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The Application of Next Generation Sequencing Maturity Onset Diabetes of the Young Gene Panel in Turkish Patients from Trakya Region

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What is already known on this topic?

Maturity-onset diabetes of the young (MODY) cases represent 1-2% of all diabetes cases. Since the clinical findings of MODY patients are similar to type 1 and type 2 diabetes, the majority of these patients are mistakenly diagnosed with type 1 or type 2 diabetes mellitus. Despite all the advances of molecular tests, mutations in any known gene may still not be detected in some of the individuals clinically diagnosed with MODY. This indicates that monogenic diabetes is a subject that needs to be investigated more in terms of new genes and new criterias.

What this study adds?

This is the first study of MODY variants in the Trakya region of Turkey. There was a relatively high case frequency for the population in a cohort of 61 patients, 47.5% had variants which were pathogenic/likely pathogenic and a further 18% had variants of uncertain clinical significance. In addition 12 novel variants were detected and are firstly reported here.

Abstract

Objective: The aim of this study was to investigate the molecular basis of maturity-onset diabetes of the young (MODY) by targeted-gene sequencing of 20 genes related to monogenic diabetes, estimate the frequency and describe the clinical characteristics of monogenic diabetes and MODY in the Trakya Region of Turkey.

Methods: A panel of 20 monogenic diabetes related genes were screened in 61 cases. Illumina NextSeq550 system was used for sequencing. Pathogenicity of the variants were assessed by bioinformatics prediction software programs and segregation analyses.

Results: In 29 (47.5%) cases, 31 pathogenic/likely pathogenic variants in the *GCK, ABCC8, KCNJ11, HNF1A, HNF4A* genes and in 11 (18%) cases, 14 variants of uncertain significance (VUS) in the *GCK, RFX6, CEL, PDX1, KCNJ11, HNF1A, G6PC2, GLIS3* and *KLF11* genes were identified. There were six different pathogenic/likely pathogenic variants and six different VUS which were novel.

Conclusion: This is the first study including molecular studies of twenty monogenic diabetes genes in Turkish cases in the Trakya Region. The results showed that pathogenic variants in the *GCK* gene are the leading cause of MODY in our population. A high frequency of novel variants (32.4%-12/37) in the current study, suggests that multiple gene analysis provides accurate genetic diagnosis in MODY.

Keywords: Monogenic diabetes, MODY, NGS, pathogenic variant, novel variant

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Introduction

The types of diabetes associated with single gene defects, including neonatal diabetes, syndromic diabetes and maturity onset diabetes of the young (MODY), are called monogenic diabetes (1). MODY is caused by pathogenic variants in genes responsible for the embryonic development or function of beta cells of the pancreas (2). When it was first defined, autosomal dominant inheritance, a history of diabetes in at least three generations, positive clinical findings before the age of 25, no need for insulin or needing low dose (< 0.5 U/kg), and good metabolic control were determined as diagnostic criteria for MODY (3). However, with the current understanding that MODY is a heterogeneous group and the clinical findings and treatment differ due to the underlying genetic defect, clinical suspicions for MODY pre-diagnosis have been expanded. In addition, due to the fact that some patients who are followed as type 1 or type 2 diabetes are diagnosed with MODY, diabetes autoantibody negativity and absence of insulin resistance findings in obese diabetics are also included in the criteria for MODY.

MODY is responsible for an estimated 1% of all diabetes cases in children and adolescents (2). However, it is very difficult to find the true prevalence as individuals with MODY are mistakenly classified as type 1 or type 2 diabetes. In some studies, while the rate of diagnosis of MODY clinically is 10-20% among all diabetics, pathogenic variants in the known genes are not detected in approximately 20% of patients when genetic testing is performed (2).

There are MODY subtypes related to many different gene defects. The most common pathogenic variants are in the *HNF4A, GCK* and *HNF1A* genes, and these three genes account for about 95% of all MODY cases (4). The clinical spectrum of individuals with MODY can vary considerably according to the underlying genetic problem. For example, GCK-MODY causes mild fasting hyperglycemia, which does not progress and does not require treatment, while HNF1A-MODY or HNF4A-MODY leads to diabetes with progressive beta cell destruction and microvascular complications. Some types of MODY, such as HNF1B-MODY or CEL-MODY, can also be classified as syndromic diabetes, as they are associated with kidney and pancreatic malformations or exocrine pancreatic insufficiency (5).

MODY due to *HNF1A, HNF4A, HNF1B* and *GCK* mutations accounts for most of all known cases (6). The next most common gene variants found in MODY are *ABCC8* and *KCNJ11* mutations, and more rarely, *INS, PDX1, NEUROD1* and *CEL* mutations. While MODY cases due to heterozygous *INS, PDX1, NEUROD1* mutations create isolated clinical diabetes in a few pedigrees, a decrease in fecal elastase and other exocrine pancreatic functions have also been reported in a small number of MODY individuals due to heterozygous *CEL* mutation (7).

