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Original Article

A Single Nucleotide Variant in HNF-1β is Associated with Maturity-Onset Diabetes of the Young in a Large Chinese Family

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Abstract

Background: Maturity-onset diabetes of the young (MODY) is a heterogeneous entity of monogenic disorders characterized by autosomal dominant inheritance. Eleven genes were related, including HNF4α, GCK, HNF1α, IPF1, and HNF-1β, and various mutations are being reported.

Methods: To help the overall understanding of MODY-related pathologic mutations, we studied a large MODY family found in 2012, in Shandong, China, which contained 9 patients over 3 generations. DNA was extracted from the periphery blood samples of (i) 9 affected members, (ii) 17 unaffected members, and (iii) 1000 healthy controls. Three pooled samples were obtained by mixing equal quantity of DNA of each individual within the each group. Totally 400 microsatellite markers across the whole genome were genotyped by capillary electrophoresis. The known MODY-related gene near the identified marker was sequenced to look for putative risk variants.

Results: Allelic frequency of marker D17S798 on chromosome 17q11.2 were significantly different ($P<0.001$) between the affected vs. unaffected members and the affected vs. healthy controls, but not between the unaffected members vs. healthy controls. MODY5-related gene, hepatocyte nuclear factor-1β (HNF-1β) on 17q12 near D17S798 became the candidate gene. A single nucleotide variant (SNV) of C77T in the non-coding area of exon 1 of HNF-1β was found to be related to MODY5.

Conclusion: This novel SNV of HNF-1β contributes to the diabetes development in the family through regulating gene expression most likely. The findings help presymptomatic diagnosis, and imply that mutations in the non-coding areas, as well as in the exons, play roles in the etiology of MODY.

Keywords: Maturity-onset diabetes of the young (MODY), Maturity-onset diabetes of the young type 5 (MODY5), Hepatic nuclear factor 1 beta (HNF1β)

Introduction

Maturity-onset diabetes of the young (MODY) was distinguished from other types of diabetes mellitus in 1970' (1). It is clinically characterized by nonketotic diabetes mellitus, a primary defect in the function of the beta cells of the pancreas, an onset before the age of 25 yr (usually in childhood or adolescence), and an autosomal dominant mode of inheritance (2). MODY seems to be an attractive model for genetic studies because its well-defined mode of autosomal dominant inher-

itance with high penetrance, and its early onset age that aids the collection of multigenerational pedigrees (3). The molecular genetic basis of MODY started in the 1990s (4-6), showed that MODY is a heterogenous group of monogenic disorders. To date, Online Mendelian Inheritance in Man (OMIM) has listed MODY1-11 based on implicated genes (Table 1) (7).

Table 1: Genetic classification and clinical phenotypes of the MODY subtypes

MODY subtype	Affected gene	Affected protein	Lqocus	Gene function	Primary defect
Type 1	HNF4 alpha	Hepatocyte nuclear factor 4 alpha	20q	Transcription factor (Nuclear factor)	Pancreas
Type 2	GCK	Glucokinase	7p15-p13	Hexokinase IV	Pancreas/Liver
Type 3	HNF1 alpha / TCF1	Hepatocyte nuclear factor 1 alpha	12q24.2	Transcription factor (Homeodomain)	Pancreas/Kidney
Type 4	IPF1	Insulin promoter factor-1	13q12.1	Transcription factor (Homeodomain)	Pancreas
Type 5	HNF1 beta / TCF2	Hepatocyte nuclear factor 1 beta	17q12	Transcription factor (Homeodomain)	Kidney/Pancreas
Type 6	Neuro D1	Neurogenic differentiation factor 1	2q	Transcription factor (bHLH)	Pancreas
Type 7	KLF11	Kruppel-like factor 11	2p25	Transforming growth factor-beta inducible-early growth response 2.	Pancreas
Type 8	CEL	Bile salt dependent lipase	9q34.3	The endocrine cells of pancreas synthesize insulin and are involved in the pathogenesis of diabetes mellitus and exocrine cells are involved in the pathogenesis of pancreas.	Pancreas
Type 9	PAX4	Paired domain gene 4	7q32	Transcription factor (paired domain gene 4)	Pancreas
Type 10	INS	Insulin	11p15.5.	Beta cells of the islets of Langerhans	NF-kappa-B
Type 11	BLK	Tyrosine kinase B-Lymphocyte specific	8p23-p22	Tyrosine kinase (B lymphocytes)	MIN6 beta cells

