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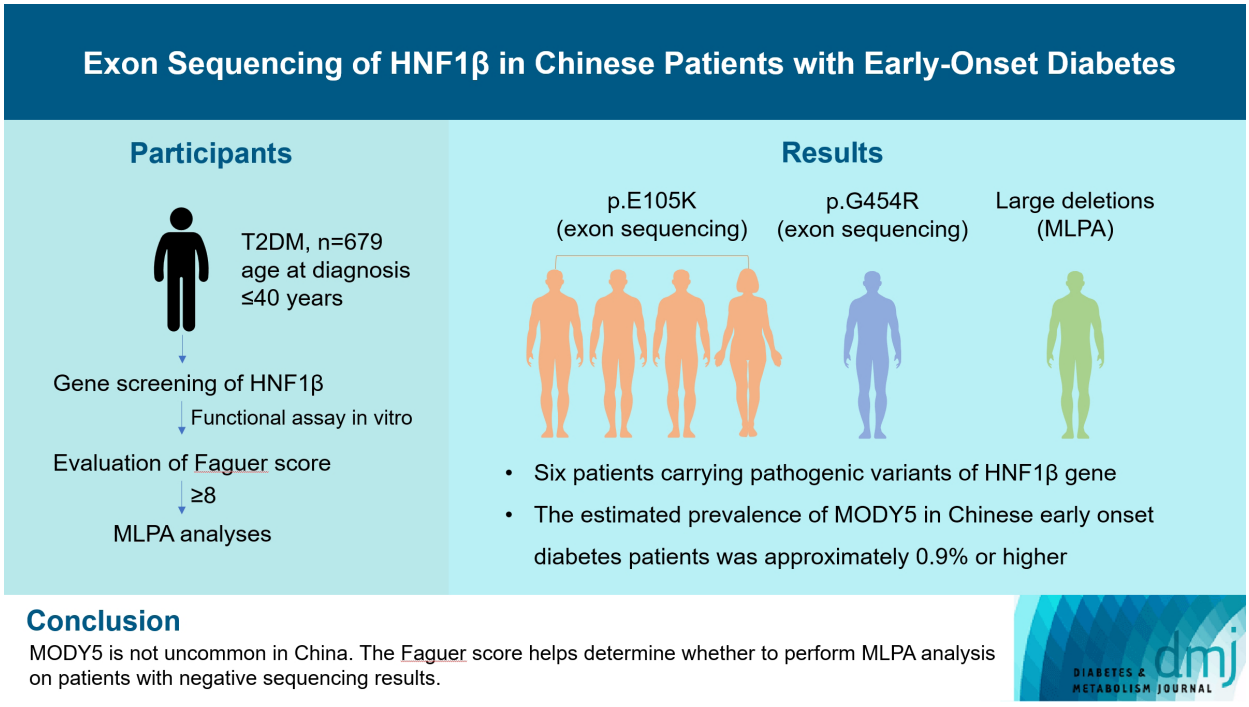

Highlights

- **MODY5 prevalence in Chinese early-onset diabetes is about 0.9% or higher.**
- **Two rare, novel HNF1β variants, E105K and G454R, were identified in the study.**
- **A Faguer score of ≥8 helps in screening patients with negative sequencing results.**

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Exon Sequencing of HNF1β in Chinese Patients with Early-Onset Diabetes

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Background: Maturity-onset diabetes of the young (MODY) due to variants of hepatocyte nuclear factor 1-beta (HNF1β) (MODY5) has not been well studied in the Chinese population. This study aimed to estimate its prevalence and evaluate the application of a clinical screening method (Faguer score) in Chinese early-onset diabetes (EOD) patients.
Methods: Among 679 EOD patients clinically diagnosed with type 2 diabetes mellitus (age at diagnosis ≤40 years), the exons of HNF1β were sequenced. Functional impact of rare variants was evaluated using a dual-luciferase reporter system. Faguer scores ≥8 prompted multiplex ligation-dependent probe amplification (MLPA) for large deletions. Pathogenicity of HNF1β variants was assessed following the American College of Medical Genetics and Genomics (ACMG) guidelines.
Results: Two rare HNF1β missense mutations (E105K and G454R) were identified by sequencing in five patients, showing functional impact *in vitro*. Another patient was found to have a whole-gene deletion by MLPA in 22 patients with the Faguer score above 8. Following ACMG guidelines, six patients carrying pathogenic or likely pathogenic variant were diagnosed with MODY5. The estimated prevalence of MODY5 in Chinese EOD patients was approximately 0.9% or higher.
Conclusion: MODY5 is not uncommon in China. The Faguer score is helpful in deciding whether to perform MLPA analysis on patients with negative sequencing results.

Keywords: Diabetes mellitus; Diabetes mellitus, type 2; Genetics, medical; Maturity onset diabetes of the young, type 5; Prevalence

INTRODUCTION

Hepatocyte nuclear factor 1-beta (HNF1β)-related diabetes mellitus is a genetic disorder caused by mutations in the *HNF1β* gene. It is a subtype of monogenic diabetes syndrome known as maturity-onset diabetes of the young subtype 5 (MODY5), or renal cyst and diabetes syndrome. This condition is characterized by diabetes, cystic kidney disease, pancreatic agenesis or dysfunction, abnormal liver function, and reproductive abnormalities. First identified in 1997 in three Japanese siblings with the *HNF1β* gene R177X mutation, the disorder's link to the *HNF1β* gene was confirmed, establishing its role in MODY5 [1].

HNF1β, a member of the transcription factor superfamily, shares significant sequence similarity with HNF1α. Crucial for endodermal development, HNF1β is extensively expressed in fetal tissues, influencing the initial development of the kidneys, pancreas, reproductive tract, and liver [2]. Mutations in the *HNF1β* gene typically lead to multisystem disorders.

Currently, defects in the *HNF1β* gene mainly involve point

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mutations and large deletions/whole-gene deletions, with the latter constituting about 50% of *HNF1β* gene abnormalities [3]. The prevalence of MODY5 varies depending on the study population and the genetic testing methods used. In cases of early-onset diabetes (EOD), MODY5 is reported to account for approximately 1.9% in Japanese individuals by direct sequencing and 4.7% in Caucasians using Sanger sequencing and large fragment deletion detection [4,5]. Within monogenic diabetes, it constitutes 13.6% in Japanese and 5.9% to 6.1% in Caucasians through direct sequencing and large fragment deletion detection [6-9].

The results of exon sequencing in a large sample of diabetes patients and the prevalence of MODY5 in the Chinese population remain unreported. Diagnosing MODY5 is challenging due to its variable phenotype and often lack of family history making genetic testing crucial. Several scholars have explored clinical screening techniques for MODY5. In 2014, Faguer et al. [10] comprehensively analyzed the clinical profiles of patients with HNF1β-related diseases from previous literature and developed the HNF1β score (Faguer score) for clinical screening. This score, which integrates information from family history, prenatal examination results, and organ involvement, aims to differentiate individuals with mutations from those without in the diabetic population. With a threshold score of 8 points, it shows high sensitivity and negative predictive value (NPV). Clissold further validated its effectiveness in 2015 [11].

Due to lack of data on MODY5 in the Chinese population and the limited research on the functional aspects of identified mutations, many pathogenic variants have been labeled as variants of uncertain significance. This classification challenge complicates understanding the actual prevalence of MODY5. Our study aims to fill this gap by exploring MODY5's prevalence and clinical characteristics in the Chinese demographic. Additionally, diagnosing MODY5 presents significant clinical challenges, emphasizing the urgent need for a practical screening system. While the Faguer score has been validated only in Caucasian populations, our study seeks to assess its utility for screening MODY5 in patients with negative DNA sequencing results in the Chinese population.

