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Evaluation of variants in maturity onset of diabetes young related genes in Balıkesir region

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ABSTRACT

Aims: Maturity-onset diabetes of the young (MODY) is an early-onset, monogenic diabetes with an autosomal dominant inheritance pattern. Single gene mutations that cause dysfunction in pancreatic beta cells are responsible for MODY etiology. In this study, we investigated the genetic variants involved in the etiopathogenesis of MODY in our region.

Methods: Between May 2018 and April 2023, 40 pediatric patients (n=25 females, n=15 males) with a clinical diagnosis of MODY were evaluated by targeted genome sequencing.

Results: Among the 40 pediatric patients included in this study, variants in MODY-associated genes were detected in 21 patients (52.5%), eight (38.09%), of which were pathogenic (38.09%), five (23.8%) were probable pathogenic, and eight (38.09%), were of uncertain significance.

Conclusion: In this study, genetic diagnostic yield (including pathogenic and likely pathogenic variants) was detected in 32.5% (13/40) patients with MODY using targeted genome sequencing analysis. This rate is consistent with other studies. However, unlike other similar studies, the MODY12 subtype was the second most frequent in our study. In addition, nine novel variants were reported, including *ABCC8* (n=3), *CEL* (n=2), *KLF11* (n=1), *GCK* (n=1), *HNF1A* (n=1), and *HNF1B* (n=1) genes. We have presented clinical findings to improve genotype-phenotype correlation in the literature for novel variants.

Keywords: MODY, Targeted genome sequencing, novel variants, *ABCC8*, *KLF11*

INTRODUCTION

Diabetes is a chronic metabolic disease that occurs mainly on the basis of genetic and environmental factors and results in hyperglycemia.[1-4] Maturity-onset diabetes of the young (MODY) is a form of diabetes characterized by high blood glucose as a result of a defect in the insulin secretion mechanism diagnosed before the age of approximately 25 years.[5] MODY, which accounts for approximately 2% of all diabetes cases, is caused by variants in a single gene.[6] This type of diabetes, which is very rare compared with Type 1 and Type 2 diabetes, has a history of diabetes in two or more generations.[5,6] In this form of diabetes, in which pancreatic beta cells are dysfunctional, molecular genetic tests are very important for appropriate treatment and genetic counseling. Because of its rarity, 90% of MODY patients are mistakenly diagnosed with Type 1 or Type 2 diabetes.[7] MODY should be considered in the differential diagnosis of patients with atypical Type 1 and Type 2 DM with negative autoantibodies.[5]

Advances in high-resolution genome sequencing technologies allow sequencing not only of a specific region of the genome, but also of the entire genome, quickly and with high accuracy. Next generation sequencing technologies (such as targeted panels, medical exome sequencing, whole exome/genome sequencing) have increased the carrier detection rate in diseases with autosomal recessive inheritance patterns. Making the correct molecular diagnosis of patients with diabetes using genetic tests enables the formation of an appropriate treatment option.[8]

The aim of this study was to determine the genetic variants associated with MODY in the Balıkesir region, evaluate the frequency of mutations, and offer appropriate treatment options to pediatric patients.

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Table 1: All variants detected by next generation sequencing

