**Name of Journal:** *World Journal of Diabetes*

**Manuscript NO:** 68300

**Manuscript Type:** ORIGINAL ARTICLE

***Observational Study***

**Comprehensive genetic screening reveals wide spectrum of genetic variants in monogenic forms of diabetes among Pakistani population**

Rafique I *et al*. Genetic variants of monogenic diabetes, Pakistan

Ibrar Rafique, Asif Mir, Shajee Siddiqui, Muhammad Arif Nadeem Saqib, Asher Fawwad, Luc Marchand, Muhammad Adnan, Muhammad Naeem, Abdul Basit, Constantin Polychronakos

**Ibrar Rafique, Asif Mir,** Department of Biological Sciences, International Islamic University, Islamabad 44000, Pakistan

**Ibrar Rafique, Luc Marchand, Constantin Polychronakos,** Departments of Pediatrics and Human Genetics, McGill University Health Centre Research Institute, Montreal H4A 3J1, Canada

**Ibrar Rafique,** Research Development and Coordination, Pakistan Health Research Council, Islamabad 44000, Pakistan

**Shajee Siddiqui,** Department of Medicine, Pakistan Institute of Medical Sciences, Islamabad 44000, Pakistan, Pakistan

**Muhammad Arif Nadeem Saqib,** Department of Medical Laboratory Technology, National Skills University, Islamabad, 44000, Pakistan

**Asher Fawwad,** Department of Biochemistry, Baqai Institute of Diabetology and Endocrinology, Baqai Medical University, Karachi 74600, Sindh, Pakistan

**Muhammad Adnan,** PHRC Research Centre, FJMU, Pakistan Health Research Council, Lahore 54000, Pakistan

**Muhammad Naeem,** Department of Biotechnology, Quaid-I-Azam University, Islamabad 44000, Pakistan

**Abdul Basit,** Department of Medicine, Baqai Institute of Diabetology and Endocrinology, Baqai Medical University, Karachi 74600, Sindh, Pakistan

**Author contributions:** Rafique I, Mir A, Saqib MAN, and Naeem M designed the research study; Siddiqui S, Fawwad A, Adnan M, and Basit A conducted and supervised the data collection; Marchand L analyzed the data. Polychronakos C supervised the exome analysis, revised the draft, and added intellectual content to the study; All authors critically reviewed and approved the final manuscript as submitted.

**Supported by** Canadian Institutes of Health Research, No. PJT-159715.

**Corresponding author: Constantin Polychronakos, MD, Professor,** Departments of Pediatrics and Human Genetics, McGill University Health Centre Research Institute, 1001 Decarie Boulevard, Montreal H4A 3J1, Canada. constantin.polychronakos@mcgill.ca

**Received:** May 18, 2021

**Revised:** July 14, 2021

**Accepted:** October 27, 2021

**Published online:** November 15, 2021

**Abstract**

BACKGROUND

Monogenic forms of diabetes (MFD) are single gene disorders. Their diagnosis is challenging, and symptoms overlap with type 1 and type 2 diabetes.

AIM

To identify the genetic variants responsible for MFD in the Pakistani population and their frequencies.

METHODS

A total of 184 patients suspected of having MFD were enrolled. The inclusion criterion was diabetes with onset below 25 years of age. Brief demographic and clinical information were taken from the participants. The maturity-onset diabetes of the young (MODY) probability score was calculated, and glutamate decarboxylase ELISA was performed. Antibody negative patients and features resembling MODY were selected (*n* = 28) for exome sequencing to identify the pathogenic variants.

RESULTS

A total of eight missense novel or very low-frequency variants were identified in 7 patients. Three variants were found in genes for MODY, *i.e.* *HNF1A* (c.169C>A, p.Leu57Met), *KLF11* (c.401G>C, p.Gly134Ala), and *HNF1B* (c.1058C>T, p.Ser353Leu). Five variants were found in genes other than the 14 known MODY genes, *i.e.* *RFX6* (c.919G>A, p.Glu307Lys), *WFS1* (c.478G>A, p.Glu160Lys) and *WFS1* (c.517G>A, p.Glu173Lys), *RFX6* (c.1212T>A, p.His404Gln) and *ZBTB20* (c.1049G>A, p.Arg350His).

CONCLUSION

The study showed wide spectrum of genetic variants potentially causing MFD in the Pakistani population. The MODY genes prevalent in European population (*GCK, HNF1A, and HNF4a*) were not found to be common in our population. Identification of novel variants will further help to understand the role of different genes causing the pathogenicity in MODY patient and their proper management and diagnosis.

**Key Words:** MODY; Diabetes; Genetics; Monogenic diabetes; Monogenic forms of diabetes

**©The** **Author(s) 2021.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Citation:** Rafique I, Mir A, Siddiqui S, Saqib MAN, Fawwad A, Marchand L, Adnan M, Naeem M, Basit A, Polychronakos C. Comprehensive genetic screening reveals wide spectrum of genetic variants in monogenic forms of diabetes among Pakistani population. *World J Diabetes* 2021; 12(11): 1957-1966

**URL:** https://www.wjgnet.com/1948-9358/full/v12/i11/1957.htm

**DOI:** https://dx.doi.org/10.4239/wjd.v12.i11.1957

**Core Tip:** There was a lack of data on monogenic forms of diabetes (MFD) from Pakistan, therefore this study was designed to determine the genetic variants responsible for MFD in the country. The study identified wide spectrum of genetic variants potentially causing MFD. The identification of novel variants paved the way for better understanding of genetic landscape and risk factors of MFD in the country.

**INTRODUCTION**

Monogenic forms of diabetes (MFD) result from changes in single gene. Maturity-onset diabetes of the young (MODY) is a monogenic form of diabetes. It is inherited in an autosomal dominant pattern[1] and is often misdiagnosed as type 1 or type 2 diabetes[2]. It is estimated that MODY accounts for 1%-2% of all the diabetic cases[3]. There are fourteen sub-types of MODY listed in OMIM(On-Line Mendelian Inheritance in Man) (#606391). Most common of them are *GCK, HNF1A,* and *HNF4A*.The other listed genes are *PDX1, HNF1B, NEUROD1, KLF11, CEL, PAX4, INS, BLK, ABCC8, KCNJ11,* and *APPL1*[1]. Mutations in a number of additional genes are also known to cause diabetes.

