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Screening of Mutations in Maturity-onset Diabetes of the Young-related Genes and *RFX6* in Children with Autoantibody-negative Type 1 Diabetes Mellitus

Enver Şimşek[1,2], Oğuz Çilingir[3], Tülay Şimşek[4], Sinem Kocagil[3], Ebru Erzurumluoğlu Gökalp[3], Meliha Demiral[1], Çiğdem Binay[1]

[1]Eskişehir Osmangazi University Faculty of Medicine, Department of Pediatrics, Division of Pediatric Endocrinology, Eskişehir, Turkey
[2]The Health Ministry of Turkish Republic, Ankara Training and Research Hospital, Clinic of Pediatrics, Ankara, Turkey
[3]Eskişehir Osmangazi University Faculty of Medicine, Department of Medical Genetics, Eskişehir, Turkey
[4]Eskişehir Osmangazi University Faculty of Medicine, Department of Ophthalmology, Eskişehir, Turkey

What is already known on this topic?

Maturity-onset diabetes of the young (MODY) subtypes differ in terms of the age of diabetes onset, the pattern of hyperglycemic presentation, the response to treatment, and the association with extra pancreatic manifestations. Although genetically identifying the MODY subtype has benefits, a large number of patients have not been tested. Mutations in *GCK, HNF1A,* and *HNF4A* are the most common causes of MODY.

What this study adds?

This study established three additional novel mutations in different *MODY* genes. The etiology has not yet been elucidated in 71% patients diagnosed with autoantibodies-negative type 1 diabetes mellitus.

Abstract

Objective: Maturity-onset diabetes of the young (MODY) is the most common type of monogenic diabetes. To date, mutations have been identified in 14 different genes of patients with a clinical diagnosis of MODY. This study screened mutations in 14 MODY-related genes and the regulator factor X6 (*RFX6*) gene in children

Methods: The presence of clinical features of MODY and negative results for three autoantibody markers of type 1 diabetes mellitus (T1DM) in children and adolescents were used as inclusion criteria for genetic testing. The screening panel for next-generation sequencing included 14 MODY-related genes (*GCK*, *HNF4A*, *HNF1A*, *HNF1B*, *PDX1*, *NEUROD1*, *KLF11*, *CEL*, *PAX4*, *INS*, *BLK*, *ABCC8*, *KCNJ11*, and *APPL1*) and the *RFX6* gene.

Results: Twenty-four different variants in MODY-related genes were identified in 49 children diagnosed with autoantibody-negative T1DM. Twelve variants were classified as pathogenic/likely pathogenic (P/LP) while 12 were interpreted as variant of unknown significance. Nine of the P/LP variants were found in *GCK*, two in *HNF1B*, and one in *ABCC8*. Three variants were novel, and one was a *de novo* variant. All but one of the variants exhibited heterozygotic inheritance.

Conclusion: The frequencies of the MODY subtypes differed from previous reports. Although GCK-MODY was the most frequent mutation in Turkish children, similar to previous studies, the second most prevalent MODY subtype was HNF1B-MODY. This study also established three additional novel mutations in different *MODY* genes.

Keywords: Diabetes mellitus, mutation, MODY, *RFX6*, Turkish children

Address for Correspondence: Enver Şimşek MD, Eskişehir Osmangazi University Faculty of Medicine, Department of Pediatrics, Division of Pediatric Endocrinology, Eskişehir, Turkey
E-mail: enversimsek06@hotmail.com **ORCID:** orcid.org/0000-0003-0120-9976