Adapted from Attiya et al (41)

MODY-related genes that have been most commonly studied are the hepatic nuclear factor 4 alpha (HNF4α) (MODY1), glucokinase (GCK) (MODY2), the hepatic nuclear factor 1 alpha (HNF1α) (MODY3), the insulin promoter factor-1 (IPF1) (MODY4) and the hepatic nuclear factor 1 beta (HNF1β) (MODY5). They account for the majority of MODY cases. For example, around 32%, 52%, 10% and 6% of cases in the UK were due to GCK, HNF1α, HNF4α, and HNF1β, respectively (8). To adding the heterogeneity of MODY, diverse mutations were reported in each pathogenic gene. For GCK only, more than 600 mutations have been described so far, leading to both hypoglycemia and hyperglycemia (9). Furthermore, 16–45% of the cases of MODY cannot be sorted into these 11 subtypes, and be named MODY-X. More studied are needed to discover the unknown MODY locus or loci (10).

Population studies revealed that heterogeneity also exists within MODY patients of different ethnic groups (11). For example, MODY2 (GCK) and MODY3 (HNF-1a) were found to account for more than 80% of cases in a cohort of U.K. Caucasians (12). However, in Hong Kong Chinese, these two forms of MODY were found to be responsible for a mere 4% and 5%, respectively (13). Except for the subtype heterogeneity, the mutations of the same MODY subtype that has been reported in different populations are usually different and hard to be confirmed (11).

To help the overall understanding of MODY-related pathologic mutations, we studied a large Chinese MODY family. We use whole genome scan

and candidate gene sequencing to identify the MODY subtype of the family, and to find the related gene mutation.

Materials and Methods

Pedigree assessment

A large Han pedigree in 2012 in Shandong Province, northern China, was identified through a proband with typical MODY features (Fig. 1).

The pattern of inheritance within the family is consistent with autosomal-dominant inheritance. In total, 9 individuals in this three-generation family met MODY diagnostic criteria (14) and showed a broad spectrum of clinical diabetic phenotypes. The other 21 family members are unaffected. This study obtained signed informed consent from 9 affected and 17 unaffected family members or their guardians, and 1000 adult voluntary blood donors.

This study was approved by the Ethics Committee of the Institute of Basic Medicine of Shandong Academy of Medical Sciences.