The subtypes of MODY differ with respect to hyperglycemia, age of disease onset, treatment pattern, and complications reported[[4](#_ENREF_4)].Therefore timely diagnosis is of vital importance in MFD. Previously, most common genes, *i.e.* *GCK, HNF1A,* and *HNF4A* were generally sequenced for suspected cases, but n ow with the advent of latest technologies, targeted panel sequencing or exome sequencing are standard[[5](#_ENREF_5),[6](#_ENREF_6)].

Pakistan has a huge burden of diabetes. The recent surveys showed that one out of every four people in the general population is suffering from diabetes in the country[[7](#_ENREF_7),[8](#_ENREF_8)]. Being a resource poor country, advanced diagnostic facilities are not available to the public. The literature from Pakistan is scarce on MFD[[9](#_ENREF_9),[10](#_ENREF_10)]. Therefore, this study was designed to enroll suspected MFD patients and assess the causal variants involved. To our knowledge, this was the first comprehensive study to be conducted in Pakistan on MFD.

**MATERIALS AND METHODS**

A total of 184 patients with diabetes onset before 25 years of age were considered for participation in the study. The participants were chosen from the sources indicated below:

***National Diabetes Survey (n = 80)***

The record of patients who had an onset of disease before 25 years of age was obtained from National Diabetes Survey data. This was a population-based survey carried out all over the four provinces of Pakistan. The detailed methodology is described elsewhere[[7](#_ENREF_7)]. According to World Health Organization, the patients were diagnosed as diabetic if having fasting glucose level > 126 mg/dL (7.0 mmol/L) or HbA1c > 6.5% (48 mmol/mol). Clinical information in 34 cases suggested MFD [young onset, low body mass index (BMI), mild hyperglycemia]. The glutamate decarboxylase 65 (GAD-65) ELISA testing was negative in 5 cases, which were selected for exome sequencing.

***Prospective enrollment from Lahore (n = 15)***

A total of 15 patients were enrolled from The Diabetes Clinic at PHRC Research Centre, Fatima Jinnah Medical University, Lahore. The inclusion criteria were onset of diabetes before 25 years of age with preferential first-degree family history of diabetes. The demographic information along with history of disease (onset, complications, treatment details and family history) was collected on proforma. On the basis of BMI and preserved fasting C-peptide, GAD-65 ELISA was performed on three samples where serum was available. The two that were found negative for GAD-65 and, along with another three patients having clinical features like low BMI but for whom serum was not available, were finally selected for exome sequencing. All the selected subjects had normal c-peptide values (range: 0.8-3.8 ng/mL)

***Prospective enrollment from Karachi (n = 89)***

The patients coming to the diabetes clinic for type-1 and type 2 diabetes treatments were enrolled if onset of the disease was below 25 years of age, with family history of diabetes as preference. Information on demography, treatment, and diagnosis was taken on pre-designed proforma. Height and weight were recorded for BMI. The GAD-65 ELISA was performed on 59 patients. Patients that were GAD-65 negative and additional patients with low BMI but no available serum for testing were selected for exome sequencing (*n* = 18) (Figure 1).

A total of 28 patients were selected for exome sequencing. Their median age at diagnosis was 18 years and median BMI was 22. Among them, 17 were taking treatment (14 insulin and 3 were taking OHA agent) and 5 were not taking any treatment.

The DNA was extracted using a QIAamp DNA mini kit (QIAGEN). The GAD-65 autoantibody test was done by using a KRONUS Elisa kit. C-peptide test was commercially tested in a diagnostic laboratory. Exome sequencing was performed by Genome Quebec, Canada. Exome sequencing was carried out with 50 Mb Agilent Sure select array and sequencing on Illumina Hi-seq at 50 × depth.

Written informed consent was taken from participants/parents/guardians prior to enrollment. The Ethical Clearance was taken from Institutional Bioethics Committee of Pakistan Health Research Council and Ethical Review Board of Pakistan Institute of Medical Sciences, Shaheed Zulfiqar Ali Bhutto Medical University, Islamabad.

***Data analysis***

For bioinformatics analysis, the FastQ files were processed using the best practices recommendations of the genome analysis tool kit (GATK). The reads were mapped to human reference genome GRCh37 using Burrow-Wheeler alignment (BWA-MEM). The Picard tool was used to mark and remove duplicate alignments and then indel realignment and base quality score recalibration was done. The gVCFs were generated with GATK HaplotypeCaller and joint variant calling was done. The variants with low map reads (DP < 20) and low genotype quality (GQ < 20) were discarded. Annovar was used to annotate the variants. UTR, synonymous, and intronic (unless splicing) variants were discarded by standard procedure to focus on protein altering ones. These variants were filtered for frequency in three public databases (Exome Aggregation Consortium, 1000 genomes, and gnomAD version v2.1.1). For dominant genes, maximal allele frequency cutoff was 0.0001 in any population while it was 0.005 for recessive genes (ACMG PM2 criterion). The missense variants were selected only if predicted disease-causing by the majority of 10 algorithms used (see legend to Table 1) which satisfies PP3 by ACMG/AMP criteria). The computational evidence supports deleterious effects on the gene[[11](#_ENREF_11)]. Analysis of the exome focused on the genes listed in the University of Chicago monogenic diabetes panel (<https://dnatesting.uchicago.edu/tests/monogenic-diabetes-panel>). Variant coordinates were searched for functional domains in uniport.org (results shown in Table 1).

**RESULTS**

A total of 80 cases selected from the National Diabetes Survey with the criteria of having diabetes and onset of disease before 25 years of age. The median age was 24 years. Most of them (62%) were females and average BMI was 28 kg/m2 (Table 2). Of them, based on clinical criteria and negativity for antibody, five were selected for exome sequencing. The results revealed three novel missense variants identified in three patients. Two of the variants belong to the OMIM-listed MODY genes, *i.e.* *HNF1A* (c. 169C>A, pLeu57Met) and *KLF11* (c. 401G>C, p.Gly134Ala) and one in *RFX6* (c. 919G>A, p.Glu307Lys), a gene recently described as mutated in dominant, MODY-like diabetes.

