

Evaluation and Long-term Monitoring of Patients with MODY, and Description of Novel Mutations

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ABSTRACT

Objective: Maturity-onset diabetes of the youth (MODY) is a genetically and clinically heterogeneous group of diseases which is often misdiagnosed as type 1 diabetes or type 2 diabetes. The aim of this study is to identify the occurrence of mutations in subjects classified clinically as having MODY, and to determine phenotypic features and their long-term monitoring consequences.

Methods: Eighteen probands were selected based on the clinical criteria of MODY. Firstly, in patients with mild stable fasting hyperglycemia who did not progress, Sanger sequencing of GCK gene was performed as GCK-MODY was the most common cause of persistent and incidental hyperglycemia in the pediatric population. Patients without a GCK gene mutation or without mild fasting hyperglycemia were analysed by using targeted next-generation sequence for seven known monogenic genes of diabetes (ABCC8, GCK, HNF1A, HNF1B, HNF4A, INS, KCNJ11) to identify the molecular pathology.

Results: We identified 11 GCK, 2 HNF1A, 2 KCNJ11 mutations in 18 probands. Eleven of them (73%) were previously reported and 4 of them (27%) were assessed as novel mutations. In two patients who were treated with insulin before the molecular analysis, insulin was switched to sulfonylurea and glibenclamide, after determination of pathogenic variants in HNF1A and KCNJ11, respectively. Retinopathy or nephropathy was not detected among the patients.

Conclusion: The MODY has a large spectrum of clinical presentations. We detected 4 novel mutations among our cohort. Although GCK-MODY was the most frequent type of our study population, identification of rare MODY types and follow-up of these patients would help us better understand monogenic diabetes.

Keywords: Diabetes, MODY, next-generation sequencing

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INTRODUCTION

Maturity onset diabetes of the youth (MODY), which is estimated to account for 1-2% of all patients with diabetes, is a monogenic form of diabetes that is inherited in an autosomal dominant manner. It encompasses a genetically and clinically heterogeneous group of diseases affecting pancreatic β -cells and resulting in impaired insulin secretion (1,2). At least 14 MODY subtypes with distinct genetic etiologies have been identified to date (GCK, HNF1A, HNF4A, HNF1B, PDX1, NEUROD1, KLF11, CEL, PAX4, INS, BLK, KCNJ11, ABCC8, APPL1) (3).

The MODY may be misdiagnosed as type 1 diabetes (T1DM) or type 2 diabetes (T2DM) and there can be a significant overlap in clinical features (4). The importance of diagnosing MODY includes the application of optimal treatment. It will be possible to switch to sulfonylurea therapy in patients with some MODY types such as GCK-MODY previously misdiagnosed as T1DM and in whom insulin therapy has been started. The diagnosis of MODY can also positively affect glycemic control, prognosis and quality of life. Moreover, it would be possible to determine the family members under risk because the disease is inherited in an autosomal dominant manner (5). Targeted next-generation sequencing (tNGS) is reported to be utilized in diagnosis of monogenic diabetes with up to 100% sensitivity (6).

The aim of this study is to identify the occurrence of both reported and novel mutations in subjects classified clinically as having MODY by using tNGS, and to determine phenotypic variability, clinical and metabolic features and their long-term monitoring consequences.

METHODS

Patients who fulfilled at least two criteria mentioned below were included in the study: 1-a family history of diabetes in one parent,

2-negative antibodies for glutamic acid decarboxylase or islet cells, 3-low (<0.5 IU/kg/day) insulin requirements 3 years after the diagnosis and with C-peptide level ≥ 0.6 ng/mL, 4-persistent but nonprogressive fasting hyperglycemia, and 5-clinical presentation resembling T2DM with normal fasting C-peptide levels but without obesity, acanthosis nigricans, or insulin resistance. Eighteen children with a clinical diagnosis of MODY aged between 1 and 18 years, who were followed up at three different pediatric endocrinology clinics between January 2015 and December 2019 were included. Sanger sequencing of GCK was performed in 16 patients with a fasting glucose level between 99 and 145 mg/dL, and with HbA1c level $\leq 7.5\%$. Other patients who did not have mild fasting hyperglycemia or without GCK mutation were analysed by using tNGS for seven known monogenic diabetes genes. The genetic testing strategy is illustrated in Figure 1.

