

# Molecular Genetic Study in a Cohort of Iranian Families Suspected to Maturity-Onset Diabetes of the Young, Reveals a Recurrent Mutation and a High-Risk Variant in the *CEL* Gene

## Abstract

**Background:** Diabetes mellitus (DM) is a group of metabolic disorders in the body, accompanied with increasing blood sugar levels. Diabetes is classified into three groups: Type 1 DM (T1DM), Type 2 DM (T2DM), and monogenic diabetes. Maturity-onset diabetes of the young (MODY) is a monogenic diabetes that is frequently mistaken for T1D or T2D. The aim of this study was to diagnose MODY and its subtype frequency in a diabetic population in Iran. **Materials and Methods:** In this study among ten diabetic families that were highly suspected to MODY by nongenetic biomarkers and without any pathogenic mutation in *GCK* and *HNF1A* genes, two patients from two unrelated families were examined via whole-exome sequencing (WES) in order to detect the causative gene of diabetes. Co-segregation analysis of the identified variant was performed using Sanger sequencing. **Results:** In this study, no pathogenic variant was found in *GCK* and *HNF1A* genes (MODY2 and MODY3), while these two types of MODY were introduced as the most frequent in other studies. By using WES, a pathogenic variant (p.I488T) was found in one of the patients in *CEL* gene causing MODY8 that its frequency is very rare in other studied populations. A high-risk variant associated with diabetes was found in another patient. **Conclusion:** WES was applied in this study to reveal the cause of MODY in 1 family. This pathogenic mutation was previously reported as a disease causing mutation.

**Keywords:** Carboxyl ester lipase, maturity-onset diabetes of the young, pathogenic variant, whole-exome sequencing

## Introduction

Diabetes mellitus (DM) is a heterogeneous group of metabolic disorders, characterized by high levels of blood glucose.<sup>[1]</sup> Usually, diabetes occurs as a result of  $\beta$ -cell dysfunction and/or deficiency of insulin secretion or its action.<sup>[2]</sup> There are two common types of diabetes: Type 1 DM (T1DM) and type 2 DM (T2DM). T1DM is an autoimmune disorder, and increased levels of anti-pancreatic  $\beta$ -cells autoantibodies are seen in this type of diabetes. T1DM patients must receive exogenous insulin in an appropriate dose daily.<sup>[3]</sup> In T2DM, partial insulin deficiency is the notable feature of the disease, and it is due to impaired insulin secretion or insulin resistance.<sup>[4]</sup>

Monogenic types of diabetes account for about 2%–5% of all types of diabetes. Usually, mutations in genes related to  $\beta$ -cell functions can lead to monogenic types of

diabetes. Maturity-onset diabetes of the young (MODY) and neonatal diabetes are monogenic types of diabetes. Syndromic diabetes are also classified in monogenic diabetes categories.<sup>[5]</sup> One of the most common concerns of health-care providers is misdiagnosis of MODY types of diabetes with common types of diabetes (T1D and T2D), especially HNF1A-MODY is usually misclassified as T1D.<sup>[6]</sup> Therefore, improper management of blood sugar control may be adopted. In some subtypes of MODY such as MODY1 (HNF4A mutations) and MODY3 (HNF1A mutations), proper management of hyperglycemia is so important to avoid micro/macro vascular complications of eyes, kidney, and heart. Whereas, in some of the MODY subtypes such as MODY2 (GCK mutations), the

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blood sugar levels are slightly higher than normal, and so that, simple physical activity can prevent the outcomes of hyperglycemia.<sup>[7]</sup>

MODY accounts for 2%–5% of all types of diabetes. For the first time in 1974, Tattersall reported a “mild” type of diabetes in three families from King’s College Hospital in London.<sup>[8]</sup> He discovered that it is a mild form of diabetes because many patients do not need necessarily insulin therapy and they had milder diabetes complications than expected.<sup>[9]</sup> Albeit Tattersall *et al.* recognized that these patients “have a type of diabetes that it is onset in young and it is different from T1D and T2D,” they did not use the term “maturity-onset diabetes of the young” for these types of diabetes.<sup>[10]</sup> MODY has several specific features that is use to distinguish it from other types of diabetes: Early onset of diabetes (<25 years), early onset diabetes in at least 2 or ideally three family generation, autosomal dominant mode of inheritance, noninsulin dependence (not requiring insulin even after 3 years of diagnosis) in most of its types, and nonketotic forms of diabetes.<sup>[11]</sup>