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Introduction

Maturity-onset diabetes of the young (MODY) is characterized by an autosomal dominant genetic defect in beta-cell function with at least two consecutively affected generations of diabetes, typically before the age of 25-years, and the absence of beta-cell autoimmunity (1). Heterozygous mutations in various transcription factors that play a role in development and maturation of pancreatic beta-cells and mutations in enzymes acting on glucose sensing of the beta-cell result in MODY. MODY is currently categorized into 14 subtypes, each caused by mutations in different genes (2). Regulatory factor X6 (RFX6) is a transcription factor encoded by the *RFX6* gene located on chromosome 6 that is expressed in pancreatic beta-cells and small-intestinal cells. It plays a key role in the differentiation of pancreatic beta cells in mice and human, and regulates insulin secretion by modulating Ca^{2+} homeostasis in human beta-cells. Homozygous mutations in this gene are associated with Mitchell-Riley syndrome, which is characterized by neonatal diabetes with pancreatic hypoplasia, duodenal or jejunal atresia, and gall bladder agenesis. However, heterozygous *RFX6* mutations, which are associated with MODY with reduced penetrance and without intestinal atresia, have been reported (3,4).

The MODY subtypes differ in terms of the age of diabetes onset, the pattern of hyperglycemic presentation, the response to treatment, and the association with extra pancreatic manifestations. Management of MODY is subtype-specific and includes diet, oral antidiabetic drugs (OADs), or insulin. Therefore, the correct genetic diagnosis is important for lifelong treatment and patient prognosis. Although genetically identifying the MODY subtype has benefits, a large number of patients have not been tested (5).

This study screened mutations in the 14 MODY-related genes and the regulatory factor X6 (*RFX6*) gene in Turkish children diagnosed with autoantibody-negative type 1 diabetes mellitus (T1DM).

Methods

This was a prospective cross-sectional study performed between January 2005 and January 2022. The following criteria were used to diagnose diabetes mellitus (6): fasting plasma glucose or random plasma glucose levels ≥126 mg/dL or ≥200 mg/dL, respectively, glycated hemoglobin (HbA1c) ≥6.5%, and C-peptide <0.6 ng/mL. The clinical diagnosis of MODY was made using the classical criteria of impaired fasting glucose or the development of diabetes before the age of 25-years, negative results for T1DM markers [islet cell antibodies (ICAs), glutamic acid decarboxylase antibodies (GADAs), and insulin autoantibodies (IAAs)] and a family history of diabetes mellitus for at least two consecutive generations. The 14 MODY-related genes and *RFX6* were screened in all patients with a clinical diagnosis of MODY. Blood samples were collected to obtain fasting or random glucose measurements with concomitant C-peptide, HbA1c%, and three antibody tests (ICA, GADA, and IAA) at the time of the T1DM diagnosis.

Peripheral blood samples were collected into EDTA tubes for genetic testing. Genomic DNA was isolated from peripheral blood samples using the MagPurix robotic system (Zinexts, New Taipei city, Taiwan). Primary quality control of isolated DNA samples was performed using a NanoDrop spectrophotometer (Peqlab Biotechnologie GmbH, Erlangen, Germany) and samples with an A260/280 value between 1.8 and 2 were included in the study.

Twenty-seven patients with clinically diagnosed MODY were included in next-generation sequencing (NGS) analyses. A panel consisting of the 14 MODY-related genes (*GCK*, *HNF1A*, *HNF4A*, *HNF1B*, *ABCC8*, *KCNJ11*, *INS*, *NEURD1*, *CEL*, *APPL1*, *PDX1*, *KLF11*, *PAX4*, and *BLK*), and the *RFX6* gene was designed using Ion Ampliseq Designer software. The MODY panel contained 384 amplicons and was optimized as two pools by Thermo Fisher Scientific (Waltham, MA, USA). Amplicons prepared using the Ion Ampliseq Library v2.0 commercial kit were loaded into the Ion Chef (Ion torrent, Thermo Fisher Scientific) instrument. Template creation, enrichment, and chip loading stages were performed automatically by the Ion Chef device. The sequencing reaction step was performed on the Ion S5 (Ion Torrent, Thermo Fisher Scientific) NGS device. As a result of sequencing, "single-end array" raw data (*fast or UBAM) were used in the NGS platform within the scope of a bioinformatics analysis.