Until recently, the diagnosis of MODY was made by scanning the intended candidate gene for point mutations or small insertions/deletions by Sanger sequencing. This method could result in both high cost and a limited number of genes being sequenced. In addition, this method could miss large insertions and deletions. To date, with the advent of the next generation sequencing (NGS) method, many genes can be analyzed together at the same time and at lower cost. Thus, many gene panels have been created and 7-29 genes are analysed simultaneously (8). However, accurate evaluation of the results is very important. In the current study, we aimed to analyse 20 different genes (*ABCC8, BLK, CEL, GCK, HNF1A, HNF1B, HNF4A, INS, KCNJ11, KLF11, NEUROD1, NKX2-2, PAX4, PDX1, RFX6, ZFP57, GLIS3, FOXP3, NEUROG3, G6PC2*) in cases with a pre-diagnosis of monogenic diabetes using NGS method.

Methods

The patient files of 61 cases with a clinical diagnosis of monogenic diabetes, mostly MODY, who were examined in Pediatric Endocrinology clinic or Medical Genetics clinic were included. For the clinical diagnosis of MODY, the following criterias were considered: diabetes mellitus in a parent and a history of diabetes mellitus in first-degree relatives for at least two generations; negativity for two or more type 1 diabetes mellitus associated autoantibodies [anti-glutamic acid decarboxylase (GAD) antibody, insulin antibody and/or islet cell antibody (ICA)]; low insulin requirement (< 0.5 U/kg/day) and measurable C-peptide level. Written informed consent was obtained from the parents of the patients.

EDTA-blood samples were collected from participants. Genomic DNA was isolated from peripheral blood mononuclear cells using EZ1 DNA Investigator Kit (Qiagen, Hilden, Germany). Primary quality control of the isolated DNA samples was performed using NanoDrop (Thermo Fisher Scientific, Waltham, MA), and samples having A260/280 values between 1.8-2.0 were included in the study.

QIAseq Targeted DNA Panel (Qiagen, Hilden, Germany) was used to analyse 20 genes (*ABCC8, BLK, CEL, GCK, HNF1A, HNF1B, HNF4A, INS, KCNJ11, KLF11, NEUROD1, NKX2-2, PAX4, PDX1, RFX6, ZFP57, GLIS3, FOXP3, NEUROG3, G6PC2*). Libraries were prepared according to manufacturer's instructions. Quality control of the prepared libraries was done with Qubit dsDNA BR Assay system (Invitrogen, Carlsbad, CA, USA). Fastq generation was performed on

Illumina NextSeq550 (Illumina Inc., San Diego, CA, USA). Libraries covering the target genes were prepared according to the QIAseq Targeted DNA Panel protocol (Qiagen, Hilden, Germany). Following the target enrichment process, libraries were sequenced on the Illumina NextSeq550 (Illumina Inc., San Diego, CA, USA). QCI analysis (Qiagen, Hilden, Germany) was used for Quality control and ordering Variant Call Format file. Variant analysis was performed with Ingenuity software (Qiagen, Hilden, Germany).

For segregation analysis, primers were designed for all needed regions and Sanger sequencing was performed using an ABI 3130 (Applied Biosystems, USA) capillary electrophoresis system.

The American College of Medical Genetics 2015 (9) guidelines were followed for the classification of all the variants, recommendations of the Human Genome Variation Society (10) were followed to describe the novel variants. ClinVar (11), HGMD-Professionel 2020 database and literature information were considered for collecting the information about known variants.

Descriptive analysis was done with patient numbers and percentages in the current study.

This study was approved by the Ethical Committee of the Trakya University (approval number: 2020/263, date: 10.08.2020) and performed in consonance with the principles of the Declaration of Helsinki.

Results

Twenty different genes were analysed in 61 unrelated cases with a pre-diagnosis of MODY. The cases had a mean age of 14.93 years and included 33 female cases (mean age 15.1 years) and 28 male cases (mean age 14.7 years). Thirty-two of the patients (52.4%) had an affected family member.

Thirty-one pathogenic/likely pathogenic variants in 29 (47.5%) cases and 14 variants of uncertain significance (VUS) in 11 (18%) cases were detected. Pathogenic/likely pathogenic variants were distributed among the target genes as follows: *GCK* n=24; *ABCC8* n=3; *KCNJ11* n=2; *HNF1A* n=1; and *HNF4A* n=1. Similarly, VUS were identified in: *GCK* n=2; *RFX6* n=3; *CEL* n=2; *PDX1* n=2; *KCNJ11* n=1; *HNF1A* n=1; *G6PC2* n=1: *GLIS3* n=1; and *KLF11* n=1 (Table 1). In total 37 different variants were identified in this study (Table 2).

Six novel pathogenic/likely pathogenic variants, five in the *GCK* gene and one in the *ABCC8* gene were found. In addition, six novel VUS (*RFX6* n=3, *HNF1A* n=1, *CEL* n=1, and *GLIS3* n=1) were detected. Thus, 12 of 37 (32.4%) variants found were novel (Table 2). All variants in this study had been inherited in heterozygously.