In the prospective enrollment from Lahore, 15 patients were enrolled. The mean age for onset of disease in patients was 23 years and mean BMI was 21.5 kg/m2. The fasting blood glucose on average was 239.26 mg/dL. The HbA1c ranged from 6% to 11% (Supplementary Table 1). The exome analysis revealed a variant in *HNF1B* (c. 1058C>T, p.Ser353Leu) for one patient.

In the prospective enrollment from Karachi, 89 diabetic patients were enrolled. The mean age of onset of disease was 16.5 years and the current age was 26.2 years. Mean BMI was 24.3 kg/m2. Most of them (95%) had family history of diabetes. Among them, 93% were taking treatment for diabetes and 68% were taking insulin. About 25% of the patients had a MODY probability score more than 75%. The serum was available for 59 patients. The GAD-65 autoantibody test was conducted on all patients and 15 of them were negative. The patients whose serum was not available were shortlisted on the basis of low BMI, family history, and HbA1c levels.

Among them, 18 were selected for exome sequencing, which revealed potentially causal variants in three patients (Table 1). One had a variant in *RFX6* (c. 1212T>A, p.His404Gln) and a second one was a compound heterozygote (Supplementary Figure 1) for *WFS1* (c. 478G>A, p.Glu160Lys and c. 517G>A, p.Glu173Lys). Recessive *WFS1* mutations cause Wolfram syndrome, but non-syndromic diabetes alone is also seen[[12](#_ENREF_12)]. A third patient had a variant in *ZBTB20* (c. 1049G>A, p.Arg350His).

The mutation in this gene is responsible for causing primrose syndrome[[13](#_ENREF_13)]. The patient did not have the other manifestations of this syndrome.

All variants reported here were missense, all satisfied the PP3 and PM2 criteria but, being novel and not having previously been reported, all classified as VUS. Nevertheless, our extremely low cut-off for allele frequency of 0.0001 (more than two orders of magnitude lower than the ACMG/AMP cut-off for a VUS), minimizes the probability of spurious variants. The MODY probability score was calculated for all the participants. More than half of the patients in National Diabetes Survey, all patients from Lahore and one fourth of patients from Karachi had probability scores more than 75%. The probability score was calculated by MODY probability calculator developed for Caucasian population.

**DISCUSSION**

Exome sequencing of 28 suspected patients for MFD identified missense novel variants in 7 patients (with the caveat that *KLF11, BLK,* and *PAX4* are not universally accepted as genes whose mutation causes diabetes). Previous studies from Pakistan have discussed the importance of diagnosing MFD in Pakistan[[9](#_ENREF_9),[10](#_ENREF_10)]. However, to the best of our knowledge, this is the first comprehensive study from the country to enroll suspected MFD patients for exome sequencing.

We enrolled diabetic patients with early onset of disease below 25 years of age with clinical features suggestive of MFD and negative (or unknown) for GAD65 auto antibodies. The probability score was calculated by using the MODY probability calculator developed for the Caucasian population[[14](#_ENREF_14)]. However there is a need to validate this with South Asian populations[[15](#_ENREF_15)].

In determining the type of MFD, ethnic differences play an important role. There is wide variation of prevalence of different MFD types in different areas. The *HNF1A* MODY type was reported to be more prevalent in northern Europe while *GCK* MODY types in southern Europe[[16](#_ENREF_16)]. There were similar findings from United States regarding the three major prevalent types of MODY in their population[[17](#_ENREF_18)]. The study on Russian children with non-type 1 diabetes showed that the most prevalent MODY type was *GCK,* with only 18% of variants in other than known MODY genes. The studies from China reported that *HNF4A* MODY types were relatively less common as compared to Europeans[18,19]. A study from Korea also has a similar finding that common MODY types prevalent in Europe were not common there, but instead they found variants in three new genes, including the *WFS1* gene[20]. The MODY landscape in India is also complex, reporting MODY types other than common genes known in European population[21,22]. A study from Oman reported that variants were not found in three common MODY genes[23]. Similar findings were also reported from Tunisia that common MODY types were not found there and concluded that other genes might be responsible for young onset diabetes in their population[24,25]. These discrepant results may be only partially explained by different methodologies and different selection criteria for testing.

We found in our study in a total 28 screened patients that 3 of the patients tested had variants in OMIM-listed MODY genes while 4 had variants were in other genes also known to be mutated in MFD. Similar findings were also reported from Norway[26], France[27] and Sweden[28]. Two novel missense variants were found in *RFX6* (c.G919A, p.E307K). In addition to Europeans[29], variants from this gene was also reported in studies from India[22] and Japan[30]. One patient was compound heterozygous at *WFS1*, the gene mutated in Wolfram syndrome and also responsible for non-syndromic diabetes. *WFS1*variants have been reported from India[22], China[[12](#_ENREF_12),31], Korea[[20](#_ENREF_21),32,33], Russia[[6](#_ENREF_6)], and European ancestry[[34](#_ENREF_35)]. Finally, one variant was identified in *ZBTB20*, a transcription factor that regulates the function of beta cells and glucose homeostasis[35-37]. In humans, dominant *ZBTB20* mutations cause Primrose syndrome.

The three variants in OMIM-listed MODY genes were found in *HNF1A, KLF11,* and *HNF1B*. The *HNF1A* (MODY 3) is most common type prevalent in some European and Asian countries[[38](#_ENREF_39)] and variants in this gene have been reported from various countries all around the world[[5](#_ENREF_5),39,40]. The patients with *HNF1A* variants respond well to sulfonylurea therapy[41]. The other variant was found in the *KLF11* (MODY 7)[42]; variants from this gene have been reported from France[43] and Japan[44], although recent literature disputes this gene as true MODY gene. One variant was found in *HNF1B* (MODY 5) as reported to account for 2%-6% of all the diagnosed MODY cases[45]. This type was generally found to be associated with kidney dysfunction[46]. Variants in this gene have been reported from different countries[47-49]. Although it has been considered that *KLF11*, *BLK*, and *PAX4* are not MODY causing genes, the OMIM and recent literature reported it as involved in MODY[[3](#_ENREF_3),[6](#_ENREF_6)].