Variant Analyses

Sequenced reads were aligned to the reference genome (GRCh37/hg19) using the Ion Torrent platform-specific pipeline software Torrent Suite 4.2. The Ion Reporter 4.0 (Thermo Fisher Scientific), Integrative Genomics Viewer, and Varsome (http://www.varsome.com) (7) software were used to analyze the data. Variants with a minor allele frequency >0.1% in the Genome Aggregation Database (http://gnomad.broadinstitute.org/) were filtered out. Mutation Taster (http://www.mutationtaster.org/), prediction of effects of human nsSNPs (http://genetics.bwh.harvard.edu/pph2/), and scale-invariant feature transform (http://sift.jcvi.org/) were used to evaluate the effect of nonsynonymous variants on protein function and structure. Human Splicing Finder

(http://www.umd.be/HSF/) was used to predict the effect of the mutations on splicing. We interpreted the variants using The Human Gene Mutation Database (http://www.hgmd.cf.ac.uk/ac/), ClinVar (http://www.ncbi.nlm.nih.gov/clinvar/) and a literature search. The variants were named according to Human Genome Variants Society (http://www.hgvs.org) nomenclature. The variants were classified as "pathogenic," "likely pathogenic," "uncertain significance," "likely benign," or "benign" following the American College of Medical Genetics and Genomics Standards and Guidelines (ACMG) (8).

Informed consent was obtained from all subjects or their parents. This study was performed according to the Declaration of Helsinki and approved by the Ethical Committee of the Eskişehir Osmangazi University (approval number: E-2022-263, date: 20.12.2022).

Statistical Analysis

The Statistical Package for the Social Sciences (SPSS), version 18.0, (SPSS Inc., Chicago, IL, USA) was used for descriptive data analysis. Descriptive statistics of the clinical and laboratory findings are expressed as mean ± standard deviation, numbers, or percentages. Comparative statistics were not performed because the number of patients in the MODY subgroups was limited and not homogeneously distributed.

Results

The three antibodies (GADA, ICA, and IAA) were negative in 49 (12%) of 408 patients diagnosed with T1DM between January 2005 and January 2022. These 49 patients were screened for the 14 MODY-related genes and *RFX6*. The MODY diagnosis was confirmed by NGS in 14 of 49 (29%) patients, as pathogenic (P) or likely pathogenic (LP). The clinical and genetic characteristics of the MODY patients are given in Table 1 and Table 2, respectively. The mean age of the MODY patients was 9.5 ± 4.3 years (range, 3.5-17.8 years). The mean HbA1c level of MODY patients was 6.9 ± 1.1% (range, 6.2-9.8%). The lowest mean HbA1c level was seen in patients with GCK-MODY [mean, 7.01 ± 0.34%, (range, 6.6-7.8%)]. The clinical and genetic characteristics of 13 patients diagnosed with autoantibody-negative T1DM and genetic identification with variants of uncertain significance (VUS) are given in Table 3 and Table 4, respectively. The mean age of the children with VUS variants in MODY related genes and in *RFX-6* was 6.4 ± 4.0 years (range, 2.5-14 years). The mean HbA1c level in these patients was 8.1 ± 1.1% (range, 6.9-10.2%). A heterogeneous clinical presentation was observed according to the *MODY* genes. Patients with GCK- and ABCC8-MODY presented with hyperglycemia, whereas, HNF1B-MODY presented with diabetic ketoacidosis and multicystic kidneys. A heterogeneous clinical presentation was also seen in diabetic patients with VUS variants in MODY related genes and in *RFX-6*. Patients with VUS variations in *HNF1A-*, *HNF4A-*, *BLK-*, and *ABCC8* presented

Table 1. Clinical characteristics of 14 patients diagnosed with confirmed maturity-onset diabetes of the young (MODY)