METHODS

Study overview

The study protocol was approved by the Ethics Committee of Peking University People's Hospital ([2013] No. 12).

Study populations

A total of 679 unrelated Chinese patients (age ≥18 years) with EOD who had been diagnosed with type 2 diabetes mellitus (T2DM) before the age of 40 years in the Endocrinology and Metabolism Department of the Peking University People's Hospital between September 2013 and June 2019 were included. Written informed consent was obtained from all the study participants. Diabetes diagnosis followed the 1999 World Health Organization criteria [12]. Patients exhibiting typical features of type 1 diabetes mellitus or specific forms of diabetes (such as chronic pancreatitis) and those who were positive for anti-glutamic acid decarboxylase antibodies, anti-islet cell antibodies, and anti-insulin antibodies (among individuals not receiving insulin) were excluded from this study. Patients who met these inclusion and exclusion criteria were all included in the study. The criteria for selecting the patients and the clinical and biochemical indicators of these participants were described in detail before [13,14].

Gene screening of *HNF1β* gene mutations

Genomic DNA was extracted from peripheral blood obtained from the patients. Subsequently, whole exome sequencing or target sequencing was conducted using the Roche NimbleGen human exon V2 capture chip (Madison, WI, USA) Illumina Hiseq2500 system and Hiseq4000 platform (San Diego, CA, USA). These sequencing procedures yielded data coverage (approximately 100×) exceeding 99% and a depth exceeding 200 bp, ensuring high-quality results. Data were filtered using public databases to find rare nonsynonymous variants (minor allele frequency [MAF] <0.005). Identified variants were confirmed through Sanger sequencing, using specific primers listed in Supplementary Table 1. The public databases included Exome Aggregation Consortium (ExAc, http://exac.broadinstitute.org/), 1000 Genomes Project (1000G, http://browser.1000genomes.org), Single Nucleotide Polymorphism Database (dbSNP; http://www.ncbi.nlm.nih.gov/snp), China Metabolic Analytics Project (ChinaMAP) database (http://www.mBiobank.com).

The pathogenicity of rare HNF1β variants was assessed following the guidelines outlined by the American College of Medical Genetics and Genomics (ACMG) [15]. Four computational tools (PolyPhen-2 [http://genetics.bwh.harvard.edu/pph2/index.shtml], MutationTaster [http://www.mutation-

taster.org], SIFT and CADD [http://cadd.gs.washington.edu]) were utilized for evaluation. The diagnostic criteria for MODY5 were patients with diabetes and carrying a pathogenic or likely pathogenic variant of HNF1β.

Evaluations were conducted on patients with EOD utilizing the Faguer score [10]. To identify large deletions/duplications in the *HNF1β* gene, multiplex ligation-dependent probe amplification (MLPA) (SALSA MLPA Probe mix P241-E1 MODY, MRC-Holland, Amsterdam, Netherlands) was performed on 22 patients with a Faguer score of 8 or higher (Supplementary Table 2). The obtained data were analyzed using Cofalyser.Net software.

Functional analysis of rare variants of *HNF1β* gene

The wild-type HNF1β overexpression plasmid was constructed by inserting a full-length human HNF1β transcript 1 (NM_001304286) into the pcDNA3.1 expression plasmid (LKL, Beijing, China). HNF1β variants (G454R and E105K) were generated by polymerase chain reaction and digested with *Haemophilus influenzae* Rd III (HindIII) and *Escherichia coli* RY13 I (EcoRI), and ligated into pcDNA3.1 plasmid. All constructs were verified by Sanger sequencing. The positive controls plasmid containing the variant S148W, which was previously reported [16] to experimentally validated the reduced transcriptional activity of HNF1β *in vitro*, was constructed using the same method. HNF1β serves as a transcription factor, regulating the expression of glucose transporter 2 (GLUT2). To comprehensively understand these mutation's impact, we conducted functional experiments, evaluating how HNF1β mutations influence the GLUT2 promoter's transcriptional activity. Concurrently, the INS and GLUT2 promoter sequence was respectively integrated into the PGL4.1 and PGL3 plasmid to generate two reporter plasmid. HEK-293T cells were co-transfected with the HNF1β expression plasmid and INS/GLUT2 reporter plasmid using lipo3000 (Thermofisher, Waltham, MA, USA). Cells were harvested 36 to 48 hours post-transfection for subsequent functional assays. The impact of HNF1β on INS or GLUT2 promoter activity was assessed using the Dual-Luciferase Reporter Assay System (E1910, Promega, Madison, WI, USA), following the manufacturer's instructions. The plasmid insertion sequence and Sanger sequencing results are presented in Supplementary Fig. 1.

Statistical analysis

Statistical analyses were conducted using SPSS version 22.0 for Mac (IBM Co., Armonk, NY, USA). Continuous variables are presented as mean±standard deviation (SD) for normally distributed data, and as median (interquartile range) for non-normally distributed data. Categorical variables are represented as numbers and percentages. Ranges are provided when fewer than five data points were available. Clinical characteristics between cases and controls were compared using chi-square tests, Fisher's exact tests, Student's *t*-tests, and Mann–Whitney tests.

Results from all functional experiments are expressed as mean±SD from three independent assays performed in duplicate. Statistical differences were assessed using Student's *t*-test, with P values <0.05 considered statistically significant.

RESULTS

Exon sequencing of *HNF1β* gene in the Chinese early-onset diabetes population

Screening 679 Chinese EOD patients revealed a total of five

Table 1. Rare variants in *HNF1β* gene detected in this study

Position	Exon	Base change	Protein change	dbSNP[a]	ACMG[b]	MAF[c]			
						1000G-EA	ExAc Asian	ChinaMAP	This study
36104563	Exon1	GAG>AAG	E105K	rs199572129	LP	0.003	0.0006	NA	0.0029 (4/1,358)
36047297	Exon7	GGA>CGA	G454R	NA	LP	NA	NA	NA	0.0015 (2/1,358)

RefSeq:NM_001304286.2.

HNF1β, hepatocyte nuclear factor 1-beta; dbSNP, Single Nucleotide Polymorphism Database; ACMG, American College of Medical Genetics; MAF, minor allele frequency; 1000G-EA, 1000 Genomes Project East Asian; ExAc, Exome Aggregation Consortium; ChinaMAP, China Metabolic Analytics Project; LP, likely pathogenic; NA, not applicable.

[a]NA indicates that the variant had not reported in dbSNP database, [b]The classification of rare variants identified in this study according to the standards and guidelines recommended by the ACMG, detailed in Supplementary Table 3, [c]NA indicates that the variant has no MAF in the ExAc Asian database, 1000G-EA databases, and ChinaMAP database.

cases with two rare *HNF1β* gene variants (Table 1): E105K and G454R. One patient had homozygous G454R, while four patients had heterozygous E105K (Supplementary Fig. 2). Both variants were predicted as deleterious and MAF <0.005 in the 1000G, ExAc, and ChinaMAP database (Table 1).

Results from dual-luciferase reporter gene assays indicated that the G454R and E105K mutants significantly increased GLUT2 promoter transcriptional activity compared to wild-type HNF1β, while, consistent with previous study [16], the S148W mutant decreased GLUT2 promoter transcriptional activity (Fig. 1). The same results were obtained using INS promoter plasmid (Fig. 1). Following ACMG guidelines, the G454R and E105K mutations were classified as variants of 'likely pathogenic' (Table 1, Supplementary Table 3). Diabetic patients carrying pathogenic or likely pathogenic variants in *HNF1β* gene could be diagnosed with MODY5.