Pakistan, being a developing country, is facing a huge burden of diabetes as evident from the recent survey findings that 26% of adults in the general population were suffering from diabetes[[7](#_ENREF_7)]. There is a need to identify the genetic basis of the diabetes in Pakistan, with large-scale efforts of screening. As Pakistan is a limited-resource society, it is important to develop sensitive and population-specific criteria. We propose our paper contributes as the first step in this direction.

A study reported that 56% of the marriages were consanguineous, and among them, over 49% were first cousin marriages[50,51], suggesting unknown recessive genes, such as seen in non-syndromic *WFS1* cases. Studies from Pakistan on MFD were very scarce. According to American Diabetes Association, the diagnosis of MFD should be considered when there is a family history of diabetes with atypical features of diabetes, such as lacking obesity[52]. There is a need to conduct large scale genetic studies on young onset diabetes to understand the genetic aspects from our country.

**CONCLUSION**

A wide spectrum of genetic variants involved in MFD was identified from this study. The common genes prevalent in European countries were not found common in this study. The genes other than commonly known MODY genes were identified. There is need for large scale genetic studies on early onset of diabetes in the country.

**ARTICLE HIGHLIGHTS**

***Research background***

The data on monogenic forms of diabetes (MFD) was lacking from Pakistan.

***Research motivation***

The identification of MFD from Pakistan will paved the way for better diagnostics and treatment for patients

***Research objectives***

To identify the genetic variants for MFD.

***Research methods***

Exome sequencing was used.

***Research results***

The wide spectrum of genetic variants was identified.

***Research conclusions***

The MODY genes prevalent in other countries, like those in Europe, were not found common in our population

***Research perspectives***

More studies are required, keeping in view the consanguinity rate in Pakistan

**ACKNOWLEDGEMENTS**

The authors thank all the participants and their guardians for participating in the study. Thanks to Ms. Raheela and Bilal Tahir from Baqai Institute of Diabetology and Endocrinology for assistance in patient enrollment and all National Diabetes Survey of Pakistan members.

**REFERENCES**

1 **Urakami T**. Maturity-onset diabetes of the young (MODY): current perspectives on diagnosis and treatment. *Diabetes Metab Syndr Obes* 2019; **12**: 1047-1056 [PMID: 31360071 DOI: 10.2147/DMSO.S179793]

2 **Thanabalasingham G**, Pal A, Selwood MP, Dudley C, Fisher K, Bingley PJ, Ellard S, Farmer AJ, McCarthy MI, Owen KR. Systematic assessment of etiology in adults with a clinical diagnosis of young-onset type 2 diabetes is a successful strategy for identifying maturity-onset diabetes of the young. *Diabetes Care* 2012; **35**: 1206-1212 [PMID: 22432108 DOI: 10.2337/dc11-1243]

3 **Firdous P**, Nissar K, Ali S, Ganai BA, Shabir U, Hassan T, Masoodi SR. Genetic Testing of Maturity-Onset Diabetes of the Young Current Status and Future Perspectives. *Front Endocrinol (Lausanne)* 2018; **9**: 253 [PMID: 29867778 DOI: 10.3389/fendo.2018.00253]

4 **Murphy R**, Ellard S, Hattersley AT. Clinical implications of a molecular genetic classification of monogenic beta-cell diabetes. *Nat Clin Pract Endocrinol Metab* 2008; **4**: 200-213 [PMID: 18301398 DOI: 10.1038/ncpendmet0778]

5 **Ağladıoğlu SY**, Aycan Z, Çetinkaya S, Baş VN, Önder A, Peltek Kendirci HN, Doğan H, Ceylaner S. Maturity onset diabetes of youth (MODY) in Turkish children: sequence analysis of 11 causative genes by next generation sequencing. *J Pediatr Endocrinol Metab* 2016; **29**: 487-496 [PMID: 26669242 DOI: 10.1515/jpem-2015-0039]

6 **Glotov OS**, Serebryakova EA, Turkunova ME, Efimova OA, Glotov AS, Barbitoff YA, Nasykhova YA, Predeus AV, Polev DE, Fedyakov MA, Polyakova IV, Ivashchenko TE, Shved NY, Shabanova ES, Tiselko AV, Romanova OV, Sarana AM, Pendina AA, Scherbak SG, Musina EV, Petrovskaia-Kaminskaia AV, Lonishin LR, Ditkovskaya LV, Zhelenina LА, Tyrtova LV, Berseneva OS, Skitchenko RK, Suspitsin EN, Bashnina EB, Baranov VS. Whole‑exome sequencing in Russian children with non‑type 1 diabetes mellitus reveals a wide spectrum of genetic variants in MODY‑related and unrelated genes. *Mol Med Rep* 2019; **20**: 4905-4914 [PMID: 31638168 DOI: 10.3892/mmr.2019.10751]

7 **Basit A**, Fawwad A, Qureshi H, Shera AS; NDSP Members. Prevalence of diabetes, pre-diabetes and associated risk factors: second National Diabetes Survey of Pakistan (NDSP), 2016-2017. *BMJ Open* 2018; **8**: e020961 [PMID: 30082350 DOI: 10.1136/bmjopen-2017-020961]

8 **Aamir AH**, Ul-Haq Z, Mahar SA, Qureshi FM, Ahmad I, Jawa A, Sheikh A, Raza A, Fazid S, Jadoon Z, Ishtiaq O, Safdar N, Afridi H, Heald AH. Diabetes Prevalence Survey of Pakistan (DPS-PAK): prevalence of type 2 diabetes mellitus and prediabetes using HbA1c: a population-based survey from Pakistan. *BMJ Open* 2019; **9**: e025300 [PMID: 30796126 DOI: 10.1136/bmjopen-2018-025300]

9 **Kanwal A FS,** Asghar S, Naeem M. Mody genes; linkage analysis and subgroup discovery from text documents. *Professional Med J* 2013; **20**: 623-633

10 **Ali FB**, Sohail S, Majid Z. MODY (maturity onset diabetes of the young). *J Pak Med Assoc* 2013; **63**: 1327 [PMID: 24392575]

11 **Richards S**, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E, Voelkerding K, Rehm HL; 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; **17**: 405-424 [PMID: 25741868 DOI: 10.1038/gim.2015.30]