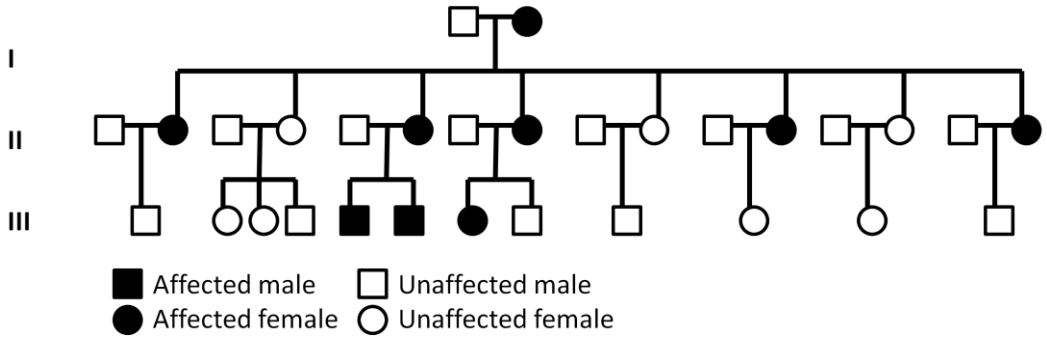

Fig. 1: Pedigree of the MODY family from Changqing, Shandong, China

DNA extraction and sample pooling

Peripheral venous blood samples were collected from 9 MODY patients in the family (2 males and 7 females, age 15-54), 17 unaffected family members (8 males and 9 females, age 7-77) and 1000 apparently healthy blood donors randomly selected from the Blood Center of Shandong province (702 males and 298 females, aged 17-55). Genomic DNA was extracted by a modified phenol-chloroform method as described before (15). The pooled DNA samples of (i) MODY patients in the family (n=9), (ii) unaffected family members (n=17), and (iii) unrelated population controls (n=1000), were obtained by combining equal amounts of DNA from each individual (16).

Genotyping and statistics

A panel of 400 microsatellite marker loci was genotyped in three pooled DNA samples, using ABI PRISM Linkage Mapping Set MD-10 (ABI). The set has fluorescence-labeled PCR primer pairs for 400 highly polymorphic dinucleotide-repeat microsatellite markers chosen from the Geneth on human genetic linkage map on both autosomal and sex chromosomes, with an average spacing of 10cM (17). Polymerase Chain Reaction (PCR), electrophoresis and genotyping analysis were as described before (15). Fifteen-µL PCR mixture contained 20 ng of genomic DNA, 1.5mM MgCI2, 10x reaction buffer, 0.2mM of each dNTP, 0.3 µM of each primer, and 1U of Taq polymerase. Amplification was performed on a Gene Amp 9700 thermo cycler (Applied Bio systems) with the following parameters: 95 ℃ for 12 min initial denaturation, followed by 94 ℃ 15 sec, 55 ℃ 15 sec, 72 ℃ 30 sec, 10 cycles; then 89 ℃ 15 sec, 55 ℃ 15 sec, 72 ℃ 30 sec, 20 cycles, then 72 ℃ 10 min, and 4 ℃ hold. Electrophoresis was carried out on ABI PRISM 3100 Avant Genetic Analyzer, by mixing up to 9 PCR products simultaneously,

which had no overlap of products of the same size range labelled with the same dye. Genotyping was performed with Gene Mapper 3.5 software (Applied Biosystems).

The predicted allele frequencies of microsatellites in each DNA pool were calculated as Allele Frequency=fluorescence peak height of each allele/the sum of fluorescence peak height of all allele at the marker. Allele Frequency was converted into allele counts and the statistical significance was calculated with CLUMP24, a DOS executable with C source code based on Monte Carlo method for assessing significance of case-control association studies with multi-allelic markers (http://www.davecurtis.net/dcurtis/software.html) (18). To reduce false positive error, the threshold of fluorescence signal was set as 30 units. A threshold of $P<0.01$ was considered statistically significant.

Sequencing and alignment for HNF-1β

Primers for nine exons of HNF-1β (listed in Table 2) were designed using Primer Premier 5.0 software (http://www.premierbiosoft.com). PCR in a 50 µl volume was performed with 5 µl genomic DNA (20 ng/µl), 5 µl of each primer (5 µM) and 25 µl of Multiplex PCR Mix (Takara) was performed on a GeneAmp9700 thermo cycler (ABI) with the following parameters: 94 ℃ for 5 min initial denaturation, followed by 94 ℃ 30 sec, 59 ℃ 30 sec, 72 ℃ 60 sec, 45 cycles; then 72 ℃ 7 min, and 4 ℃ hold. After spin column purification, the reaction products was sequenced using BigDye® Terminator v3.1 Cycle Sequencing Kit (ABI). The purified labeled DNA fragments were sequenced by Applied Bio systems 3130 Genetic Analyzer (ABI) and analyzed by Sequencing Analysis Software 5.2 (ABI). Sequence alignment was performed using DNA man software.

