# Identification of novel pathogenic variants in genes related to pancreatic β cell function: A multi-center study in Chinese with young-onset diabetes

**Fan Yu[1], Yinfang Tu[1], Yanfang Zhang[2], Tianwei Gu[3], Haoyong Yu[1], Xiangyu Meng[4], Si Chen[1], Fengjing Liu[1], Ke Huang[2], Tianhao Ba[5], Siqian Gong[5], Danfeng Peng[1], Dandan Yan[1], Xiangnan Fang[6], Tongyu Wang[1], Yang Hua[1], Xianghui Chen[1], Hongli Chen[1], Jie Xu[1], Rong Zhang[1], Linong Ji[5], Yan Bi[3], Xueyao Han[5], Hong Zhang[1], Cheng Hu[1,7]**

[1]Shanghai Diabetes Institute, Department of Endocrinology and Metabolism, Shanghai Key Laboratory of Diabetes Mellitus, Shanghai Clinical Centre for Diabetes, Shanghai Sixth People's Hospital Affiliated to Shanghai Jiao Tong University of Medicine, Shanghai 200233, China;
[2]Department of Endocrinology, Luoyang Central Hospital Affiliated to Zhengzhou University, Luoyang, Henan 471000, China;
[3]Department of Endocrinology, Drum Tower Hospital affiliated to Nanjing University Medical School, Branch of National Clinical Research Centre for Metabolic Diseases, Nanjing, Jiangsu 210008, China;
[4]Department of Endocrinology, The First Affiliated Hospital of Xinxiang Medical University, Xinxiang, Henan 453100, China;
[5]Department of Endocrinology and Metabolism, Peking University People's Hospital, Peking University Diabetes Center, Beijing 100044, China;
[6]Department of Endocrinology, First Affiliated Hospital of Gannan Medical University, Ganzhou, Jiangxi 341000, China;
[7]Institute for Metabolic Disease, Department of Endocrinology and Metabolism, Fengxian Central Hospital Affiliated to Southern Medical University, Shanghai 201499, China.

*To the Editor*: Maturity-onset diabetes of the young (MODY) is characterized as a non-autoimmune form of diabetes with an autosomal dominant inheritance pattern. Due to the overlapping clinical phenotypes among MODY, such as type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM), over 80% of MODY patients are initially misdiagnosed.[1] In recent decades, the advent and development of next-generation sequencing technology have facilitated a deeper understanding of MODY. High-throughput sequencing has remarkably expanded our knowledge of the pathogenic patterns of MODY across diverse populations.[2,3] Given that the prevalence of MODY varies by ethnicity, clinical or research setting, selection criteria for identifying cases, and sequencing methods, the detection rate of causative genes differs among various study populations.[4] Since the majority of previous studies on monogenic diabetes have primarily focused on populations of European descent, particularly in pediatric groups, a lack of comprehensive understanding of the clinical and genetic architecture of MODY in Chinese patients constrains the development of relevant genetic services within the health care system. Therefore, in this multi-center study, we aimed to identify novel pathogenic variants in MODY genes in three cohorts from three centers, and thereby obtain essential information for effective implementation of risk assessment, genetic counseling, and personalized therapies for those with MODY in China.

A total of 834 patients with early onset diabetes, including three cohorts and two new pedigrees, were included in this multi-center study [Supplementary Figure 1, http://links.lww.com/CM9/C343]. With reference to previous monogenic diabetes,[1] 23 genes related to MODY were included [Supplementary Table 1, http://links.lww.com/CM9/C343]. The criteria for selecting participants, high-throughput sequencing, genetic variation analysis, and pathogenicity assessment, and Sanger sequencing can be found in Supplementary Methods, http://links.lww.com/CM9/C343. This study was approved by the Ethics Committee of Shanghai Sixth People's Hospital (No. 2022-KY-164 [K]).

Access this article online

Quick Response Code:

Website: www.cmj.org

DOI: 10.1097/CM9.0000000000003514

Fan Yu, Yinfang Tu, Yanfang Zhang, and Tianwei Gu contributed equally to this work.

