



Genetic diagnosis of maturity-onset diabetes of the young (MODY) in northeast Turkey

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Abstract

ARTICLE INFO

Keywords:

MODY

Next generation sequencing

Mutation

Received: May 17, 2021

Accepted: Sep 24, 2021

Available Online: Apr 29, 2022

DOI:

[10.5455/annalsmedres.2021.05.409](https://doi.org/10.5455/annalsmedres.2021.05.409)

Aim: Maturity-onset diabetes of the young (MODY) is a group of monogenic diabetes mellitus with autosomal dominant inheritance, usually present in adolescence or young adulthood, resulting from beta cell dysfunction. To date, 14 genes have been identified for the majority of all MODY cases. The correct MODY diagnosis is important for appropriate treatment and improvement of the patients' life quality. Despite various strategies in clinical assessment, molecular genetic studies are required for the definitive diagnosis of MODY. The aim of this study was to define MODY gene mutations in the northeastern part of Turkey and contribute to the mutational spectrum of MODY. Also, the importance of molecular diagnosis of MODY and testing strategies were discussed.

Material and Methods: In this study sequencing analyses of MODY-related genes were performed using next generation sequencing (NGS) platform in 46 unrelated Turkish patients.

Results: Disease causing variant was found in 30.4% (n=14) of the patients. Ten different variants were detected in 14 patients. Nine variants in *GCK*, one in *HNF1A* genes were found and six mutations were novel among them.

Conclusion: Mutations in the *GCK* gene are the leading cause of MODY in Turkish population. Besides, six novel mutations were identified which enriches mutation spectrum of *GCK* gene and contributes to genotype-phenotype correlation of MODY. Also, we suggest that NGS is a practical and useful tool in the first step of genetic analysis.



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Introduction

Maturity onset diabetes of the young (MODY) is a clinically heterogeneous disease, defined as the most common type of monogenic diabetes with autosomal dominant inheritance. Its prevalence is estimated to be 1-5% of all diabetes cases. To date, 14 genes (*ABCC8*, *APPL1*, *BLK*, *CEL*, *GCK*, *HNF1A*, *HNF1B*, *HNF4A*, *INS*, *KCNJ11*, *KLF11*, *NEUROD1*, *PAX4*, *PDX1*) associated with MODY have been reported. All these genes are related to beta cells and mutations lead to subtypes of MODY. The most common subtypes are HNF1A-MODY, GCK-MODY and HNF4A-MODY. Clinical findings are variable based on the responsible gene [1].

MODY is frequently misdiagnosed as type 1 or type 2 diabetes. Treatment and management of MODY is subtype

specific and identification of the genetic cause allows personalized treatment. In addition, screening of family members is important for detecting asymptomatic and/or misdiagnosed relatives. The use of targeted next generation sequencing (NGS) method for diagnosis of MODY is becoming widespread. NGS provides rapid and cost effective diagnosis compared to Sanger sequencing [2].

We aimed to define MODY gene mutations in the northeastern part of Turkey and contribute to the mutational spectrum of MODY. Besides, testing algorithms were discussed for improving cost effectiveness.

Material and Methods

Forty-six patients with the prediagnosis of MODY who were consulted to Department of Medical Genetics from 2017 to 2019 were taken to this study. This work was approved by the Institutional Ethics Committee (Number: 2019/24 by University of Health Sciences Trabzon Kanuni

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Training and Research Hospital). Informed written consents were obtained from patients' parents or guardians.

Peripheral blood samples were collected from the patients and their parents, if possible. DNAs were extracted by QIAcube® automated DNA isolation system (Qiagen) according to the manufacturer's instructions.

The *ABCC8*, *APPL1*, *BLK*, *CEL*, *GCK*, *HNF1A*, *HNF1B*, *HNF4A*, *INS*, *KCNJ11*, *KLF11*, *NEUROD1*, *PAX4*, *PDX1* genes custom primers were used targeting their coding regions, and the intron-exon boundaries. The libraries were prepared with the NexteraXT kit (Illumina Inc.), according to the manufacturer's instructions. The sequencing reactions were performed with MiSeq® NGS system (Illumina Inc., USA). Reads were analyzed using the Integrative Genomics Viewer program and were aligned according to the hg19/GRCh37 human reference genome. The minimum depth of coverage was 100x. Common variants were excluded by minor allele frequency (MAF > 1%) score by using 1000 Genomes Project (<http://www.1000genomes.org/>) and dbSNP database.