Family members of cases 2, 3, 4, 10, 12, 15, 17, and 18 who also had the same variants with the reported cases also had clinical findings consistent with MODY (Table 1). They were all diagnosed as diabetes mellitus 1 or 2 previously. However, due to these moleular results, the diagnoses of these family members could be reconsidered.

Cases 4 and 5 each had two different likely pathogenic variants. Case 4 had *GCK* and *KCNJ11* likely pathogenic variants, which were maternally inherited and *de novo*, respectively. He had been diagnosed with type 1 diabetes mellitus initially. After revealing the maternal history, he had been tested for MODY, and had the correct diagnosis. It is interesting that he had two likely pathogenic variants for MODY type 2 and type 13. However, during follow-up, neither he nor his mother had a complication related with diabetes. Case 5 had *ABCC8* and *GCK* likely pathogenic variants, related with the phenotypes of MODY type 2 and diabetes mellitus, noninsulin dependent. She was diagnosed with incidentally detected high serum glucose level and she had been taking unnecessary insulin because of the diabetes mellitus diagnosis. A MODY test was planned due to the strong family history of diabetes with her mother, sister and grandmother all affected. During follow-up, case 5 also never exhibited complication associated with diabetes.

Discussion

MODY is a rare form of diabetes that has genetic, metabolic and clinical differences and is autosomal dominantly inherited (12). MODY should be considered in the differential diagnosis of patients diagnosed with type 1 or type 2 diabetes but who have clinically atypical findings. MODY diagnosis should be kept in mind in patients diagnosed with type 1 diabetes but with negative pancreatic autoantibodies and/or measurable C-peptide level at the time of diagnosis and/or good glycemic control with low-dose insulin therapy. In addition, the diagnosis of MODY should be considered in patients without obesity and acanthosis nigricans, followed by a diagnosis of type 2 diabetes in the laboratory without signs of insulin resistance. Diagnosis of diabetes in three generations or similar findings in the family is also suggestive for MODY.

Many MODY-related genes have been identified. The most common of these genes are *HNF4A* (MODY1), *GCK* (MODY2), *HNF1A* (MODY3), *IPF1* (MODY4), *HNF1B* (MODY5), *NEUROD1* (MODY6), and *CEL* (MODY8). Glucokinase, the protein product of the *GCK* gene (also called pancreatic β-cell glucose sensor) acts as the key regulatory enzyme in glucose-

induced insulin release. The heterozygous loss-of-function mutation in the *GCK* gene, which may be diagnosed at any age, leads to a slight increase in the glucose threshold value in the glucose-insulin release curve. This results in fasting glucose levels of between 100-153 mg/dL and HbA1c levels do not exceed 7.5% while the increase in glucose level at 0 and 120 min on oral glucose tolerance test (OGTT) does not exceed 90 mg/dL in 95% of cases (13).

In our study, twenty-four cases had a *GCK* pathogenic variant; all were diagnosed due to incidental hyperglycemia. The youngest patient was 9 months old (case 14). Only one patient (case 2) was obese [body mass index (BMI) > 95 P] and the other cases had normal weight. All these cases were negative for ICA although two of them were positive for GAD antibody (cases 18 and 20). At diagnosis, the lowest HbA1c was 6.1% and the highest was 6.7%, (normal range 3.6-5.8). The same mutation was detected in one of the parents of six patients with *GCK* pathogenic variants. Three of the parents (mothers of cases 2, 17 and 18) were diagnosed with gestational diabetes and had to use insulin. All our patients have been managed with a controlled carbohydrate diet. None of them needed medical treatment.

Haliloglu et al (14) selected cases for *GCK* analysis with a pre-diagnosis of MODY in a Turkish population. In 11 cases of 21 probands (52%) a pathogenic/likely pathogenic variant was identified. In our study, *GCK* pathogenic/likely pathogenic variants were detected 39.3% of cases. Pathogenic variants in the *GCK* gene are the most frequent in the literature (15). Since hyperglycemia is mild in these patients, microvascular complications are not observed. Therefore, confirming molecular diagnosis in these patients will prevent unnecessary insulin treatment. Although there is no long-term data on macrovascular complications, it is thought that cardiovascular risk does not increase in these patients (16). In a study, it was reported that GCK-MODY patients' BMI and blood glucose level increased and insulin sensitivity decreased during follow-up (17). Therefore, HbA1c is recommended to be observed once a year for GCK-MODY patients.

The presence of GCK-MODY in pregnant women may also be a clinical concern. A genetically unaffected baby of a mother with GCK-MODY will be born macrosomically. In cases where both mother and baby are GCK-MODY, starting treatment for the mother may lead to the birth of the baby at low weight (18). For this reason, identifying GCK-MODY during pregnancy is important for the problems which may occur for either the mother or the baby.