**Correspondence to:** Xueyao Han, Department of Endocrinology and Metabolism, Peking University People's Hospital, Peking University Diabetes Center, No. 11, Xizhimen South Avenue, Beijing 100044, China
E-Mail: xueyaohan@bjmu.edu.cn;
Hong Zhang, Shanghai Diabetes Institute, Department of Endocrinology and Metabolism, Shanghai Key Laboratory of Diabetes Mellitus, Shanghai Clinical Centre for Diabetes, Shanghai Sixth People's Hospital Affiliated to Shanghai Jiao Tong University of Medicine, 600 Yishan Road, Shanghai 200233, China
E-Mail: mingyi-hong@163.com;
Cheng Hu, Shanghai Diabetes Institute, Department of Endocrinology and Metabolism, Shanghai Key Laboratory of Diabetes Mellitus, Shanghai Clinical Centre for Diabetes, Shanghai Sixth People's Hospital Affiliated to Shanghai Jiao Tong University of Medicine, 600 Yishan Road, Shanghai 200233, China
E-Mail: alfredhc@sjtu.edu.cn



Chinese Medical Journal 2025;138(9)
**Received:** 21-10-2024; **Online:** 03-04-2025 **Edited by:** Jinjiao Li

In cohort 1, 57 rare variants (minor allele frequency [MAF] <0.1% in East Asians) of MODY genes were detected in 56 patients (27.3%, 56/205), and no rare variants were detected in the remaining 149 patients. The clinical characteristics of patients with or without MODY gene diabetes are summarized in Supplementary Table 2, http://links.lww.com/CM9/C343. According to the American College of Medical Genetics and Genomics (ACMG) guidelines, 24 rare variants were categorized as pathogenic (P). Among them, patients P18 and P137 harbored the same pathogenic variant p.E227K in *KCNJ11*. In addition, 13 variants were categorized as likely pathogenic (LP), and 20 as variants of uncertain significance (VUS) [Supplementary Table 3, http://links.lww.com/CM9/C343]. These variants included 46 missenses, three nonsenses, three frames shift deletion/insertion, four in-frame deletion/insertion, and one splice site [Supplementary Figure 2, http://links.lww.com/CM9/C343]. According to the final variant classification, 25 P and 13 LP were identified in 56 patients, and defined as "Positive cases ($N$ = 38)", achieving a diagnostic rate of 18.5% (38/205) [Supplementary Figure 3A, http://links.lww.com/CM9/C343]. Additionally, 18 patients with VUS were defined as "VUS cases ($N$ = 18)", and the remaining 149 patients with no rare variants were defined as "Negative cases ($N$ = 149)". Moreover, seven patients had rare variants in two different MODY genes (*KCNJ11* and *FOXA2*, *HNF1A* and *ABCC8*, *GCK* and *INS*, *HNF4A* and *RFX6*, *APPL1* and *RFX6*, *NEUROD1* and *BLK*, *ABCC8* and *ONECUT1*) [Supplementary Table 4, http://links.lww.com/CM9/C343]. In terms of the number of rare variants detected in this cohort, *GCK* had the most pathogenic variants. Notably, *RFX6* had the most abundant rare variants among the potential MODY genes [Figure 1]. Information regarding the identification of rare variants in cohort 1 is detailed in Supplementary Results, http://links.lww.com/CM9/C343. We reanalyzed the rare variants of *RFX6* in 205 patients after expanding the threshold of MAF in East Asians (MAF <0.5%) in cohort 1, and identified five different *RFX6* variants, including three-LP (p.H148R, p.G238E, and p.R752W) and two VUS (p.Q296K and p.S906R). Besides, two rare variants c.2398G>A (p.G800R) and c.566+5G>A were identified in two new pedigrees from Shanghai [Supplementary Table 5, http://links.lww.com/CM9/C343]. Detailed clinical information about these patients harboring *RFX6* rare variants is listed in Supplementary Table 6, http://links.lww.com/CM9/C343.

In cohort 2, the overall clinical characteristics of 70 patients from Nanjing who underwent targeted sequencing are shown in Supplementary Table 7, http://links.lww.com/CM9/C343. A total of 130 genes involved in monogenic diabetes are listed in Supplementary Table 8, http://links.lww.com/CM9/C343, and the detection rate of rare variants in MODY genes (MAF <0.1% in East Asians) was 24.3% (17/70). According to the ACMG guidelines, 10 rare variants were categorized as P, three as LP, and six as VUS [Supplementary Figure 3B and Supplementary Table 9, http://links.lww.com/CM9/C343]. Additionally, 10 P and two LP were identified in 17 patients based on the final variant classification [Supplementary Table 9, http://links.lww.com/CM9/C343], achieving a total diagnostic rate of 17.1% (12/70) [Supplementary Figure 3A, http://links.lww.com/CM9/C343]. Besides, two patients had rare variants in two different MODY genes (*GCK* and *APPL1*, *HNF1B* and *RFX6*). Notably, we identified three rare variants in *RFX6*: p.F495V and p.P660R were classified as LP, and p.L533F was classified as VUS [Supplementary Tables 5 and 9, http://links.lww.com/CM9/C343]. Detailed clinical information about the three patients harboring *RFX6* rare variants is provided in Supplementary Table 10, http://links.lww.com/CM9/C343.