No	*MODY* gene	Age at diagnosis (year)	Gender	Family history for MODY/generations	Clinical presentation	HbA1c % level at diagnosis	Follow-up treatment
1	*GCK*	14.2	Male	+/3	Hyperglycaemia	6.3	Diet
2	*GCK*	6.9	Male	+/3	Hyperglycaemia	6.6	Diet
3	*GCK*	7.5	Male	+/2	Hyperglycaemia	6.4	Diet
4	*GCK*	14.3	Male	+/3	Hyperglycaemia	6.3	Diet
5	*GCK*	7.1	Male	+/3	Hyperglycaemia	6.5	Diet
6	*GCK*	8.4	Female	+/3	Hyperglycaemia	6.3	Diet
7	*GCK*	6.1	Male	+/3	Hyperglycaemia	6.2	Diet
8	*GCK*	10.4	Male	NA	Hyperglycaemia	6.8	Diet
9	*GCK*	17.8	Female	+/3	Hyperglycaemia	6.2	Diet
10	*GCK*	3.8	Female	-/1	Hyperglycaemia	6.3	Diet
11	*GCK*	8.5	Female	+/2	Hyperglycaemia	6.8	Diet
12	*HNF1B*	9.5	Male	+/2	Diabetic ketoacidosis Multicystic kidneys	8.8	Insulin
13	*HNF1B*	13,5	Female	+/3	Diabetic ketoacidosis Multicystic kidneys	9.8	Insulin
14	*ABCC8*	3.5	Female	+/2	Hyperglycaemia	7.6	Sulfonylurea

OADs: oral antidiabetic drugs, NA: not available

Table 2. Mutation analysis screening of the *MODY* genes in Turkish children diagnosed with autoantibody negative type 1 diabetes mellitus

Gene OMIM ID MODY type	cDNA rs ID Types of mutation	Protein	Zygosity	*In silico* prediction	MAF %	ACMG class	N
GCK 125851/MODY2	c.109C > T rs762263694 missense	p.(Arg37Trp)	Hetero	PbD/Del/DC	0.00119	P	2
GCK	c.679G > A rs148311934 missense	p.(Val227Met)	Hetero	PbD/Del/DC	0.000796	P	2
GCK	c.116A > C rs1064794268 missense	p.(Gln39Pro)	Hetero	PbD/Del/DC	NA	LP	1
GCK	c.575G > A rs886042610 missense	p.(Arg192Gln)	Hetero	PbD/Del/DC	NA	LP	1
GCK	c.686C > T rs80356655 missense	p.(Thr229Met)	Hetero	PbD/Del/DC	0.000399	P	1
GCK	c.1181T > C missense	p.(Met394Thr)	Hetero	PD/Del/DC	NA	P	1
GCK	c.45 + 1G > C novel splice site	p.?	Hetero	NA/NA/DC	NA	LP	1
GCK	c.1090_1091insGCTGCGACCCTCGACCACCG novel insertion	p.(Asp364Glyfs Ter46)	de-novo	NA/NA/DC	NA	LP	1
GCK	c.536G > A rs886039380 missense	p.(Gly179Glu)	Hetero	PbD/Del/DC	NA	P	1
HNF1B 137920/MODY5	c.1024T > C rs1282596664 missense	p.(Ser342Pro)	Hetero	PD/Tol/DC	0.000402	LP	1
HNF1B	c.1045 + 1G > A novel splice site	p.?	Hetero	NA/NA/DC	NA	LP	1
ABCC8	c.4369G > A rs72559717 missense	p.(Ala1457Thr)	Hetero	PbD/Del/DC	NA	LP	1

In silico prediction: PolyPhen-2, SIFT: mutation tester, respectively; Polyphen predictions: B: bening, PD: possibly damaging, PbD: probably damaging, SIFT predictions: Del: deleterious, Tol: tolerated, mutation tester predictions: DC: disease-causing, Poly: polymorphism, NA: not available, ACMG Class, P: pathogenic, LP: likely pathogenic
Transcripts: GCK(NM_033507.3), HNF1B(NM_000458.4), ABCC8(NM_000352.6)

with hyperglycemia, with *KLF11* and *RFX6* variants with diabetic ketosis, and in *HNF1B-*, *PAX4-*, and *INS* variants with diabetic ketoacidosis.