Data about presenting complaints, age at diagnosis, duration of diabetes, medical history of the proband and family, diagnosis if any before MODY diagnosis, treatment plans, duration of follow-up, baseline anthropometric measurements, and physical examination findings were obtained from the hospital records. Laboratory results including fasting blood glucose (FBG), urine glucose-ketone, venous blood gases, serum insulin, C-peptide and/or insulin, HbA1c levels in admission and diabetes autoantibodies were recorded. Standard deviation (SD) scores of weight, height and body mass index (BMI) of patients were calculated by using reference values for Turkish children (7). According to age and gender, patients with BMI between the 85th and 95th percentiles was considered as overweight, and patients with BMI $\geq 95^{\text{th}}$ percentile was considered as obese. Diabetic ketoacidosis was biochemically defined as venous pH <7.3 or serum bicarbonate concentration <15 mmol/L, and serum glucose concentration >200 mg/dL together with ketonemia, glucosuria, and ketonuria (8). All patients were evaluated by the ophthalmologist with direct ophthalmoscopy for diabetic retinopathy 2 years after diagnosis.

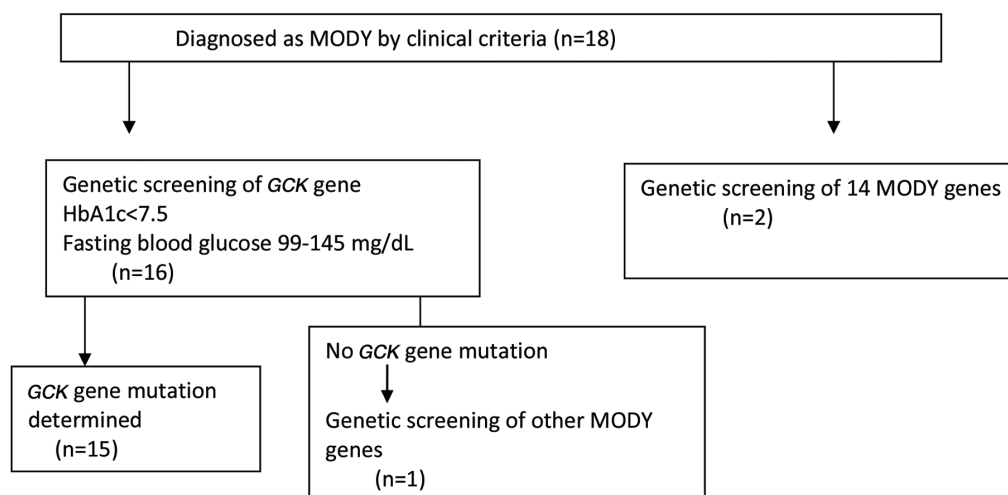


Figure 1. Genetic testing algorithm

Urinary albumin/creatinine ratio (ACR) was analyzed. According to the 2018 International Society for Pediatric and Adolescent Diabetes guidelines, patients with ACR value above 30 mg/g (spot urine) were evaluated for diabetic nephropathy (9).

Genetic Analysis

After obtaining written informed consent, blood samples were obtained from the patients and available parents. Automatic DNA isolation was performed in accordance with the standard protocols of the QIAamp DNA Mini (Qiagen) kit from EDTA-anticoagulated peripheral blood samples.

Sanger sequencing: Sanger sequencing of all coding exons of GCK was performed using BigDye terminator chemistry 3.1 on the 3130 Genetic Analyzer (Applied Biosystems). Primer sequences and PCR conditions are available on request.

Targeted NGS: Within the scope of the test, the sequencing was done on Miseq (Illumina) Next Generation Sequencing platform using SOPHIA Clinical Exome Solution using Illumina V2 chemicals. The targeted gene panel consisted of 7 (*ABCC8*, *GCK*, *HNF1A*, *HNF1B*, *HNF4A*, *INS*, *KCNJ11*) genes associated with MODY. Sequence analysis covered coding regions of each gene, including all coding exons, +/- 10 base pairs of adjacent intronic sequences and each nucleotide was read at a depth of at least 50X. Any variants that fall outside these regions and exonic variants with a minor allele frequency of less than 10% were considered as false positives and not analyzed. With this analysis, copy number variations were not examined. The DNA sequences were aligned to the NCBI Build36 (hg18) version of the human genome. Variant calling and data analysis were performed by Sophia-DDM-V5.2 bioinformatics analysis program.