Mutations in at least 14 different genes are known to cause MODY.<sup>[12]</sup> The carboxyl ester lipase protein (CEL) is a pancreatic enzyme hydrolyzing cholesteryl esters and other dietary lipids. *CEL* or *BSSL* (MIM:114840) is located on human chromosome 9 (9q34.13), containing 11 exons and encodes a protein that is a glycoprotein secreted from the pancreas into the digestive tract.<sup>[13]</sup> Mutations in *CEL* have been associated with MODY8 and chronic pancreatitis.<sup>[14]</sup> *CEL* is expressed in the acinar cells of the exocrine pancreas and along with a variety of other lipases, released into the gut to break down complex lipids for absorption.<sup>[15]</sup> Recent evidence shows that mutations in the *CEL* gene can cause protein aggregation in the pancreas, which could induce tissue damage and lead to the symptoms of pancreatic dysfunction; it most likely results in MODY8.<sup>[16]</sup>

Despite its low prevalence, efforts in diagnosing and classification of MODY patients is important because it may clarify the understanding of genetic susceptibility in families predisposed to high risk of the disease. According to autosomal dominant mode of inheritance, every patient has 50% chance to have an affected child.<sup>[17]</sup>

The purpose of this study was to identify and diagnose patients with MODY among Isfahan province diabetic patients and determining the frequency of different types of MODY based on the mutated gene.

## Materials and Methods

### Subjects

The medical files of about 2000 diabetic patients with early-onset diabetes were checked to find MODY patients. Of them, about sixty cases having MODY criteria (onset age under 25, no insulin consumes or low dose usage, strong history of diabetes in the family, and

no history for keto-acid attacks) were selected for more evaluations. Written informed consent and informational questionnaires were taken from all the individuals. Five milliliters of peripheral blood were drawn from affected and healthy members of selected families, and conserved in ethylenediaminetetraacetic acid-containing tubes.

### Clinical examination

There are some nongenetic biomarkers that are important in distinguishing MODY from T1D and T2D such as anti-pancreatic beta cell autoantibodies and serum C-peptide levels.<sup>[17,18]</sup> A few case reports described antibody-positive MODY patients with a prevalence of <1%.<sup>[19]</sup> Thus, we measured anti-insulin II, anti-GAD65 autoantibodies, and serum C-peptide levels in all the patients. Antibody positivity and undetectable levels of C-peptide were used as exclusion criteria for MODY in the present study. Finally, 35 families were selected for the study.

### Molecular analysis

#### DNA extraction

DNA was extracted from peripheral blood lymphocytes using Cinnagen DNA extraction kit (E × 6071, Cinnacolon Company, Iran). A NanoDrop 2000 spectrophotometer (Thermo Scientific Inc., Wilmington, DE, USA) was used to determine DNA concentration and purity.

#### Polymerase chain reaction-sequencing and mutation screening of *GCK* and *HNF1A* genes

Based on previous studies in different populations, mutations in *GCK* (MODY2) and *HNF1A* (MODY3) genes account for about 30%–80% of MODY cases.<sup>[20,21]</sup> Primers for all the 11 exons of the *GCK* gene and 10 exons of the *HNF1A* gene, encompassing at least 60 flanking nucleotides, were designed by primer3 ver. 0.4.0 (<http://frodo.wi.mit.edu/primer3/>). All the exons of these two genes were amplified by polymerase chain reaction (PCR). DNA sequencing of the PCR products were carried out by an ABI 3730XL automated sequencer (Applied Biosystems, Macrogen, South Korea) using the same forward primers. Reaction conditions to amplify exons in 30 µl was as follows: 0.5 µl of each of the primers (10 µM), 4 µl DNA sample, 15 µl Ampliqon PCR master mix (Taq 2x Master Mix RED. 1.5 mM MgCl<sub>2</sub>, ID: 5200300-1250) and 10 µl ddH<sub>2</sub>O. All the variants in these two genes were analyzed.

#### Whole-exome sequencing

About 300 ng of genomic DNA was sent to Macrogen (South Korea) and was subjected to whole-exome sequencing (WES) using a NovaSeq 4000 platform (Illumina, US). The mean depth of coverage was × 100, and 92% of targeted regions were covered. After performing WES, the dataset was analyzed. The released raw data were converted to the FASTQ file.