Results

Genotyping of 400 microsatellite markers in three pooled DNA samples revealed D17S798 as a positive marker to MODY

Of all the 400 microsatellite marker loci in ABI PRISM Linkage Mapping Set MD-10 (ABI), D17S798 on chromosome 17q11.2 was related with MODY in the family. Allele frequencies for D17S798 were found to differ significantly ($P<0.001$) between the affected and unaffected family members, the affected and the healthy controls, but not between unaffected family members and the healthy controls ($P=0.94$) (Fig. 2). No microsatellite markers on sex chromosomes showed significant differences for frequency between any of the DNA pooling samples, indicating an autosomal dominant inheritance that is one of the MODY criterions.

Table 2: Primers for nine exons of HNF-1β

Exon	Forward Primer	Reverse Primer
Exon1-a	CCAGGTCTTCTGCTCTCCAG	GTGGCCGTTGGTGAGAGTAT
Exon1-b	TTTTCCGTCCTTGGAAAATG	GACTTCTCTGGTGGGAAACG
Exon2	TTTTGGCCTCATGTCTACCC	AGAGGGCAAAGGTCACTTCA
Exon3	TCGTCCGTTGTCTGTCTGTC	GGTGAGCTTCTGGTGGTGTT
Exon4	ACTCCCAACCAAGACTGCTG	TAAGATCCGTGGCAAGAACC
Exon5	ATTTCTTGTGGGTGGACAGG	ATCAGCTCCAGAGCGACAAT
Exon6	TCCCAAAGTGCTGGGATTAC	CCCAAGTTTTCCAACCAAGA
Exon7	TCCCATGGAATCTCCTGTGT	ACCCAGAGAGGGAAAGTGGT
Exon8	AGATGGGAGCTATGGTGTGG	AACAACAGGGAGCCTCAGAA
Exon9-a	TTGGGCATCATCTCCCTTAG	TTTTCGCATCAGTTTGTTCG
Exon9-b	CTCCCACGATGTCAAGGACT	AGAGGACAAGGGGCTTCACT
Exon9-c	GTGCAATTTCCCCTCTGTGT	GAACCATGGCAGGGAAAGTA

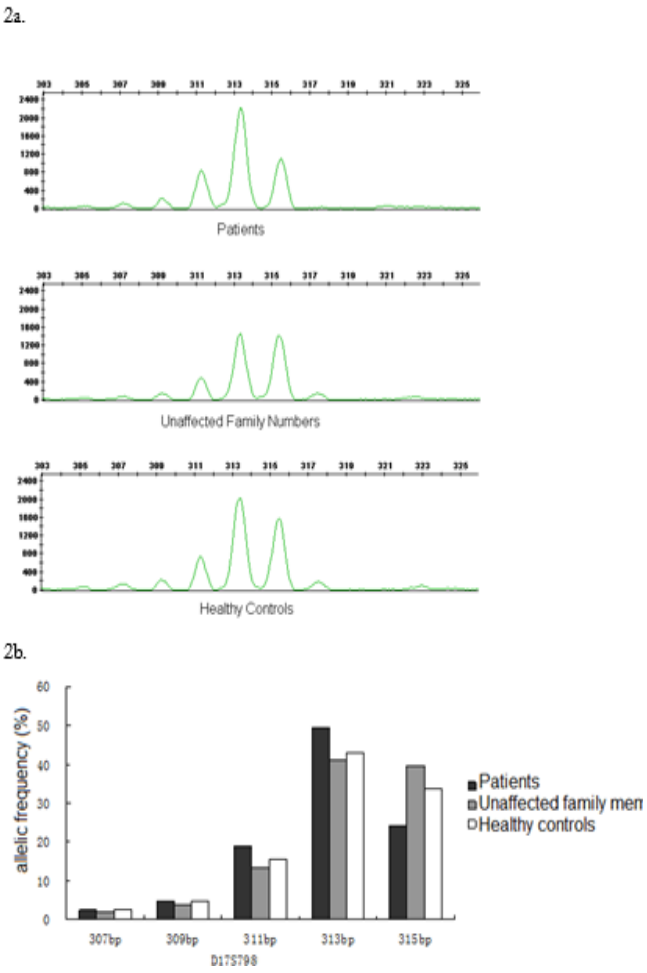

Fig. 2: Genotyping for microsatellite marker loci D17S798 in the three DNA pools
2a. Peak heights of D17S798
2b. Allele frequency estimates for D17S798