12 **Li M**, Wang S, Xu K, Chen Y, Fu Q, Gu Y, Shi Y, Zhang M, Sun M, Chen H, Han X, Li Y, Tang Z, Cai L, Li Z, Shi Y, Yang T, Polychronakos C. High Prevalence of a Monogenic Cause in Han Chinese Diagnosed With Type 1 Diabetes, Partly Driven by Nonsyndromic Recessive *WFS1* Mutations. *Diabetes* 2020; **69**: 121-126 [PMID: 31658956 DOI: 10.2337/db19-0510]

13 **Cleaver R**, Berg J, Craft E, Foster A, Gibbons RJ, Hobson E, Lachlan K, Naik S, Sampson JR, Sharif S, Smithson S; Deciphering Developmental Disorders Study, Parker MJ, Tatton-Brown K. Refining the Primrose syndrome phenotype: A study of five patients with ZBTB20 de novo variants and a review of the literature. *Am J Med Genet A* 2019; **179**: 344-349 [PMID: 30637921 DOI: 10.1002/ajmg.a.61024]

14 **Shields BM**, McDonald TJ, Ellard S, Campbell MJ, Hyde C, Hattersley AT. The development and validation of a clinical prediction model to determine the probability of MODY in patients with young-onset diabetes. *Diabetologia* 2012; **55**: 1265-1272 [PMID: 22218698 DOI: 10.1007/s00125-011-2418-8]

15 **Ma RC**, Chan JC. Type 2 diabetes in East Asians: similarities and differences with populations in Europe and the United States. *Ann N Y Acad Sci* 2013; **1281**: 64-91 [PMID: 23551121 DOI: 10.1111/nyas.12098]

16 **Kleinberger JW**, Pollin TI. Undiagnosed MODY: Time for Action. *Curr Diab Rep* 2015; **15**: 110 [PMID: 26458381 DOI: 10.1007/s11892-015-0681-7]

17 **Bennett JT**, Vasta V, Zhang M, Narayanan J, Gerrits P, Hahn SH. Molecular genetic testing of patients with monogenic diabetes and hyperinsulinism. *Mol Genet Metab* 2015; **114**: 451-458 [PMID: 25555642 DOI: 10.1016/j.ymgme.2014.12.304]

18 **Liang H**, Zhang Y, Li M, Yan J, Yang D, Luo S, Zheng X, Yang G, Li Z, Xu W, Groop L, Weng J. Recognition of maturity-onset diabetes of the young in China. *J Diabetes Investig* 2021; **12**: 501-509 [PMID: 32741144 DOI: 10.1111/jdi.13378]

19 **Xu A**, Lin Y, Sheng H, Cheng J, Mei H, Ting TH, Zeng C, Liang C, Zhang W, Li C, Li X, Liu L. Molecular diagnosis of maturity-onset diabetes of the young in a cohort of Chinese children. *Pediatr Diabetes* 2020; **21**: 431-440 [PMID: 31957151 DOI: 10.1111/pedi.12985]

20 **Shim YJ**, Kim JE, Hwang SK, Choi BS, Choi BH, Cho EM, Jang KM, Ko CW. Identification of Candidate Gene Variants in Korean MODY Families by Whole-Exome Sequencing. *Horm Res Paediatr* 2015; **83**: 242-251 [PMID: 25765181 DOI: 10.1159/000368657]

21 **Chapla A**, Mruthyunjaya MD, Asha HS, Varghese D, Varshney M, Vasan SK, Venkatesan P, Nair V, Mathai S, Paul TV, Thomas N. Maturity onset diabetes of the young in India - a distinctive mutation pattern identified through targeted next-generation sequencing. *Clin Endocrinol (Oxf)* 2015; **82**: 533-542 [PMID: 25041077 DOI: 10.1111/cen.12541]

22 **Mohan V**, Radha V, Nguyen TT, Stawiski EW, Pahuja KB, Goldstein LD, Tom J, Anjana RM, Kong-Beltran M, Bhangale T, Jahnavi S, Chandni R, Gayathri V, George P, Zhang N, Murugan S, Phalke S, Chaudhuri S, Gupta R, Zhang J, Santhosh S, Stinson J, Modrusan Z, Ramprasad VL, Seshagiri S, Peterson AS. Comprehensive genomic analysis identifies pathogenic variants in maturity-onset diabetes of the young (MODY) patients in South India. *BMC Med Genet* 2018; **19**: 22 [PMID: 29439679 DOI: 10.1186/s12881-018-0528-6]

23 **Woodhouse NJ**, Elshafie OT, Al-Mamari AS, Mohammed NH, Al-Riyami F, Raeburn S. Clinically-Defined Maturity Onset Diabetes of the Young in Omanis: Absence of the common Caucasian gene mutations. *Sultan Qaboos Univ Med J* 2010; **10**: 80-83 [PMID: 21509085]

24 **Ben Khelifa S**, Martinez R, Dandana A, Khochtali I, Ferchichi S, Castaño L. Maturity Onset Diabetes of the Young (MODY) in Tunisia: Low frequencies of GCK and HNF1A mutations. *Gene* 2018; **651**: 44-48 [PMID: 29408271 DOI: 10.1016/j.gene.2018.01.081]

25 **Dallali H**, Pezzilli S, Hechmi M, Sallem OK, Elouej S, Jmel H, Ben Halima Y, Chargui M, Gharbi M, Mercuri L, Alberico F, Mazza T, Bahlous A, Ben Ahmed M, Jamoussi H, Abid A, Trischitta V, Abdelhak S, Prudente S, Kefi R. Genetic characterization of suspected MODY patients in Tunisia by targeted next-generation sequencing. *Acta Diabetol* 2019; **56**: 515-523 [PMID: 30656436 DOI: 10.1007/s00592-018-01283-5]

26 **Johansson BB**, Irgens HU, Molnes J, Sztromwasser P, Aukrust I, Juliusson PB, Søvik O, Levy S, Skrivarhaug T, Joner G, Molven A, Johansson S, Njølstad PR. Targeted next-generation sequencing reveals MODY in up to 6.5% of antibody-negative diabetes cases listed in the Norwegian Childhood Diabetes Registry. *Diabetologia* 2017; **60**: 625-635 [PMID: 27913849 DOI: 10.1007/s00125-016-4167-1]