MLPA analyses for patients with EOD and high-scoring Faguer score

All 679 EOD patients were evaluated by Faguer score and 22 patients had scores of 8 or higher. For these 22 patients, MLPA analyses were performed, and one patient (patient F-01) was identified to carry a heterozygous whole-gene deletion of HNF1β. This deletion spanned exons 1–9, ranging from chr17: 33121445 to 33178946 (Supplementary Fig. 3). The clinical characteristics and Faguer scores of the 22 patients are presented in Supplementary Table 2.

Clinical features of patients with MODY5 identified in this study

We identified a total of five patients with likely pathogenic point mutations and one patient with large deletion in the *HNF1β* gene among 679 patients with EOD, indicating a MODY5 prevalence of 0.9% within the Chinese EOD population.

Among the four carriers with the heterozygous E105K variant, patient 02-01 presented renal ultrasonographic abnormalities, notably hydronephrosis, accompanied by hyperuricemia. Despite insulin therapy, this patient struggled with suboptimal blood sugar control (glycosylated hemoglobin [HbA1c] >7%), reduced levels of high-density lipoprotein cholesterol, and elevated high-sensitivity C-reactive protein. Patient 03-01 exhibited elevated blood urea nitrogen levels and was prescribed oral antidiabetic medication (metformin); however, blood sugar control remained unsatisfactory (HbA1c >7%). Patient

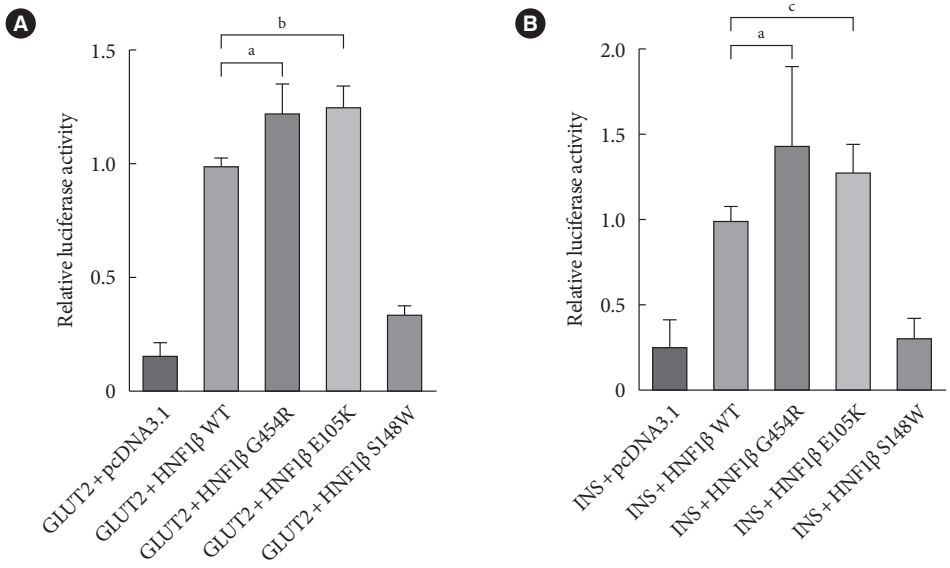

Fig. 1. Dual-luciferase reporter assay of constitutive hepatocyte nuclear factor 1-beta (HNF1β) variants. (A) Detection of transcriptional activity of different variants of HNF1β using glucose transporter 2 (GLUT2) as the promoter. (B) Detection of transcriptional activity of different variants of HNF1β using insulin (INS) as the promoter. Following the procedures outlined in the methods section, 293T cells were transfected with expression vectors containing both wild-type of HNF1β, rare variants of HNF1β screened out in this study, and the INS or GLUT2 reporter plasmid. The luciferase activity was measured relative to the GLUT2-pcDNA3.1 or INS-pcDNA3.1 control group. S148W variants is a loss-of-function mutation in *HNF1β* gene reported before, serving as a positive control in this assay. [a]$P<0.05$, [b]$P<0.01$, [c]$P<0.001$.

Table 2. Clinical characteristics of patients with MODY5 screened out in Chinese with early-onset type 2 diabetes mellitus

Characteristic	01-01	02-01	03-01	04-01	05-01	F-01
Mutation	E105K	E105K	E105K	E105K	G454R	Large deletions
Type	Het	Het	Het	Het	Homo	Het
Sex	Female	Male	Male	Male	Male	Male
Duration of diabetes, yr	22	0.1	4	0.1	1	7
Age at examination, yr	45	31	34	27	30	42
Family history of diabetes	ND	Yes	Yes	Yes	No	Yes
Therapy[a]	OHD (glibenclamide)	Insulin	OHD (metformin)	No	OHD (metformin)	Insulin
BMI, kg/m^2	28.7	34.4	26.7	37.7	24.7	14.4
WC, cm	92	117	88	116	92	ND
SBP, mm Hg	130	140	110	110	133	87
DBP, mm Hg	ND	100	60	83	88	65
CHD	No	No	No	No	No	No
Stroke	No	No	No	No	No	No
Hypertension	No	Yes	No	No	No	No
DR	No	No	Yes	No	No	No
DKD	No	No	No	Yes	No	No
FPG, mmol/L	7.80	7.70	11.37	9.34	5.86	8.71
FCP, ng/mL	2.92	2.61	ND	4.14	1.19	0.54
Fins, μU/mL	17.85	ND	ND	21.18	5.06	2
HbA1c, % (mmol/mol)	6.8 (50.8)	12.1 (108.7)	10.2 (88.0)	8.8 (72.7)	6.7 (49.7)	16.5 (156.8)
ALT, U/L	ND	46	22	89	20	48
AST, U/L	ND	37	18	44	14	85
Urea, mmol/L	ND	3.64	8.95	4.33	4.13	7.28
Scr, μmol/L	ND	73	70	86	63	71
UA, μmol/L	ND	514	265[b]	446	445	531
TC, mmol/L	3.44	2.38	5.91	4.81	4.06	5.07
TG, mmol/L	1.02	1.11	1.77	2.15	1.24	1.27
LDL-C, mmol/L	1.74	0.86	3.71	2.57	2.57	2.62
HDL-C, mmol/L	1.03	0.58	1.11	1.04	1.08	1.72
Potassium, mmol/L	ND	3.43	ND	ND	ND	3.20
Magnesium, mmol/L	ND	0.87	ND	ND	ND	0.63
hs-CRP, mg/L	ND	13.06	1.10	7.09	37.43	1.05
CK, U/L	ND	116	148	113	53	43
eGFR, mL/min/1.73 m^2	ND	117	124	100.36	145	117.48
UACR, mg/g	1.24	3.09	2.03	91.26	4	12.26
Renal ultrasound	ND	Normal size and structure, left kidney has hydronephrosis	ND	ND	ND	Left renal cyst and right renal hypoplasia

MODY5, maturity-onset diabetes of the young subtype 5; Het, heterozygote; Homo, homozygote; ND, not determined; OHD, oral hypoglycemic drug; BMI, body mass index; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; CHD, coronary heart disease; DR, diabetic retinopathy; DKD, diabetic kidney disease; FPG, fasting plasma glucose; FCP, fasting C-peptide; Fins, fasting insulin; HbA1c, glycosylated hemoglobin; ALT, alanine transaminase; AST, aspartate transaminase; Scr, serum creatinine; UA, uric acid; TC, total cholesterol; TG, triglyceride; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; hs-CRP, high-sensitivity C-reaction protein; CK, creatine kinase; eGFR, estimated glomerular filtration rate; UACR, urinary albumin/creatinine ratio.
[a]Therapy, family history of diabetes, [b]The patient has a history of hyperuricemia and is currently undergoing treatment with uric acid-lowering medication.