The mutation of C77T in exon 1 of HNF-1β gene was associated with MODY5 in the family

Close to D17S798 (17q11.2), there is a known MODY-related gene on 17q12, hepatocyte nuclear factor-1β (HNF-1β). The differences of allele frequencies for the D17S798 mentioned above implicated that the affected family members can be sorted as the MODY5 subtype. A single nucleotide variant (SNV) of C77T in the 5′-terminal non-coding region of exon 1 of HNF-1β was related to MODY5 in the proband by sequencing and alignment analysis (Fig. 3).

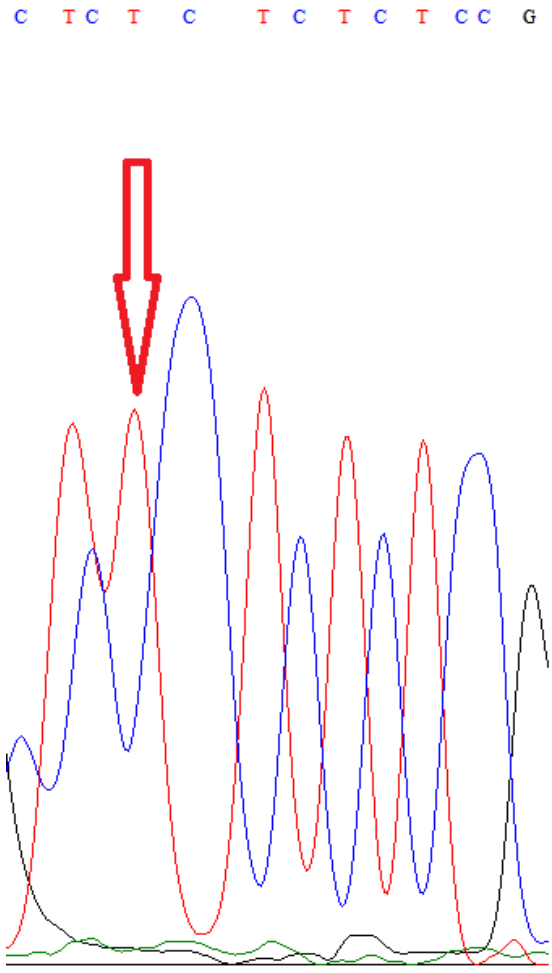

Fig. 3: Sequence analysis of exon 1 of HNF-1β gene in the proband.
The arrow indicates C77T in exon 1 of HNF-1β gene

Discussion

In the current study, allelic frequency of marker D17S798 on chromosome 17q11.2 was found to be related to MODY in the Chinese family. The known MODY-related gene near D17S798 is HNF-1β, implicating the patients in the family were MODY5 subtype. Further sequencing and alignment analysis found that a single nucleotide variant (SNV) of C77T in the 5′-terminal non-coding region of exon 1 of HNF-1β was related to MODY5 in the proband.

MODY is an entity of several monogenic disorders, and 11 genes have been associated to different MODY subtypes: MODY1-HNF4α, MO-