27 **Donath X**, Saint-Martin C, Dubois-Laforgue D, Rajasingham R, Mifsud F, Ciangura C, Timsit J, Bellanné-Chantelot C; Monogenic Diabetes Study Group of the Société Francophone du Diabète. Next-generation sequencing identifies monogenic diabetes in 16% of patients with late adolescence/adult-onset diabetes selected on a clinical basis: a cross-sectional analysis. *BMC Med* 2019; **17**: 132 [PMID: 31291970 DOI: 10.1186/s12916-019-1363-0]

28 **Carlsson A**, Shepherd M, Ellard S, Weedon M, Lernmark Å, Forsander G, Colclough K, Brahimi Q, Valtonen-Andre C, Ivarsson SA, Elding Larsson H, Samuelsson U, Örtqvist E, Groop L, Ludvigsson J, Marcus C, Hattersley AT. Absence of Islet Autoantibodies and Modestly Raised Glucose Values at Diabetes Diagnosis Should Lead to Testing for MODY: Lessons From a 5-Year Pediatric Swedish National Cohort Study. *Diabetes Care* 2020; **43**: 82-89 [PMID: 31704690 DOI: 10.2337/dc19-0747]

29 **Patel KA**, Kettunen J, Laakso M, Stančáková A, Laver TW, Colclough K, Johnson MB, Abramowicz M, Groop L, Miettinen PJ, Shepherd MH, Flanagan SE, Ellard S, Inagaki N, Hattersley AT, Tuomi T, Cnop M, Weedon MN. Heterozygous RFX6 protein truncating variants are associated with MODY with reduced penetrance. *Nat Commun* 2017; **8**: 888 [PMID: 29026101 DOI: 10.1038/s41467-017-00895-9]

30 **Akiba K**, Ushijima K, Fukami M, Hasegawa Y. A heterozygous protein-truncating RFX6 variant in a family with childhood-onset, pregnancy-associated and adult-onset diabetes. *Diabet Med* 2020; **37**: 1772-1776 [PMID: 31001871 DOI: 10.1111/dme.13970]

31 **Wang Y**, Zhang J, Zhao Y, Wang S, Zhang J, Han Q, Zhang R, Guo R, Li H, Li L, Wang T, Tang X, He C, Teng G, Gu W, Liu F. *COL4A3* Gene Variants and Diabetic Kidney Disease in MODY. *Clin J Am Soc Nephrol* 2018; **13**: 1162-1171 [PMID: 30012629 DOI: 10.2215/CJN.09100817]

32 **Kwak SH**, Jung CH, Ahn CH, Park J, Chae J, Jung HS, Cho YM, Lee DH, Kim JI, Park KS. Clinical whole exome sequencing in early onset diabetes patients. *Diabetes Res Clin Pract* 2016; **122**: 71-77 [PMID: 27810688 DOI: 10.1016/j.diabres.2016.10.005]

33 **Park SS**, Jang SS, Ahn CH, Kim JH, Jung HS, Cho YM, Lee YA, Shin CH, Chae JH, Kim JH, Choi SH, Jang HC, Bae JC, Won JC, Kim SH, Kim JI, Kwak SH, Park KS. Identifying Pathogenic Variants of Monogenic Diabetes Using Targeted Panel Sequencing in an East Asian Population. *J Clin Endocrinol Metab* 2019 [PMID: 30977832 DOI: 10.1210/jc.2018-02397]

34 **Marchand L**, Li M, Leblicq C, Rafique I, Alarcon-Martinez T, Lange C, Rendon L, Tam E, Courville-Le Bouyonnec A, Polychronakos C. Monogenic Causes in the Type 1 Diabetes Genetics Consortium Cohort: Low Genetic Risk for Autoimmunity in Case Selection. *J Clin Endocrinol Metab* 2021; **106**: 1804-1810 [PMID: 33538814 DOI: 10.1210/clinem/dgab056]

35 **Hattersley AT**, Greeley SAW, Polak M, Rubio-Cabezas O, Njølstad PR, Mlynarski W, Castano L, Carlsson A, Raile K, Chi DV, Ellard S, Craig ME. ISPAD Clinical Practice Consensus Guidelines 2018: The diagnosis and management of monogenic diabetes in children and adolescents. *Pediatr Diabetes* 2018; **19** Suppl 27: 47-63 [PMID: 30225972 DOI: 10.1111/pedi.12772]

36 **Stellacci E**, Steindl K, Joset P, Mercurio L, Anselmi M, Cecchetti S, Gogoll L, Zweier M, Hackenberg A, Bocchinfuso G, Stella L, Tartaglia M, Rauch A. Clinical and functional characterization of two novel ZBTB20 mutations causing Primrose syndrome. *Hum Mutat* 2018; **39**: 959-964 [PMID: 29737001 DOI: 10.1002/humu.23546]

37 **Cordeddu V**, Redeker B, Stellacci E, Jongejan A, Fragale A, Bradley TE, Anselmi M, Ciolfi A, Cecchetti S, Muto V, Bernardini L, Azage M, Carvalho DR, Espay AJ, Male A, Molin AM, Posmyk R, Battisti C, Casertano A, Melis D, van Kampen A, Baas F, Mannens MM, Bocchinfuso G, Stella L, Tartaglia M, Hennekam RC. Mutations in ZBTB20 cause Primrose syndrome. *Nat Genet* 2014; **46**: 815-817 [PMID: 25017102 DOI: 10.1038/ng.3035]

38 **Rafique I,** Saqib MAN, Mir A, Naeem M. Maturity onset diabetes of the young–an overview of common types. a review. *Romanian J Diabetes Nutr Metab Dis* 2018; **25**: 209-213 [DOI: 10.2478/rjdnmd-2018-0024]

39 **Kyithar MP**, Bacon S, Pannu KK, Rizvi SR, Colclough K, Ellard S, Byrne MM. Identification of HNF1A-MODY and HNF4A-MODY in Irish families: phenotypic characteristics and therapeutic implications. *Diabetes Metab* 2011; **37**: 512-519 [PMID: 21683639 DOI: 10.1016/j.diabet.2011.04.002]

