DOI: 10.4274/jcrpe.galenos.2021.2020.0285

Original article

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

YALCINTEPE S et al. Targeted Gene Analysis for MODY Cases

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What is already known on this topic?
The genetic etiology of MODY is being known, clinical findings are being threated.

What this study adds?
The pathogenic variants in MODY etiology have been differing according to regions. This is the first study introducing Trakya region MODY variants with a relatively high case frequency for the population of this region. 12 novel variants were detected in our study, these variants will be firstly reported.

Abstract
Objective: The aim of this study was to investigate the molecular basis of maturity-onset diabetes of the young by targeted-gene sequencing of 20 genes related to monogenic diabetes and estimate the frequency and describe the clinical characteristics of monogenic diabetes and MODY in Trakya Region of Turkey.
Methods: In 61 cases, a panel of 20 monogenic diabetes related genes were screened. Illumina NextSeq550 system was used for sequencing of the genes. 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 in the *GCK, RFX6, CEL, PDX1, KCNJ11, HNF1A, G6PC2, GLIS3* and *KLF11* genes were identified. 6 different pathogenic/likely pathogenic variants and 6 different variants of uncertain significance were detected as novel.
Conclusions: This is the first study including molecular studies of twenty monogenic diabetes genes in Turkish cases in Trakya Region. The results of this study 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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30.11.2020
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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 3 generation of pedigree, 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, nowadays with 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 were 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 of MODY.

MODY is responsible for 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 only *GCK* and *HNF1A* pathogenic variants are responsible for about 70% of all MODYs[4]. 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 result in 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 as mentioned[6]. Subsequently, there are frequent *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 diabetes clinics in a few defined families, a decrease in fecalelastase 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 costly and limited number of genes sequenced. In addition, this method could bypass large insertions and deletions. Today, with the next generation sequencing (NGS) method, many genes can be analyzed together at the same time and lower costs. For this purpose, 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 the cases with a pre-diagnosis of monogenic diabetes and/or fasting plasma level differencies using NGS method.

Material and Methods

The patient files of 61 cases with a clinical diagnosis of monogenic diabetes, mostly MODY, who were examined in Child Endocrinology clinic or Medical Genetics clinic were included in this study. For the clinical diagnosis of MODY we considered: Diabetes mellitus in a parent and its first-degree relative presence of history (at least 2 generations), autoantibody negativity (in the results of 2 or more observed antibodies), low insulin requirement (<0.5 U / kg / g) and measurable C-peptide level. The written informed consent forms were obtained from the parents of the patients.

EDTA-blood samples were collected after the informed concent forms were taken from the patients or from their legal guardians. Genomic DNA was isolated from peripheral blood samples of the patients by 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) kit was performed 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 instructions of manufacturer's instructions. Quality control of the prepared libraries was done with Qubit dsDNA BR Assay system (Invitrogen, Carlsbad, CA). Fastq generation was performed on Illumina NextSeq550 (Illumina Inc., San Diego, CA, ABD). 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, ABD). 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. ACMG-2015 (American College of Medical Genetics)[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.

This study is approved by the Ethical Committee of our university with the number of 2020/263 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 (33 female cases with a mean age of 15,1, 28 male cases with a mean age of 14,7). Thirty-two of the patients (52,4%) had an affected family member.

31 pathogenic/likely pathogenic variants in 29 (47,5%) cases, 14 variants of uncertain significance (VUS) in 11 (18%) cases were detected in our study. Pathogenic/likely pathogenic variants were in the *GCK* gene-24 variants, *ABCC8* gene-3 variants, *KCNJ11* gene: 2 variants, *HNF1A* gene: 1 variant, *HNF4A* gene: 1 variant. VUS were in the *GCK* gene: 2 variants, *RFX6* gene: 3

variants, *CEL* gene: 2 variants, *PDX1* gene: 2 variants, *KCNJ11* gene: 1 variant, *HNF1A* gene: 1 variant, *G6PC2* gene: 1 variant, *GLIS3* gene: 1 variant, *KLF11* gene: 1 variant (Table 1). Totally 37 different variants were identified in this study (Table 2).

6 novel pathogenic/likely pathogenic variants (*GCK*: 5, *ABCC8*: 1), 6 novel VUS (*RFX6*: 3, *HNF1A*: 1, *CEL*: 1, *GLIS3*: 1) were detected. 12 different variants (32.4%) of 37 in this study were novel (Table 2). All variants in this study were heterozygously detected.

