*	N	-
P11	F	9	Ketoacidosis	Insulin at 1.05 U/kg	Father	*CEL*	c.670-1G > A	18
P12	M	25	Pdips	OAD	Father	*ABCC8*	NA	30
P13	M	5	Puria, Pdips	Insulin at 0.5 U/kg	Mother	*ABCC8*	p.Arg648His	15

P: Patient **Puria**: Polyuria **Pdips**: Polydipsia **OAD**: Oral Antidiabetic Drugs **Seg Anly Res Par**: Segregation Analysis Results of Parents **N**: Not Detected **NA**: Not Applicable **MT**: Mody Type **DM**: Diabetes Mellitus

splice sites of target genes (*GCK*, *HNF1A*, *HNF4A*, *HNF1B*, *PDX1*, *NEUROD1*, *CEL*, *INS*, *ABCC8* and *KCNJ11*) using via Illumina custom enrichment panel. 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×. 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 0.1% 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[15]. All variants identified by NGS were confirmed by Sanger sequencing, and patients and their parents were tested to determine whether the identified variants were *de novo* or inherited, albeit with no maternity and paternity analyses. 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 the 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/;[16], 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/; [17]. The PyMOL Molecular Graphics System, Version 1.8 (Schrödinger LLC, Portland, OR, USA) was used to visualize and analyze protein structures and related data.

The research data were analyzed via the Statistical Package for Social Sciences, or SPSS, version 23.0 (IBM Corp., Armonk, NY, USA). The Shapiro–Wilk test was used to inspect the distribution of data. Because the data were not normally distributed, the Mann–Whitney U test was used as the test of choice for the pairwise comparison of groups. $p < 0.05$ was accepted as statistically significant.

Results

A total of 40 patients were included in the study. There were 18 males (45%) and 22 females (55%), with a mean a mean age at diagnosis of 12.3 ± 7.928 years (minimum: 1,5; maximum: 25). The average insulin requirement of patients and the mean level of C-peptide were 0.735 ± 0.197 U/kg/day (minimum: 0.19; maximum: 1.25) and 0.608 ± 0.408 ng/mL (minimum:0.10; maximum: 2.41), respectively. The mean BMI, HbA_{1c} at diagnosis and level of fasting blood glucose were 19.693 ± 4.243 kg/m^2 (minimum: 13.80; maximum: 34.50), 9.187 ± 2.631 per cent (minimum: 5.80; maximum: 15) and 265.475 ± 114.756 mg/dL (minimum: 100; maximum: 485), respectively. Clinical presentation included incidental identification of hyperglycemia in 13 patients (32.5%), ketoacidosis in 3 patients (7.5%), polyuria and polydipsia in 17 patients (42.5%), polyuria in 4 patients (10%), polydipsia in 3 patients (7.5%). All these cases were negative for ICA, with one of them being positive for anti-GAD.

The clinical and laboratory characteristics belonging to patients who were carrying a pathogenic/likely pathogenic variant are given in Tables 2 and 3. Ten different genes were analyzed in 40 unrelated cases with a pre-diagnosis of MODY. After excluding all benign and likely benign variants, pathogenic or likely pathogenic variants in 10 (25%) cases, variants of uncertain significance (VUS) in 3 (7.5%) cases were detected in our study. The distribution of detected pathogenic/likely pathogenic variants were as follows: five variants in the *GCK* gene (5/10 or 50%), three variants in the *HNF1A* gene (3/10 or 30%), one variant in the *HNF1B* gene (1/10 or 10%) and one variant in the *CEL* gene (1/10 or 10%). VUS were detected in the following numbers: two variants in the *ABCC8* gene and one variant in the *HNF1A* gene. Overall, a total of 13 different variants were identified in this study (Table 1). We were able to identify a novel pathogenic variant in *HNF1A* gene. All variants detected in this study were heterozygous, and the pathogenicity assessments of them are provided in Table 1.