Another common cause of MODY is heterozygous mutations in the *HNF1A* gene. The *HNF1A* gene is a transcription factor that plays a role in the development, proliferation and death of beta cells, as well as regulating insulin secretion (19). For this reason, individuals with HNF1A-MODY are born normoglycemic, but with advancing age (usually puberty and after) the clinical spectrum progresses due to the onset of beta cell destruction and obvious diabetes develops. With a high penetration, 63% of those carrying the mutation develop diabetes before the age of 25 years and 96% before the age of 55 years. The type and location of the mutation affects the age of onset of diabetes (20). A mutation in the dimerization or binding region of *HNF1A* leads to the development of diabetes 10 years earlier than a mutation in the transactivation region. Although the clinical features of cases varies according to the mutation, there can be phenotypic variability in the same family with genotypic homogeneity. Although ketoacidosis is rare, approximately 25% of patients can present with a type 1 diabetes-like clinical picture. Most present type 2 diabetes-like clinical findings, but patients are generally not obese and there are no signs of insulin resistance (21).

We identified *HNF1A* pathogenic variant in one case (case 12), and *HNF1A* variant of unknown significance in a further case (case 31). Case 31 was 12 years old, at the beginning of the pubertal period, and case 12 was 16 years and 11 months old at the end of the pubertal period. Case 31 had no positive family history of diabetes. In the OGTT test, this patient, had a blood glucose level in the normal range and the HbA1c level was 6%. Case 31 is managed with a controlled carbohydrate diet. Case 12 had a typical positive three-generation family history of diabetes mellitus. These two cases were negative for ICA, GAD antibodies and insulin auto-antibody. At diagnosis, case 12 had an HbA1c level of 8.5% and high blood sugar (fasting blood sugar > 100 mg/dL, postprandial blood sugar > 200 mg/dL), and she is managed with sulfonylurea treatment.

HNF4A-MODY is rarer than HNF1A-MODY and is responsible for approximately 5-10% of all MODY cases. *HNF4A* is also a transcription factor, abnormality of which leads to progressive beta cell destruction, similar to a dysfunctional heterozygous mutation in the *HNF1A* gene. Clinical findings are the same as HNF1A-MODY, and 50% of individuals with *HNF4A* heterozygous mutation have a history of macrosomic delivery, and about 15% have neonatal hyperinsulinemic hypoglycaemia that is diazoxide responsive. Extra-pancreatic laboratory findings in HNF4A-MODY are low high-density lipoprotein, low triglyceride and high low-density lipoprotein (22). We identified a pathogenic variant of *HNF4A* in one case (case 23) with HbA1c of 12% and fasting blood sugar of 237 mg/dL at diagnosis. He was diagnosed as type 1 diabetes and treated with insulin before molecular analysis by MODY panel.

Table 1. Genotypes and phenotypes of the cases and segregation results in the study

Case	Age/Gender	Gene	Variant
1	16/F	*GCK*	ENST00000403799.3:c.387C > A
2	13/F	*GCK*	NM_033507.3:c.1256 + 1G > T (c.1253 + 1G > T)
3	5/F	*GCK*	ENST00000403799.3:c.387C > A
4	16/M	*GCK*	NM_000162.5:c.943C > T
		KCNJ11	NM_000525.3:c.668C > T
5	21/F	*ABCC8*	NM_000352.6:c.3517G > A
		GCK	NM_000162.5:c.943C > T
6	11/F	*GCK*	ENST00000403799.3:c.398T > A
7	7/F	*GCK*	NM_033507.3:c.133G > A
8	3/F	*GCK*	NM_033507.3:c.537delG
9	18/F	*GCK*	NM_000162.5:c.943C > T
10	13/F	*GCK*	ENST00000403799.3:c.387C > A
11	11/F	*GCK*	NM_033507.3:c.867-1G > A
12	16/F	*HNF1A*	NM_000545.8:c.864delGinsCC (c.872dupC)
13	5/F	*ABCC8*	NM_000352.6:c.4014G > A
14	9 months/M	*GCK*	NM_000162.5:c.943C > T
15	3/F	*GCK*	NM_000162.5:c.880G > C
16	8/F	*GCK*	NM_000162.5:c.1248C > G
17	14/M	*GCK*	NM_000162.5:c.746G > A
18	1/F	*GCK*	NM_000162.5:c.667G > A
19	21/M	*GCK*	NM_000162.5:c.506A > G
20	12/F	*GCK*	NM_000162.5:c.565A > G
21	14/M	*GCK*	NM_000162.5:c.565A > G
22	1/M	*GCK*	NM_000162.5:c.617C > T
23	14/M	*HNF4A*	NM_000457.4:c.844G > A
24	33/F	*GCK*	NM_000162.5:c.943C > T
25	28/M	*GCK*	NM_000162.5:c.1222G > T
26	16/M	*ABCC8*	NM_000352.6:c.2768T > G
27	12/F	*KCNJ11*	NM_000525.3:c.481G > A
28	1/M	*GCK*	NM_000162.5:c.115_117delAAG
29	12/F	*GCK*	NM_000162.5:c.214G > A
30	29/M	*KCNJ11*	NM_000525.3:c.1117G > A
31	12/F	*HNF1A*	NM_000545.8:c.1769-3C > T
32	33/M	*G6PC2*	NM_021176.3:c.89C > T
33	13/F	*CEL*	NM_001807.6:c.2184_2216del
		PDX1	NM_000209.4:c.226G > A
34	13/M	*RFX6*	NM_173560.4:c.428G > A
		GCK	NM_000162.5:c.863 + 3A > G
35	33/F	*GCK*	NM_000162.5:c.863 + 3A > G
36	8/M	*CEL*	NM_001807.6:c.2049_2082del
		RFX6	NM_173560.4:c.1072G > A
37	12/F	*RFX6*	NM_173560.4:c.246C > G
38	14/F	*PDX1*	NM_000209.4:c.97C > A
39	18/F	*GLIS3*	NM_001042413.2:c.589G > T
40	2/M	*KLF11*	NM_003597.5:c.1447C > T