04-01, a recently diagnosed with diabetes, displayed features such as obesity, elevated liver enzymes, hyperuricemia, and an elevated urinary albumin/creatinine ratio, partially matching the characteristics of MODY5.

Patient 05-01, bearing the homozygous G454R variant, with a non-overweight status, demonstrated significantly diminished fasting insulin and C-peptide levels, hyperuricemia, and elevated high-sensitivity C-reactive protein levels (Table 2).

Patient F-01, harboring a large deletion in the *HNF1β* gene, exhibited elevated transaminases at 32 years old and was diagnosed with T2DM at 36. At diagnosis, the patient had a low body mass index (BMI), reduced C-peptide levels, hyperuricemia, and was undergoing insulin therapy. At age 37, the patient was admitted to the gastroenterology department due to recurring vomiting and elevated liver enzymes. Comprehensive investigations ruled out drug-induced hepatitis, viral hepatitis, autoimmune liver diseases, and other potential causes. A liver biopsy performed at another hospital resulted in a diagnosis of 'diabetic hepatopathy.' Abdominal computed tomography (CT) scans revealed the absence of the pancreatic tail, a significantly reduced and non-functional right kidney, multiple small cysts in the left kidney, and no apparent abnormalities in liver size or morphology (Supplementary Fig. 4).

DISCUSSION

This study has successfully identified six MODY5 patient among 679 EOD patients, indicating a MODY5 prevalence of 0.9% in the Chinese EOD population. Among these patients, four carried the E105K heterozygous variant and one carried the G454R homozygous variant. The clinical features of these five probands were nearly indistinguishable from those of other T2DM patients. Furthermore, one patient with a complete *HNF1β* gene deletion was diagnosed using MLPA, exhibiting nearly all phenotypic characteristics of MODY5. If only considering the patient with large deletion in the *HNF1β* gene for estimating prevalence, the prevalence of MODY5 is discerned to be 0.1%. To evaluated the pathogenicity of the two novel missense variants in HNF1β, we employed *in vitro* reporter gene assays. Using a previously reported mutation known to decrease HNF1β transcriptional activity as a positive control, our findings revealed elevated transcriptional activity for these two variants.

Previous reports on MODY5 patients predominantly focused on individuals of Caucasian and Japanese descent, with limited representation from China and South Asia. The two point mutations discovered in this study were validated as gain-of-function variants through *in vitro* functional assays. Studies in in Japanese [4] and European [17,18] MODY5 families have confirmed that gain-of-function mutations in HNF1β can cause MODY5. For instance, in the Japanese MODY5 family [4], the proband, diagnosed with diabetes at 17, carries a gain-of-function mutation (S36F) and has renal cysts but normal serum creatinine levels. Both her daughter and mother also carry the mutation and were diagnosed with diabetes at 14 and 60 years of age, respectively, all treated with insulin. In the European MODY5 family [17], the proband, who carries a frameshift and gain-of-function mutation (P328L329fsdelCCTCT), was diagnosed with diabetes at 21 during pregnancy, has renal cysts, moderate impairment of creatinine clearance and is treated with insulin. Her son also carries the mutation and has renal cysts. Although the pathogenic mechanisms of gain-of-function mutations differ from loss-of-function mutations, their impact on insulin secretion remains unclear. This study identified patients with gain-of-function mutations who did not exhibit typical MODY5 features, such as renal cysts, differing from previously reported. Further research is needed to understand the pathogenic mechanisms of these gain-of-function mutations.

In this study, the prevalence of MODY5 in EOD populations in China is about 0.9%. Earlier research [3] suggested an approximately equal detection rate between large deletions and point mutations in MODY5 patients. Traditional direct sequencing methods lack the capability to detect gene copy number variations, potentially leading to the oversight of many patients with MODY5-like symptoms. Accurate diagnosis necessitates gene dosage analysis to identify large deletions/whole-gene deletions in the *HNF1β* gene. Notably, MLPA testing was exclusively conducted on patients scoring 8 or higher according to the Faguer score in this study. Consequently, the true prevalence of MODY5 in China is likely underestimated.

Historically, all reported MODY5 patients have exhibited heterozygous mutations, with no instances of homozygous mutations recorded. Given HNF1β's crucial role in endoderm development, homozygous mutations might result in early embryonic lethality. Previous research has shown that mice with homozygous deletions of the *HNF1β* gene perish at early embryonic stages [19]. However, within our cohort of individuals with EOD, we identified one patient with the G454R homozygous mutation. This patient lacked a family history of di-

abetes, exhibited a non-obese physique, and achieved optimal blood glucose control through oral hypoglycemic medication. Coupled with the functional assay results for the G454R mutation, it is conceivable that mildly detrimental biallelic gene variations may cumulatively affect the patient's phenotype.

Additionally, we identified four individuals within the Chinese EOD population who carried the E105K heterozygous mutation. These patients displayed an incomplete MODY5 phenotype and lacked uniform clinical features, making it challenging to distinguish them from typical T2DM cases. Current research lacks definitive evidence linking specific clinical features to particular pathogenic variants within HNF1β [20]. The reasons for phenotypic variability are unclear, potentially involving different genetic abnormalities, random *HNF1β* gene expression fluctuations during early development, or additional genetic and environmental factors [21]. *HNF1β* deficiency's diversity might result from irregularities in proteins related to its signaling pathway.

Previous studies do not provide definitive evidence regarding phenotypic differences between patients with complete *HNF1β* gene deletions and those with point mutations, suggesting that these functional impairments are due to gene dosage defects (haploinsufficiency). However, previous research in MODY5 cohorts [22] indicated that patients with complete gene deletions had a lower incidence of chronic kidney disease stage 3–4 or end-stage renal disease compared to patients with point mutations. In a pediatric HNF1β-related kidney disease cohort [23], patients with point mutations exhibited higher uric acid levels than those with complete gene deletions, indicating a greater impact of HNF1β point mutations on the kidneys. This may be because mutated proteins from point mutations induce endoplasmic reticulum stress, causing tubular cell damage, renal interstitial fibrosis, and impaired kidney function. Complete gene deletions do not lead to abnormal protein accumulation in the endoplasmic reticulum, making it less likely to induce endoplasmic reticulum stress and kidney damage. In our study, one patient with point mutation showed mild proteinuria (20%, 1/5), and three have hyperuricemia (75%, 3/4). In contrast, the patient with complete gene deletions exhibited kidney hypoplasia and hyperuricemia but did not show other renal functional abnormalities. The cohort study [22] also found that patients with complete gene deletions were leaner and more frequently required insulin therapy compared to those with HNF1β point mutations. This study observed similar characteristics in the patient with complete gene deletions: extremely low BMI, continuous insulin use, and poor glucose control, while patients with point mutations were generally overweight or obese, with only one patient requiring insulin therapy due to significantly elevated glucose levels newly diagnosed. This illustrates the varying mechanisms of pathogenicity associated with different types of mutations, leading to distinct MODY5 phenotypes.