Family members of the cases 2, 3, 4, 10, 12, 15, 17, and 18 who have also the same variants with the reported cases had also MODY clinical findings (Table 1). They were all diagnosed as diabetes mellitus before, and with our moleular results, these family members had also the correct diagnosis.

Cases 4 ve 5 each had two different likely pathogenic variants. Case 4 had *GCK* and *KCNJ11* likely pathogenic variants, maternally inherited and *de novo*, respectively. He had been diagnosed with type 1 diabetes mellitus, before. With learning maternal story, 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. But in the follow-up period, he and his mother had never 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 taken unnecessary insulin treatment with diabetes mellitus diagnosis. MODY test was planned due to, her mother, sister and grandmother had diabetes mellitus. In the follow-up, case 5 had also never a complication about diabetes.

Discussion

MODY is a rare form of diabetes that has genetic, metabolic and clinical differences and inherited as autosomal dominant[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 same 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 significant for MODY.

There is a large number of MODY-related genes known. The most common of these genes are *HNF4A* (MODY1), *GCK* (MODY2), *HNF1A* (MODY3), *IPF1* (MODY4), *HNF1B* (MODY5), *NEUROD1* (MODY6), *CEL* (MODY8). *GCK* (Glucokinase), 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 leads to a slight increase in the glucose threshold value in the glucose-insulin release curve. Accordingly, it is between 100-153 mg/dl in fasting glucose levels. They can be diagnosed at any age. HbA1c levels do not exceed 7.5%, and the increase in glucose level at 0 and 120 min in OGTT does not exceed 90 mg/dl in 95%[13].

In our study, we had a diagnostic rate of 47,5% for MODY, twenty-four cases had a *GCK* pathogenic variant, and they all were diagnosed due to incidental hyperglycemia. The youngest patient was 9 months old (Case 14). Only one patient (case 2) was obese (BMI> 95 P) and the other cases were normally weighed. All these cases were negative for ICA (islet cell antibody) and two of them were positive for GADA (glutamic acid decarboxylase 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, 18) were diagnosed with gestational diabetes and had to use insulin. All our patients have been followed up with a controlled carbohydrate diet. None of them needed medical treatment.

In a study, the cases were selected for *GCK* analysis with a pre-diagnosis of MODY, 11 cases of 21 probands (52%) were identified with a pathogenic/likely pathogenic variant[14]. 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 intake of patients. 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' body mass index 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 is also an issue to be considered. 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, getting to know GCK-MODY during pregnancy is important for the problems which may occur for the mother and 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 age 25 and 96% before age 55. The type and location of the mutation affects the age of onset of diabetes[20]. A mutation in the dimerization or binding section of *HNF1A* leads to the development of diabetes 10 years earlier than a mutation in the transactivation section. Although the clinic of cases varies according to the mutation, the same mutation can lead to different clinical results in the same family. Although ketoacidosis is rare, approximately 25% of patients can apply like a type 1 diabetes clinic. Most of them give 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 of the cases (case 12), and *HNF1A* variant of unknown significance in one of the cases (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, whose blood glucose level is within the normal range and the HbA1c level is 6% (3.6-5.8), is followed up 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, GADA and IAA (insulin auto-antibody). At diagnosis, case 12 had a HbA1C level 8.5% and high blood sugar (fasting blood sugar>100 mg/dl, postprandial blood sugar>200 mg/dl), and she is followed up with sulfonylurea treatment.

HNF4A-MODY is rarer than HNF1A-MODY and is responsible for approximately 5-10% of all MODY individuals. *HNF4A* is also a transcription factor, which leads to progressive beta cell destruction, such as 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% of neonatal hyper insulinemic hypoglycaemia with diazoxide response. Extrapancreatic laboratory findings suggesting HNF4A-MODY are low HDL, low triglyceride and high LDL (22). We identified a pathogenic variant of *HNF4A* in one case (case 23) with HbA1c:12%, fasting blood sugar:237 mg/dl at diagnosis. He was diagnosed as diabetes and treated with insülin before molecular analysis of MODY panel.

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 hyper insulinism. A mutation in the *ABCC8* or *KCNJ11* gene, which creates a serious clinical situation in the neonatal period, may develop a lighter clinic, 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 the *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 was applied with abdominal pain, nausea, and vomiting. Hyperglicemia was detected in her blood tests (214 mg/dl). She had also postprandial blood sugar near to highest limit and diabetes mellitus family history. Case 26 had diabetes mellitus clinical findings, but his mother, grandmother, mother's uncles and aunts also had diabetes mellitus diagnosis.