An investigation of family history revealed that 23 of 27 (85%) patients (including MODY patients and patients with VUS) had one or two consecutive affected generations. Overall, 11 of the 27 diabetic patients whose family history revealed only mild hyperglycemia in two generations and who had not required insulin therapy, were presumptively diagnosed with GCK-MODY and the diagnosis was confirmed by genetic testing. GCK-MODY patients were managed with diet treatment only, ABCC8-MODY patients with oral sulfonylurea treatment only, whereas HNF1B-MODY were managed with insulin treatment. In patients with VUS in MODY related genes; HNF1A- and ABCC8-MODY were managed by sulfonylurea therapy only, whereas all remaining patients with VUS in *MODY* genes were managed by insulin treatment.

All MODY patients, except one, were heterozygotes, and one was a *de novo* mutation. The distribution of mutations according to the MODY related genes was as follows: nine different P/LP variants in the *GCK* gene, and one LP in *ABCC8* (Table 2). Seven of the nine mutations in the *GCK* gene were missense and two were novel splice site and insertion mutations. One of two LP mutations in *HNF1B* was a missense mutation, and the other was a novel splice-site mutation. The distribution of VUS variants according to genes is shown in Table 4. Two of the 12 different VUS variants in MODY related genes and *RFX6* were novel variants, and the other 10 variants were reported previously (Table 4).

Table 3. Clinical characteristics of 13 patients diagnosed with autoantibody-negative type 1 diabetes mellitus and genetic identification with variants of uncertain significance detected according to According to the American College of Medical Genetics and Genomics

No	*MODY* gene	Age at diagnosis (year)	Gender	Family history for MODY/ generations	Clinical presentation	HbA1c % level at diagnosis	Follow-up treatment
1	*HNF1A*	10.1	Male	+ /3	Hyperglycaemia	8.4	Diet, sulfonylurea
2	*HNF1A*	14	Female	+ /3	Hyperglycaemia	7.1	Diet, sulfonylurea, insulin
3	*HNF1B*	4.0	Male	-/1	Diabetic ketoacidosis	8.9	Insulin
4	*HNF1B*	8.3	Female	NA	Diabetic ketoacidozis	9.4	Insulin
5	*HNF4A*	2.3	Male	+ /3	Hyperglycaemia	6.9	Diet, sulfonylurea, insulin
6	*BLK*	1.5	Male	+ /2	Hyperglycaemia	7.4	Diet, sulfonylurea, insulin
7	*BLK*	10	Female	No	Hyperglycaemia	10.2	Diet, sulfonylurea, insulin
8	*PAX4*	5.5	Female	+ /2	Diabetic ketoacidosis	8.8	Diet, sulfonylurea, insulin
9	*INS*	10.5	Female	+ /2	Diabetic ketoacidosis	9.2	Diet, diet, sulfonylurea, insulin
10	*KLF11*	8.5	Male	+ /2	Diabetic ketosis	7.7	Diet, sulfonylurea, insulin
11	*ABCC8*	2.4	Male	+ /2	Hyperglycaemia	7.1	Sulfonylurea
12	*ABCC8*	4.2	Male	+ /2	Hyperglycaemia	7.3	Sulfonylurea
13	*RFX6*	2.4	Male	No	Diabetic ketosis	7.4	Diet, sulfonylurea, insulin

OADs: oral antidiabetic drugs, NA: not available, HbA1c: glycated hemoglobin

Table 4. Genetic identification of the cases with variants of uncertain significance detected according to According to the American College of Medical Genetics and Genomics