The phenotype of MODY5 extends beyond diabetes and is predominantly marked by kidney disease. Virtually all documented MODY5 patients exhibit non-diabetic kidney conditions, primarily manifesting as structural abnormalities, with renal cysts being the most prevalent, accounting for approximately 73.4% of cases. Additionally, some MODY5 patients experience hypomagnesemia, hyperuricemia, and hypokalemia. Pancreatic abnormalities are also frequently observed in MODY5 patients. A previous study reported that 15 out of 20 fetuses with HNF1β mutations exhibited pancreatic hypoplasia, a condition not detectable through prenatal ultrasound [24]. This highlights the importance of considering CT scans or magnetic resonance imaging when necessary. In our study, the patient with an *HNF1β* gene deletion displayed isolated kidney and pancreatic hypoplasia, underscoring the essentiality of abdominal imaging in EOD patients.

Existing literature indicates that around half of MODY5 patients exhibit elevated liver enzymes, though the precise mechanism remains unclear. While some genes regulated by HNF1β have been identified (e.g., those linked to cystic diseases and cilia-related proteins) through candidate gene approaches, the comprehensive downstream gene set controlling HNF1β's physiological and pathological functions remains unknown. The signaling cascade governing liver phenotype development remains entirely unexplored [25]. It is speculated that elevated liver enzymes could be linked to liver insulin resistance and may also be associated with fatty liver and chronic liver damage. MODY5 patients with concurrent liver function abnormalities should avoid hepatotoxic drugs. Some MODY5 patients might have a history of neonatal cholestasis, and adults with MODY5 could experience intermittent cholestasis. The absence of cilia in bile duct epithelial cells due to developmental abnormalities is linked to cholestasis [26]. We identified two patients with point mutations (50%, 2/4) and the patient with an *HNF1β* gene deletion had elevated liver enzymes. Despite extensive examinations, including liver biopsy, a definitive diagnosis remained elusive. This case underscores the substantial value of genetic diagnosis in determining the cause of

liver damage.

The primary clinical features of HNF1β-related diabetes include renal cysts, renal and pancreatic hypoplasia, hyperuricemia, hypomagnesemia, renal dysfunction, and unexplained elevated liver enzymes. Some patients solely present with diabetes without other extrapancreatic symptoms, making diagnosis challenging and necessitating heightened clinical vigilance. In practical clinical settings, pancreatic and renal imaging tests, along with blood magnesium tests, are frequently overlooked in EOD patients. These aspects should receive greater attention in routine examinations.

Faguer et al. [10] introduced the Faguer score in 2014 as a clinical tool for HNF1β-related diseases, based on a review of previous studies. It includes 17 indicators covering family history, prenatal results, and organ involvement (kidneys, pancreas, liver, reproductive tract). The score effectively distinguishes *HNF1β* gene mutation cases, achieving 98.2% sensitivity and 41.1% specificity with a cutoff score of 8 points. Subsequently, its clinical utility was validated in a UK renal disease population, showing an receiver operating characteristic (ROC) area under the curve of 0.72 and an 85% NPV [11]. However, the Faguer score lacks certain indicators (such as blood glucose levels, BMI, pancreatic β-cell function, and response to antidiabetic medications) and the defined MODY criteria (diagnosis of diabetes at ≤25 years of age, non-obesity, and a family history of diabetes) might not perfectly fit MODY5. Consequently, its efficiency in identifying MODY5 cases among EOD patients in this study was suboptimal. The clinical data of patients carrying the two identified missense mutations in this study lack certain Faguer scoring indicators, notably electrolyte levels and abdominal imaging results. Consequently, while acknowledging the ongoing need for further refinement of the Faguer score, it is imperative to underscore the necessity of augmenting clinical data for precise Faguer scoring, particularly in individuals with EOD.

This study has its limitations: firstly, not all patients with EOD underwent large gene deletion testing, preventing an accurate determination of the true disease prevalence. Secondly, as this study's samples were drawn from a single center and did not cover different regions and populations, the representativeness of the calculated prevalence of MODY5 in the Chinese EOD population is limited. Thirdly, the data in the case study were not comprehensive, with some patients lacking renal ultrasound data and incomplete biochemical test results, including missing values for blood potassium and magnesium levels. This led to an underestimation of the Faguer score for some patients, reducing its diagnostic sensitivity in this study. Moreover, the identification of an insufficient number of cases with positive gene diagnoses posed a challenge in calculating the sensitivity of Faguer score for screening MODY5. Additionally, the lack of large deletion testing for all sequencing-negative patients hinders the computation of the score's specificity. Fourthly, this study exclusively conducted *in vitro* functional experiments to validate the identified variants. Further assessments regarding the pathogenicity of these variants and exploration of the underlying mechanisms are still needed.

In summary, MODY5 affects at least 0.9% of patients with EOD in China. Abdominal and urological imaging are essential for EOD patients. Patients with renal, pancreatic, or genital organ abnormalities, as well as those with unexplained elevation of liver enzymes, should undergo both *HNF1β* gene sequencing and large deletion analysis for a definitive diagnosis. The Faguer score holds some value in identifying MODY5, especially for patients with negative sequencing results, yet its diagnostic efficacy is not yet optimal and requires further enhancement.

SUPPLEMENTARY MATERIALS

Supplementary materials related to this article can be found online at https://doi.org/10.4093/dmj.2024.0159.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

AUTHOR CONTRIBUTIONS

Conception or design: X.H., L.J.
Acquisition, analysis, or interpretation of data: S.G., H.L., Y.L., X.C., W.L., Y.L., M.L., S.Z., R.Z., L.Z., Y.Z., Q.R., X.Z., J.C., J.W., X.Z., X.W.
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Supplementary Table 1. *HNF1β* gene primers sequences used in Sanger sequencing

		5′-3′	Length, bp	Annealing temperature, °C
E1a	Forward	TTCTTTTTCCGTCCTTGGAA	370	60
	Reverse	CTGCGCCTACCTGAGCAT		
E1b	Forward	GCCGGTCTTCCATACTCTCA	280	60
	Reverse	GACTTCTCTGGTGGGAAACG		
E2	Forward	CTCCCACTAGTACCCTAACC	290	60
	Reverse	GAGAGGGCAAAGGTCACTTCAG		
E3	Forward	AGTGAAGGCTACAGACCCTATC	365	60
	Reverse	TTCCTGGGTCTGTGTACTTGC		
E4a	Forward	TGTGTTTTGGGCCAAGCACCA	380	60
	Reverse	AACCAGATAAGATCCGTGGC		
E4b	Forward	AACCAGACTCACAGCCTGAACC	290	60
	Reverse	TCACAGGGCAATGGCTGAAC		
E5	Forward	TGCCGAGTCATTGTTCCAGG	270	60
	Reverse	CCTCTTATCTTATCAGCTCCAG		
E6	Forward	CTGCTCTTTGTGGTCCAAGTCC	280	60
	Reverse	GAGTTTGAAGGAGACCTACAG		
E7	Forward	ATCCACCTCTCCTTATTCCAG	340	60
	Reverse	ACTTCCGAGAAAGTTCAGACC		
E8	Forward	TTTGCCTGTGTATGCACCTTG	260	60
	Reverse	GCCGAGTCCATGCTTGCCAC		
E9	Forward	CTTTGCTGGTTGAGTTGGGC	200	60
	Reverse	TTCCATGACAGCTGCCCAGAG		

HNF1β, hepatocyte nuclear factor 1-beta.