GCK-MODY

We detected one pathogenic and four likely pathogenic variants in the *GCK* gene, with the entire set of variants being previously described in the literature. The corresponding patients (P1, P2, P3, P4, and P5) are shown in Tables 1 and 2, along with the clinical and molecular findings in them. P1 was first noticed to have high fasting blood glucose levels six years ago incidentally at the age of 8, and at diagnosis her HbA_{1c} levels were mildly elevated at 6.6%. A heterozygous c.534delG (p.Asn179ThrfsTer25) variant was detected in P1, and the results of the segregation analysis

Table 2 The pathogenicity assessment of the detected variants

Patient Number	Gene	Transcript Number	Nucleotide Change	AA Change	MAF by gnomAD	Zyg	Variant Location	Variant Type	ClinVar	ACMG Class	ACMG Pat Crit
P1	*GCK*	NM_000162.5	c.534delG	p.Asn179ThrfsTer25	-	Het	Exon 5	Frms	NP	Pat	PVS1, PM2, PP4
P2	*GCK*	NM_000162.5	c.661G>A	p.Glu221Lys	-	Het	Exon 6	Mis	Pat	LP	PM1, PM2, PP2, PP3, PP4
P3	*GCK*	NM_000162.5	c.449_451delTCT	p.Phe150del	-	Het	Exon 4	Del	NP	LP	PM1, PM2, PM4, PM5, PP4
P4	*GCK*	NM_000162.5	c.1301G>T	p.Cys434Phe	-	Het	Exon 10	Mis	NP	LP	PM1, PM2, PP2, PP3, PP4
P5	*GCK*	NM_000162.5	c.790G>A	p.Gly264Ser	-	Het	Exon 7	Mis	Pat	LP	PM1, PM2, PP2, PP3, PP4
P6	*HNF1A*	NM_000545.8	c.505_506delAA	p.Lys169AlafsTer18	-	Het	Exon 2	Frms	NP	Pat	PVS1, PM2, PP4
P7	*HNF1A*	NM_000545.8	c.476G>A	p.Arg159Gln	-	Het	Exon 2	Mis	Pat	LP	PM1, PM2, PM5, PP3, PP4
P8	*HNF1A*	NM_000545.8	c.862G>A	p.Gly288Arg	0.0000461	Het	Exon 4	Mis	NP	VUS	PP3, PS2, PP4
P9	*HNF1A*	NM_000545.8	c.376 C>T	p.His126Tyr	-	Het	Exon 2	Mis	NP	LP	PM1, PM2, PP3, PS2, PP4
P10	*HNF1B*	NM_000458.3	c.704G>A	p.Arg235Gln	-	Het	Exon 3	Mis	LP	LP	PM1, PM2, PM5, PS2, PP3, PP4
P11	*CEL*	NM_001807.6	c.670-1G>A	-	-	Het	Intron 5	Splc	NP	Pat	PVS1, PM2, PP4
P12	*ABCC8*	NM_000352.6	c.3584 C>T	p.Thr1195Ile	-	Het	Exon 29	Mis	NP	VUS	PM2, PP3, PP4
P13	*ABCC8*	NM_000352.6	c.1943G>A	p.Arg648His	0.000012	Het	Exon 14	Mis	NP	VUS	PM2, PP3, PP4

AA: Amino Acid **MAF**: Minor Allele Frequency **Zyg**: Zygosity **ACMG Class**: The American College of Medical Genetics and Genomics Classification **ACMG Pat Crit**: ACMG Pathogenicity Criteria **Het**: Heterozygous **Hom**: Homozygous **Del**: Deletion **Frms**: Frameshift **Mis**: Missense **Splc**: Splicing **NP**: Not Provided **Pat**: Pathogenic **LP**: Likely Pathogenic **VUS**: Variant of Uncertain Significance

Table 3 Clinical and laboratory characteristics of patients harboring a pathogenic or likely pathogenic variant and patients with VUS or no variants at all