In cohort 3, the overall clinical characteristics of 557 patients from Beijing who underwent whole-exome sequencing (WES) are shown in Supplementary Table 7, http://links.lww.com/CM9/C343. Nine rare *RFX6* variants (p.Q106E, p.T263M, p.Q296K, p.I321T, p.P612S, p.P660R, p.N768D, p.G815R, and p.D839N) were identified, in which p.Q106E, p.I321T, and p.P660R were classified as LP, and the remaining were classified as VUS [Supplementary Table 5, http://links.lww.com/CM9/C343]. These variants were further confirmed through Sanger sequencing [Supplementary Figure 4, http://links.lww.com/CM9/C343]. Comprehensive clinical data of patients harboring *RFX6* rare variants are shown in Supplementary Table 11, http://links.lww.com/CM9/C343. The identification of *RFX6* variants in different cohorts

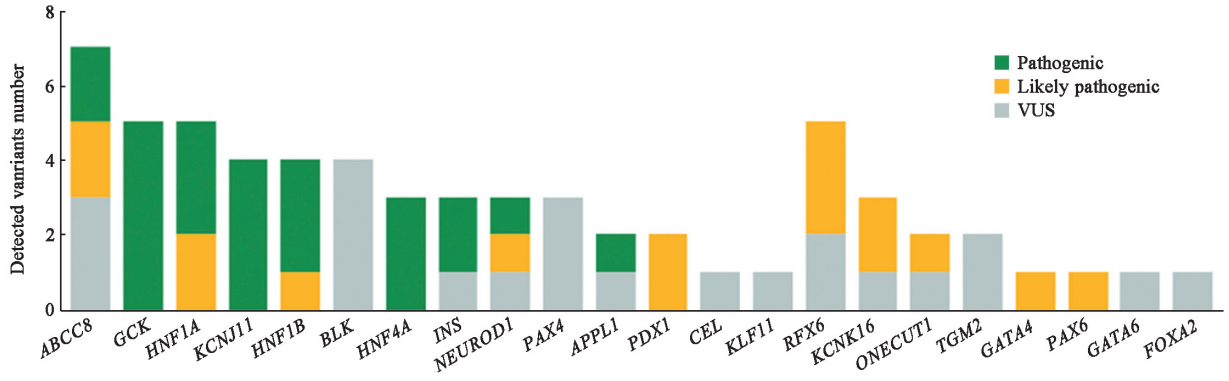


**Figure 1:** The distribution of detected rare variants in cohort 1 is classified as Pathogenic, Likely pathogenic, and VUS. Cohort 1 consisted of 205 patients with young-onset diabetes. According to the American College of Medical Genetics and Genomics guidelines, the detected rare variants for 14 common MODY genes and 8 candidate MODY genes were conducted pathogenicity assessment. VUS: Variants of uncertain significance.

is elucidated in Supplementary Results, http://links.lww.com/CM9/C343.

Furthermore, we conducted pedigree analysis on *RFX6* variants in family P82, P136, P160, P120, S1, and S2 [Supplementary Figure 5A, http://links.lww.com/CM9/C343]. Sanger sequencing validation is presented in Supplementary Figure 6, http://links.lww.com/CM9/C343. We found that the proband, carrying a variant in *RFX6* and another diabetes-related gene from each parent, developed diabetes at an earlier age, probably due to the combined effect of dual-genetic variants. We also mapped the rare variants of *RFX6* onto a protein structure pattern diagram [Supplementary Figure 5B, http://links.lww.com/CM9/C343]. The conservatism of *RFX6* variants were shown in Supplementary Figure 7, http://links.lww.com/CM9/C343. Detailed information of *RFX6* varaints and pedigree analysis for each family are provided in Supplementary Results, http://links.lww.com/CM9/C343.

A previous study with 36 European patients identified that *RFX6* heterozygous nonsense variants (p.L292X and p.K351X) were associated with MODY with reduced penetrance.[5] This multi-center study of 834 individuals with young-onset diabetes, from various hospitals, enabled the identification of 17 novel rare variants of *RFX6*, including 15 missenses and two splicings. The majority of these variants are highly conserved evolutionarily, strongly indicating the deleterious pathogenicity in MODY. To advance molecular diagnostics for MODY, this multi-center study systematically investigated the spectrum of *RFX6* rare variants in the Chinese population with early-onset diabetes. The high detection rate of *RFX6* rare variants in those with early onset diabetes is further evidence for considering *RFX6* as a candidate MODY gene, and for its inclusion in routine molecular diagnosis of MODY.