Interpretation of Mutations

The interpretation of the variants were performed according to the 2015 ACMG standards and guidelines (10). Since there were not enough genome and exome databases for the Turkish population, 1000 genome projects, dbSNP, ExAC, GnomAD data were used as control population. The effects of the variants on protein function were investigated by using prediction programs such as SIFT, Polyphen, and MutationTaster. Human Gene Mutation Database (HGMD) and ClinVar databases were used to investigate mutations which were previously associated with MODY.

Statistical Analysis

Statistical analyses in our study were performed using the SPSS (Version 22.0, SPSS Inc., Chicago, IL, USA) package program. The normality distribution of the retrospective data was evaluated with the Kolmogorov-Smirnov test. Descriptive statistics for continuous variables were presented as mean $\pm$ SD in normally distributed data, median (minimum-maximum) in non-normally distributed data, and categorical data as numbers and percentages (%).

The study was approved by Taksim Training and Research Hospital Clinical Research Ethics Committee (decision no: 83, date: 12.06.2019) and was performed in accordance with the Helsinki Declaration.

RESULTS

Clinical Features of the Patients

A total of 18 patients, 12 females (66.6%), 6 males (33.3%), diagnosed as having MODY by using clinical criteria were included in the study. The mean age of the patients at diagnosis was 13.9 ± 5.4 years (range 5-17). The mean BMI was 18.2 ± 1.9 kg/m² (range 15.5-26.5), and all patients had normal weight, except for patient 8 (GCK-MODY) who was overweight (26.5 kg/m²).

The presenting complaints of two patients (11%) were polydipsia, polyuria, whereas the remaining 16 (89%) patients had incidental hyperglycemia. Mean duration of follow-up was 40.1 ± 10.6 months (range 9-156 months). None of the patients were diagnosed as having ketoacidosis at the initial evaluation or during the follow-up. Mean FBG, HbA1c, C-peptide and insulin levels at presentation were 138.7 ± 15.9 mg/dL, $6.7 \pm 0.4\%$, 1.5 ± 0.8 ng/mL, and 7.3 ± 1.2 IU/mL, respectively. Glucosuria was detected in P17 (HNF1A-MODY) and P18 (KCNJ11-MODY). Treatment consisting of medical nutrition therapy alone was given to 15 (83.3%) patients, whereas insulin was used in 1 (5.5%) patient, sulfonylurea in 2 (11.1%) patients. None of the patients had retinopathy or nephropathy during follow-up. Clinical characteristics of the patients are summarized in Table 1.

Molecular Genetic Test Results

We identified 15 patients with GCK variants (5 pathogenic and 10 likely pathogenic), 2 patients with HNF1A variants (1 pathogenic, 1 variant), and 1 patient with 2 different KCNJ11 mutations (variant/pathogenic). Two patients who had GCK and HNF1A mutations also had novel ABCC8 mutations classified as variant of uncertain significance (VUS) (Table 2). The frequency of GCK-MODY, HNF1A-MODY and KCNJ11-MODY was 83.3% (15/18), 11.1% (2/18) and 5.5% (1/18), respectively in our cohort. All variants were heterozygous. There were 4 novel GCK gene mutations (c.74T>C, c.106_108delAGAGinsTGG, c.534delG, c.583G>A). Genetic characteristics are summarized in Table 2. Segregation analysis could be performed only in P15 and P17.

Characteristics of Patients with GCK Mutation

In patients with a GCK mutation (10 females and 5 males), mean age was 11 ± 4.4 years (range 4.8-17 years) and mean duration of follow-up was 3.8 years (1-13 years). Mean FBG, HbA1c, C-peptide and insulin levels were 120.3 ± 13 mg/dL, $6.5 \pm 0.2\%$, 1.4 ± 0.7 ng/mL, and 7.5 ± 1.2 IU/mL, respectively. Patients 12 and 13 were siblings. All six patients (P2, P3, P4, P10, P12, P13) had been diagnosed before puberty. While P7 and P14 had been diagnosed due to polyuria and polydipsia, others had been diagnosed due to