	Pathogenicity		
	LP/P (n = 10)	**Others (n = 30)**	***p-value***
Age at diagnosis (years)	11.750 ± 8.344	10.833 ± 4.945	0.950
C-peptide level (ng/ml)	0.559 ± 0.284	0.624 ± 0.444	0.876
Insulin requirement (U/kg)	0.710 ± 0.272	0.738 ± 0.189	0.800
BMI at diagnosis (kg/m^2)	21.525 ± 1.504	18.921 ± 4.797	**0.019***
HbA_{1c}at diagnosis (%)	7.510 ± 1.857	9.786 ± 2.6231	**0.010***
Fasting blood glucose at diagnosis (mg/dl)	187.300 ± 68.14	291.533 ± 116.014	**0.010***

BMI: Body Mass Index **Y**: Yes **N**: No **LP/P**: Likely Pathogenic/Pathogenic

* Statistically significant

demonstrated that P1 inherited the variant from her father. The father's fasting blood glucose 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 P2 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 P3 were between 120 and 180 mg/dL and monitored by diet. In the family history, the fasting blood glucose of the mother of P3 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.Phe150del) variant.

Fasting blood glucose levels in P4 were 90–160 mg/dL and monitored by diet. The same variant (c.1301G > T [p.Cys434Phe]) was also detected in the patient's father by Sanger sequencing. In the last routine follow-up visit of P4, who has a history of diabetes first diagnosed when he was 3 years old, HbA_{1c} was measured as 6.3%, body mass index as 22 kg/m^2 and C-peptide as 0.8 ng/mL.

A heterozygous c.790G > A (p.Gly264Ser) variant was detected in P5 and the patient was first noticed to have high fasting blood glucose levels a year ago incidentally at the age of 6. Today, the patient does not use any medication and fasting blood glucose levels vary between 80 and 160 mg/dL. Targeted mutation analysis showed that the patient's father also carries the same variant. 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 in an α-helical region (helix 5; residues 180–193), while Phe150 is in a β-stranded region (strand 5; residues 145–150) (Fig. 1 A). 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). Nevertheless, the importance of Phe150 for glucokinase structure/function has been demonstrated in several reports correlating the presence of the missense mutation p.Phe150Ser with the incidence of GCK-MODY [18–23]. Similarly, Asn180 itself is not engaged in favorable contacts with glucose. Most 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. The three other affected residues, namely Glu221, Gly264 and Cys434, are even farther away from the enzyme's glucose-binding site than Phe150 and Asn180. Glu221 (strand 9; residues 220–226) is a negatively charged hydrophilic residue exposed to the cytosol. It is in sufficiently close proximity (i.e. <5 Å) to Arg250 for establishing an electrostatic interaction bridging the terminals of two separate beta-strands, which can possibly contribute to the conformational stability and hence catalytic activity of glucokinase. The introduction of an oppositely charged lysine residue at the same position will interrupt this salt bridge. Gly264 is in a loop connecting a short (residues 256–260) and a longer α-helix (residues 266–280), where the protein backbone makes a sharp turn (bend). Substituting serine with its bulkier side chain for Gly264 is likely to disallow flexible dihedral angles (φ/ψ) via increasing low levels of internal steric hindrance typical of glycine residues. This can possibly result in structural damage to the enzyme. Cys434 (strand 13; residues 434–440) does not form a disulfide bridge with any neighboring residue of the same type. Therefore, the p.Cys434Phe substitution is likely to exert its potentially damaging effect on the enzyme by a mechanism other than disulfide bond breakage. Indeed, the significance of near- and far-active site cysteine residues for the stability/catalytic activity of human pancreatic beta-cell glucokinase have been demonstrated before [24].

ABCC8-MODY

We detected the c.1943G > A (p.Arg648His) variant as "VUS" in the *ABCC8* gene of our 7-year-old male patient, P13, having elevated blood glucose 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