Bioinformatics analysis included using burrows-wheeler aligner for read mapping to the reference genome (hg19, NCBI Build 38), Picard for removal of duplicate reads, and GATK for variant calling. Variant filtering was performed based on MAF <1% (MAF: Minor Allele Frequency) in dbSNP version 147, 1000 genomes project phase 3 database, NHLBI GO exome sequencing project, exome aggregation consortium (ExAC), and Iranome (local database) for missense, nonsense, splice site, stop loss, start codon change, frame-shift, and in-frame indels. Then, all the variants were analyzed and in order to validate any of them, MutationTaster2, MutPred, SIFT, Mutation Assessor, PolyPhen-2, and PROVEAN were applied. The highest probable predictions were filtered. Then, the pathogenicity of these variants was evaluated by ACMG guideline.<sup>[22]</sup> The effect of variants on protein stability was assessed using mCSM.<sup>[23]</sup> Co-segregation analysis was done using forward and reverse primers for 10 that cover the variant region. The sequence of used primers was F: GGGTCTCAGCTTCCCTTCC and R: GGGGCTCTGCCAGTAACTCT.

## Results

### Clinical findings

Many studies show that most of the MODY patients are misdiagnosed as T1D patients. Patients with detectable C-peptide levels and no high levels of pancreatic autoantibodies are suspected to be MODY (with a higher probability than 95%). From a population of about 2000 diabetic patients with a duration of 3–7 years of diabetes and onset of disease before 25 years old, 35 families were suspected to MODY by clinical check out (pancreatic antibodies and C-peptide)

### GCK and HNF1A genes sequencing results

Among ten families in whom all the exons of *GCK* and *HNF1A* genes were sequenced in all their members (five for *GCK* and five for *HNF1A*), several nonpathogenic polymorphisms were found. dbSNP (<https://www.ncbi.nlm.nih.gov/snp/>), clinvar (<https://www.ncbi.nlm.nih.gov/>

clinvar/), ExAC (<http://exac.broadinstitute.org>), and 1000 genome databases were searched for detected variants, and any of them was not disease causing mutation.

### Whole-exome sequencing results

After WES analysis in two patients, a missense mutation (p.Ilu488Thr) in the exon 10 of the *CEL* gene was detected in one of them [Table 1]. Mutations in the *CEL* gene can cause MODY8.<sup>[14]</sup> The proband (Isf-12) was a 24-year-old girl with a diabetes duration of 6 years. She was from a consanguineous family (first cousins) with a strong history of diabetes in the family [pedigree is shown in Figure 1]. While the proband and her older diabetic brother were treated with oral hypoglycemic drugs at first, both of them and also their father, were receiving insulin for about 4–6 years after the onset of the disease. The variant (p.Ilu488Thr) was previously reported as a pathogenic variant in 2014.<sup>[24]</sup> The frequency of this variant is 0.01 in Iranian population ([www.iranome.ir](http://www.iranome.ir)) [Table 2].

Co-segregation analysis showed variant segregation with diabetes in heterozygous state in all affected members of the family. The electropherogram of the variant region in the proband, her diabetic father, and her healthy mother is shown in Figures 1 and 2.

The second proband was a 16-year-old girl suffering from diabetes for 4 years. Her father, grandmother, 2 uncles, aunt, and 1 cousin were diabetic too

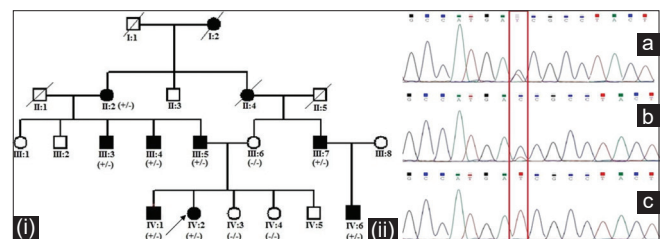


Figure 1: (i) The pedigree of family Isf-12. Co-segregation of the variant in the members of the family is shown by +: being positive for the variant and -: being negative for the variant. (ii) The electropherogram of the mutation co-segregation in the family. (a) The proband with c.1463T>C heterozygously, (b) her diabetic father with the same variant heterozygously, and (c) her healthy mother with T nucleotide in that position homozygously