The 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 get rid of unnecessary insulin treatment once they get diagnosed and achieve better metabolic control with oral sulfanylurea. In addition, genetically diagnosed as MODY enables early diagnosis of individuals who have not yet been diagnosed by performing a family scanning and the patients are 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 in *HNF4A* or ABCC8 / *KCNJ11* genes, it also allows genetic counseling to be given in terms of MODY screening of the family and the possibility of developing a newborn in the future[25]. In our study 3 pathogenic/likely pathogenic variants in *ABCC8* gene, 2 pathogenic/likely pathogenic variants in *KCNJ11* gene. Case 4 had both *GCK* and *KCNJ11*, case 5 had both *GCK* and *ABCC8* pathogenic/likely pathogenic variants. Case 4 and 5 had a diagnosis of diabetes mellitus type 1 before. Mother of case 4; mother, sister and grandmother of case 5 had also diabetes mellitus diagnosis. Both case 4 and case 5 had been observed with a mild clinical follow-up period for diabetes.

The diagnosis of HNF1B-MODY by an individual who is followed up with the diagnosis of diabetes gives an idea to be careful about the extrapancreatic findings that accompany and/or may occur and provides a multidisciplinary planning of their follow-up[26]. In our cases, there was not any *HNF1B* pathogenic variant.

Six of our cases had been diagnosed with novel pathogenic variants in *GCK* and *HNF1A* genes. If only known variants would be analyzed in suspicious cases, it means that many patients could not be diagnosed. In our study, analyzing the all exons of genes increased the rate of diagnosis.

There are known 14 genes for MODY (*HNF4A, GCK, HNF1A, PDX1, HNF1B, NEUROD1, KLF11, CEL, PAX4, INS, BLK, ABCC8, KCNJ11, APPL1*) [27]. We analysed these genes except *APPL1*, and 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 than the subtypes. Molecular analysis can also be planned in asymptomatic cases with a family history depending on permission of them. Genetic counselling was given to the cases and their families in this study.

Study Limitations: The current study analysing 20 genes including 13 MODY-associated genes was designed with 61 cases. The limitations of this study:

- The number of cases should be higher to take comprehensive results. This is a cross-sectional study, which was designed in a low populated region of our country. The case group of this study states the Trakya region of Turkey.
- MODY type 14 could not be determined due to *APPL1* gene was not included in the targeted gene panel in the current study. The APPL1 gene causes less than 1% of all MODY types.

Conclusion

As new genes are identified with developing molecular testing possibilities, the class of monogenic diabetes is expected to expand gradually. Thinking of monogenic diabetes in patients diagnosed with type 1 and type 2 diabetes mellitus but showing atypical course, and confirming the diagnosis with appropriate molecular tests plays a key role in the treatment of diabetes. Molecular diagnosis is of great importance in terms of choosing the appropriate treatment that will affect the prognosis of the patient, giving genetic counseling and screening individuals at risk.

Author Contributions

SY, FOC was responsible for study design, protocol development, acquisition and interpretation of data, drafting the article, and writing the manuscript; HG—for study design, acquisition, interpretation of data; SD—for acquisition and interpretation of data; EIA—for study design and protocol development; EA—for acquisition and interpretation of data; DE—for acquisition of data; FTK—for revising the manuscript for important intellectual content and interpretation of data; SY, HG—for study design, project coordination, writing the manuscript, and approval of the final version of the manuscript.

Acknowledgments

We would like to thank the subjects who participated in the study.

Declaration of interest: There is no conflict of interest.

Research funding: None.

References

1- Szopa M, Ludwig-Gałęzowska A, Radkowski P, Skupień J, Zapała B, Płatek T, et al. Genetic testing for monogenic diabetes using targeted next-generation sequencing in patients with maturity-onset diabetes of the young. Pol Arch Med Wewn 2015;125(11):845-51. doi: 10.20452/pamw.3164. Epub 2015 Nov 9.

2- Kleinberger JW, Pollin TI. Undiagnosed MODY: Time for Action. Curr Diab Rep 2015 Dec;15(12):110. doi: 10.1007/s11892-015-0681-7.

3- Xu A, Lin Y, Sheng H, Cheng J, Mei H, Ting TH, et al. Molecular diagnosis of maturity-onset diabetes of the young in a cohort of Chinese children. Pediatr Diabetes 2020 May;21(3):431-440. doi: 10.1111/pedi.12985. Epub 2020 Jan 28.

4- Tatsi EB, Kanaka-Gantenbein C, Scorilas A, Chrousos GP, Sertedaki A. Next generation sequencing targeted gene panel in Greek MODY patients increases diagnostic accuracy. Pediatr Diabetes 2020 Feb;21(1):28-39. doi: 10.1111/pedi.12931. Epub 2019 Nov 10.