The widespread application of high-throughput sequencing has greatly enhanced our comprehension of the dual-gene pathogenicity. In our study, the detection rate of dual-genetic variants was 3.4% (7/205) in the entire cohort 1, and 12.5% (7/56) among patients carrying rare MODY gene variants within this cohort. Meanwhile, the detection rate of dual-genetic variants was 2.9% (2/70) in the entire cohort 2, and 11.8% (2/17) among the positive cases of cohort 2. In addition to the dual-genetic variants of *RFX6* and another diabetes-related gene, we collected samples only from the probands for other dual-genetic variants, which limited our ability to assess phenotype differences or severity within their families. Due to limited sample sizes, consistent phenotypes or patterns among dual-genetic variant carriers could not be identified. Further research on larger cohorts is warranted to elucidate the precise pathogenic patterns of dual-genetic variants in MODY. We speculate that individuals carrying dual-genetic variants might display earlier onset or greater severity of diabetes (such as an increased likelihood of complications) compared to those with single gene variants.

In summary, these findings expand the known repertoire of pathogenic variants in MODY genes, offering insights into their prevalence among patients with young-onset diabetes in various Chinese cohorts. The identification of novel pathogenic variants, particularly in *RFX6*, enhances our understanding of the genetic basis of early onset diabetes and lays the foundation for improved diagnostic procedures, personalized treatment strategies, and enhanced genetic counseling services. We look forward to collaborating with other diabetes research centers in China to enroll a more comprehensive and larger cohort of early onset diabetes, which will provide deeper insights into the pathogenic patterns of dual-genetic variants.

### Acknowledgment

The authors are grateful to the patients who participated in this study and would like to acknowledge the medical staff at the Department of Endocrinology and Metabolism in Shanghai Sixth People's Hospital, Drum Tower Hospital affiliated with Nanjing University Medical School, and Peking University People's Hospital.

### Funding

This work was supported by grants from the National Natural Science Foundation of China for Distinguished Young Scholars (No. 82325010), Medical-Engineering Cross Foundation of Shanghai Jiao Tong University (No. YG2021ZD20), Shuguang Project (No. 21SG11), Innovative Research Team of High-level Local Universities in Shanghai (No. SHSMU-ZDCX20212700), Shanghai Sixth People's Hospital Grant (No. ynhg202204), National Natural Science Foundation of China for Young Scientists (No. 82300891), Shanghai Research Center for Endocrine and Metabolic Diseases (No. 2022ZZ01002), National Key Clinical Specialty (No. Z155080000004), and Shanghai Municipal Key Clinical Specialty.

### Conflicts of interest

None.

## References

1. Thanabalasingham G, Owen KR. Diagnosis and management of maturity onset diabetes of the young (MODY). BMJ 2011;343:d6044. doi: 10.1136/bmj.d6044.
2. Maltoni G, Franceschi R, Di Natale V, Al-Qaisi R, Greco V, Bertorelli R, *et al*. Next generation sequencing analysis of MODY-X patients: A case report series. J Pers Med 2022;12:1613. doi: 10.3390/jpm12101613.
3. Yu H, Yu H, Zhang R, Peng D, Yan D, Gu Y, *et al*. Targeted gene panel provides advantages over whole-exome sequencing for diagnosing obesity and diabetes mellitus. J Mol Cell Biol 2023;15:mjad040. doi: 10.1093/jmcb/mjad040.
4. Bonnefond A, Unnikrishnan R, Doria A, Vaxillaire M, Kulkarni RN, Mohan V, *et al*. Monogenic diabetes. Nat Rev Dis Primers 2023;9:12. doi: 10.1038/s41572-023-00421-w.
5. Patel KA, Kettunen J, Laakso M, Stančáková A, Laver TW, Colclough K, *et al*. Heterozygous RFX6 protein truncating variants are associated with MODY with reduced penetrance. Nat Commun 2017;8:888. doi: 10.1038/s41467-017-00895-9.

**How to cite this article:** Yu F, Tu YF, Zhang YF, Gu TW, Yu HY, Meng XY, Chen S, Liu FJ, Huang K, Ba TH, Gong SQ, Peng DF, Yan DD, Fang XN, Wang TY, Hua Y, Chen XH, Chen HL, Xu J, Zhang R, Ji LN, Bi Y, Han XY, Zhang H, Hu C. Identification of novel pathogenic variants in genes related to pancreatic β cell function: A multi-center study in Chinese with young-onset diabetes. Chin Med J 2025;138:1129–1131. doi: 10.1097/CM9.0000000000003514