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Genetic and clinical characteristics of Turkish children with Maturity Onset Diabetes of the Young Type 2 (MODY2): A single center experience

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Abstract

Objective: The aim of the study was to investigate the clinical and molecular genetic characteristics of children with maturity-onset diabetes of the youth-glucokinase (MODY-GCK, MODY type 2).
Method: Medical files of 21 patients with suspected MODY-GCK were reviewed retrospectively. The file records of the clinical findings, laboratory results and the suspected clinical diagnoses of MODY were based on (1) asymptomatic fasting hyperglycemia (glucose ≥100mg/dl, HbA1c < 7.5% (at least twice measurement) 2) parents with a history of diabetes without complications or mild fasting hyperglycemia (100-144mg/dL).
Results: The mean age at diagnosis was 11.5±4.3 years (min-max, 1.9-17.2). The mean (SD) fasting blood glucose level was 119.1 (9.8) mg/dL. The mean (SD) fasting C-peptide level was 1.3 (0.7) ng/mL, the mean (SD) insulin level was 5.9 (2.3) IU/ml, and the mean (SD) HbA1c level at diagnosis was 6.2 (0.5) %. Among 12 variants detected in the GCK gene, 8 were missense mutation, 2 were non-sense mutation, 1 of them was splice site and 1 of them was frameshift mutation. Eight of them (p. Val227Met, p. Ser282Ala, p.Val183Met, p.Met239Thr, p.Arg304Gln, p.Thr229Met, p.Gly163Asp, p.Cys130Ter) have been previously reported in the literature and 4 variants (c.582+4delA, p.Glu436Ter, p.His106ThrfsTer11, p.Asp133Gly) were novel.
Conclusion: We found similar phenotype characteristic of children with GCK-MODY among the children with different variants. The most common mutation type was missense and followed by nonsense, splice site and frameshift mutations. Detection of the molecular defect in patients with MODY is vital for the implementation of appropriate treatment approaches.
Keywords: Children; Maturity Onset Diabetes of Youth; Youth-Glucokinase; Next Generation Sequencing

INTRODUCTION

Maturity-onset diabetes of the young (MODY) is a rare, insulin independent, autosomal dominantly inherited type of diabetes typically diagnosed before 25 years of age and that results from heterozygous mutations in various transcription factors acting in the development and maturation of pancreatic β-cells (1). MODY is reported to be the most common form of monogenic diabetes and it is estimated to account for about 1–5% of all cases of diabetes (2-4). MODY2 (also referred as Glucokinase (GCK)-MODY), which is caused by heterogenous inactivating mutations in the glucokinase (GCK) gene, is one of the most common types of MODY (5). GCK mutations (MODY2) have been reported to be the most common cause of MODY in Germany, Spain, France, Poland, Austria, the Czech Republic, Italy, and Greece (6).

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GCK catalyzes the rate limiting reaction of the glucose metabolism and therefore, it is the key enzyme in regulation of insulin release in pancreatic β-cells (7). The GCK gene is expressed in liver and beta cells (8). The GCK gene is located at chromosome 7p15.3–p15.1. Until now, missense, nonsense, frameshift, splice site, promoter mutations and deletions have been reported in GCK gene (7). While homozygous or compound heterozygous mutations in the GCK gene lead to permanent neonatal diabetes mellitus, heterozygote-inactivating mutations of the GCK gene cause mild, subclinical, non-progressive hyperglycemia, which is generally present at birth (8, 9). Typically, fasting glucose in MODY2 patients is between 100 and 144 mg/dL, and hemoglobin A1c (HbA1c) is between 5.6-7.3%. Patients with MODY2 are usually asymptomatic and most of them are diagnosed by random blood glucose measurements.

In this study, we aimed to present the clinical features of the patients with suspected MODY2 diagnosis and to identify genetic variations in GCK genes.

METHOD

Subjects

A total of 21 Turkish children and adolescents suspected MODY2 between 2019 and 2022 in Behçet Uz Child Disease and Pediatric Surgery Training and Research Hospital were enrolled. The file records of the clinical findings, laboratory results and the suspected clinical diagnoses of MODY were based on (1) asymptomatic fasting hyperglycemia (glucose ≥100mg/dl, HbA1c < 7.5%) (at least twice measurement), 2) parents with a history of diabetes without complications or mild fasting hyperglycemia (100-144mg/dL). The cases with insulin resistance, stress hyperglycemia, and those with diseases or using medication that may cause hyperglycemia were excluded from the study.