5- Ming-Qiang Z, Yang-Li D, Ke H, Wei W, Jun-Fen F, Chao-Chun Z, et al. Maturity onset diabetes of the young (MODY) in Chinese children: genes and clinical phenotypes. J Pediatr Endocrinol Metab 2019 Jul 26;32(7):759-765. doi: 10.1515/jpem-2018-0446.

6- McDonald TJ, Ellard S. Maturity onset diabetes of the young: identification and diagnosis. Ann Clin Biochem 2013 Sep;50(Pt 5):403-15. doi: 10.1177/0004563213483458. Epub 2013 Jul 22.

7- Naylor R, Philipson LH. Who should have genetic testing for maturity-onset diabetes of the young? Clin Endocrinol (Oxf) 2011 Oct;75(4):422-6. doi: 10.1111/j.1365-2265.2011.04049.x.

8- Campbell MR. Review of current status of molecular diagnosis and characterization of monogenic diabetes mellitus: a focus on next-generation sequencing. Expert Rev Mol Diagn 2020 Apr;20(4):413-420. doi: 10.1080/14737159.2020.1730179. Epub 2020 Mar 1.

9- Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al; ACMG Laboratory Quality Assurance Committee. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015 May;17(5):405-24. doi: 10.1038/gim.2015.30. Epub 2015 Mar 5.

10- den Dunnen JT, Dalgleish R, Maglott DR, Hart RK, Greenblatt MS, McGowan-Jordan J, et al. HGVS Recommendations for the Description of Sequence Variants: 2016 Update. Hum Mutat 2016 Jun;37(6):564-9. doi: 10.1002/humu.22981. Epub 2016 Mar 25.

11- Landrum MJ, Lee JM, Benson M, Brown GR, Chao C, Chitipiralla S, et al. ClinVar: improving access to variant interpretations and supporting evidence. Nucleic Acids Res 2018 Jan 4;46(D1):D1062-D1067. doi: 10.1093/nar/gkx1153.

12- Jang KM. Maturity-onset diabetes of the young: update and perspectives on diagnosis and treatment. Yeungnam Univ J Med 2020 Jan;37(1):13-21. doi: 10.12701/yujm.2019.00409. Epub 2020 Jan 9.

13- Urbanova J, Brunerova L, Broz J. Hypoglycemia and antihyperglycemic treatment in adult MODY patients - A systematic review of literature. Diabetes Res Clin Pract 2019 Dec;158:107914. doi: 10.1016/j.diabres.2019.107914. Epub 2019 Nov 2. PMID: 31682881.

14- Haliloglu B, Hysenaj G, Atay Z, Guran T, Abali S, Turan S, et al. GCK gene mutations are a common cause of childhood-onset MODY (maturity-onset diabetes of the young) in Turkey. Clin Endocrinol (Oxf) 2016 Sep;85(3):393-9. doi: 10.1111/cen.13121. Epub 2016 Jul 5.

15- Ağladıoğlu SY, Aycan Z, Çetinkaya S, Baş VN, Önder A, Peltek Kendirci HN, et al. Maturity onset diabetes of youth (MODY) in Turkish children: sequence analysis of 11 causative genes by next generation sequencing. J Pediatr Endocrinol Metab 2016 Apr;29(4):487-96. doi: 10.1515/jpem-2015-0039.