Supplementary Table 2. Clinical characteristics and scoring reults of 22 cases of high Faguer-score patients

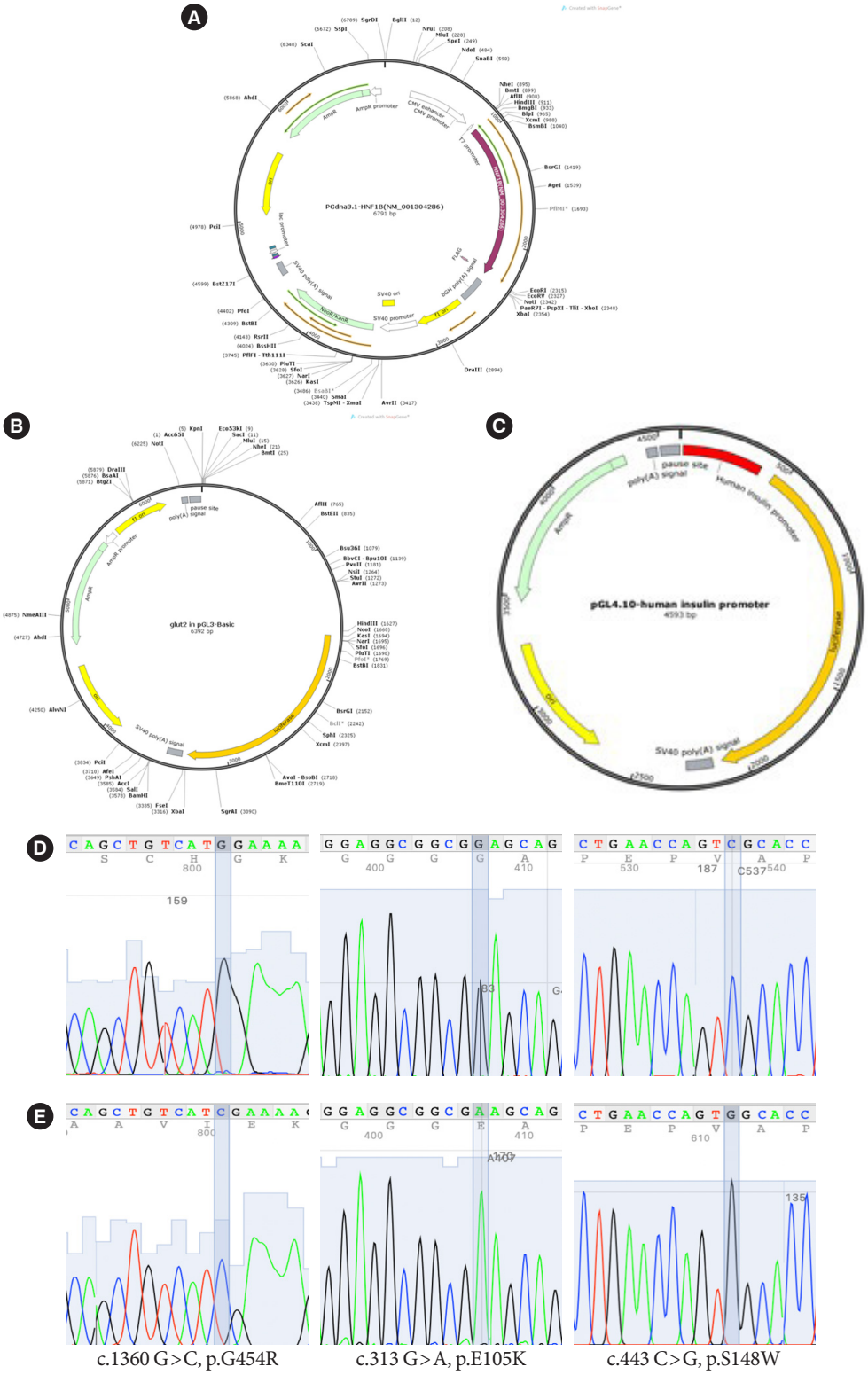
Charateristic	F-01	F-02	F-03	F-04	F-05	F-06	F-07	F-08	F-09	F-10	F-11	F-12	F-13	F-14	F-15	F-16	F-17	F-18	F-19	F-20	F-21	F-22
Sex	Male	Female	Male	Male	Male	Female	Female	Male	Female	Male	Male	Male	Female	Male	Male	Male	Male	Male	Male	Male	Male	Female
Duration of diabetes, yr	7	30	3	0	10	0.3	1	6	6	14	11	15	27	5	13	0.08	20	23	10	4	22	4
Age at examination, yr	42	60	34	30	28	27	34	30	34	47	43	55	53	29	47	31	48	57	49	40	62	34
Age at diagnosis, yr	35	30	31	30	18	27	33	24	28	33	32	40	26	24	34	30.92	28	34	39	36	40	30
History	No	Yes	Yes	No	Yes	ND	Yes	Yes	No	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
BMI, kg/m^2	14.4	23.6	27.7	25.4	25.5	27.5	20.5	26.4	25.4	28.4	26.9	25.3	26.1	25.3	25.7	21.3	27.3	27.3	27.8	27.7	26	26.6
SBP, mm Hg	87	145	ND	ND	ND	130	ND	130	149	110	121	158	141	107	144	129	140	163	143	169	152	ND
DBP, mm Hg	65	69	ND	ND	ND	85	ND	90	84	74	75	96	72	65	87	87	80	101	93	113	94	ND
Therapy	Insulin	OHA, insulin	OHA	No	OHA, insulin	OHA	No	OHA	OHA, insulin	OHA, insulin	OHA	OHA	OHA, insulin	OHA	OHA, insulin	No	Insulin	OHA, insulin	OHA, insulin	ND	ND	ND
ALT, U/L	48	16	76	62	40	31	24	12	16	13	65	24	40	14	77	132	25	59	46	29	13	9
AST, U/L	85	19	49	36	28	23	23	16	24	19	31	20	35	10	61	69	19	34	32	24	12	10
Urea, mmol/L	7.28	13.31	ND	4.05	ND	3.71	5.21	17.88	5.44	6.45	4.89	4.76	9.11	4.53	5.28	4.22	4.78	5.36	5.10	4.05	5.35	4.98
Scr, μmol/L	71	223	83	55	106	34	68	364	140	55	63	57	76	46	76	57	61	94	51	70	56	78
UA, μmol/L	531	392	536	304	606	424	583	638	300	462	350	435	435	335	377	336	371	350	424	521	208	594
TC, mmol/L	5.07	3.60	3.96	3.54	4.87	6.99	5.57	4.77	8.95	4.80	3.08	2.93	5.19	4.27	4.32	5.49	3.51	5.56	3.07	5.95	3.19	5.27
TG, mmol/L	1.27	2.16	1.45	1.32	10.78	1.44	2.57	2.21	3.48	3.48	1.99	1.74	2.29	1.48	1.86	6.80	1.75	5.20	3.34	2.46	0.92	1.10
LDL-C, mmol/L	2.62	1.96	2.77	2.59	1.16	4.95	3.67	3.16	6.16	2.94	1.85	1.91	3.69	2.83	2.95	3.39	2.2	3.49	1.32	3.96	1.71	3.61
HDL-C, mmol/L	1.72	1.21	1.16	0.67	0.72	0.97	1.20	0.90	1.51	1.04	0.78	0.82	0.94	0.81	0.92	0.97	0.96	1.01	0.94	1.13	1.08	1.21
hs-CRP, mg/L	1.05	ND	0.65	3.21	ND	20.78	2.67	0.69	14.90	0.34	0.84	2.38	0.69	2.78	2.67	0.95	0.94	2.90	ND	1.55	ND	1.20
CK, U/L	43	121	ND	64	ND	174	101	94	484	71	49	90	127	47	117	79	101	89	56	116	59	563
eGFR, mL/min/1.73 m^2	117.48	20.03	100.62	ND	77.04	249.19	101.19	16.60	42.08	117.96	114.81	109.65	77.03	143.55	103.17	129.88	112.33	77.92	119.98	112.21	105.38	85.72
FPG, mmol/L	8.71	6.97	6.38	10.63	5.32	5.67	6.73	ND	12.63	6.61	3.87	6.28	11.01	10.46	5.48	11.54	6.28	7.26	8.48	5.43	6.29	4.56
FCP, ng/mL	0.54	1.77	ND	0.85	2.98	3.28	1.71	ND	5.09	1	0.66	1.94	3.29	1.19	1.84	1.90	1.66	1.63	1.83	3.26	1.95	ND
Fins, μU/mL	2	9.42	1.51	ND	22.94	ND	4.35	ND	12.24	1.55	7.11	5.85	13.55	8.64	163.10	ND	5.90	5.80	16.70	ND	ND	ND
HbA1c, %	16.5	7	ND	11.2	6.2	6.1	6.9	5.3	ND	8.7	7.9	6.5	8.1	13.9	9.4	11.6	8.6	6.8	7.9	8.1	7.8	8.7
UACR, mg/g	12.26	3,307.13	20.79	5.54	190.84	7.13	12.43	ND	7,586.65	4.70	47.64	7.36	5,829.91	5.13	9.52	17.13	268.62	984.72	13.08	10.26	ND	ND
Potassium, mmol/L	ND	ND	ND	ND	ND	ND	ND	ND	4.05	3.44	3.79	3.88	4.10	3.67	3.52	3.03	4.22	3.61	4.20	3.76	4.02	4.66
CHD	No	No	No	No	Yes	No	No	No	No	No	No	No	No	No	No	No	0	0	0	0	0	0
Stroke	No	No	No	No	Yes	No	No	No	No	Yes	No	No	No	No	No	No	Yes	No	No	No	No	No
Hypertension	No	Yes	No	No	Yes	No	No	Yes	Yes	No	No	Yes	Yes	No	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes
DR	No	Yes	No	Yes	No	No	No	Yes	Yes	No	No	No	Yes	Yes	No	No	Yes	No	No	No	No	No
DKD	No	Yes	No	No	Yes	No	ND	Yes	Yes	No	No	No	Yes	No	No	No	Yes	Yes	No	No	No	No
Hyperuricemia	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	No	No	Yes	No	No	Yes	Yes	No	Yes
Other phenotypes	Congenital right renal hypoplasia, pancreatic tail agenesis	Chronic kidney disease	No	ND	No	Uterine and vaginal malformation, elevated transaminases	Bicornuate and arcuate uterus	No	No	Hepatic cyst	Left renal artery stenosis	Hepatic cyst	No	No	Hepatic cyst	Elevated transaminases	No	No	Coronary artery myocardial bridge, hepatic cyst	No	No	No
Renal ultrasound	Left renal cyst and right renal hypoplasia	Bilateral renal cysts	Normal	Right renal cysts	Right renal cysts, increased echogenicity of kidneys	ND	Normal	Increased echogenicity of kidneys	ND	Bilateral renal cysts	Left renal cysts	Bilateral renal cysts	Bilateral renal cysts	Left renal cysts	Left renal cysts	Left renal cysts	Left renal cysts	Bilateral renal cysts	Right renal cysts	Right renal cysts	Bilateral renal cysts	Right renal cysts
Faguer-score	12	12	8	10	16	10	8	12	8	8	10	12	8	8	10	11	8	12	8	8	12	8