Table 1. Clinical characteristics of the patients

Patient no.	Age at diagnosis, year	Symptoms at diagnosis	Parents with diabetes	Affected relatives	Fasting blood glucose (mg/dL)	HbA1c at admission (%)	Urine glucose	Previous diagnosis	Treatment
P1	12.6	Coincidental		Positive	123	6.6	Negative		Medical nutrition therapy
P2	10	Coincidental	Mother	Positive	116	6.7	Negative		Medical nutrition therapy
P3	4.8	Coincidental		Positive	114	6.4	Negative		Medical nutrition therapy
P4	6	Coincidental	Father	Positive	115	6.2	Negative		Medical nutrition therapy
P5	15	Coincidental	Mother	Positive	117	6.3	Negative		Medical nutrition therapy
P6	5.1	Coincidental	Father	Positive	142	6.1	Negative		Medical nutrition therapy
P7	15	Polyuria, polydipsia	Father	Positive	120	6.1	Negative		Medical nutrition therapy
P8	17.2	Coincidental	Father	Positive	115	6	Negative		Medical nutrition therapy
P9	8.4	Coincidental	GD in mother	Positive	126	6.2	Negative		Insulin (0.1 U/kg/day)
P10	6.6	Coincidental	GD in mother	Positive	116	6	Negative		Medical nutrition therapy
P11	17	Coincidental		Positive	132	6.9	Negative		Medical nutrition therapy
P12	8.4	Coincidental	GD in mother	Positive	122	6.1	Negative	T2DM	Medical nutrition therapy
P13	7.1	Coincidental	GD in mother	Positive	118	6.1	Negative	T2DM	Medical nutrition therapy
P14	15.8	Polyuria, polydipsia		Positive	113	6.4	Negative		Medical nutrition therapy
P15	12.6	Coincidental	Mother	Positive	116	6.6	Negative	T1DM	Medical nutrition therapy
P16	11	Coincidental	GD in mother	Positive	107	6.2	Negative		Medical nutrition therapy
P17	17.3	Coincidental	Father	Positive	4635	11.7	Positive	T1DM	Sulfonylure
P18	4.5	Coincidental	GD in mother	Positive	178	9.7	Positive	T1DM	Glibenclamide

GD: Gestational diabetes, T1DM: type 1 diabetes mellitus, T2DM: type 2 diabetes mellitus

Table 2. Genetic characteristics of the patients

Patient no.	Gene	Status	c.DNA	Mutation type	Protein effect	Mutation taster	Polyphen	SIFT prediction/score	HGMD
P1	GCK	Novel	c.74T>C	Missense	p.Leu26Pro	Disease causing	Probably damaging	Damaging /0	NA
P2	GCK	Known	c.506A>G	Missense	p.Lys169Arg	Disease causing	Probably damaging	Damaging /0	CM141531 (DM)
P3	GCK	Known	c.676G>A	Missense	p.Val226Met	Disease causing	Probably damaging	Damaging /0	CM970636 (DM)
P4	GCK	Novel	c.106_108delAGinsTGG	Indel	p.Arg36Trp	Disease causing	Probably damaging	Damaging	NA
P5	GCK	Known	c.506A>G	Missense	p.Lys170Arg	Disease causing	Probably damaging	Damaging /0	CM141531 (DM)
P6	GCK	Novel	c.534delG	Frameshift	p.Asn180ThrfsTer25	Disease causing	Probably damaging	Damaging	NA
P7	GCK	Known	c.661G>A	Missense	p.Glu221Lys	Disease causing	Probably damaging	Tolerated/0.116	CM970635 (DM)
P8	GCK	Known	c.1178T>C	Missense	p.Met394Thr	Disease causing	Possibly damaging	Damaging /0.002	CM096945 (DM)
P9	GCK	Known	c.1178T>C	Missense	p.Met394Thr	Disease causing	Possibly damaging	Damaging /0.002	
P10	GCK	Known	c.676G>A	Missense	p.Val226Met	Disease causing	Probably damaging	Damaging /0	CM970636 (DM)
P11	GCK	Known	c.898G>A	Missense	p.Glu300Lys	Disease causing	Probably damaging	Damaging /0.001	CM930305 (DM)
P12	GCK	Novel	c.583G>A	Missense	Asp195Asn	Disease causing	Probably damaging	Damaging /0.025	NA
P13	GCK	Novel	c.583G>A	Missense	Asp195Asn	Disease causing	Probably damaging	Damaging /0.025	NA
P14	GCK	Known	c.898G>A	Missense	p.Glu300Lys	Disease causing	Probably damaging	Damaging /0.001	CM930305 (DM)
P15	GCK/ ABCC8	Known/ Novel	c.544G>A/ c.2888A>G	Missense/ Missense	p.Val182Met p.Asp963Gly	Disease causing/ Disease causing	Probably damaging/benign	Tolerated/ Tolerated	CM930298 (DM) /-
P16	HNF1A	Known	c.716C>T	Missense	p.Ala239Val	Disease causing	Probably damaging	Damaging	CM023588 (DM)
P17	HNF1A/ ABCC8	Known/ Novel	c.872dupC c.1996C>T	Frameshift /Synonym	p.Gly292Argfs*25 p.Arg657Trp	Disease causing/ Disease causing	Possibly damaging/ Benign	Damaging/ Tolerated	CI962354 (DM)/ NA
P18	KCNJ11	Known	c.1054G>C / c.965A>C	Missense/ Missense	p.Asp352His/ p.Glu322Ala	Disease causing/ Disease causing	Possibly damaging/ Probably damaging	Tolerated/ Damaging	CM1111083(DM)/ CM1111082 (DM)