DY2-GCK, MODY3-HNF1α, MODY4-PDX1, MODY5-HNF1β, MODY6-NEUROD1, MODY7-KLF11, MODY8-CEL, MODY9-PAX4, MODY10-INS, MODY11-BLK (7). MODY-related genes are being found continuingly, eg. MODY12-ABCC8 (19) and MODY13-KCNJ11 (20). Still, a large part of MODY cases are labeled as "MODYX", which means their genetic factors remain unclear and wait to be found. Another research interest in the MODY studies is heterogeneous across populations. The relative prevalence of each subtypes varied (21, 22). In European populations, MODY2 (the most prevalent form in French families) (21) and MODY3 (the most prevalent form in British families) (22) are the two most prevalent forms. In Danish kindred clinically diagnosed as MODY, a relative prevalence of 36% of MODY3, 10% of MODY2 and 3% of MODY1 were reported (23). In Spain, relative frequencies in the MODY group were 80% MODY2, 8.5% MODY3 and 1% MODY5 (24). The other MODY subtypes are rarely described in European studies. However, the studies in other continents reported that MODY2 and MODY3 are less prevalent. For example, MODY2 and MODY3 mutations were accounted for 13% and 8.7% in 23 Brazilian families only (25). Similarly, in Asia, MODY2 and MODY3 do not explain the majority of MODY cases (26-28). Yorifuji et al. found in 79 MODY Japanese patients, type 2 and type 3 accounts for about 36.7% (11 plus 18). Next most prevalent were MODY 5 (6 patients) and MODY 1 (3 patients) (26). In a cohort of 27 MODY Korean patients and 17 patients with early onset type 2 diabetes, MODY2 and MODY 3 variants were found in only 3 patients (including 1 MODY patient) (27). In China particularly, a comprehensive study on 146 Hong Kong Chinese MODY families found 13 families had MODY3 mutations (9%), 2 had MODY2 mutations (1%). No MODY1 mutation was found. The authors concluded that the majority of Chinese MODY patients are due to yet unknown genes (29). Compared to MODY 1-3, MODY 5 is less studied. Wang et al. screened in 154 unrelated probands from early onset and multiple affected diabetes Chinese pedigrees and found only two patients carried mutation of HNF-1β gene (30). Here, we reported a large MODY5 family found in the northern China, as a supplement to the above two Chinese study which are based in the southern China.

HNF-1β gene (Gene ID: 6928) is located in chromosome 17:37686432-37745247, encoded a protein of 557 amino acids in length (http://asia.ensembl.org/). Many different mutations in HNF-1β have been associated with MODY5 (31-33). Missense mutations that caused protein sequence changes were reported in different populations, e.g. M160V in Australian (31), P239R in Romanian (34), L264S in Japanese patients (32), P159L in Korean patients (35) and S36F in Chinese patients (30). A frame-shift mutation, c.C1304del was found in Italy patients, which resulted in a truncated protein (36). Monoallelic loss of the entire HNF1B gene were reported in Merman patients who showed more severe clinical symptoms other than diabetes, such as pancreas dysplasia, exocrine insufficiency, genital defects and mental retardation (37). In the systematic review of HNF-1β anomalies related to MODY5 (38), the detection rate of HNF-1β anomalies was 1.4% among MODY cases (1.4%) and 41.2% among MODY cases with renal structure anomalies. Mutations were strikingly located within the DNA binding domain and spread among exons of the DNA binding domain with exons 2 and 4 were the hottest spots. However, in our MODY family an exon 1 mutation was implicated. This novel SNV is located in the 5′-terminal non-coding region of exon 1. The function of the 5′ non-coding region of eukaryotic mRNA and its binding proteins has not been extensively studied. Generally, the 5′ non-coding region plays a crucial role in the protein translation by forming secondary structure and providing binding sites for the ribosome and translation factors (39). Methylation of this region also affects translation (40). Therefore, we hypothesize that the change from a "methylate-able" base C to a "non-methylate-able" base T in the 5′non-coding region of HNF-1β may change transcription levels by altering the mRNA secondary structure and influence the binding sites for ribosome and/or translation factors.

Conclusion

The SVP of C77T in the 5′non-coding region of HNF-1β was related to MODY5 in the Chinese family studied. Previously detected mutations mainly indicated pathology resulting from the relation between HNF-1β protein structure/function and primary insulin secretory defects. As this novel mutation is in a non-coding region of the gene it suggests that regulation of the gene may be worthy of attention in studying the pathological mechanism of MODY or diabetes.

Ethical considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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