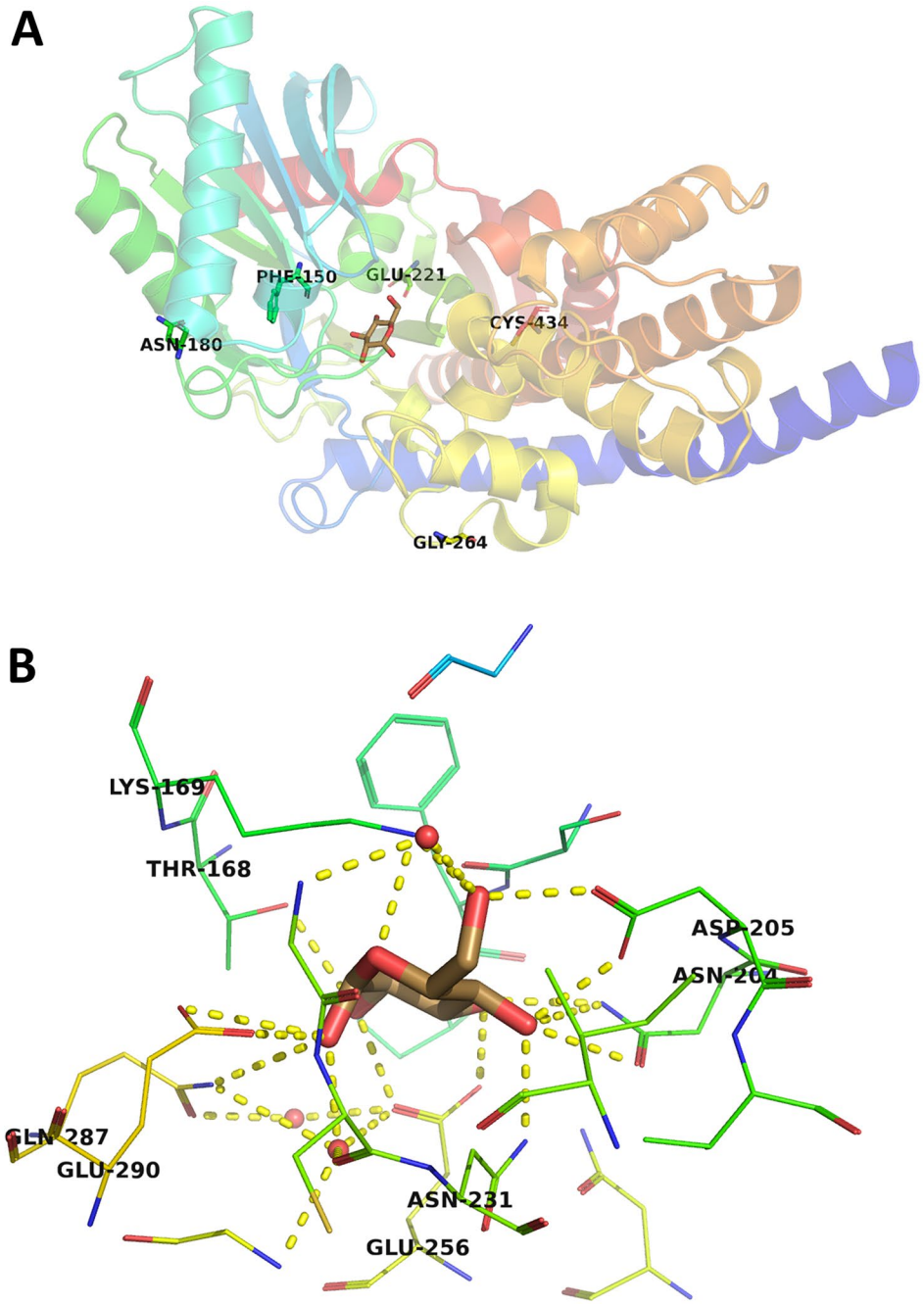

Fig. 1 (**A**) A ribbon diagram showing the three-dimensional structure of human glucokinase (PDB ID: 3IDH). The bound glucose substrate is shown in brown sticks. The relative positions of the affected Phe150, Asn180, Glu221, Gly264 and Cys434 residues are also indicated. (**B**) The polar interaction 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)

visit of P13, 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/m^2 and C-peptide was 0.87 ng/mL. In the family history, it was noted that the patient's mother had high blood glucose 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 *ABCC8*-MODY.