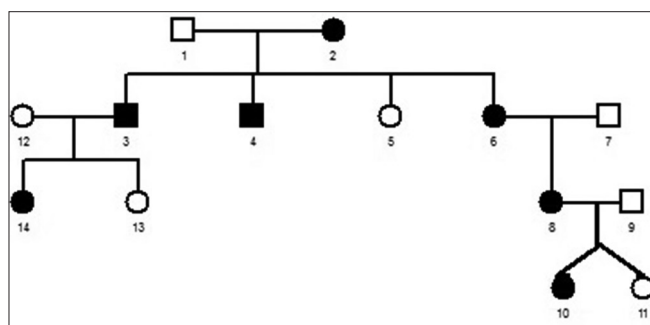
Table 1: *In silico* analysis of identified variants in the carboxyl ester lipase gene

Variant/genomic location	Exon	Amio-acid alteration	MAF	Software	Mutation Taster2.0	Polyphen-Pred	PROVEAN	SIFT	REVEL	PANTHER	Predicted ΔΔG (Kcal/mol)
c.1463T>C	10	p.I488T	C=0.0180	Prediction Score	Disease causing NA	Probably Damaging 0.995	Deleterious -3.124	Deleterious 0.00	damaging 0.615	Probably Damaging PSEP: 456 MY	Highly destabilizing -2.801
c.1235C>T	9	p.T412I	T=0.3698	Prediction Score	Disease Causing NA	Probably damaging 1.00	Deleterious -4.506	Deleterious 0.00	Pathogenic 0.901	Probably Damaging PSEP: 455 MY	Destabilizing -0.453

NA: Not available, MY: Million years, MAF: Minor allele frequency

**Table 2: Population frequency of p.I488T in carboxyl ester lipase gene in different populations of Iran**

Population	Allele count	Allele number	Number of homozygotes	Number of heterozygotes	Homozygous genotype frequency	Heterozygous genotype frequency	Allele frequency
Turkmen	2	200	0	2	0.0	0.02	0.01
Persian Gulf Islander	1	200	0	1	0.0	0.01	0.005
Persian	4	200	0	4	0.0	0.04	0.02
Lur	2	200	0	2	0.0	0.02	0.01
Kurd	1	200	0	1	0.0	0.01	0.005
Baloch	2	200	0	2	0.0	0.02	0.01
Azeri	3	200	0	3	0.0	0.03	0.015
Arab	1	200	0	1	0.0	0.01	0.005
Total	16	1600	0	16	0.0	0.02	0.01

**Figure 2: The pedigree of family Isf-9. The proband in this study was number 14**

[pedigree is shown in Figure 2]. Metformin was prescribed for glycemic control as a choice drug. The affected father suffering from hyperglycemia, hypercholesterolemia, and high blood pressure is receiving insulin now, after treating with glibenclamide for 5 years. WES detected a missense variant in the exon 9 of *CEL* gene. The variant was named as p.Thr412Ilu by MutationTaster and its Reference ID is Rs62576769. It was not found in the 1000 Genome project, but it has a frequency of 5599 in the ExAC database in heterozygous state (0 allele homozygous state). The prediction was disease causing in this online software because it is situated in a highly conserved region of the protein. SIFT and PolyPhen scores described it as a deleterious variant [Table 1]. Co-segregation analysis of this variant was also done and confirmed that it only presents in the diabetic members in heterozygous state and it was absent in the healthy members of the family. Although there were lots of supporting evidence to confirm its pathogenicity, its MAF score and the high prevalence in the Iranian population [ $>0.05$ ; Table 3] is a strong reason for its rejection as a pathogenic mutation ([www.iranome.ir](http://www.iranome.ir)).

## Discussion

MODY accounts for at least 2%–5% of all diabetes cases.<sup>[25]</sup> To date, heterozygous mutations in 14 genes have been reported for MODY subtypes [Table 4].<sup>[12]</sup> Early onset of diabetes (before the age of 25), strong family history for diabetes, autosomal dominant pattern of inheritance,

and noninsulin dependent diabetes are the most important features of MODY.<sup>[11]</sup> Considering that most of MODY cases are misdiagnosed as T1D or T2D, and due to the importance of diagnosing this type of diabetes, the aim of this study was to identify and determine the subtypes of MODY in a cohort of diabetic patients in Isfahan province of Iran.