16- Hunter JD, Staton H, Constantacos C, Walsh ET, Crudo DF. Pathogenicity of a glucokinase gene mutation and description of its clinical phenotype. Pediatr Diabetes 2020 May 29. doi: 10.1111/pedi.13058. [Epub ahead of print].
17- Martin D, Bellanné-Chantelot C, Deschamps I, Froguel P, Robert JJ, Velho G. Long-term follow-up of oral glucose tolerance test-derived glucose tolerance and insulin secretion and insulin sensitivity indexes in subjects with glucokinase mutations (MODY2). Diabetes Care 2008;31:1321-3.
18- Monsonego S, Clark H, Karovitch A, O'Meara P, Shaw T, Malcolm J. Management and Outcomes of Maturity-Onset Diabetes of the Young in Pregnancy. Can J Diabetes 2019 Dec;43(8):647-654. doi: 10.1016/j.jcjd.2019.07.004. Epub 2019 Aug 2.
19- Malikova J, Kaci A, Dusatkova P, Aukrust I, Torsvik J, Vesela K, et al. Functional Analyses of HNF1A-MODY Variants Refine the Interpretation of Identified Sequence Variants. J Clin Endocrinol Metab 2020 Apr 1;105(4):dgaa051. doi: 10.1210/clinem/dgaa051.
20- Fu J, Wang T, Zhai X, Xiao X. Primary hepatocellular adenoma due to biallelic HNF1A mutations and its co-occurrence with MODY 3: case-report and review of the literature. Endocrine 2020 Mar;67(3):544-551. doi: 10.1007/s12020-019-02138-x. Epub 2019 Nov 21.
21- Valkovicova T, Skopkova M, Stanik J, Gasperikova D. Novel insights into genetics and clinics of the HNF1A-MODY. Endocr Regul 2019 Apr 1;53(2):110-134. doi: 10.2478/enr-2019-0013.
22- Yahaya TO, Ufuoma SB. Genetics and Pathophysiology of Maturity-onset Diabetes of the Young (MODY): A Review of Current Trends. Oman Med J 2020 May 28;35(3):e126. doi: 10.5001/omj.2020.44.
23- Alkorta-Aranburu G, Carmody D, Cheng YW, Nelakuditi V, Ma L, Dickens JT, et al. Phenotypic heterogeneity in monogenic diabetes: the clinical and diagnostic utility of a gene panel-based next-generation sequencing approach. Mol Genet Metab 2014 Dec;113(4):315-320. doi: 10.1016/j.ymgme.2014.09.007. Epub 2014 Sep 28.
24- Bishay RH, Greenfield JR. A review of maturity onset diabetes of the young (MODY) and challenges in the management of glucokinase-MODY. Med J Aust 2017 Aug 4;207(5):223. doi: 10.5694/mja16.01467.
25- Hasbaoui BE, Elyajouri A, Abilkassem R, Agadr A. Congenital hyperinsulinsim: case report and review of literature. Pan Afr Med J 2020 Feb 24;35:53. doi: 10.11604/pamj.2020.35.53.16604.
26- Raile K, Klopocki E, Holder M, Wessel T, Galler A, Deiss D, et al. Expanded clinical spectrum in hepatocyte nuclear factor 1b-maturity-onset diabetes of the young. J Clin Endocrinol Metab 2009 Jul;94(7):2658-64. doi: 10.1210/jc.2008-2189. Epub 2009 May 5.
27- Urakami T. Maturity-onset diabetes of the young (MODY): current perspectives on diagnosis and treatment. Diabetes Metab Syndr Obes 2019 Jul 8;12:1047-1056. doi: 10.2147/DMSO.S179793.