The heterozygous c.3584 C>T (p.Thr1195Ile) variant, which was predicted to be "VUS", was detected in the *ABCC8* gene of P12. The high levels of fasting blood glucose in the patient were first noticed at the age of 25, and the patient is currently using 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, however, did not have the opportunity to perform a 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 in a

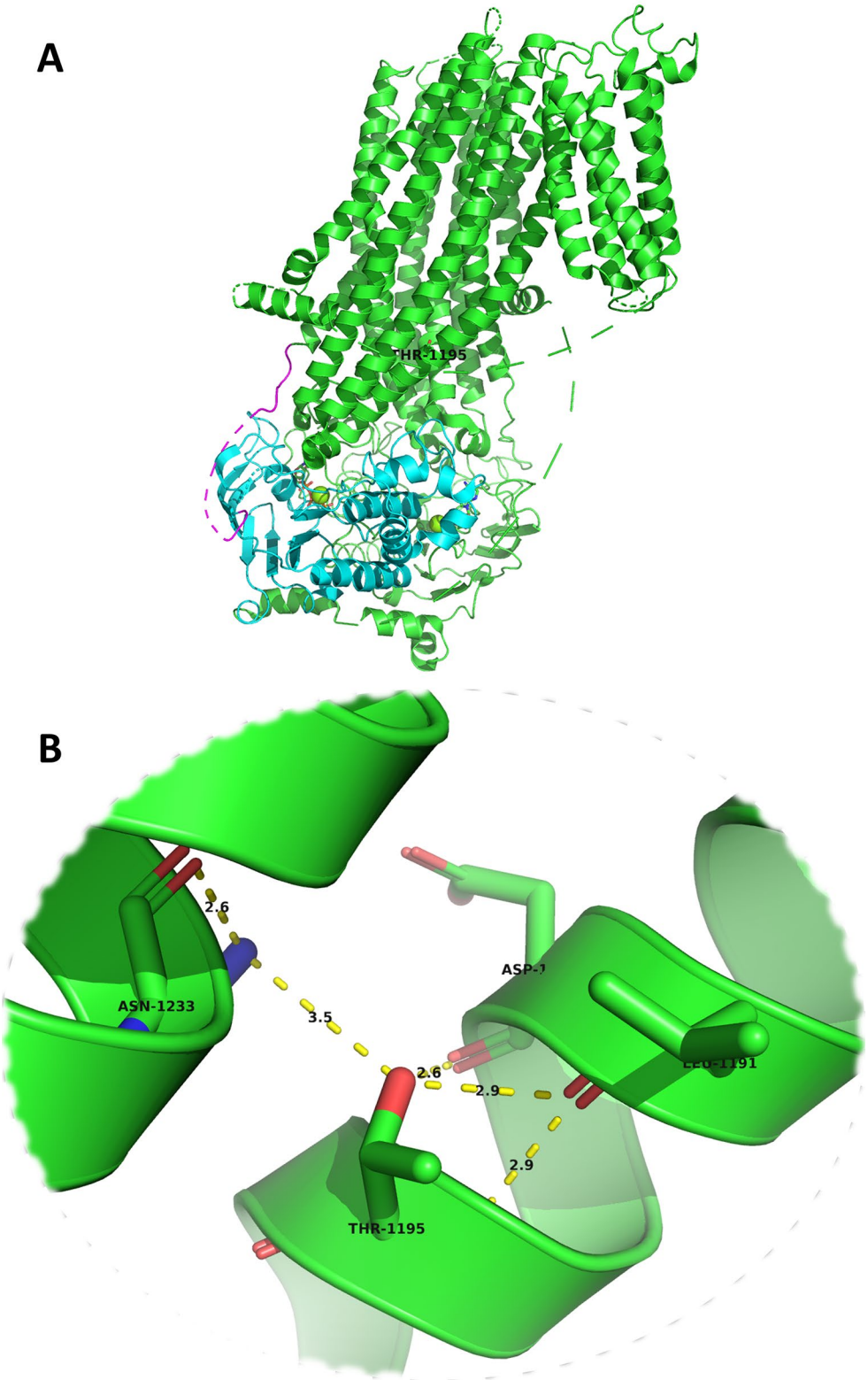

Fig. 2 (**A**) A ribbon diagram showing the three-dimensional structure of human sulfonylurea receptor 1 (PDB ID: 6C3O). The relative position of Thr1195 is indicated. The affected Arg648 residue is in a cytosolic loop structure (magenta; mostly missing in the crystal structure due to high flexibility) between the eleventh transmembrane helix (green) and the first nucleotide-binding domain (cyan) of the receptor. (**B**) The hydrogen bond network around Thr1195. Thr1195 lies within the fifteenth transmembrane helix, while Asn1233 lies within the sixteenth transmembrane helix. These images were rendered using the PyMOL Molecular Graphics System, Version 1.8 (Schrödinger LLC, Portland, OR, USA)

cytosolic loop structure between the eleventh transmembrane helix (TM11) and the first nucleotide-binding domain (NBD1) of SUR1 (Fig. 2 A). 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 [25]. 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. Thr1195 is situated in the fifteenth transmembrane helix (TM15), and its hydroxyl group falls within a distance of 3.5 Å from the amide group of Asn1233 from the sixteenth transmembrane helix (TM16) (Fig. 2B). The proposed hydrogen bond between these two amino acid residues could be important for the stability and dynamics of SUR1. Although isoleucine is sufficiently small to replace threonine at the same position, its non-polar character disrupts hydrogen-bonding interactions with the surrounding residues. Although loss of this hydrogen bond network around Thr1195 might be involved in MODY, the precise mechanism of action of p.Thr1195Ile is yet to be determined experimentally.

HNF1A-MODY