Family	Case	Age	Gender	Gene	Exon	Inheritance	Zygosity	Nucleotide variation	Amino acid variation	Mutation type	ACMG classification	Associated phenotype	Treatment
1	1	5y	F	*GCK* (NM_033507.3)	3	AD	Heterozygous	c.316del (na)	p.His106ThrfsTer11	missense	Likely pathogenic	MODY2	Insulin
2	2	7 y	F	*GCK* (NM_033507.3)	10	AD	Heterozygous	c.1361C>T (na)	p.Ser454Leu	missense	Pathogenic	MODY2	Dietary therapy
3	3	7 y	F	*GCK* (NM_033507.3)	10	AD	Heterozygous	c.1361C>T (pat)	p.Ser454Leu	missense	Pathogenic	MODY2	Dietary therapy
	4	13 y	M										Dietary therapy
4	5	14 y	M	*GCK* (NM_033507.3)	10	AD	Heterozygous	c.1361C>T (mat)	p.Ser454Leu	missense	Pathogenic	MODY2	Dietary therapy
5	6	18 y	F	*GCK* (NM_033507.3)	10	AD	Heterozygous	c.1361C>T (mat)	p.Ser454Leu	missense	Pathogenic	MODY2	Dietary therapy
6	7	18 y	F	*GCK* (NM_033507.3)	10	AD	Heterozygous	c.1361C>T (mat)	p.Ser454Leu	missense	Pathogenic	MODY2	Insulin
7	8	17 y	M	*HNF1A* (NM_000545.8)	3	AD	Heterozygous	c.687_707del (de novo)	p.Glu230_Cys236del	in frame	Likely pathogenic	MODY3	Insulin
8	9	17 y	F	*HNF1B* (NM_000458.4)	4	AD	Heterozygous	c.947A>G (na)	p.Asp316Gly	missense	Likely pathogenic	MODY5	Oral antidiabetic
9	10	14 y	M	*KCNJ11* (NM_000525.4)	1	AR	Homozygous	c.405dup (na)	p.Arg136AlafsTer5	frameshift	Pathogenic	MODY13	Insulin
	11	9 y	M										Dietary therapy
10	12	9 y	F	*ABCC8* (NM_000352.6)	7	AD	Heterozygous	c.1168G>T (na)	p.Ala390Ser	missense	Uncertain significance	MODY12	Dietary therapy
11	13	17 y	F		10	AD	Heterozygous	c.1616A>G (de novo)	p.Tyr539Cys	missense	Uncertain significance		Insulin
12	14	16 y	M		22	AD	Heterozygous	c.2578G>A (na)	p.Asp860Asn	missense	Uncertain significance		Insulin
13	15	16 y	F		39	AD	Heterozygous	c.4673A>T (na)	p.Glu1558Val	missense	Likely pathogenic		Oral antidiabetic
14	16	18 y	F	*CEL* (NM_001807.6)	4	AD	Heterozygous	c.355C>T (pat)	p.Pro119Ser	missense	Uncertain significance	MODY8	Oral antidiabetic
15	17	17 y	M	*CEL* (NM_001807.6)	11	AD	Heterozygous	c.1983del (na)	p.Thr662ArgfsTer42	frameshift	Likely pathogenic	MODY8	Insulin
16	18	14 y	F	*KLF11* (NM_003597.5)	3	AD	Heterozygous	c.1095G>C (na)	p.Lys365Asn	missense	Uncertain significance	MODY7	Insulin
17	19	13 y	F	*PDX1* (NM_000209.4)	1	AD	Heterozygous	c.107T>G (mat)	p.Leu36Arg	missense	Uncertain significance	MODY4	Oral antidiabetic
18	20	13 y	F	*PAX4* (NM_001366111.1)	5	AD	Heterozygous	c.521G>A (na)	p.Arg174Gln	missense	Uncertain significance	MODY9	Insulin
19	21	4 y	F	*INS* (NM_001185097.2)	3	AD	Heterozygous	c.206G>A (na)	p.Gly69Asp	missense	Uncertain significance	MODY10	Dietary therapy

y: years; F: female; M: male; AR: Autosomal recessive; AD: Autosomal dominant; mat: maternally inherited; pat: paternally inherited; na: not available, inheritance not known; bolded novel variant

METHODS

The study was carried out with the permission of Balıkesir Atatürk City Hospital Ethics Committee (Date: 01/06/2023, Decision No: 2023/3/32). The study was evaluated as a research file, and it was decided that it was scientifically and ethically appropriate. All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

40 patients from 38 unrelated families who applied for the clinical prediagnosis of MODY between May 2018 and April 2023 were included in this study. The criteria for inclusion in this study were as follows: i) patients of both genders and age of ≤18 years diagnosed with diabetes, ii) diabetes specific autoantibody negative diabetes, and iii) a family history of diabetes in three generations. All patients who underwent targeted genome sequencing (TG-Seq) were retrospectively evaluated. Familial segregation analysis was performed using Sanger sequencing

DNA Isolation

DNA isolation was performed from lymphocytes in 200 µL peripheral venous blood using a QIAamp DNA Blood Mini Kit (Qiagen Inc., Valencia, CA, USA).