ND, not determined; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; OHA, oral hypoglycemic agent; ALT, alanine transaminase; AST, aspartate transaminase; Scr, serum creatinine; UA, uric acid; TC, total cholesterol; TG, triglyceride; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; hs-CRP, high sensitivity C-reaction protein; CK, creatine kinase; eGFR, estimated glomerular filtration rate; FPG, fasting plasma glucose; FCP, fasting C-peptide; Fins, fasting insulin; HbA1c, glycosylated hemoglobin; UACR, urinary albumin/creatinine ratio; CHD, coronary heart disease; DR, diabetic retinopathy; DKD, diabetic kidney disease.

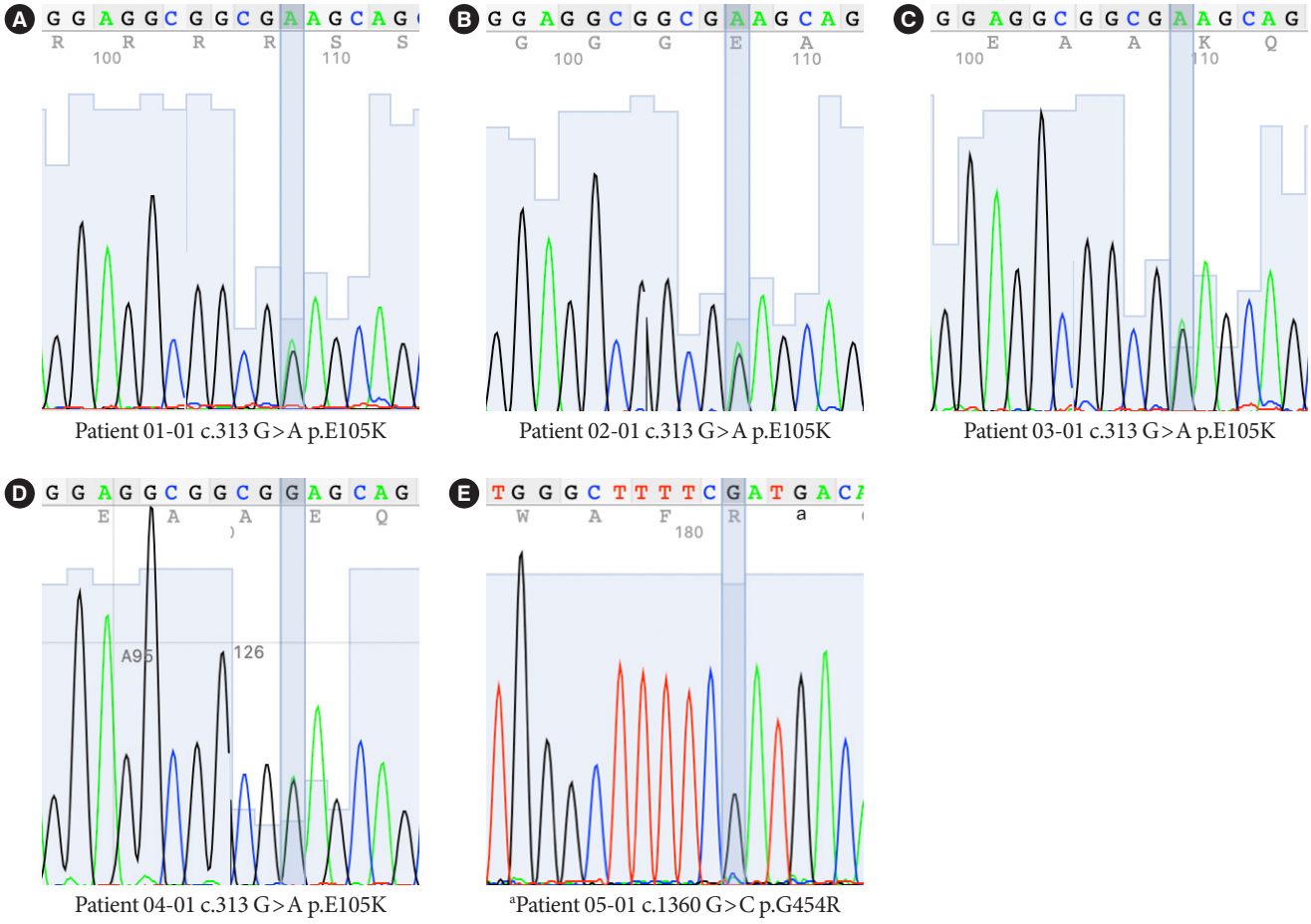
Supplementary Table 3. Pathogenicity prediction of rare variants of HNF1β identified in this study