Informed consents were obtained from all patients' parents. This study was approved by the Ethics Committee of Behçet Uz Child Disease and Pediatric Surgery Training and Research Hospital, (approval number: 640, date: 12.09.2021) and conducted after obtaining written informed consent from the patients or their guardians. Helsinki Declaration rules were followed to conduct this study.

DNA extraction and Next-generation sequencing

Peripheral blood samples were collected from these patients and the affected family members if required. Genomic DNA was extracted from leukocytes using the MagPurix kit (Zinexts Life Science Corp., New Taipei City 235, Taiwan), according to manufacturer's instructions. For the molecular genetic evaluation, NEXTflex® MODY1 Amplicon Kit (Bioo Scientific Corp., Perkin Elmer, USA) was used for all coding regions and exon-intron boundaries (±10 bases) of GCK (ENST00000345378) gene. Initially, DNA was amplified. Subsequently, ligation and indexing procedures were performed according to manufacturer's instructions. Sequencing reactions was performed with MiniSeq® NGS system (Illumina Inc., San Diego, CA, USA). FASTQ sequencing files were collected and transferred to "SEQ" variant analysis software (Genomize, Istanbul, Turkey). The Integrative Genome Viewer (IGV) (http://software.broadinstitute.org/software/igv/) was used for visualizing the status of each read alignment.

Pathogenicity Assessment

In an appropriate reference population, the pathogenic variant should have a frequency of much less than 1%. Therefore, we excluded all the common variants (minor allele frequency, MAF > 1%) reported in the following public databases: 1000 Genomes Project (http://www.1000genomes.org/) and dbSNP database. Possible variants that were not presented in Human Gene Mutation Database (HGMD, http://www.hgmd.cf.ac.uk), ClinVar (http://www.ncbi.nlm.nih.gov/clinvar/), or genetic studies in the published literature were considered as novels and included in the further analysis. Novel variants were evaluated according to 2015 publication of standards and guidelines for the clinical interpretation of sequence variants by the American College of Medical Genetics and Genomics (ACMG). The pathogenicity of the identified sequence variants was reported using an automatic variant classifier that evaluated the submitted variant according to the American College of Medical Genetics (ACMG) guidelines, classifying it as one of 'pathogenic', 'likely pathogenic', 'likely benign', 'benign' or 'uncertain significance'. (10). We also used the search engine Varsome (https://varsome.com/), which has information from 30 external databases, to investigate the pathogenicity of the novel variant. For confirmation and to detect the status of the specific familial variant in relatives, Sanger sequencing was also performed.

Statistical Analysis

Analyses were performed using the Statistical Package for the Social Sciences 18.0 (SPSS). Whether the quantitative variables were suitable for normal distribution or not was tested with the single-sample Kolmogorov Smirnov test. Mann-Whitney U test was used to compare data that was not normally distributed, and the chi-square test was used to compare categorical data between groups. Descriptive statistics for the data were given as median (minimum-maximum) for non-normally distributed parameters and mean ± SDS for normally distributed parameters. A p value of <0.05 was considered significant.

RESULTS

A total of 21 children suspected with the diagnosis of MODY were included in study. Among 21 patients, 12 variants were identified in 12 patients (12/21 patients; 57.14%). Eight out of 12 children (66.6%) were boys and the remaining (4 children,

33.3%) were girls. Body mass index SDS mean (SD) was found to be 0.2 (0.8). The mean age at diagnosis was 11.5±4.3 years (min-max, 1.9-17.2). The mean (SD) fasting blood glucose level was 119.1 (9.8) mg/dL. The mean (SD) fasting C-peptide level was 1.3 (0.7) ng/mL, the mean (SD) insulin level was 5.9 (2.3) IU/ml, and the mean (SD) HbA1c level at diagnosis was

6.2 (0.5) %. Clinically, all of the patients with GCK mutation had random hyperglycemia, none of them have glycosuria, polydipsia and polyuria, ketonemia/ketonuria, ketoacidosis, or dyslipidemia at the time of diagnosis. Five of the 12 patients had consanguinity. Clinical data on each of the 12 patients with mutations in the *GCK* gene are shown in Table 1.

Among 12 variants detected in *GCK* gene, 8 were missense mutation, 2 were non-sense mutation, 1 of them was splice site and 1 of them was frameshift mutation. Eight of 12 variants were previously reported while 4 of the rest were novel according to in ClinVar. Seven of the 8 previously reported variants were missense mutation and 1 of them was nonsense mutation, while among the novel variants there were 1 frame shift, 1 nonsense, 1 splice site and 1 missense mutations. An overview of these mutations was shown in Table 2.