Targeted Genome Sequencing

Targeted genome sequencing containing 12 genes (*HNF1A*, *HNF1B*, *HNF4A*, *GCK*, *ABCC8*, *PDX1*, *NEUROD1*, *INS*, *KCNJ11*, *BLK*, *KLF11*, *PAX4*) associated with MODY was designed. The genomic DNAs obtained were sequenced with Roche HyperCap DS CES kit and then sequenced with MGI, DNBSeq G400.

Sanger Sequence

Before sequencing, the PCR products were purified using a NucleoFast 96 PCR kit (Macherey-Nagel GmbH, Germany). After completion of the thermal cycle step, the sequence reactions were purified according to the protocol of the ZR-96 DNA Sequencing Clean-up Kit (Zymo Research Corp.) Capillary electrophoresis of the purified sequence products was performed using ABI 3130 (Applied Biosystems Inc.).

Data Analysis

GenomizeSeq (Version 6.13.1) software was used for analysis with an average read depth of 20X and coverage of 94.17%. Exon-intron junction boundaries (±10 base pair) were included in the analysis. Human Genome Mutation Database (HGMD, http://www.hgmd.cf.ac.uk/ac/index.php) and Franklin (https://franklin.genoox.com/clinical-db/home), VarSome (https://varsome.com/), ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/), Online Mendelian Inheritance in Man (OMIM, https://www.omim.org/) novel variants in the databases were checked. The pathogenicity score of new variants was interpreted using the in silico variant prediction program Mutation Taster, CADD (Combined Annotation Dependent Depletion). The data obtained were analyzed according to the American College of Medical Genetics and Genomics (ACMG) criteria.9

RESULTS

A total of 40 cases (n=25 female, n=15 male) from 38 different families were included in this study. Demographic data, variant information, and treatments are summarized in **Table 1**. The current mean age of these patients was 12.5 years (3 years-18 years). The mean age of female patients was 12.2 years, and that of male patients was 13 years. Of the 40 patients, 21 (52.5%) had molecular genetic results consistent with their clinical findings. There were 7 different pathogenic/likely pathogenic variants in thirteen (13/21=63.7%) patients. There were 8 different uncertain significance variants in eight (8/21=38.9%) patients (**Table 1**).

Pathogenic variants were found in the *GCK* (n=6 patients) and *KCNJ11* (n=2 patients) genes. Six patients had the same *GCK* gene: c.1361C>T (p.Ser454Leu) pathogenic variant. Two patients, siblings, had the c.405dup pathogenic variant in the *KCNJ11* gene. The likely pathogenic variants were *GCK* gene: c.316del (p.His106ThrfsTer11), *HNF1A* gene: c.687_707del(p.Glu230_Cys236del), *HNF1B* gene: c.947A>G (p.Asp316Gly), *ABCC8*: c.4673A>T (p.Glu1558Val), and *CEL* gene: c.1983del (p.Thr662ArgfsTer42). All these likely pathogenic variants were novel and have not been previously reported in any public database. In addition, there were a total of eight variants of uncertain significance in *ABCC8* (n=3), *CEL* (n=1), *KLF11* (n=1), *PDX1* (n=1), *PAX4* (n=1) and *INS* (n=1) genes. Among these variants of uncertain significance, the *ABCC8* gene: c.1168G>T (p.Ala390Ser), *ABCC8* gene: c.2578G>A (p.Asp860Asn), *CEL* gene: c.355C>T (p.Pro119Ser), and *KLF11* gene: c.1095G>C (p.Lys365Asn) were novel.