Position	Protein change	SIFT	Mutationtaster	CADD	Polyphen-2	ACMG evidence	ACMG[a]
36104563	E105K	Tolerated	Disease causing	28.1	Damaging	PS3PM2PP3	Likely pathogenic
36047297	G454R	Damaging	Polymorphism	12.5	Damaging	PS3PM2PP3	Likely pathogenic

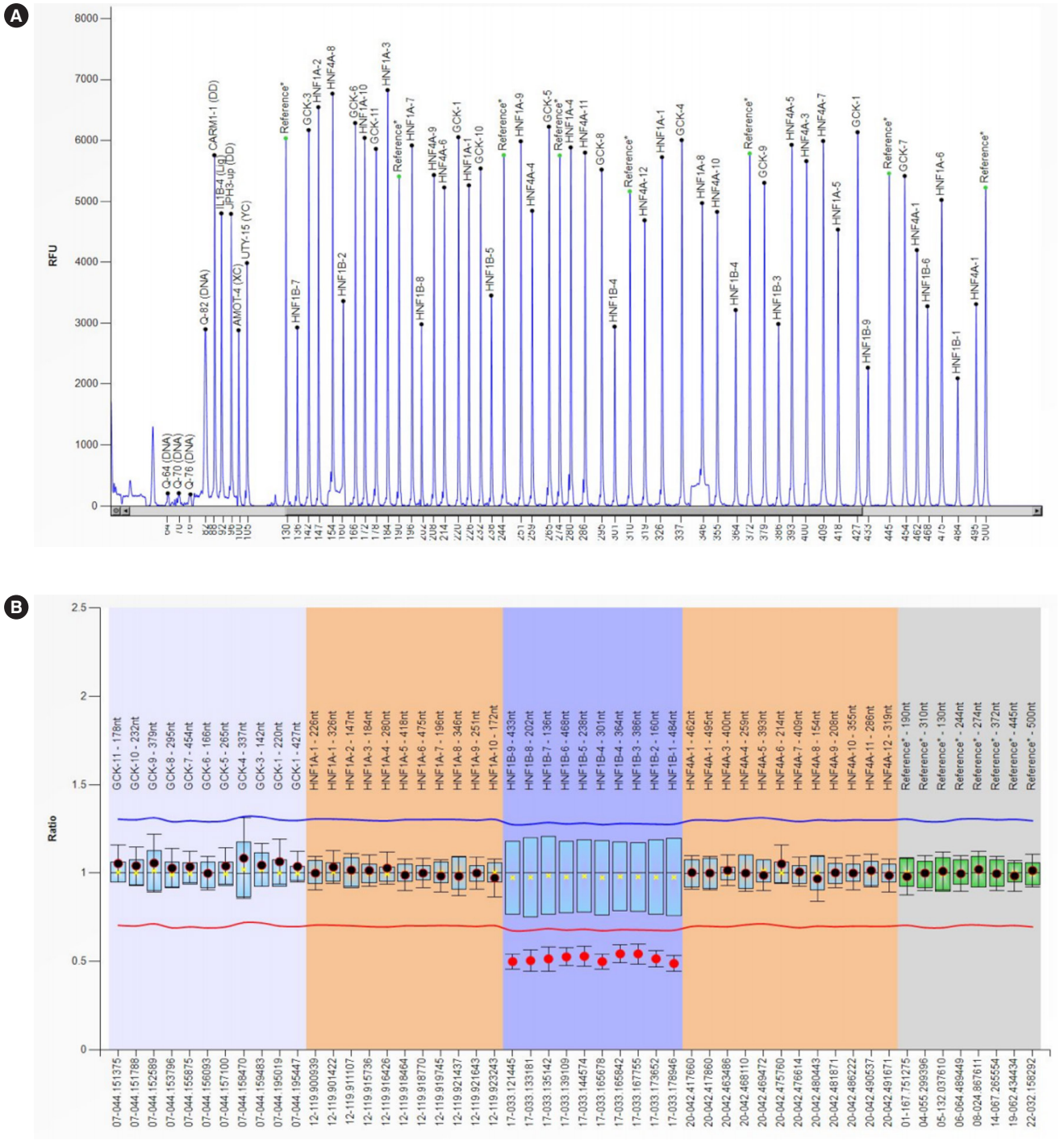
RefSeq:NM_001304286.2.
HNF1β, hepatocyte nuclear factor 1-beta; SIFT, Sorting Intolerant From Tolerant (http://cadd.gs.washington.edu); CADD, Combined Annotation Dependent Depletion (http://cadd.gs.washington.edu); ACMG, American College of Medical Genetics.
[a]The classification of rare variants identified in this study according to the standards and guidelines recommended by the ACMG.



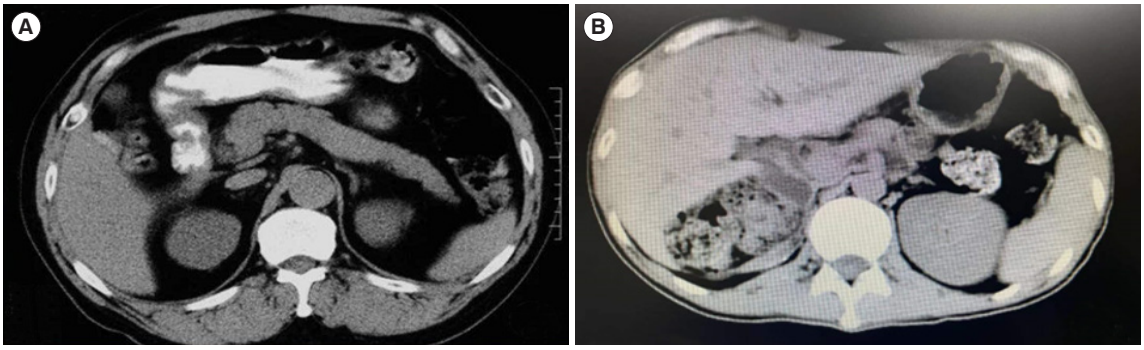
Supplementary Fig. 1. (A) The profile of hepatocyte nuclear factor 1-beta (HNF1β) overexpression plasmid. (B, C) The profile of glucose transporter 2 (GLUT2) and insulin (INS) reporter. (D, E) Sanger sequencing of wild-type and mutations in HNF1β (G454R, E105K, S148W) overexpression plasmid.



Supplementary Fig. 2. DNA sequence analysis for five patients with hepatocyte nuclear factor 1-beta (HNF1β) point mutations. (A) Sanger sequencing for Patient 01-01. (B) Sanger sequencing for Patient 02-01. (C) Sanger sequencing for Patient 03-01. (D) Sanger sequencing for Patient 04-01. (E) Sanger sequencing for Patient 05-01. [a]The sequencing diagram represents the reverse strand sequencing.



Supplementary Fig. 3. Multiple ligation probe amplification (MLPA) analysis for patient F-01. (A) Target fragment fluorescence probe signal intensity. (B) Percentage of standardized fluorescence probe signal intensity relative to baseline signal. RFU, relative fluorescence units.



Supplementary Fig. 4. (A) Abdominal computed tomography (CT) scan of a normal individual. (B) Abdominal CT scan of patient F-01 (left kidney atrophy, absence of the body and tail of the pancreas).