40 **Pavić T**, Juszczak A, Pape Medvidović E, Burrows C, Šekerija M, Bennett AJ, Ćuća Knežević J, Gloyn AL, Lauc G, McCarthy MI, Gornik O, Owen KR. Maturity onset diabetes of the young due to *HNF1A* variants in Croatia. *Biochem Med (Zagreb)* 2018; **28**: 020703 [PMID: 29666556 DOI: 10.11613/BM.2018.020703]

41 **Bacon S**, Kyithar MP, Rizvi SR, Donnelly E, McCarthy A, Burke M, Colclough K, Ellard S, Byrne MM. Successful maintenance on sulphonylurea therapy and low diabetes complication rates in a HNF1A-MODY cohort. *Diabet Med* 2016; **33**: 976-984 [PMID: 26479152 DOI: 10.1111/dme.12992]

42 **Neve B**, Fernandez-Zapico ME, Ashkenazi-Katalan V, Dina C, Hamid YH, Joly E, Vaillant E, Benmezroua Y, Durand E, Bakaher N, Delannoy V, Vaxillaire M, Cook T, Dallinga-Thie GM, Jansen H, Charles MA, Clément K, Galan P, Hercberg S, Helbecque N, Charpentier G, Prentki M, Hansen T, Pedersen O, Urrutia R, Melloul D, Froguel P. Role of transcription factor KLF11 and its diabetes-associated gene variants in pancreatic beta cell function. *Proc Natl Acad Sci U S A* 2005; **102**: 4807-4812 [PMID: 15774581 DOI: 10.1073/pnas.0409177102]

43 **Lomberk G**, Grzenda A, Mathison A, Escande C, Zhang JS, Calvo E, Miller LJ, Iovanna J, Chini EN, Fernandez-Zapico ME, Urrutia R. Krüppel-like factor 11 regulates the expression of metabolic genes via an evolutionarily conserved protein interaction domain functionally disrupted in maturity onset diabetes of the young. *J Biol Chem* 2013; **288**: 17745-17758 [PMID: 23589285 DOI: 10.1074/jbc.M112.434670]

44 **Ushijima K**, Narumi S, Ogata T, Yokota I, Sugihara S, Kaname T, Horikawa Y, Matsubara Y, Fukami M, Kawamura T; Japanese Study Group of Insulin Therapy for Childhood and Adolescent Diabetes. KLF11 variant in a family clinically diagnosed with early childhood-onset type 1B diabetes. *Pediatr Diabetes* 2019; **20**: 712-719 [PMID: 31124255 DOI: 10.1111/pedi.12868]

45 **Horikawa Y**, Enya M, Fushimi N, Fushimi Y, Takeda J. Screening of diabetes of youth for hepatocyte nuclear factor 1 mutations: clinical phenotype of HNF1β-related maturity-onset diabetes of the young and HNF1α-related maturity-onset diabetes of the young in Japanese. *Diabet Med* 2014; **31**: 721-727 [PMID: 24905847 DOI: 10.1111/dme.12416]

46 **Omura Y**, Yagi K, Honoki H, Iwata M, Enkaku A, Takikawa A, Kuwano T, Watanabe Y, Nishimura A, Liu J, Chujo D, Fujisaka S, Enya M, Horikawa Y, Tobe K. Clinical manifestations of a sporadic maturity-onset diabetes of the young (MODY) 5 with a whole deletion of HNF1B based on 17q12 microdeletion. *Endocr J* 2019; **66**: 1113-1116 [PMID: 31391355 DOI: 10.1507/endocrj.EJ19-0020]

47 **Dotto RP**, Santana LS, Lindsey SC, Caetano LA, Franco LF, Moisés RCMS, Sa JR, Nishiura JL, Teles MG, Heilberg IP, Dias-da-Silva MR, Giuffrida FMA, Reis AF. Searching for mutations in the HNF1B gene in a Brazilian cohort with renal cysts and hyperglycemia. *Arch Endocrinol Metab* 2019; **63**: 250-257 [PMID: 31066763 DOI: 10.20945/2359-3997000000138]

48 **Kim EK**, Lee JS, Cheong HI, Chung SS, Kwak SH, Park KS. Identification and Functional Characterization of P159L Mutation in HNF1B in a Family with Maturity-Onset Diabetes of the Young 5 (MODY5). *Genomics Inform* 2014; **12**: 240-246 [PMID: 25705165 DOI: 10.5808/GI.2014.12.4.240]

49 **Ozsu E**, Cizmecioglu FM, Yesiltepe Mutlu G, Yuksel AB, Calıskan M, Yesilyurt A, Hatun S. Maturity Onset Diabetes of the Young due to Glucokinase, HNF1-A, HNF1-B, and HNF4-A Mutations in a Cohort of Turkish Children Diagnosed as Type 1 Diabetes Mellitus. *Horm Res Paediatr* 2018; **90**: 257-265 [PMID: 30481753 DOI: 10.1159/000494431]

50 **Hina S**, Malik S. Pattern of consanguinity and inbreeding coefficient in sargodha district, punjab, pakistan. *J Biosoc Sci* 2015; **47**: 803-811 [PMID: 25299747 DOI: 10.1017/S0021932014000431]

51 **Pervaiz R**, Faisal F, Serakinci N. Practice of consanguinity and attitudes towards risk in the pashtun population of khyber pakhtunkhwa, pakistan. *J Biosoc Sci* 2018; **50**: 414-420 [PMID: 28502253 DOI: 10.1017/S0021932017000189]

52 **American Diabetes Association**. Classification and diagnosis of diabetes. *Diabetes Care* 2015; **38** Suppl: S8-S16 [PMID: 25537714 DOI: 10.2337/dc15-S005]

**Footnotes**

**Institutional review board statement:** The study was approved by the Institutional Bioethics Committee of Pakistan Health Research Council (4-87-1/16/IBC/RDC/3231) and Ethical Review Board of Pakistan Institute of Medical Sciences (1-1/2015/ERB/SZABMU).

**Informed consent statement:** All study participants or their legal guardian provided consent for participation in this study

**Conflict-of-interest statement:** The authors declare conflict of interest as none.

**Data sharing statement:** No additional data are available.

**STROBE statement:** The authors have read the STROBE statement – checklist of items, and the manuscript was prepared and revised according to the STROBE statement – checklist of items.