The heterozygous c.505_506delAA (p.Lys169AlafsTer18) variant was observed in P6, and the presence of high fasting blood glucose levels in this patient was first noticed by chance at the age of 25. Today, the patient's condition is controlled by low-dose insulin injections, and his fasting blood glucose levels vary between 90 and 200 mg/dL. Targeted mutation analysis showed that the patient's father also carries the same variant. It was stated that the father of P6 was a 55-year-old patient without follow-up for diabetes and had not used insulin or oral antidiabetics until today. Although we do not have clear information about the age at which the diabetes history started, it was noted that high fasting glucose levels were first detected in his 30s.

The heterozygous c.376 C>T (p.His126Tyr) variant, which was predicted to be "likely pathogenic", was assumed to be a *de novo* mutation in P9. 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 a history of diabetes which was first diagnosed when he was 24 years old, and his blood glucose levels are now controlled by oral antidiabetics.

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 P7, 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/m^2 and C-peptide concentrations to be 0.77 ng/mL. The same variant was detected also in his mother who was diagnosed with diabetes at the age of 25 and requires insulin to control her blood glucose.

In another patient (P8), who has a history of diabetes first diagnosed when he was 14 years old, the heterozygous c.862G>A (p.Gly288Arg) variant, which was predicted to be "VUS" in the *HNF1A* gene was detected. As a result of the segregation analysis, the variant was assumed to be a *de novo* variant and has an estimated allele frequency of 0.0000461 in the gnomAD genomes database and has not been reported in ClinVar before. In the last control visit of the patient BMI, HbA_{1c} levels and C-peptide concentrations were found to be 21 kg/m^2, 6.9% and 0.72 ng/mL, respectively, with negative assay results for autoantibodies. *HNF1A* encodes hepatocyte nuclear factor 1-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 [26, 27]. 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 HNF1α with coactivators. This, however, remains to be investigated by biochemical and structural approaches. To date, no human HNF1α structures containing the C-terminal transactivation domain have been deposited in the PDB.