Gene/OMIM ID/MODY type	Variants/types of mutation	dsSNP	Zygosity	*In silico* prediction	Reference
HNF1A 600496 MODY3	c.481G > A p.(Ala161Thr) missense	rs201095611	Heterozygous	pBD/Del/DC	(9,10)
HNF1A	c.517G > A p.(Val173Met) missense	NA	Heterozygous	PD/Del/DC	(11,12)
HNF1B 189907 MODY5	c.1006C > G p.(His336Asp) missense	rs138986885	Heterozygous	B/Tol/DC	(10)
HNF1B	c.1339 + 5G > A p.? splice site (novel)	NA	Heterozygous	NA/NA/DC	NA
HNF4A 125850 MODY1	c.473C > T p.(Ala158Val) missense	rs754143633	Heterozygous	PbD/Tol/DC	NA
BLK 613375 MODY11	c.497delA p.(Asp166ValfsTer8) deletion (novel)	NA	Heterozygous	NA/NA/DC	NA
BLK	c.569C > G p.(Ser190Cys) missense	rs200875749	Heterozygous	B/Del/DC	NA
PAX4 612225 MODY9	c.521G > T p.(Arg174Leu) missense	rs776151854	Heterozygous	PbD/Del/DC	(13)
INS 613370 MODY10	c.2455C > G p.(Arg819Gly) missense	rs1555738952	Heterozygous	B/Del/DC	NA
KLF11 610508 MODY7	c.673A > C p.(Ser225Arg) missense	rs200061013	Heterozygous	B/Tol/Poly	NA

Table 4. Continued

Gene/OMIM ID/Mody type	Variants/types of mutation	dsSNP	Zygosity	*In silico* prediction	Reference
ABCC8 600509 MODY12	c.2395A > G p.(Lys799Glu) missense	rs1336775990	Heterozygous	B/Del/DC	NA
RFX6 NA MODY?	c.1782C > G p.(His594Gln) missense	rs4946206	Heterozygous	B/Tol/Poly	NA

dsSNP: the Single Nucleotide Polymorphism Database, *In silico* prediction: PolyPhen-2, SIFT: mutation tester, respectively, Polyphen predictions: B: bening, PD: possibly damaging, PbD: probably damaging, SIFT predictions: Del: deleterious, Tol: tolerated, mutation tester predictions: DC: disease-causing, Poly: polymorphism ? = unknown effect; NA: not available.

Transcripts: HNF1A(NM_000545.8), HNF4A(NM_000457.5), HNF1B(NM_000458.4), BLK(NM_001715.3), PAX4(NM_001366110.1), INS(NM_000208.4), KLF11(NM_003597.5), ABCC8(NM_000352.6), RFX6(NM_173560.4)

Discussion

MODY is the most common type of monogenic diabetes, accounting for 1-6% of all pediatric diabetic cases (2). Although there are several clinical predictors for diagnosing MODY, such as a positive family history of diabetes mellitus before the age of 25-30 years, negative results for antibodies associated with T1DM, high C-peptide concentrations (14), and high levels of C-reactive protein (15) and lipids, MODY diagnosis is relatively difficult and cases are often misdiagnosed as T1DM (3). A genetic test is the most accurate and cost-effective option for diagnosing the MODY subtypes (16). However, selecting pediatric and adolescent diabetic patients for genetic testing is still controversial. A clinical diagnosis of MODY should be considered in patients who have atypical features of diabetes based on age < 25 years, negative results for antibodies associated with T1DM, the presence of neonatal hypoglycemia, and/or multiple family members with diabetes not characteristic of T1DM or T2DM (1,2,5). Epidemiological studies have shown that 10-15% of children and adolescents diagnosed with T1DM are negative for the three autoantibodies (GADA, IAA, and ICA). In the present study, 49 (12%) of 408 patients diagnosed with T1DM had negative results for these three antibodies. In our study the MODY diagnosis was confirmed in only 14 of 49 (29%) patients. However, in the remaining 35 (71%) patients, there was no P or LP mutations in any of the 14 *MODY* genes. This suggests that the etiology has not yet been elucidated in a significant group of patients diagnosed with T1DM whose autoantibodies are negative. This group of patients may be candidates for genetically inherited diabetes groups that are likely to be identified in the near future. Determining the MODY subtype is important, as the subtypes differ in terms of age of onset, clinical presentation and progression, and response to treatment (2). The current literature shows that the prevalence and frequency of MODY subgroups vary by country. Mutations in *GCK*, *HNF1A*, and *HNF4A* are the most common causes of MODY (17,18,19,20). The most common subtype in European countries (UK, Germany, The Netherlands, Norway, and Poland) is HNF1A-MODY, followed by GCK-, HNF4A-, and HNF1B-MODY (21). The most common subtypes in the UK are HNF1A-, GCK-, HNF4A-, and HNF1B-MODY (5,22). However, Chakera et al. (22) reported that the estimated prevalence of GCK-MODY is about 1 in 1,000 individuals in the UK. GCK-MODY is the most prevalent MODY in Japan (23). In Korea, only 10% of clinical MODY of childhood-onset type 2 diabetes cases harbor known MODY-related genetic defects (*HNF1A*, 5%; *GCK*, 2.5%, and *HNF1B*, 2.5%) (24). The prevalence of MODY in Middle Eastern, Asian, and African populations is unknown. Ağladıoğlu et al. (25) and Gökşen et al. (26) reported that the most prevalent MODY subtypes in Turkish children are GCK-MODY and HNF1A-MODY. Yalçıntepe et al. (27) reported 31 cases of P/LP variants (*GCK*, n = 24; *ABCC8*, n = 3; *KCNJ11*, n = 2; *HNF1A*, n = 1; and *HNF4A*, n = 1) in 61 unrelated cases with clinical diagnosed with MODY in Turkish children. The present study revealed that the most prevalent MODY subgroup in Turkish children pre-diagnosed as T1DM was GCK-MODY, as reported previously (25,26,27). Unfortunately, the number of patients in our cohort diagnosed with MODY is not sufficient to make a realistic comment about the regional MODY frequency or the distribution of MODY subgroups. This would only be possible after national-scale MODY study or studies.