Variants were checked by using HGMD [3], ClinVar [4], Varsome databases [5]. The variants that were not detected in those databases were defined as novel.

In silico bioinformatics tools, PolyPhen2 [6], SIFT [7], MutationTaster software [8] were used for the interpretation of novel variants.

Final variants were classified according to American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG) 2015 Guidelines [9].

Results

A total of 46 unrelated patients were included in the study (25 females – 21 males, age: 3-28 years). A disease causing variant was detected in 14 out of 46 patients (30.4%). Ten different variants were detected in 14 patients (Table 1). Each patient was heterozygous for aforementioned variants. Seven variants were pathogenic whereas three were likely pathogenic according to the ACMG criteria. Nine variants in *GCK* and one in *HNF1A* genes were found. Two mutations were observed in more than one patient (NM_000.162.5:*GCK*; c.584delG; p.Asn179ThrfsTer25 and NM_000.162.5:*GCK*; c.686delG; p.Gly229AlafsTer6). Six different novel mutations were detected in seven patients. Family study could be performed in eight of the patients and seven mutations were found to be inherited (5 maternal vs. 2 paternal).

In this study six novel *GCK* variants were identified. The first novel variant, NM_000.162.5: *GCK*; c.534delG; p.Asn179ThrfsTer25, was detected in two patients. It was a frameshift variant which disturbed the reading frame and lead to premature termination codon and resulted in a truncated protein (evidence of pathogenicity: PVS1, PM2, PP3). Although one patient inherited this variant from diabetic mother, the variant of other patient was *de novo*. The second novel variant, NM_000.162.5: *GCK*; c. 612T > A; p.Asn204Lys, was a missense variant that probably affect the splicing site. It was classified as likely pathogenic (evidence of pathogenicity: PM1, PM2, PP2, PP3). Family study could not be performed. But there were no similar cases in the family, hence it was assumed as *de novo*.

The third and fourth novel variants were also frameshift (NM_000.162.5: *GCK*; c.784delG; p.Asp262ThrfsTer32 and NM_000.162.5: *GCK*; c.1256delT; p.Phe419SerfsTer12) and classified as pathogenic (evidence of pathogenicity: PVS1, PM2, PP3). Although both cases had family history, family study could be performed in one. c.784delG was shown to inherited from affected father. Last two novel variants were missense (NM_000.162.5: *GCK*; c.1181G>A; p.Arg384His and NM_000.162.5: *GCK*; c.1222G>T; p.Val408Leu) and classified as likely pathogenic (evidence of pathogenicity: PM1, PM2, PP2, PP3). The change of a single base pair caused the substitution of a different amino acid, which probably altered the expression in resulting protein. Family study could be performed in one case and the c.1181G>A was shown to inherited from diabetic mother.

Discussion

Although MODY is a well-known disorder, it is frequently misdiagnosed as type 1 or type 2 diabetes. Due to the fact that the treatment of MODY can be different from type 1 and type 2 diabetes, getting the correct diagnosis is important. Besides, MODY has 14 subtypes with different phenotypic presentation and management [1].

Genetic testing is the most powerful option for genetic diagnosis. Since there are 14 responsible genes, cost of diagnosis is a challenging factor. Selection of the patient is important as well as the type of genetic method [2, 10]. MODY testing should be considered in diabetic young patients with multi generation history, non-ketotic insulin sensitive hyperglycemia, the absence of pancreatic islet autoantibodies and middle-aged patients with type 2 diabetes symptoms, autosomal dominant inheritance pattern, without obesity, insulin resistance and fatty liver [10].

Recently targeted NGS analysis is frequently used for genetic diagnosis. NGS is more cost-effective and rapid than Sanger sequencing. But still, some algorithms must be considered for saving time and using resources effectively. Most common genes (*GCK*, *HNF1A*, *HNF4A*, *HNF1B*) may be analyzed in the first step. Alternatively, personalized testing can be performed according to the clinical finding. For example, *HNF1B* should be tested in a patient with renal and urogenital problem, in addition to pancreatic dysfunction [10]. Besides, if no disease causing variant is detected by sequencing, deletion/duplication analysis could be considered especially for the genes such as *HNF1B*, in which large deletions are reported [1].