HNF1B-MODY

The heterozygous c.704G>A (p.Arg235Gln) variant was detected in P10 and was assumed to be a *de novo* variant after targeted mutation analysis in parents. The patient was diagnosed with diabetes two years ago at the age of 19, 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%. *HNF1B* encodes hepatocyte nuclear factor 1-beta, a transcription factor that is involved in the organogenesis of the kidneys, urinary tract, liver, and endocrine and exocrine pancreas. 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

In P11, who has a history of diabetes first diagnosed when she was 9 years old, a heterozygous IVS5-1G > A variant in intron 5 of the *CEL* gene was detected. *CEL* codes for bile salt-activated lipase (BAL), a secreted pancreatic enzyme that hydrolyzes a broad spectrum of lipids. 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 determined in the last visit of P11 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/m^2. 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 P11 may lead to the MODY phenotype. This, however, needs further studies to be substantiated.

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 2–5% of all diabetic cases [28–30], molecular genetic diagnosis of MODY is necessary for optimal long-term treatment, prognosis, and especially genetic counseling. To date, 14 genes thought to be responsible for the MODY phenotype have been identified in the OMIM database. Although the *BLK*, *PAX4*, and *KLF11* genes are still classified as causal genes in OMIM, we did not include them in the present study because of the recently discussed and refuted gene–disease association claims[4]. 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 [31]. Moreover, the frequency of disease-causing variants in MODY is likely to show differences among different ethnic groups[12, 32, 33] depending on ethnicity and the molecular analysis methods employed for its diagnosis. In studies on MODY diabetes, it was shown that a possible pathogenic/pathogenic (i.e. high-risk) MODY-related variant was detected in 6–48% of patients [3, 31, 34–39]. Since the age of onset of the disease in MODY subtypes may vary, the age of the individuals in the studies may also affect the mutation distribution [40]. It is believed that many genes associated with MODY have not been identified yet and unidentified MODY genes may play a role in populations with low detection rates [32].

Patel et al. analyzed 50 known dominant and recessive genes of monogenic diabetes in a Turkish cohort of 236 children with low risk of type 1 diabetes and found that 34 patients had monogenic diabetes and found that 41% (14/34) of these patients had autosomal recessive causes [41]. Current clinical guidelines for childhood-onset monogenic diabetes other than infancy are focused primarily on identifying and testing for predominantly MODY genes that are dominantly inherited. From this point of view, we can say that recessive inherited mutations are a common cause of monogenic diabetes in populations with high inbreeding rates, and current MODY-oriented genetic testing strategies are insufficient to identify affected individuals. It is possible to study monogenic diabetes-related genes quickly and simultaneously and to identify new genes with next-generation sequencing technology so the number of patients with monogenic diabetes can be expected to increase rapidly.

In the current study, we detected a likely pathogenic/pathogenic (i.e., high-risk) MODY-related variant in 25% of our patients and made a comprehensive description of the clinical and molecular characteristics of these patients. Ten variants in ten patients are of high-risk type, with five patients (50%) harboring a *GCK* variant, three (30%) harboring a *HNF1A* variant, one (10%) harboring a *HNF1B* variant and one (10%) harboring a *CEL* variant. For physicians, the most current challenge in the molecular genetic diagnosis of MODY is perhaps the assessment of variants of uncertain significance (VUS), which represents a barrier to clinical interpretation. Consequently, segregation analysis studies in family members will be particularly helpful and even vital in some cases. In addition, comprehensive *in silico* analyses on the structural and functional impact of each genetic alteration on the protein product may prove useful to some. We believe that our cases will contribute to the development of the relevant scientific literature on MODY. Clinically, there are no differences between MODY-suspected patients (i.e. patients carrying a VUS or no variants at all) and MODY-confirmed patients (i.e. patients carrying a likely pathogenic/pathogenic variant) except for BMI, HbA_{1c} values, and fasting blood glucose levels (Table 3).

None of the patients who are carrying a likely pathogenic/pathogenic variant had ketonemia/ketonuria and ketoacidosis at the time of diagnosis or during follow-up, except for P11 who carries a *CEL* variant. Heterozygous pathogenic variants detected in the *CEL* gene are characterized by early pancreatic atrophy and subsequent exocrine insufficiency [42], and diabetes in these patients seems to require oral antidiabetics or insulin in treatment [43]. Our patient, in

whom we detected a pathogenic variant in the *CEL* gene, is currently using insulin at 1.05 U/kg.