incidental hyperglycemia. None of the patients had ketonemia, ketoacidosis at the time of diagnosis or during follow-up. Hyperglycemia was not detected in the parents of P1, P3, P11 and P14, however they had affected relatives. Mothers of two siblings (P12-P13), P9 and P10 had gestational diabetes. All of the patients were consuming low carbohydrate diet. P9 needed low dose of insulin (0.1 U/kg). Before the molecular diagnosis of MODY, P15 was followed up for 9 months diagnosed as having T1DM, and P12 and P13 (two siblings) were diagnosed as having T2DM for 5 years. After the genetic diagnosis, insulin and antidiabetic drug were discontinued.

Eleven different heterozygous mutations were identified in 15 patients. P3 and P10, P8 and P9, P12 and P13, P11 and P14 had same variants. There were 9 (82%) missense mutations, 1 (9%) frameshift mutation and 1 (9%) indel variants. Seven of the variants had already been reported (c.506A>G, c.676G>A, c.506A>G, c.661G>A, c.1178T>C, c.898G>A, c.544G>A). The remaining four variants (c.74T>C, c.106_108delAGAGinsTGG, c.534delG, c.583G>A) were not listed in HGMD, ClinVar or PUBMED and were classified as novel mutations. In P15, a heterozygous missense ABCC8(c.2888A>G) VUS variant was also identified along with the likely pathogenic variant in GCK. Mother of P15 was heterozygous for the variant in ABCC8 and the father was heterozygous for the variant in GCK.

Characteristics of Patients with HNF1A Mutation

The mean age at diagnosis was 12.2±3.6 years (8.6-17 years). All patients were diagnosed during adolescence. None of them was obese. The mean HbA1c level at diagnosis was 8.5±2.5% (6-11%). They had been diagnosed due to incidental hyperglycemia. One of patients had positive family history of diabetes and one of the mothers was diagnosed during pregnancy. None of the patients had ketosis at the time of diagnosis or during follow-up. Before the diagnosis of MODY, P17 was followed up for 1 year with a diagnosis of T1DM. After the genetic diagnosis, insulin therapy was switched to sulfonylurea treatment. P16 was monitored without any treatment. Two different mutations in *HNF1A* gene were identified in 2 patients. In P16, a previously reported missense VUS variant (c.716C>T) was detected. P17 had a known a frameshift variant in *HNF1A* (c.872dupC) and a synonymous variant in *ABCC8* (c.1996C>T) classified as pathogenic and VUS, respectively. Father of P17 was also heterozygous for *HNF1A* (c.872dupC) variant.

Characteristics of Patient with KCNJ11 Gene

A *KCNJ11* mutation was detected in a boy. Incidental hyperglycemia was detected at the age of 14. Before the diagnosis of MODY, he was followed up for 3 years with the diagnosis of T1DM. A two-generation positive family history was noted. The FBG, HbA1c, C-peptide and insulin levels were 178 mg/dL, 9.2%, 3.22 ng/mL, and 7.8 IU/mL, respectively at diagnosis. Urine glucose was positive. He did not have ketosis or ketoacidosis. Two

known missense mutations were detected in the *KCNJ11* gene (c.1054G>C and c.965A>C) classified as VUS and pathogenic, respectively. After the genetic diagnosis, insulin treatment was switched to glibenclamide. He has been followed up for 4 years, so far, he has no retinopathy or nephropathy.