ACMG: American College of Medical Genetics, dbSNP: The Single Nucleotide Polymorphism Database, VUS: variants of unknown significance, NA: not applicable, M: male, F: female

Protein	Pathogenicity (ACMG-2015)	Segregation analysis	Phenotype
p.(Cys129Ter)	Pathogenic	NA	MODY type 2
	Pathogenic	Inherited from the affected mother	MODY type 2
p.(Cys129Ter)	Pathogenic	Affected sister has the same variant	MODY type 2
p.(Leu315Phe)	Likely pathogenic	Inherited from the affected mother	MODY type 2
p.(Thr223Ile)	Likely pathogenic	*de novo*	MODY type 13
p.(Val1173Met)	Likely pathogenic	NA	Diabetes mellitus, noninsulin dependent
p.(Leu315Phe)	Likely pathogenic	NA	MODY type 2
p.(Phe133Tyr)	Likely pathogenic	NA	MODY type 2
p.(Gly45Ser)	Likely pathogenic	NA	MODY type 2
p.(Asn180ThrfsTer25)	Pathogenic	NA	MODY type 2
p.(Leu315Phe)	Likely pathogenic	NA	MODY type 2
p.(Cys129Ter)	Pathogenic	Inherited from the affected mother	MODY type 2
	Pathogenic	NA	MODY type 2
p.(Gly292ArgfsTer25)	Pathogenic	Inherited from the affected mother	MODY type 3
p.(Trp1338Ter)	Pathogenic	NA	Diabetes mellitus, noninsulin dependent
p.(Leu315Phe)	Likely pathogenic	NA	MODY type 2
p.(Gly294Arg)	Likely pathogenic	Inherited from the affected mother	MODY type 2
p.(His416Gln)	Likely pathogenic	NA	MODY type 2
p.(Gly249Asp)	Likely pathogenic	Inherited from the affected mother	MODY type 2
p.(Gly223Ser)	Pathogenic	Inherited from the affected mother	MODY type 2
p.(Lys169Arg)	Likely pathogenic	NA	MODY type 2
p.(Ile189Val)	Likely pathogenic	NA	MODY type 2
p.(Ile189Val)	Likely pathogenic	NA	MODY type 2
p.(Thr206Met)	Likely pathogenic	NA	MODY type 2
p.(Asp282Asn)	Likely pathogenic	NA	
p.(Leu315Phe)	Likely pathogenic	NA	MODY type 2
p.(Val408Leu)	Likely pathogenic	NA	MODY type 2
p.(Leu923Arg)	Likely pathogenic	NA	Diabetes mellitus, noninsulin dependent
p.(Ala161Thr)	Likely pathogenic	NA	MODY type 13
p.(Lys39del)	Likely pathogenic	NA	MODY type 2
p.(Gly72Arg)	Pathogenic	NA	MODY type 2
p.(Val373Met)	VUS	NA	MODY type 13
	VUS	NA	MODY type 3
p.(Ser30Phe)	VUS	NA	NA
p.(Gly729_Thr739del)	VUS	NA	MODY type 8
p.(Asp76Asn)	VUS	NA	MODY type 4
p.(Cys143Tyr)	VUS	NA	Mitchell-Riley syndrome
	VUS	NA	MODY type 2
	VUS	NA	MODY type 2
p.(Thr684ArgfsTer9)	VUS	NA	MODY type 8
p.(Val358Ile)	VUS	NA	Mitchell-Riley syndrome
p.(Asn82Lys)	VUS	NA	Mitchell-Riley syndrome
p.(Pro33Thr)	VUS	NA	MODY type 4
p.(Asp197Tyr)	VUS	NA	Diabetes mellitus, neonatal, with congenital hypothyroidism
p.(Pro483Ser)	VUS	NA	MODY type 7

Table 2. ***In silico*** **predictions and previous database access number information of each variant identified in this study**