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

Case	Age/Gender	Gene	Variant	Protein	Pathogenicity (ACMG-2015)	Segregation analysis	Phenotype
1-EHK	16/F	GCK	ENST00000403799.3:c.387C>A	p.(Cys129Ter)	Pathogenic	NA	MODY Type 2
2-SO	13/F	GCK	NM_033507.3:c.1256+1G>T (c.1253+1G>T)		Pathogenic	inherited from the affected mother	MODY Type 2
3-ET	5/F	GCK	ENST00000403799.3:c.387C>A	p.(Cys129Ter)	Pathogenic	Affected sister has the same variant	MODY Type 2
4-EC	16/M	GCK	NM_000162.5:c.943C>T	p.(Leu315Phe)	Likely pathogenic	inherited from the affected mother	MODY Type 2
		KCNJ11	NM_000525.3:c.668C>T	p.(Thr223Ile)	Likely pathogenic	*de novo*	MODY Type 13
5-GO	21/F	ABCC8	NM_000352.6:c.3517G>A	p.(Val1173Met)	Likely pathogenic	NA	Diabetes Mellitus, noninsulin dependent
		GCK	NM_000162.5:c.943C>T	p.(Leu315Phe)	Likely pathogenic	NA	MODY Type 2
6-ST	11/F	GCK	ENST00000403799.3:c.398T>A	p. (Phe133Tyr)	Likely pathogenic	NA	MODY Type 2
7-EM	7/F	GCK	NM_033507.3:c.133G>A	p. (Gly45Ser)	Likely pathogenic	NA	MODY Type 2
8-ET	3/F	GCK	NM_033507.3:c.537delG	p. (Asn180ThrfsTer25)	Pathogenic	NA	MODY Type 2
9-BB	18/F	GCK	NM_000162.5:c.943C>T	p. (Leu315Phe)	Likely pathogenic	NA	MODY Type 2
10-SS	13/F	GCK	ENST00000403799.3:c.387C>A	p. (Cys129Ter)	Pathogenic	inherited from the affected mother	MODY Type 2
11-EA	11/F	GCK	NM_033507.3:c.867-1G>A		Pathogenic	NA	MODY Type 2
12-IK	16/F	HNF1A	NM_000545.8:c.864delGinsCC (c.872dupC)	p.(Gly292ArgfsTer25)	Pathogenic	inherited from the affected mother	MODY Type 3
13-BA	5/F	ABCC8	NM_000352.6:c.4014G>A	p.(Trp1338Ter)	Pathogenic	NA	Diabetes Mellitus, noninsulin dependent
14-EC	9 months/M	GCK	NM_000162.5:c.943C>T	p.(Leu315Phe)	Likely pathogenic	NA	MODY Type 2
15-AEO	3/F	GCK	NM_000162.5:c.880G>C	p.(Gly294Arg)	Likely pathogenic	inherited from the affected mother	MODY Type 2
16-HSE	8/F	GCK	NM_000162.5:c.1248C>G	p.(His416Gln)	Likely pathogenic	NA	MODY Type 2
17-OTG	14/M	GCK	NM_000162.5:c.746G>A	p.(Gly249Asp)	Likely pathogenic	inherited from the affected mother	MODY Type 2
18-EZS	1/F	GCK	NM_000162.5:c.667G>A	p.(Gly223Ser)	Pathogenic	inherited from the affected mother	MODY Type 2
19-HT	21/M	GCK	NM_000162.5:c.506A>G	p.(Lys169Arg)	Likely pathogenic	NA	MODY Type 2
20-SY	12/F	GCK	NM_000162.5:c.565A>G	p.(Ile189Val)	Likely pathogenic	NA	MODY Type 2
21-MS	14/M	GCK	NM_000162.5:c.565A>G	p.(Ile189Val)	Likely pathogenic	NA	MODY Type 2
22-YAS	1/M	GCK	NM_000162.5:c.617C>T	p.(Thr206Met)	Likely pathogenic	NA	MODY Type 2
23-BSO	14/M	HNF4A	NM_000457.4:c.844G>A	p.(Asp282Asn)	Likely pathogenic	NA	

24-HH	33/F	GCK	NM_000162.5:c.943C>T	p.(Leu315Phe)	Likely pathogenic	NA	MODY Type 2
25-MC	28/M	GCK	NM_000162.5:c.1222G>T	p.(Val408Leu)	Likely pathogenic	NA	MODY Type 2
26-HO	16/M	ABCC8	NM_000352.6:c.2768T>G	p.(Leu923Arg)	Likely pathogenic	NA	Diabetes Mellitus, noninsulin dependent
27-FO	12/F	KCNJ11	NM_000525.3:c.481G>A	p.(Ala161Thr)	Likely pathogenic	NA	MODY Type 13
28-OC	1/M	GCK	NM_000162.5:c.115_117delAAG	p.(Lys39del)	Likely pathogenic	NA	MODY Type 2
29-ENM	12/F	GCK	NM_000162.5:c.214G>A	p.(Gly72Arg)	Pathogenic	NA	MODY Type 2
30-HC	29/M	KCNJ11	NM_000525.3:c.1117G>A	p.(Val373Met)	VUS	NA	MODY Type 13
31-MO	12/F	HNF1A	NM_000545.8:c.1769-3C>T		VUS	NA	MODY Type 3
32-SD	33/M	G6PC2	NM_021176.3:c.89C>T	p.(Ser30Phe)	VUS	NA	NA
33-AB	13/F	CEL	NM_001807.6:c.2184_2216del	p.(Gly729_Thr739del)	VUS	NA	MODY Type 8
		PDX1	NM_000209.4:c.226G>A	p.(Asp76Asn)	VUS	NA	MODY Type 4
34-BY	13/M	RFX6	NM_173560.4:c.428G>A	p.(Cys143Tyr)	VUS	NA	Mitchell-Riley syndrome
		GCK	NM_000162.5:c.863+3A>G		VUS	NA	MODY Type 2
35-AK	33/F	GCK	NM_000162.5:c.863+3A>G		VUS	NA	MODY Type 2
36-OAB	8/M	CEL	NM_001807.6:c.2049_2082del	p.(Thr684ArgfsTer9)	VUS	NA	MODY Type 8
		RFX6	NM_173560.4:c.1072G>A	p.(Val358Ile)	VUS	NA	Mitchell-Riley syndrome
37-SK	12/F	RFX6	NM_173560.4:c.246C>G	p.(Asn82Lys)	VUS	NA	Mitchell-Riley syndrome
38-BC	14/F	PDX1	NM_000209.4:c.97C>A	p.(Pro33Thr)	VUS	NA	MODY Type 4
39-SG	18/F	GLIS3	NM_001042413.2:c.589G>T	p.(Asp197Tyr)	VUS	NA	Diabetes mellitus, neonatal, with congenital hypothyroidism
40-MAC	2/M	KLF11	NM_003597.5:c.1447C>T	p.(Pro483Ser)	VUS	NA	MODY Type 7