DISCUSSION

In this study, 18 patients were selected with the clinical criteria of MODY. We assessed mutation distribution, novel mutations, clinical characteristics, and prognosis. Today, tNGS is routinely performed in all newly diagnosed patients. Early diagnosis and correct diagnostic approach are essential for appropriate treatment and for patients who have a milder phenotype. GCK mutations were the most frequent in our cohort. We detected GCK mutations in 15 of the 18 patients with MODY (83.3%). The frequency of GCK-MODY varies according to different healthcare systems. In countries which perform routine blood glucose screening such as Italy, France, and Spain, GCK mutations are the most common cause of MODY. However, in the United Kingdom, where the studies mainly included symptomatic patients, *HNF1A* mutations were more common than GCK mutations (11). Similar to our study, variants were most frequently found in the GCK gene in several studies in Turkey (12-15). Heterozygous loss-of-function mutations in the GCK gene cause GCK-MODY. Mutations in GCK usually presents with mild hyperglycemia. Almost 15% of all patients with incidental hyperglycemia may be caused by GCK mutations (16). In our study, except 2 patients, all the other patients with a GCK mutation were diagnosed due to incidental hyperglycemia. In the remained 2 patients (P7 and P14), polyuria and polydipsia were the presenting symptoms. These osmotic symptoms are rarely observed in patients with GCK-MODY (17). Patients with GCK-MODY usually have a lower HbA1c level and good metabolic control. In support of this view, among patients with GCK-MODY in our cohort, HbA1c levels were between 6.1-6.9%. Normal levels of blood glucose were achieved with only medical nutrition therapy in 14 of 15 patients. The remained one patient needed low dose insulin (0.1 U/kg) therapy because of non-compliance to diet and HbA1c level rising up to 7.1%. We discontinued oral antidiabetic drugs in 2 siblings (P12 and P13) who were misdiagnosed as having T2DM before the molecular diagnosis. Therapy was continued with only medical nutrition therapy in such patients. The similar incidence of microvascular complications compared with controls without diabetes was reported in patients with GCK-MODY (18). Based on this, none of our patients with GCK-MODY in our cohort had any microvascular complications. In one patient (P15), a VUS variant in the *ABCC8* gene was detected in addition to the GCK variant. However, we did not consider that the *ABCC8* variant to have a modifying effect, since this patient's clinical status was not different from patients with GCK mutations.

In *HNF1A*-MODY, response of insulin secretion to blood glucose

levels is severely reduced. However, our patients (P16 and P17) with HNF1A mutations had been diagnosed with incidental hyperglycemia. This situation may be related to the pathogenicity of mutations. We detected 2 different HNF1A mutations in 2 patients. Variant in P16 was classified as VUS and this patient had mild hyperglycemia, and so far, did not need treatment. P17 was misdiagnosed as having T1DM and blood glucose levels were high even with the insulin treatment before the molecular diagnosis. In addition to the pathogenic variant in HNF1A, there was an additional synonym VUS variant in ABCC8 in this patient and we considered that this variant had no contribution to the phenotype.

Heterozygous mutations in KCNJ11 which encode the 2 subunits (Kir6.2 and SUR1, respectively) of the pancreatic β -cell ATP sensitive potassium (K ATP) channels have been shown to cause a wide spectrum of phenotypes, ranging from neonatal diabetes to MODY (19). Moreover, heterozygous KCNJ11 mutations were identified in a Chinese family with early onset T2DM (20). In our study, two KCNJ11 variants were detected only in P18. Among heterozygous KCNJ11 mutations, c.965A>C (p.Glu322Ala) variant was classified as pathogenic and c.1054G>C (p.Asp352His) variant was classified as VUS. In previous studies, c.965A>C (p.Glu322Ala) variant was identified in two patients with transient neonatal diabetes and it was reported that sulfanylurea was effective in the treatment of these patients (21,22). P18 was misdiagnosed as T1DM and treatment was switched to glibenclamide after the molecular diagnosis. Sulfanylurea was also effective in our patient. He was using glibenclamide for four years and HbA1c level decreased after the treatment change.

Study Limitations

There are some limitations in this study. Segregation analysis could not be performed in all parents of the patients. So, we could not detect if the mutation was *de novo* or not. We also could not perform functional analyses of the new mutations.

CONCLUSION

Since a wide spectrum of phenotypes of MODY has been shown, identification of new mutations and experiences of the clinics are very important. This study provides molecular-clinical features of MODY in three different centers.

Ethics Committee Approval: The study was approved by Taksim Training and Research Hospital Clinical Research Ethics Committee (decision no: 83, date: 12.06.2019) and was performed in accordance with the Helsinki Declaration.

Informed Consent: After obtaining written informed consent, blood samples were obtained from the patients and available parents.

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