Gene (transcript ID)	Variant	Variant type	Chr position (hg19)	dbSNP
GCK	NM_000162.5:c.115_117delAAG p.(Lys39del)	In frame	7:44192991	NA
	NM_033507.3:c.133G > A p.(Gly45Ser)	Missense	7:44192978	rs267601516
	NM_000162.5:c.214G > A p.(Gly72Arg)	Missense	7:44192019	rs193922289
	ENST00000403799.3:c.387C > A p.(Cys129Ter)	Nonsense	7:44190651	rs1583601365
	ENST00000403799.3:c.398T > A p.(Phe133Tyr)	Missense	7:44190640	NA
	NM_000162.5:c.506A > G p.(Lys169Arg)	Missense	7:44189641	NA
	NM_033507.3:c.537delG p.(Asn180ThrfsTer25)	Frameshift	7:44189613	NA
	NM_000162.5:c.565A > G p.(Ile189Val)	Missense	7:44189582	rs757978639
	NM_000162.5:c.617C > T p.(Thr206Met)	Missense	7:44189421	rs1441649062
	NM_000162.5:c.667G > A p.(Gly223Ser)	Missense	7:44189371	rs1360415315
	NM_000162.5:c.746G > A p.(Gly249Asp)	Missense	7:44187366	NA
	NM_000162.5:c.863 + 3A > G	Splicing	7:44187246	rs193922334
	NM_033507.3:c.867-1G > A	Splicing	7:44186218	rs1167675604
	NM_000162.5:c.880G > C p.(Gly294Arg)	Missense	7:44186201	NA
	NM_000162.5:c.943C > T p.(Leu315Phe)	Missense	7:44186138	rs1583594350
	NM_000162.5:c.1222G > T p.(Val408Leu)	Missense	7:44185127	NA
	NM_000162.5:c.1248C > G p.(His416Gln)	Missense	7:44185101	NA
	NM_033507.3:c.1256 + 1G > T	Splicing	7:44185095	NA
ABCC8 **(NM_000352.6)**	c.2768T > G p.(Leu923Arg)	Missense	11:17429991	NA
	c.3517G > A p.(Val1173Met)	Missense	11:17426099	rs141322087
	c.4014G > A p.(Trp1338Ter)	Nonsense	11:17418568	NA
HNF1A **(NM_000545.8)**	c.864delGinsCC p.(Gly292ArgfsTer25)	Frameshift	12:121432117	rs1593058932
	c.1769-3C > T	Splicing	12:121438865	NA
KCNJ11 **(NM_000525.3)**	c.481G > A p.(Ala161Thr)	Missense	11:17409158	rs1363707190
	c.668C > T p.(Thr223Ile)	Missense	11:17408971	rs561086953
	c.1117G > A p.(Val373Met)	Missense	11:17408522	rs770375846

ClinVar variation ID	HGMD	SIFT	DANN	GERP	Mutation taster	Classification (ACMG-2015)
NA	CD191970	NA	NA	5.07	NA	Likely pathogenic (PM1, PM2, PM4, PP3)
76898	CM013265	Damaging	0.9989	5.07	Disease causing	Pathogenic (PS1, PM1, PM2, PM5, PP2, PP3, PP5)
36209	CM023383	Damaging	0.9983	4.67	Disease causing	Pathogenic (PS1, PM1, PM2, PP2, PP3, PP5)
804846	CM012111	NA	0.9939	4.94	NA	Pathogenic (PVS1, PM2, PP3, PP5)
Novel	Novel	Damaging	0.9906	5.05	Disease causing	Likely pathogenic (PM1, PM2, PM5, PP2, PP3)
NA	CM141531	Damaging	0.9991	5.69	Disease causing	Likely pathogenic (PM1, PM2, PP2, PP3, PP5)
Novel	Novel	NA	NA	5.79	NA	Pathogenic (PVS1, PM2, PP3)
NA	NA	Tolerated	0.9986	5.82	Disease causing	Likely pathogenic (PM1, PM2, PP2, PP3)
NA	CM012122	Damaging	0.9993	5.96	Disease causing	Pathogenic (PM1, PM2, PM5, PP2, PP3, PP5)
435306	CM012123	Damaging	0.9992	6.17	Disease causing	Pathogenic (PS1, PM1, PM2, PP2, PP3, PP5)
Novel	Novel	Tolerated	0.9986	5.23	Disease causing	Likely pathogenic (PM1, PM2, PP2, PP3)
36261	NA	NA	0.9673	5.5	NA	VUS (PM2, BP4)
804861	NA	NA	0.9943	4.59	Disease causing	Pathogenic (PVS1, PM2, PP3, PP5)
Novel	Novel	NA	0.9991	4.76	Disease causing	Likely pathogenic (PM1, PM2, PP2, PP3)
804863	CM064013	Damaging	0.9985	4.59	Disease causing	Likely pathogenic (PM1, PM2, PM5, PP2, PP3)
NA	CM171422	Damaging	0.9955	5.57	Disease causing	Likely pathogenic (PM1, PM2, PP2, PP3)
Novel	Novel	Damaging	0.994	5.57	Disease causing	Likely pathogenic (PM1, PM2, PM5, PP2, PP3)
NA	CS032698	NA	0.9949	5.57	Disease causing	Pathogenic (PVS1, PM2, PP3)
Novel	Novel	Damaging	0.9981	6.03	Disease causing	Likely pathogenic (PM1, PM2, PP2, PP3)
35609	NA	Tolerated	0.9944	5.32	Disease causing	Likely pathogenic (PM1, PM2, PP2, PP5)
NA	CM994652	NA	0.9945	4.76	Disease causing	Pathogenic (PVS1, PM2, PP3)
817605	CX1310026	NA	NA	4.15	NA	Pathogenic (PVS1, PM2, PP5)
Novel	Novel	NA	0.9708	6.05	NA	VUS (PM2)
NA	NA	Damaging	0.9993	4.92	Disease causing	Likely pathogenic (PM1, PM2, PP2, PP3)
NA	NA	Damaging	0.9988	5.28	Disease causing	Likely pathogenic (PS2, PM2, PP2, PP3)
RCV001280332.1	NA	Tolerated	0.9367	5.42	Disease causing	VUS (PM2, PP2, BP4)