The GCK-MODY phenotype is characterized by lifelong nonprogressive fasting hyperglycemia (2,28,29). Patients are often asymptomatic and diagnosed incidentally during pregnancy or routine examinations, and the majority of patients do not require pharmacotherapy. Although patients with GCK-MODY have long-standing hyperglycemia, they have a low prevalence of micro- and macro-vascular complications (30). However, Kawakita et al. (31) reported that 7 of 55 patients with GCK-MODY were treated with OADs, and the authors concluded that these seven patients required OADs because they consumed carbohydrate-rich foods and had sedentary lifestyles. Insulin treatment is also required in pregnant patients with GCK-MODY to prevent maternal

hyperglycemia and reduce the risk for the development of overweight fetuses (32). In the present study, 11 cases from nine families had nine different mutations in *GCK*. Two cases had novel mutations; one was a splice site type mutation (c45 + 1G > C) and the other was an insertional type and a *de novo* mutation (c.1090_1091insGCTGCGACCCTCGACCACCG, p.Asp364GlyfsTer6). All cases presented with mild hyperglycemia and were followed up with only a low carbohydrate diet.

Heterozygous mutations in *HNF1B* cause HNF1B-MODY (formerly referred to as MODY-5), which is characterized by early-onset diabetes, pancreas hypoplasia, and multicystic kidney dysplasia (33). In patients with diabetes mellitus, the presence of cystic kidneys and elevated liver enzymes may be used as predictors of an *HNF1B* mutation. In the present study, two cases had *HNF1B* mutations and both presented with diabetic ketoacidosis. One of these two mutations was previously reported as a missense mutation (c.1024T > C), and the other was a novel splice site mutation (c.1045 + 1G > A). Both cases were diagnosed with cystic renal disease in the first decade of life. In one of the patients, cystic renal disease was identified by ultrasonography performed in the intensive care unit, where she presented with diabetic ketoacidosis. The other case was followed up by the pediatric nephrology department for polycystic renal disease. The pediatric nephrology department consulted pediatric endocrinology when the patient was diagnosed with fasting hyperglycemia and developed a high HbA1c level. Both patients were followed up with an intensive insulin regimen protocol. Unlike previous studies (13,14,15,21), the present study found that the *HNF1B* mutations were more prevalent in Turkish children than the *HNF1A* or *HNF4A* mutations. However, our cohort size is small so to confirm this hypothesis, more prospective and comprehensive studies are needed with larger cohorts. We suggest that, in the presence of renal abnormalities in young patients with diabetes mellitus, *HNF1B* mutations should be initially suspected and may be screened before the investigation of other MODY subgroups. The major complications in HNF1B-MODY patients have been related to the kidneys, such as chronic kidney disease (34).