To date, 14 different types of MODY have been reported until now. MODY8 (OMIM 609812, CEL-MODY) is the result of heterozygous mutations in the *CEL* gene, which accounts for <1% of all MODY cases.<sup>[27]</sup> This gene is located on chromosome 9q34.13 and consists of 11 exons. The *CEL* gene is not transcribed in beta cells, but is mostly expressed in pancreatic acinar cells<sup>[28,29]</sup> and lactating mammary glands.<sup>[30]</sup> CEL is a CEL product that is secreted from the pancreas as a glycoprotein into the digestive tract, and it hydrolyzes dietary fat, cholesteryl esters, and fat-soluble vitamins in the duodenum.<sup>[31]</sup> Symptoms of *CEL* mutations/MODY8 are pancreatic lipomatosis (fatty replacement of pancreatic parenchyma) and fecal elastase levels manifesting before the age of 25, followed by the development of diabetes and pancreatic cysts later in life and lipomatosis.<sup>[14]</sup> The CEL protein represents 4%–8% of the total secreted protein in human pancreatic juice.<sup>[32]</sup> The mutant protein forms intracellular and extracellular aggregates, and misfolding of the protein leads to MODY8.<sup>[33]</sup>

Pathogenic gain-of-function effect of the altered protein has been shown as the cause of the diabetes by activation of maladaptive cell signaling pathways.<sup>[34,35]</sup> Vesterhus *et al.* in 2010 showed that mice with knocked-out *CEL* gene do not show diabetes or exocrine dysfunction, indicating that MODY in *CEL* mutated cases is not due to haploinsufficiency.<sup>[36]</sup> In another study, patients with MODY8 indicated that the mutant CEL protein causes inflammatory changes in pancreatic tissue, expressing a cytotoxic link between mutant CEL and cellular proteins.<sup>[37]</sup>

The most common type of mutation that causes MODY in this gene is missense/nonsense (6 of 13 described in HGMD-professional 2019.1) (<http://www.hgmd.cf.ac.uk>). Six of these 13 mutations cause MODY and/

**Table 3: Population frequency of p.T412I in carboxyl ester lipase gene in different populations of Iran**

Population	Allele count	Allele number	Number of homozygotes	Number of heterozygotes	Homozygous genotype frequency	Heterozygous genotype frequency	Allele frequency
Turkmen	13	126	1	11	0.0159	0.1746	0.1032
Persian Gulf Islander	29	186	1	27	0.0108	0.2903	0.1559
Persian	20	154	1	18	0.013	0.2338	0.1299
Lur	31	162	2	27	0.0247	0.3333	0.1914
Kurd	39	196	2	35	0.0204	0.3571	0.199
Baloch	28	194	0	28	0.0	0.2887	0.1443
Azeri	31	176	1	29	0.0114	0.3295	0.1761
Arab	33	182	1	31	0.011	0.3407	0.1813
Total	224	1376	9	206	0.0130814	0.2994186	0.1628

**Table 4: Molecular and clinical characteristics of maturity-onset diabetes of the young subtypes**

Type of MODY	Gene	Chromosome	Frequency (%)	Pathophysiology
MODY 1	<i>HNF4A</i>	20q12	5	Neonatal hyperinsulinemia, low triglycerides, $\beta$ -cell dysfunction
MODY 2	<i>GCK</i>	7p15	25-80	$\beta$ -cell dysfunction, fasting hyperglycemia
MODY 3	<i>HNF1A</i>	12q24	20-50	$\beta$ -cell dysfunction, glycosuria
MODY 4	<i>IPF1/PDX1</i>	13q12	<1	$\beta$ -cell dysfunction, pancreatic agenesis
MODY 5	<i>HNF1A</i>	17q21	5	$\beta$ -cell dysfunction, renal anomalies, genital anomalies, pancreatic hypoplasia
MODY 6	<i>NEUROD1</i>	2q32	<1	$\beta$ -cell dysfunction
MODY 7	<i>KLF11</i>	2p25	<1	$\beta$ -cell dysfunction
MODY 8	<i>CEL</i>	9q34	<1	Pancreas endocrine and exocrine dysfunction, exocrine insufficiency, lipomatosis
MODY 9	<i>PAX4</i>	7q32	<1	$\beta$ -cell dysfunction, ketoacidosis
MODY 10	<i>INS</i>	11p15	<1	Can also present PNDM
MODY 11	<i>BLK</i>	8p23	<1	Insulin secretion defect
MODY 12	<i>ABCC8</i>	11p15	<1	PNDM (homozygote) or TNDM (heterozygote)
MODY 13	<i>KCNJ11</i>	11p15	<1	NDM
MODY 14	<i>APPL1</i>	3p14	<1	Recently described <sup>[26]</sup>