ACMG : American College of Medical Genetics, dbSNP: The Single Nucleotide Polymorphism Database, VUS: Variants of unknown significance, NA: Not applicable

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	ClinVar Variation ID	HGMD	SIFT	DANN	GERP	Mutation Taster	Classification (ACMG-2015)
GCK	NM_000162.5:c.115_117delAAG p.(Lys39del)	in frame	7:44192991	NA	NA	CD191970	NA	NA	5.07	NA	Likely pathogenic (PM1, PM2, PM4, PP3)
	NM_033507.3:c.133G>A p. (Gly45Ser)	missense	7:44192978	rs267601516	76898	CM013265	Damaging	0.9989	5.07	Disease causing	Pathogenic (PS1, PM1, PM2, PM5, PP2, PP3, PP5)
	NM_000162.5:c.214G>A p.(Gly72Arg)	missense	7:44192019	rs193922289	36209	CM023383	Damaging	0.9983	4.67	Disease causing	Pathogenic (PS1, PM1, PM2, PP2, PP3, PP5)
	ENST00000403799.3:c.387C>A p.(Cys129Ter)	nonsense	7:44190651	rs1583601365	804846	CM012111	NA	0.9939	4.94	NA	Pathogenic (PVS1, PM2, PP3, PP5)
	ENST00000403799.3:c.398T>A p. (Phe133Tyr)	missense	7:44190640	NA	novel	novel	Damaging	0.9906	5.05	Disease causing	Likely pathogenic (PM1, PM2, PM5, PP2, PP3)
	NM_000162.5:c.506A>G p.(Lys169Arg)	missense	7:44189641	NA	NA	CM141531	Damaging	0.9991	5.69	Disease causing	Likely pathogenic (PM1, PM2, PP2, PP3, PP5)
	NM_033507.3:c.537delG p. (Asn180ThrfsTer25)	frameshift	7:44189613	NA	novel	novel	NA	NA	5.79	NA	Pathogenic (PVS1, PM2, PP3)
	NM_000162.5:c.565A>G p.(Ile189Val)	missense	7:44189582	rs757978639	NA	NA	Tolerated	0.9986	5.82	Disease causing	Likely pathogenic (PM1, PM2, PP2, PP3)
	NM_000162.5:c.617C>T p.(Thr206Met)	missense	7:44189421	rs1441649062	NA	CM012122	Damaging	0.9993	5.96	Disease causing	Pathogenic (PM1, PM2, PM5, PP2, PP3, PP5)
	NM_000162.5:c.667G>A p.(Gly223Ser)	missense	7:44189371	rs1360415315	435306	CM012123	Damaging	0.9992	6.17	Disease causing	Pathogenic (PS1, PM1,

											PM2, PP2, PP3, PP5)
	NM_000162.5:c.746G>A p.(Gly249Asp)	missense	7:44187366	NA	novel	novel	Tolerated	0.998 6	5.23	Disease causing	Likely pathogenic (PM1, PM2, PP2, PP3)
	NM_000162.5:c.863+3A>G	splicing	7:44187246	rs193922334	36261	NA	NA	0.967 3	5.5	NA	VUS (PM2, BP4)
	NM_033507.3:c.867-1G>A	splicing	7:44186218	rs116767560 4	804861	NA	NA	0.994 3	4.59	Disease causing	Pathogenic (PVS1, PM2, PP3, PP5)
	NM_000162.5:c.880G>C p.(Gly294Arg)	missense	7:44186201	NA	novel	novel	NA	0.999 1	4.76	Disease causing	Likely pathogenic (PM1, PM2, PP2, PP3)
	NM_000162.5:c.943C>T p.(Leu315Phe)	missense	7:44186138	rs158359435 0	804863	CM064013	Damaging	0.998 5	4.59	Disease causing	Likely pathogenic (PM1, PM2, PM5, PP2, PP3)
	NM_000162.5:c.1222G>T p.(Val408Leu)	missense	7:44185127	NA	NA	CM171422	Damaging	0.995 5	5.57	Disease causing	Likely pathogenic (PM1, PM2, PP2, PP3)
	NM_000162.5:c.1248C>G p.(His416Gln)	missense	7:44185101	NA	novel	novel	Damaging	0.994	5.57	Disease causing	Likely pathogenic (PM1, PM2, PM5, PP2, PP3)
	NM_033507.3:c.1256+1G>T	splicing	7:44185095	NA	NA	CS032698	NA	0.994 9	5.57	Disease causing	Pathogenic (PVS1, PM2, PP3)
ABCC8 (NM_000352.6)	c.2768T>G p.(Leu923Arg)	missense	11:17429991	NA	novel	novel	Damaging	0.998 1	6.03	Disease causing	Likely pathogenic (PM1, PM2, PP2, PP3)
	c.3517G>A p.(Val1173Met)	missense	11:17426099	rs141322087	35609	NA	Tolerated	0.994 4	5.32	Disease causing	Likely pathogenic (PM1, PM2, PP2, PP5)
	c.4014G>A p.(Trp1338Ter)	nonsense	11:17418568	NA	NA	CM994652	NA	0.994 5	4.76	Disease causing	Pathogenic (PVS1, PM2, PP3)