The diagnosis of ABCC8-MODY was confirmed in one of the patients initially diagnosed with T1DM but with negative autoantibodies. The ATP-binding cassette transporter subfamily C member 8 (*ABCC8*) gene is expressed in the pancreas where it controls the expression of the sulfonylurea receptor 1 subunit of the ATP-sensitive potassium channel found on the beta cell membrane (35). Heterozygous mutations in the *ABCC8* gene damage the normal function of potassium channels leading to impaired insulin secretion. ABCC8-MODY (formerly referred to as MODY-12) is characterized by congenital hyperinsulinemic hypoglycemia, a transient or permanent form of neonatal diabetes mellitus, or adulthood-onset diabetes mellitus (2). Patients with ABCC8-MODY respond to sulfonylurea treatment (2). Our patient presented with hyperglycemia at 3.5 years old and was managed with sulfonylurea treatment. As seen in this case, by identifying MODY subgroups, correct treatment options can be chosen from younger ages.

In the present study, genetic testing identified 12 different VUS variants in MODY-related genes in 13 of 49 patients diagnosed with autoantibody-negative T1DM, but all patients had a clinical diagnosis of MODY. Two of 12 VUS variants were novel, and the others were reported previously (9,10,11,12,13). A clearer interpretation can be made by comparing the results obtained after segregation analyzes in our patients and previously reported studies related to these VUS variants. Though computational prediction tools and conservation analyses suggest that these VUS variants are not predictive enough to determine pathogenicity, given the current lack of comprehensive MODY-variant classification expertise, current ACMG-based classification should be interpreted cautiously and these VUS variants may be subject to change in the future.

Study Limitations

This study was conducted with a small number of cases, except for patients with GCK-MODY, so the reported frequency of the rare MODY subgroups may be unreliable. Segregation could not be tested in all families for VUS variants. Large-scale prospective studies are needed to allow for a stronger interpretation of the frequencies of rare MODY subgroups in Turkish children.

Conclusion

Clinical evaluation and genetic testing can be used to make a correct MODY diagnosis and subgrouping. The incidence and prevalence of MODY vary between countries. A timely and accurate diagnosis of MODY may prevent some subgroups from unnecessarily treatment with long-term insulin therapy. Therefore, all autoantibody-negative T1DM cases should be screened for known *MODY* genes, and the treatment of patients should be individualized, based on the appropriate identified MODY subgroup. This study established three additional novel mutations in different *MODY* genes. Our study also revealed that the 14 currently known *MODY* genes or *RFX6* were not reported to be P or LP in 71.5% (35/44) autoantibody-negative T1DM cases, indicating that this group may have novel MODY subtypes.

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Ethics

Ethics Committee Approval: This study was performed according to the Declaration of Helsinki and approved by the Ethical Committee of the Eskişehir Osmangazi University (approval number: E-2022-263, date: 20.12.2022).

Informed Consent: All patients with autoantibody-negative type 1 diabetes mellitus were routinely screened from the aspects of MODY and a separate consent form was not issued.

Authorship Contributions

Surgical and Medical Practices: Enver Şimşek, Tülay Şimşek, Meliha Demiral, Çiğdem Binay, Concept: Enver Şimşek, Oğuz Çilingir, Sinem Kocagil, Ebru Erzurumluoğlu Gökalp, Design: Enver Şimşek, Data Collection or Processing: Enver Şimşek, Tülay Şimşek, Meliha Demiral, Çiğdem Binay, Analysis or Interpretation: Enver Şimşek, Oğuz Çilingir, Sinem Kocagil, Ebru Erzurumluoğlu Gökalp, Literature Search: Enver Şimşek, Oğuz Çilingir, Sinem Kocagil, Ebru Erzurumluoğlu Gökalp, Meliha Demiral, Çiğdem Binay, Writing: Enver Şimşek, Oğuz Çilingir, Tülay Şimşek.

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