Table 2. Continued

Gene (transcript ID)	Variant	Variant type	Chr position (hg19)	dbSNP
HNF4A **(NM_000457.4)**	c.844G > A p.(Asp282Asn)	Missense	20:43048468	rs1236613475
CEL **(NM_001807.6)**	c.2049_2082del p.(Thr684ArgfsTer9)	Frameshift	9:135946938	NA
	c.2184_2216del p.(Gly729_Thr739del)	In frame	9:135947057	rs756606428
PDX1 **(NM_000209.4)**	c.97C > A p.(Pro33Thr)	Missense	13:28494372	rs192902098
	c.226G > A p.(Asp76Asn)	Missense	13:28494501	rs137852783
RFX6 **(NM_173560.4)**	c.246C > G p.(Asn82Lys)	Missense	6:117198981	NA
	c.428G > A p.(Cys143Tyr)	Missense	6:117201754	NA
	c.1072G > A p.(Val358Ile)	Missense	6:117240349	NA
G6PC2 **(NM_021176.3)**	c.89C > T p.(Ser30Phe)	Missense	2:169757930	rs142189264
GLIS3 **(NM_001042413.2)**	c.589G > T p.(Asp197Tyr)	Missense	9:4125741	NA
KLF11 **(NM_003597.5)**	c.1447C > T p.(Pro483Ser)	Missense	2:10192542	rs761563032

VUS: variants of uncertain significance, NA: not applicable, dbSNP: The Single Nucleotide Polymorphism Database

Gain of function mutations in the *ABCC8* and *KCNJ11* gene are known to cause temporary or permanent neonatal diabetes. In addition, dysfunctional mutations lead to congenital hyperinsulinism. However, some children have been shown to develop diabetes years after remission of neonatal hyperinsulinism. A mutation in the *ABCC8* or *KCNJ11* gene, which results in a serious clinical situation in the neonatal period, may exhibit a more moderate clinical picture, such as type 2 diabetes, gestational diabetes, or impaired glucose tolerance in other family members carrying the same mutation (23). Moreover, patients with mild hyperglycemia due to *ABCC8* pathogenic variants and no family history or neonatal diabetes history were also identified. The mechanism underlying these two gene-dependent variable clinical situations is still unknown. Case 13 presented with abdominal pain, nausea, and vomiting. Hyperglycemia was detected at 214 mg/dL. She had also a high postprandial blood sugar and a diabetes mellitus family history. Case 26 had clinical findings indicative of diabetes mellitus, but there was a very strong family history with his mother, grandmother, mother's uncles and aunts all having a diagnosis of diabetes mellitus.

A diagnosis of MODY has many clinical benefits for both the family and the patient. Diagnosing GCK-MODY will eliminate unnecessary treatment (insulin or oral antidiabetic) due to accidental classification of type 1 diabetes, or mostly type 2 diabetes in adults, and will affect the patient's quality of life. In addition, the absence of complications in GCK-MODY will prevent unnecessary visits by providing more comfortable follow-up for the patient's diabetes.

Individuals with *HNF1A* or HNF4A-MODY can stop unnecessary insulin treatment once the diagnosis is confirmed and achieve better metabolic control with oral sulfanylurea. In addition, genetic diagnosis with MODY enables early diagnosis of affected individuals in the family and all patients may be closely followed up in terms of complications. Again, individuals with HNF1A-MODY have a 5-10% risk of hepatic adenomatosis (24). Therefore, genetic diagnosis enables the patient to be followed in this respect.