The recommended first-line therapy for *HNF1A*-MODY is low-dose sulfonylurea that acts on K_{ATP} channels to stimulate insulin release in part through a glucose-dependent mechanism [44]. In our case, however, most of the patients were receiving unnecessary insulin treatment at the time they were referred to our center (Table 2). We do not have information regarding their treatment after molecular genetic diagnosis, but according to the literature such patients can be switched from insulin therapy to sulfonylurea therapy [43, 45]. It is also notable that two of the four variants identified in the *HNF1A* gene are *de novo* variants. Studies suggesting that de novo MODY mutations may be more common than previously thought in probands without a family history of diabetes are reported [46]. Therefore, molecular genetic testing for MODY can be considered in patients who do not have a family history of diabetes but meet other diagnostic criteria for MODY.

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 [47]. The family history of the disease is not specific as pathogenic variants in the *HNF1B* gene very often occur *de novo*[48]. However, in the abdominal MRI examination of our patient with the MODY phenotype (P21), in whom we detected a *de novo* likely pathogenic variant in the *HNF1B* gene, no additional features, such as the commonly encountered renal cysts, were observed.

In *ABCC8*-MODY patients, the clinical phenotype is usually characterized by congenital hypoglycemic hyperinsulinism, transient or permanent neonatal diabetes mellitus, or adult-onset diabetes mellitus [49]. *ABCC8* variant-induced phenomena, such as incomplete penetrance and clinical heterogeneity, have been already described in the literature by others [50]. Both of our patients (P12 and P13), for whom we detected variants in the *ABCC8* gene, showed no neonatal features typical of the previously mentioned phenotypes. Therefore, we think that segregation analyses in families may help clarify the causal role of the identified variants.

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 one pathogenic and four likely pathogenic variants in the *GCK* gene. If translated, c.534del (p.Asn179ThrfsTer25) 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 (Phe-150Leu, 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 [51], 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 Mg^{2+} cofactor through a mediating water molecule [52]. Therefore, it is tempting to speculate that ΔPhe150 may lead to a loss of Mg^{2+} coordination, affecting the catalytic properties of glucokinase. p.Glu221Lys appears to result in breakage of a solvent-exposed salt bridge, while p.Gly264Ser introduces a residue with disallowed φ/ψ angles. p.Cys434Phe affects glucokinase by a mechanism other than disulfide bond breakage. 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 [39, 53–55].

The findings of the present study should be considered in the light of various limitations. First, the *APPL1* gene, which is associated with MODY, 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 [56]. Next, some disease-causing variants might be hiding in the non-coding regions (intronic, regulatory and promoter regions) of the examined genes, which were not sequenced here. 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. Given the possibility that there are still unknown genes associated with MODY, it may be best not the exclude the diagnosis of MODY in patients who test negative for documented likely pathogenic or pathogenic mutations in the genes that were mentioned in this study.

Conclusions

Large-scale genetic studies are needed to understand the genetic aspects of MODY diabetes in our country and other populations. Overall, 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 10 causal

MODY-related genes. In the current study, we detected a likely pathogenic/pathogenic (i.e. high-risk) MODY-related variant in 25% of our patients and besides, we identified a novel pathogenic variant in *HNF1A* (c.505_506delAA [p.Lys169AlafsTer18]) gene. Confirmatory genetic testing in patients with suspected MODY allows for definitive diagnoses which, may guide management and provide rationales for screening other family members pre-symptomatically. In studies conducted with NGS method as in our study, through advancing molecular testing and identification of new genes, the number of patients with monogenic diabetes can be expected to rise rapidly. It is reasonable and appropriate to conclude here that an early molecular genetic diagnosis can lead to major changes or modifications in the treatment of MODY.

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Declarations

Conflict of interest The authors declare that they have no conflicts of interest.

Ethics approval This study was approved by Duzce University Faculty of Medicine Non-Invasive Clinical Research Ethics Committee (approval no.: 2016/24). Informed consents were obtained from the patients or their parents who participated in the study and whose data were used for analyses. All authors gave consent to the final draft before it was published in *Molecular Biology Reports*.

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