The variants detected in this study were associated with the phenotypes of maturity-onset diabetes of the young, type 2 (MODY2, OMIM # 125851), maturity-onset diabetes of the young, type 3 (MODY3, OMIM # 600496), maturity-onset diabetes of the young, type 5 (MODY5), maturity-onset diabetes of the young, type 13 (MODY13, OMIM # 616329), maturity-onset diabetes of the young, type 12 (MODY12), maturity-onset diabetes of the young, type 8, with exocrine dysfunction (MODY8, OMIM # 609812), maturity-onset diabetes of the young, type 7 (MODY7, OMIM # 610508), maturity-onset diabetes of the young, type 4 (MODY4, OMIM # 606392)), maturity-onset diabetes of the young, type 9 (MODY9, # 612225), and maturity-onset diabetes of the young, type 10 (MODY10, # 613370) phenotypes.

DISCUSSION

In our study presenting the genetic variants of MODY pediatric patients in our region, the yield of molecular genetic diagnosis was 52.5% (n=21 patients; 21/40). In twenty-one patients with positive genetic results by TG-Seq, there were 15 different variants (n=2 pathogenic, n=5 likely pathogenic, n=8 of uncertain significance). Of the 15 variants, 9 (9/15=60%) have not been reported in the relevant scientific literature, such as gnomAD (https://gnomad.broadinstitute.org), Leiden Open Variation Database (LOVD, https://www.lovd.nl/), and ClinVar.

Mutations in the *HNF4A* (MODY, type I), *GCK* (MODY, type II), and *HNF1A* (MODY, type III) genes are responsible for approximately 90% of MODY cases.[10-12] In our study, where we did not detect any pathogenic/likely pathogenic variant in the *HNF4A* gene, this rate was approximately 40%. In our study, the most common *GCK* (MODY, type II) gene variants were observed in seven patients (n=7), including two patients (case 4, case 5) from the same family. Among the *GCK* gene mutations, the most common variant was c.1361C>T (p.Ser454Leu). When evaluated together with our previous study, this mutation was the most common *GCK* variant in all age groups in our region.[13] *ABCC8* gene mutations were the second most common in this study. According to other scientific studies, *ABCC8* gene variants have been implicated in approximately 1% of all MODY.[14,15] Here MODY12 was seen in 19.4% of all MODY. This finding was the most remarkable feature that distinguished our study from other similar literature.[5,14-17]

Homozygous *KCNJ11* gene: NM_000525.4: c.405dup (p.Arg136AlafsTer5) variant was detected in family 9. Two siblings (Case 10, Case11) from family 9 with the same variant exhibited different clinical progressions. Case 10 suffered from hyperinsulinemic hypoglycemia in the neonatal period and required pancreatectomy. Therefore, the patient uses insulin in the treatment. However, Case 11 was controlled with the help of diet therapy, and he had no insulin requirement.

Although mutations in the *BLK*, *PAX4*, and *KLF11* genes have not yet been clearly associated with MODY, these genes are still being screened in studies.[17] In our study, there were patients with variants of uncertain significance in the *KLF11* (MODY7) and *PAX4* (MODY9) genes. All these patients had negative diabetes specific autoantibodies and a low insulin dose requirement. Although we could not make a definitive diagnosis, these findings were consistent with MODY.

Our diagnostic yield of genetic diagnosis was found to be 32.5% (13/40) according to pathogenic/likely pathogenic variants. In previous similar studies, a possible pathogenic/pathogenic variant was found in 6-48% of patients.[17-20] In this study, we obtained results consistent with these studies.

Our study has some limitations. It contains only one province ‘s data. No further genetic testing, such as a whole genome sequencing or medical exome sequencing, was performed in patients with negative test results.

CONCLUSION

MODY is responsible for 2-5% of all diabetes, but a better understanding of the genetic spectrum of MODY is needed. Patients with a family history of diabetes and atypical diabetes manifestations should be screened for mutations in MODY-related genes. These results also suggest that the MODY genotype may have regional differences in our country. In addition, this study reports 9 (9/15=60%) different novel variants that were not previously reported in similar scientific literature.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Balıkesir Atatürk City Hospital Ethics Committee (Date: 01/06/2023, Decision No: 2023/3/32).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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