If the pathogenic variants of individuals with neonatal hyperinsulinemic hypoglycemia is found in *HNF4A* or *ABCC8/KCNJ11* genes, it also allows genetic counseling to be given in terms of MODY screening of the family and the risk

ClinVar variation ID	HGMD	SIFT	DANN	GERP	Mutation taster	Classification (ACMG-2015)
NA	NA	Damaging	0.9993	4.98	Disease causing	Likely pathogenic (PM1, PM2, PP2, PP3)
Novel	Novel	NA	NA	1.34	NA	VUS (PVS1)
NA	NA	NA	NA	2.67	NA	VUS (PM2)
36414	CM056344	Damaging	0.9977	5.46	Disease causing	VUS (PM2, PP2, PP3)
8859	CM992901	Damaging	0.9982	4.96	Disease causing	VUS (PM2, PP2)
Novel	Novel	Tolerated	0.9171	5.36	Polymorphism	VUS (PM2)
Novel	Novel	Damaging	0.9981	5.61	Disease causing	VUS (PM1, PM2, PP3, BP1)
Novel	Novel	Tolerated	0.9951	6.07	Disease causing	VUS (PM2, BP1)
NA	NA	Damaging	0.9982	5.42	Disease causing	VUS (PP3, BS1)
Novel	Novel	Damaging	0.9885	5.26	Disease causing	VUS (PM2, PP3, BP1)
NA	NA	Tolerated	0.9953	5.78	Disease causing	VUS (PM2)

for future pregnancies (25). In our study there were three pathogenic/likely pathogenic variants in *ABCC8* gene and two pathogenic/likely pathogenic variants in *KCNJ11* gene. Case 4 had both *GCK* and *KCNJ11* and case 5 had both *GCK* and *ABCC8* pathogenic/likely pathogenic variants. Notably, cases 4 and 5 had both received a diagnosis of type 1 diabetes mellitus previously. The mother of case 4 and the mother, sister and grandmother of case 5 also had a diagnosis of diabetes mellitus. Both case 4 and case 5 had shown mild clinical signs while being followed-up for diabetes.

A diagnosis of HNF1B-MODY in an individual who was being followed-up with the diagnosis of diabetes suggests that care should be taken when assessing for extrapancreatic findings that accompany and/or may occur and requires multidisciplinary planning of their follow-up (26). There were no *HNF1B* pathogenic variants detected in our cohort.

Six of our cases were found to harbor novel pathogenic variants in *GCK* and *HNF1A* genes. If only known variants are analyzed in suspicious cases, many novel variants are at risk of being missed. In our study, analyzing all the exons of the genes increased the rate of diagnosis.

There are 14 known genes for MODY (*HNF4A, GCK, HNF1A, PDX1, HNF1B, NEUROD1, KLF11, CEL, PAX4, INS, BLK, ABCC8, KCNJ11, APPL1*) (27). We analysed these genes, with the exception of *APPL1*, and included extra seven genes (*NKX2-2, RFX6, ZFP57, GLIS3, FOXP3, NEUROG3, G6PC2*) to exclude similar phenotypes.

After determining the molecular diagnosis in the index case, first and second degree relatives should be informed that they are at risk for monogenic diabetes, this type of diabetes can affect their general health, and that their treatment is different depending on the subtype. Molecular analysis can also be planned in asymptomatic cases with a family history, depending on consent. Genetic counselling was given to all cases and their families in this study.

Study Limitations

A number of limitations of the present study should be noted. Firstly, the number of cases should be higher to provide more robust results. This was a cross-sectional study, which was performed in a region of our country with a low density population. Secondly, MODY type 14 could

not be investigated due to the *APPL1* gene was not included in the targeted gene panel in the current study. However, variants in the *APPL1* gene cause less than 1 % of all MODY types.

Conclusion

As new genes are identified through developing more widespread and more comprehensive molecular testing, the category of monogenic diabetes is expected to expand gradually. Suspicion of monogenic diabetes in patients diagnosed with type 1 or type 2 diabetes mellitus but showing atypical course, and confirming the diagnosis with appropriate molecular tests plays a key role in providing appropriate treatment for their condition. Molecular diagnosis is of great importance in terms of identifying the most appropriate treatment, and this will affect the prognosis of the patient, allow provision of genetic counseling and prompt screening of individuals at risk.

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Ethics

Ethics Committee Approval: The study were approved by the Trakya University of Local Ethics Committee (approval number: 2020/263, date: 10.08.2020).

Informed Consent: The written informed consent forms were obtained from the parents of the patients.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Sinem Yalçıntepe, Fatma Özgüç Çömlek, Hakan Gürkan, Filiz Tütüncüler Kökenli, Concept: Sinem Yalçıntepe, Fatma Özgüç Çömlek, Hakan Gürkan, Design: Sinem Yalçıntepe, Hakan Gürkan, Filiz Tütüncüler Kökenli, Data Collection or Processing: Sinem Yalçıntepe, Fatma Özgüç Çömlek, Selma Demir, Emine İkbal Atlı, Engin Atlı, Damla Eker, Filiz Tütüncüler Kökenli, Analysis or Interpretation: Sinem Yalçıntepe, Hakan Gürkan, Selma Demir, Emine İkbal Atlı, Engin Atlı, Damla Eker, Literature Search: Sinem Yalçıntepe, Writing: Sinem Yalçıntepe, Fatma Özgüç Çömlek.

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