Table1. Clinical and genetic characteristics of the patients with GCK-MODY related gene variations

Patient number	Age/ Gender	Gestational week	DKA	Weight (kg)/ Weight SDS	Height (cm)/ Height SDS	BMI/BMI SDS	Fasting Glucose (mg/ dl)	Fasting İnsulin (IU/ml)	C-peptide (ng/Ml)	HbA1c	Ketonemia /ketonuria	Consanguinity
P1	15.97/M	37	No	61/-0.52	164/-1.41	22.68/0.28	118	10.1	2.1	6.41	-	Yes
P2	4.51/F	39	No	16/-0.58	104/-0.45	14.7/-0.46	124	2.2	0.31	6.23	-	No
P3	7.44/M	38	No	44.3/3.34	133.8/1.94	24.7/2.95	124	10.2	2.7	7.03	-	Yes
P4	16.01/F	36	No	63.5/0.9	158.6/-0.6	25.2/1.4	129	6.5	1.9	6.1	-	Yes
P5	10.8/M	39	No	43/0.7	152.1/1.4	18.8/0.28	136	5.1	1.8	6.6	-	Yes
P6	13.36/M	38	No	39/-1.42	157.6/-0.35	15.7/-1.74	139	4.3	0.9	6.6	-	Yes
P7	11.44/F	37	No	54/1.29	157/1.49	21.9/1.09	108	2.2	1.1	6.15	-	No
P8	9.11/M	34	No	42.3/1.41	142.2/0.86	20.9/1.20	108	4.4	1.6	6.5	-	No
P9	10.85/M	38	No	34/-0.42	132/-1.7	19.51/0.5	124	7.2	2.2	7.01	-	No
P10	14.62/F	39	No	59/0.63	153/-1.4	25.2/1.46	121	11.6	3.8	6.03	-	No
P11	11.01/M	36	No	58.8/1.95	152.6/1.35	25.25/1.78	124	11.7	2.56	6.57	-	No
P12	1.9/M	38	No	12.2/-0.2	85.3/-0.54	16.7/0.22	114	6.93	0.36	6.93	-	No

P: Patient, DKA: Diabetic ketoacidosis, SDS: Standard deviation score, NA: not available, M: male, F: female, kg: kilogram

Table 2. Glucokinase (GCK) gene mutations in 12 patients and their family members

Patient Number	Zygosity	cDNA	Protein	Type of mutation	Inheritance	Reported/Novel	Pathogenicity (ACGM 2015)	SIFT	Mutation Taster
P1	HT	c.679G>A	p. Val227Met	Missense	From the mother	Reported	Pathogenic	Damaging	Disease causing
P2	HT	c.582+4delA	-	Splice site	NA	Novel	Likely Pathogenic	NA	Disease causing
P3	HT	c.844T>G	p. Ser282Ala	Missense	From the mother	Reported	Pathogenic	Damaging	Disease causing
P4	HT	c.1306G>T	p. Glu436Ter	Nonsense	From both of the parents	Novel	Likely Pathogenic	NA	Disease causing
P5	HT	c.316delC	p. His106ThrfsTer11	Frameshift	From the father	Novel	Likely Pathogenic	NA	Disease causing
P6	HT	c.547G>A	p. Val183Met	Missense	De novo	Reported	Pathogenic	Damaging	Disease causing
P7	HT	c.398A>G	p. Asp133Gly	Missense	From the mother	Novel	Likely Pathogenic	Damaging	Disease causing
P8	HT	c.716T>C	p. Met239Thr	Missense	From the father	Reported	Pathogenic	Tolerated	Disease causing
P9	HT	c.911G>A	p. Arg304Gln	Missense	NA	Reported	Pathogenic	Damaging	Disease causing
P10	HT	c.686C>T	p. Thr229Met	Missense	NA	Reported	Pathogenic	Damaging	Disease causing
P11	HT	c.488G>A	p. Gly163Asp	Missense	NA	Reported	Pathogenic	Damaging	Disease causing
P12	HT	c.390C>A	p. Cys130Ter	Nonsense	NA	Reported	Pathogenic	NA	Disease causing

HT: heterozygous, NA: not available

DISCUSSION

In this study, we aimed to investigate the clinical and molecular spectrum of patients with *GCK*-MODY in pediatric population. GCK mutation is a common cause of incidental hyperglycemia and individuals with *GCK*-MODY are generally first diagnosed during routine investigations or from blood glucose measurements performed to investigate another complaint. The ratio of *GCK*-MODY has been reported as 40–50% among children with asymptomatic or coincidental hyperglycemia (6, 11, 12). Our study was conducted in patients who had randomly detected hyperglycemia. We found that the fasting blood glucose levels between 106-139 mg/dl and HbA1C levels between 6.1-7.03 ng/ml at the first admission and these values were consistent with the literature. GCK mutations have been reported as account for 8%-56% of MODY, and it also reported as the most common cause of MODY in European countries (13, 14, 15). The prevalence of GCK mutations in Turkish population has been reported by Ağladıoğlu et al. (16), Anik et al. (17) and Aykut et al. (18) as 64%, 25% and 25.42 %, respectively. In our study, the frequency of the patients with *GCK* gene mutations was also 57.14%. In none of our patients with GCK-MODY pharmacological treatment including oral anti-diabetic medicines and insulin regimen was recommended and in all of them diet alone is sufficient to achieve metabolic control.