MODY: Maturity-onset diabetes of the young, NDM: Neonatal diabetes mellitus, TNDM: Transient NDM, PNDM: Permanent NDM

hypercholesterolemia, three cause only MODY, two cause diabetes and pancreatic exocrine dysfunction, and the other two cause high level of low-density lipoprotein (LDL)-cholesterol and chronic pancreatitis. These data and the clinical examination of our first proband and his family were clues that the variant is pathogenic. The father, the grandmother, and the affected uncles of the proband had high levels of LDL, but the proband (25 years old girl) and her 31-year-old brother, have not shown evidence of high LDL levels yet.

The most common types of MODY were reported as MODY2 and MODY3 in many studies in populations other than Iran.<sup>[38]</sup> However, there are few studies on MODY to provide data concerning the frequency of different types of MODY in Iran. Moghbeli *et al.*, in 2017 reported that among 34 Iranian families of MODY patients, only 2% of them harbored mutations in *HNF1A* gene.<sup>[39]</sup> Whereas, in our previous study in 2019, we reported a frequency of 20% for *HNF1A* gene mutation from a population of ten patients with MODY criteria in Iran.<sup>[40]</sup> Thus, it seems that the frequency of different types of MODY in Iran is different to other populations and more studies on larger

populations are needed. Similarly, in a study in 2015 in China, the authors showed that the frequency of mutated genes among Asian and other populations was different.<sup>[41]</sup> In our study, no pathogenic variant in *GCK* and *HNF1A* genes was found. However, using Sanger sequencing of all exons of these corresponding genes, several single-nucleotide polymorphisms (SNPs) were identified which have been associated with T2D. For example, George *et al.*, in 2014 showed that the T nucleotide in the position of rs2908274 in exon 6 of the *GCK* gene has association with MODY2.<sup>[42]</sup> Another variant was rs1169310. The A allele is associated with increased plasma hsCRP levels, MODY, and T2D.<sup>[43]</sup> In a study in India using WES to indicate the cause of disease in 56 MODY patients, in addition to 12 pathogenic mutations they have reported, they also found some potentially damaging variants related to diabetes in *HNF4A*, *GCK*, *HNF1A*, *PDX1*, *HNF1B*, *NEUROD1*, and *PAX4*. However, they did not meet the criteria for being categorized as pathogenic variants. This could demonstrate a much more complex landscape of MODY.<sup>[44]</sup> The p.T412I polymorphism which was found in our second proband was found to be co-segregating in

all members of the family, which was reported in another study in 2015 in China.<sup>[41]</sup> Therefore, it might be a high-risk variant contributing to diabetes. In agreement with the findings, we found that this variant fulfilled several lines of evidence, suggesting it as a high-risk variant: (1) This variant was introduced as a pathogenic variant based on its SIFT, PolyPhen, REVEL, and PANTHER score and Mutation Taster prediction [Table 1] and (2)  $\Delta\Delta G$  of this variant was assessed using a programme mCSM and it showed that the variant might result in destabilization of the protein ( $\Delta\Delta G = -0.453$ ).<sup>[23]</sup>

However, due to the high frequency of this variant, it could not be considered a pathogenic variant in our population. Therefore, our study supports the evidence that this variant is a high-risk factor for developing familial diabetes.

As in many studies on different populations, a high percentage of MODY patients did not show mutations on known genes, these so-called MODYX types should be subjected to further studies for possible identification of new genes and gene interactions.

## Conclusion

Few studies have been performed on MODY and its subtypes in the Iranian population. Our results suggest that mutations in the *CEL* gene might be involved in early-onset diabetes. The gene should be studied in a larger sample size in Iran for a more thorough understanding of its role in causing MODY. In this study, WES was employed to determine the type of MODY in one Iranian family. In the second family, another variant (p.T412I) was identified. Based on our current understanding, it does not meet the pathogenic criteria. However, it could well be a high-risk susceptibility variant. Further studies using WES are warranted to clarify the mutation profile of MODY in Iran.

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## Conflicts of interest

There are no conflict of interest.

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