Several studies from Turkey related to molecular analysis of MODY in the literature. Mutation detection rates vary between 16%-65% and *GCK* was the leading cause of the MODY according to targeted gene panel studies [11-14]. Ağlıadioğlu et al. [11] performed NGS analysis to investigate the variants in 11 MODY genes in 43 Turkish patients and identified disease causing variants in 28 (65%). Similarly, Anık et al. [12] performed molecular analysis of 11 genes associated with MODY in 42 Turkish patients and genetic diagnosis was made in 12 (29%) patients. Yalçıntepe et al. [13] performed NGS analysis to investigate the variants in 20 MODY genes in 61 Turkish patients and identified disease causing variants in 29 (47.5%). Özdemir et al. [14] investigated mutation

Table 1. List of detected disease causing variants

Case No	Age/Sex	Age at diagnosis	Family history	Gene (Transcript)	Nucleotide change	Amino acid change	Reference	Inheritance	Pathogenicity (ACMG criteria)
1	17/F	14	-	HNF1A (NM_000545.8)	c.526C>T	p.Gln176X	ClinVar, HGMD	NA	Pathogenic
2	13/F	8	-	GCK (NM_000.162.5)	c.534delG	p.Asn179ThrfsTer25	Novel	de novo	Pathogenic
3	15/M	14	+	GCK (NM_000.162.5)	c.534delG	p.Asn179ThrfsTer25	Novel	maternal	Pathogenic
4	15/M	15	-	GCK (NM_000.162.5)	c.612T>A	p.Asn204Lys	Novel	NA	Likely pathogenic
5	18/M	14	+	GCK (NM_000.162.5)	c.686delG	p.Gly229AlafsTer65	Clinvar	maternal	Pathogenic
6	3/M	2	+	GCK (NM_000.162.5)	c.686delG	p.Gly229AlafsTer65	Clinvar	paternal	Pathogenic
7	24/F	14	+	GCK (NM_000.162.5)	c.686delG	p.Gly229AlafsTer65	Clinvar	maternal	Pathogenic
8	13/M	12	+	GCK (NM_000.162.5)	c.686delG	p.Gly229AlafsTer65	Clinvar	maternal	Pathogenic
9	7/F	4	+	GCK (NM_000.162.5)	c.784delG	p.Asp262ThrfsTer32	Novel	paternal	Pathogenic
10	15/F	12	+	GCK (NM_000.162.5)	c.1256delT	p.Phe419SerfsTer12	Novel	NA	Pathogenic
11	24/F	24	+	GCK (NM_000.162.5)	c.802G>T	p.Glu268Ter	HGMD	NA	Pathogenic
12	12/M	10	-	GCK (NM_000.162.5)	c.1009C>T	p.Gln337Ter	HGMD	NA	Pathogenic
13	13/F	11	+	GCK (NM_000.162.5)	c.1181G>A	p.Arg384His	Novel	maternal	Likely pathogenic
14	16/M	12	-	GCK (NM_000.162.5)	c.1222G>T	p.Val408Leu	Novel	NA	Likely pathogenic

analysis of seven MODY gene in 106 patients and genetic diagnosis was made in 17 out of 106 patients (16%). Furthermore, only *GCK* gene mutations were investigated two Turkish studies. Aykut et al. [15] evaluated *GCK* gene mutations in 177 Turkish MODY type 2 patients. Mutations in the *GCK* gene were identified in 79 out of 177 (44.6%). Similarly, Halilöglü et al [16] investigated *GCK* gene mutations in 54 Turkish MODY type 2 patients and disease causing variants were detected in 24% (13/54) of the them. In our study, disease causing variants could be detected in 30.4% of the patients by NGS. Diagnostic rate is thought to be increased by using more strict patient selection criteria. Additionally deletion-duplication analysis should be performed for undiagnosed MODY cases. *GCK* was the most mutation identified gene according to our study, which is consistent with current literature. Also, this information confirms the necessity of performing gene panels step by step.

We detected two recurrent mutations in unrelated patients in this study (NM_000.162.5:*GCK*; c.584delG; p.Asn179ThrfsTer25 and NM_000.162.5:*GCK*; c.686delG; p.Gly229AlafsTer6). This study was designed on the discussion of genetic diagnosis of MODY patients in the northeastern Turkey. The founder effect could be the reason due to the geographical extent. Besides, six novel variants were identified which were enriches mutation spectrum of *GCK* gene and contributes to genotype-phenotype correlation of MODY.

Conclusion

In conclusion, we suggest NGS as a useful tool for diagnosis. Stepwise approach should be considered for cost-effectiveness.

Ethical approval

The study was carried out with the approval of the University of Health Sciences Trabzon Kanuni Training and Research Hospital Ethics Committee (Number: 2019/24)

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