HNF1A (NM_000545.8)	c.864delGinsCC p.(Gly292ArgfsTer25)	frameshift	12:121432117	rs1593058932	817605	CX1310026	NA	NA	4.15	NA	Pathogenic (PVS1, PM2, PP5)
	c.1769-3C>T	splicing	12:121438865	NA	novel	novel	NA	0.9703	6.05	NA	VUS (PM2)
KCNJ11 (NM_000525.3)	c.481G>A p.(Ala161Thr)	missense	11:17409158	rs1363707190	NA	NA	Damaging	0.9993	4.92	Disease causing	Likely pathogenic (PM1, PM2, PP2, PP3)
	c.668C>T p.(Thr223Ile)	missense	11:17408971	rs561086953	NA	NA	Damaging	0.9988	5.28	Disease causing	Likely pathogenic (PS2, PM2, PP2, PP3)
	c.1117G>A p.(Val373Met)	missense	11:17408522	rs770375846	RCV001280332.1	NA	Tolerated	0.9367	5.42	Disease causing	VUS (PM2, PP2, BP4)
HNF4A (NM_000457.4)	c.844G>A p.(Asp282Asn)	missense	20:43048468	rs1236613475	NA	NA	Damaging	0.9993	4.98	Disease causing	Likely pathogenic (PM1, PM2, PP2, PP3)
CEL (NM_001807.6)	c.2049_2082del p.(Thr684ArgfsTer9)	frameshift	9:135946938	NA	novel	novel	NA	NA	1.34	NA	VUS (PVS1)
	c.2184_2216del p.(Gly729_Thr739del)	in frame	9:135947057	rs756606428	NA	NA	NA	NA	2.67	NA	VUS (PM2)
PDX1 (NM_000209.4)	c.97C>A p.(Pro33Thr)	missense	13:28494372	rs1929020988	36414	CM056344	Damaging	0.9977	5.46	Disease causing	VUS (PM2, PP2, PP3)
	c.226G>A p.(Asp76Asn)	missense	13:28494501	rs137852783	8859	CM992901	Damaging	0.9982	4.96	Disease causing	VUS (PM2, PP2)
RFX6 (NM_173560.4)	c.246C>G p.(Asn82Lys)	missense	6:11719898	NA	novel	novel	Tolerated	0.9171	5.36	Polymorphism	VUS (PM2)
	c.428G>A p.(Cys143Tyr)	missense	6:11720175	NA	novel	novel	Damaging	0.9981	5.61	Disease causing	VUS (PM1, PM2, PP3, BP1)
	c.1072G>A p.(Val358Ile)	missense	6:11724034	NA	novel	novel	Tolerated	0.9951	6.07	Disease causing	VUS (PM2, BP1)
G6PC2 (NM_021176.3)	c.89C>T p.(Ser30Phe)	missense	2:169757930	rs142189264	NA	NA	Damaging	0.9982	5.42	Disease causing	VUS (PP3, BS1)
GLIS3 (NM_001042413.2)	c.589G>T p.(Asp197Tyr)	missense	9:4125741	NA	novel	novel	Damaging	0.9885	5.26	Disease causing	VUS (PM2, PP3, BP1)
KLF11 (NM_003597.5)	c.1447C>T p.(Pro483Ser)	missense	2:10192542	rs761563032	NA	NA	Tolerated	0.9953	5.78	Disease causing	VUS (PM2)