**Open-Access:** This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: http://creativecommons.org/Licenses/by-nc/4.0/

**Provenance and peer review:** Unsolicited article; Externally peer reviewed

**Peer-review started:** May 18, 2021

**First decision:** July 4, 2021

**Article in press:** October 27, 2021

**Specialty type:** Genetics and heredity

**Country/Territory of origin:** Canada

**Peer-review report’s scientific quality classification**

Grade A (Excellent): 0

Grade B (Very good): 0

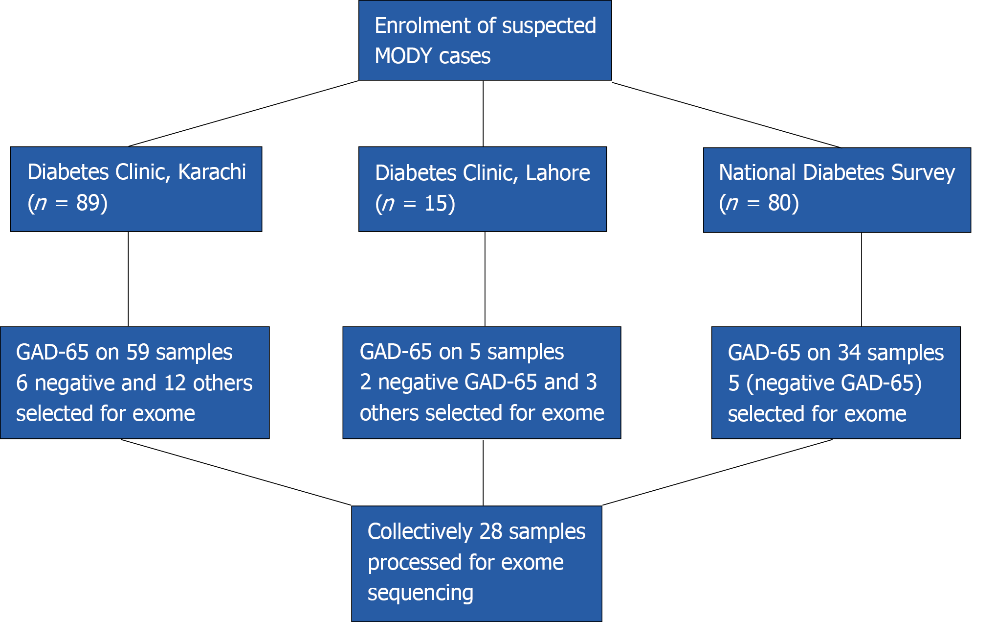
Grade C (Good): C

Grade D (Fair): D, D, D

Grade E (Poor): 0

**P-Reviewer:** Sun XD, Wen XL, Xiao X **S-Editor:** Liu M **L-Editor:** Filipodia **P-Editor:** Liu M

**Figure Legends**



**Figure 1: Flow chart for enrollment of patients.** GAD-65: Glutamate decarboxylase.

**Table 1 Rare variants in cases of monogenic forms of diabetes**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Case ID** | **Chromosome position** | **Gene symbol** | **cDNA change** | **Protein change** | **Max**  **freq.** | **In silico prediction** | **Protein region by**  **Uniport (uniport.org)** |
| 612 | 12; 1416740 | *HNF1A* | c. 169C>A | p.Leu57Met | 0 | T, NA, D, D, N, L, D, D, D, D | DNA-interacting |
| 705 | 2; 10187916 | *KLF11* | c. 401G>C | p.Gly134Ala | 0 | T, N, N, T, N, N, T, T, T, N | NA |
| 830 | 6;117237424 | *RFX6* | c. 919G>A | p.Glu307Lys | 0.0001 | T, D, D, T, N, N, T, T, D, D | NA |
| P-9 | 17; 6070581 | *HNF1B* | c. 1136C>T | p.Ser379Leu | 0.00003 | NA, D, D, NA, NA, L, D, D, D, D | NA |
| P-17 | 4; 6292941 | *WFS1* | c. 478G>A | p.Glu160Lys | 0 | T, N, N, D, N, M, D, D, D, D | NA |
| 4; 6292980 |  | c. 517G>A | p.Glu173Lys | 0 | D, D, D, D, D, M, T, T, D, D |
| P-68 | 6; 117241502 | *RFX6* | c. 1212T>A | p.His404Gln | 0.0001 | T, D, D, T, N, L, T, T, T, D | NA |
| P-87 | 3; 114069876 | *ZBTB20* | c. 1049G>A | p.Arg350His | 0 | D, D, D, T, N, N, T, T, T, D | NA |

Fathmm-MKL\_coding are listed in sequence. D: Deleterious; T: Tolerated for SIFT, LRT, MetaSVM, MetaLR\_pred, M-Cap prediction, FATHMM; N: Neutral for LRT and Provean. For mutation assessor, H: High; M: Medium; L: Low; N: Neutral; NA: Not available. SIFT: Sorting intolerant from tolerant; LRT: Likelihood ration test; Mutation taster, FATHMM: Functional Analysis through Hidden Markov Models; PROVEAN: Protein Variation Effect Analyzer; Mutation assessor, MetaSVM: Support vector machine; MetaLR: Logistic regression; M-CAP: Mendelian Clinically Applicable Pathogenicity.

**Table 2 Demographic features of patients having causal variants**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **ID** | **Age at diagnosis** | **Sex**1 | **HbA1c (%)** | **Affected**  **parent**2 |
| 612 | 25 | F | 9.2 | M and F |
| 705 | Not listed but current age 23 | M | 5.4 | NA |
| 830 | Not listed but current age 21 | F | 7.2 | NA |
| P-9 | 20 | M | 6.20 | - |
| P-17 | 15 | F | NA | M |
| P68 | 13 | M | 6.8 | M |
| P87 | 25 | M | 6.10 | M |

1M: Male; F: Female.

2M: Mother; F: Father.

徽标, 公司名称

描述已自动生成

Published by **Baishideng Publishing Group Inc**

7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA

**Telephone:** +1-925-3991568

**E-mail:** bpgoffice@wjgnet.com

**Help Desk:** https://www.f6publishing.com/helpdesk

https://www.wjgnet.com

QR 代码

描述已自动生成

**© 2021 Baishideng Publishing Group Inc. All rights reserved.**