To the best of our knowledge, to date, more than 900 mutations of the GCK gene have been documented (19). According to different studies conducted in our country, different mutations were detected. Ağladıoğlu et al. (16) identified seven novel and five known GCK mutations among 18 patients. Anık et. al (17) detected mutation in GCK gene in 8 of 42 patients and 3 of these mutations were novel. Aykut et al. (18) identified 45 different mutations in the GCK gene, 20 of which were novel. In this study, we reported 12 (57,14%) different likely pathogenic/pathogenic variants. Eight of them (p.Val227Met, p.Ser282Ala, p.Val183Met, p.Met239Thr, p.Arg304Gln, p.Thr229Met, p.Gly163Asp, p.Cys130Ter) have been previously reported in the literature and 4 variants (c.582+4delA, p.Glu436Ter, p.His106ThrfsTer11, p.Asp133Gly) were novel. While the most frequently reported mutation among GCK-MODY mutations are missense mutation, followed by nonsense, frameshift or splice site mutations; pancreatic islet promoter mutations and partial or complete gene deletions are the rare mutations that cause GCK-MODY (20, 21). In a study conducted in our country, among 45 GCK mutations, 32 were missense mutations, 5 were nonsense mutations, 6 small deletions/insertions resulting in frameshifts and 2 splice site mutation (18). Another study conducted in our country, Bolu et al (22) detected sixteen different variants in the GCK gene of the 40 cases; 33 were missense mutations, six were deletions, and one was a nonsense mutation. Consistent with the literature, in our study the most common mutation type was missense and followed by nonsense, splice site and frameshift mutations.

However, in recent studies the mutations observed in individuals with GCK-MODY have showed that the phenotype may significantly differ in patients with GCK-MODY depending on the type of the mutation, it is mostly known that the phenotypic characteristics of GCK-MODY patients are very similar with the unaffected alleles compensate for the mutations (6, 23, 24). In our study, we found similar phenotype characteristic of children with GCK-MODY among the children with different variants.

Patients with GCK-MODY have hyperglycemia due to the altered physiological set point of glucose homeostasis and they have no risk of developing long-term complications of diabetes, and they can only be treated with diet and do not require medication. Genetic confirmation of GCK-MODY will not only prevent patients from being mistakenly diagnosed with type 1 or type 2 diabetes but will also prevent unnecessary oral antidiabetic medication and insulin therapy. Children with hyperglycemia should underestimated for GCK-MODY. Clinical findings can be similar different variants.

Limitations of the Study

Our study has some limitations. The small number of patients is major limitation of the study. Second, in vitro functional studies are essential to prove the disease-causing effects of novel variants that were interpreted to be as pathogenic / likely pathogenic by various bioinformatics tools. Third, genetic analysis of all the parents of the patients with positive variant could not be performed. Finally, large copy number or deep intronic variants could not be analyzed in the current study.

CONCLUSION

In conclusion, we identified *GCK* gene variants in 12 of 21 (57.14%) patients with asymptomatic hyperglycemia and family history of diabetes. Four of these mutations were novel. We found similar phenotype characteristic of children with GCK-MODY among the children with different variants. The most common mutation type was missense and followed by nonsense, splice site and frameshift mutations. Detection of the molecular defect in patients with MODY is vital for the implementation of appropriate treatment approaches.

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Conflict of Interest

The authors declare that they have no conflict of interests regarding content of this article..

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Ethical Declaration

Ethical permission was obtained from the University of Health Sciences Turkey, Dr. BehçetUz Child Disease and Pediatric Surgery Training and Research Hospital Clinical / Human Research Ethics Committee for this study with date 12.09.2021 and number 640(2021/19-10) and Helsinki Declaration rules were followed to conduct this study.

Authorship Contributions

Concept: ÖN, BÖ, Design: ÖN, SG, Supervising: ÖN, BÖ, TK, Financing and equipment: ÖN, TK, Data collection and entry: TK, ÖN, Analysis and interpretation: SG, FH, Literature search: FH, TK, Writing: ÖN, SG, Critical review: BÖ.

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