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ORIGINAL ARTICLE

Screening of *HNF1A* and *HNF4A* mutation and clinical phenotype analysis in a large cohort of Chinese patients with maturity-onset diabetes of the young

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Abstract

Aims The study aimed to screen the *HNF1A* and *HNF4A* mutation in a large Chinese cohort of high clinical suspicion of maturity-onset diabetes of the young (MODY) patients and characterize the clinical features of those patients. The performance of hsCRP as a biomarker to differentiate MODY3 from early onset T2DM was also evaluated.

Methods A total of 74 patients with a strong clinical suspicion of MODY from 59 families and 33 newly diagnosed early-onset T2DM were included. *HNF1A* and *HNF4A* mutations were analyzed by Sanger sequencing. ROC curves were used to identify the optimal cutoff of hsCRP.

Results One novel (c.864_865insG) and six recurrent *HNF1A* mutations (R203H, R263H, P379T, L422P, P519L and c.873delC) in 17 patients from 8 families (13.6%), as well as one novel *HNF4A* (R331H) mutation were identified. Non-specific clinical presentations were observed in MODYX compared to MODY3 patients. MODY3 subjects exhibited with younger, lower BMI, TG, fasting and postprandial C-peptide, higher HDL than T2DM. Particularly, we confirmed serum hsCRP was lower in MODY3 than T2DM. ROC curve showed a good discrimination with an AUC of 0.852 and identified a cutoff hsCRP of 0.79 (75% sensitivity and 83% specificity). Good glycemic control was observed in all identified patients after switching to glimepiride therapy.

Conclusions The prevalence of *HNF1A* mutation was relatively lower in Mainland China and *HNF4A* mutation was rare. Serum hsCRP concentrations performed well in discriminating MODY3 from T2DM. Molecular diagnosis of MODY3/1 did transform management in clinical practice and facilitated the glycemic control.

Keywords Maturity-onset diabetes of the young · HNF1A · HNF4A · CRP · Mutation

Introduction

Maturity-onset diabetes of the young (MODY) is a clinically and genetically heterogeneous monogenic disorders characterized by autosomal dominant inheritance, early onset and pancreatic beta cells dysfunction [1]. Presently, at least 14 causative genes involved in insulin secretion have been identified for MODY. Mutation in genes encoding glucokinase (MODY2), hepatic nuclear factors-1A (MODY3) and *HNF4A* (MODY1) are the three most common subtypes, accounting for more than 90% MODY patients [2], but the relative prevalence varied considerably among different ethnicity [3]. Small studies from Hong Kong reported that *HNF1A* mutation was responsible for only 5–10% MODY and no MODY1 was identified in Chinese [4–6], compared to 52% and 10% MODY patients with *HNF1A* and *HNF4A* mutation, respectively, in UK [7]. However, the prevalence of MODY3 and MODY1 in Mainland China remains unknown.

In contrast with MODY2 characterized by mild fasting hyperglycemia and the absence of complications, patients

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with *HNF1A* or *HNF4A* mutation demonstrate with progressive beta cell failure and high risk of microvascular and macrovascular complications due to increasing hyperglycemia [8]. Another significantly clinical implication is that afflicted cases are extremely sensitive to sulfonylureas [9]. Thus, a definitive molecular diagnosis facilitates optimal treatment and good glycemic control. However, hitherto there were fewer reports on MODY1 and MODY3 families in Chinese population. It is noteworthy that diabetes is dramatically prevalent in China, where the number of diabetes ranks first throughout the world [10]. This clearly suggests that the vast majority of MODY3 and MODY1 individuals are currently undiagnosed. Limited clinicians experience and restrictive selection criteria for genetic testing may partly explain the low referral rate in Chinese population. In recent years, an older age of onset had been frequently observed in *HNF1A* mutation carriers [11]. Hence, an extensive genetic screening in the higher age threshold is warrant.

On the other hand, since the prevalence of young-onset diabetes and overweight is increasing [10], it becomes more challenging for clinicians to differentiate *HNF1A*/*4A* MODY from other forms of diabetes owing to the significantly overlapping clinical features. Although the correct diagnosis depends on molecular genetic testing, it is too expensive and not available in many hospitals. Consequently, a cost effective and practical biomarker that helps to pick up patients with high probability of *HNF1A*/*4A* mutation for genetic screening is desirable. A body of evidence shows that high sensitivity C reaction protein (hsCRP) is significantly lower in MODY3 patients than other types of diabetes, and hsCRP discriminates MODY3 well from young-onset type 2 diabetes mellitus (T2DM) [12, 13]. However, the diagnostic value of hsCRP in Chinese has not been investigated yet.

Combine with these, this study aimed to first screen the *HNF1A* and *HNF4A* mutation in a large Chinese cohort of high clinical suspicion of MODY3 or MODY1 patients using a wider selection criterion (up to 45 yeas) to determine the prevalence of MODY3/1 in Mainland China, and analyzed the clinical features of those patients. In addition, the study evaluated the clinical validity of hsCRP as a diagnostic marker to differentiate *HNF1A* mutation carriers from early onset T2DM.

Materials and methods

Subjects

A total of 74 patients with a strong clinical suspicion of MODY3 or MODY1 from 59 families were studied. These subjects were consecutively recruited from the outpatient clinic of Endocrinology at Peking Union Medical College Hospital (PUMCH), Beijing, China, between January 2014 and December 2016. All proband met the following criteria: (1) the age at diagnosis of diabetes ≤ 45 year; (2) family history of diabetes in at least two generations with autosomal dominant mode of inheritance; (3) the absence of pancreatic islet autoantibodies including glutamic acid decarboxylase antibody (GAD), protein tyrosine phosphatase antibody (IA2) and islet cell antibody (ICA); (4) no marked obesity or evidence of insulin resistance. We excluded patients with a renal disorder, persistently mild fasting hyperglycemia, a maternally inherited diabetes and deafness. In addition, 33 unrelated Chinese with newly identified early-onset T2DM aged less than 45 years were recruited as a control group. These patients were diagnosed with diabetes within 1 year. Subjects with hsCRP > 10 mg/L were removed.

Genetic analysis

All these patients were sequentially underwent *HNF1A* and *HNF4A* gene mutation analysis. Genomic DNA was extracted from peripheral blood lymphocytes using a DNA extraction kit (Qiagen, Germany). All exons, the intron–exon boundaries and the promoter regions of *HNF1A* and *HNF4A* gene were amplified by polymerase chain reaction (PCR). The primers were available on request. Sequencing of purified PCR products in both forward and reverse directions was performed on an ABI3730 automated sequencer (Applied Biosystems. Foster City. CA. USA) using the chain-termination method. The NCBI BLAST database was applied to identify variants by aligning with reference sequences NM_000545.6 (*HNF1A*) and NM_000457.4 (*HNF4A*). Then, three databases including Exome Variant Sever (http://evs.gs.washington.edu/EVS), dbSNP database in NCBI (http://www.ncbi.nlm.nih.gov/snp/) and UCSC genome bank were searched to exclude single nucleotide polymorphisms (SNPs). Furthermore, the identified variants should not been found in 100 healthy control. For putative novel missense mutation, SIFT (http://sift.jcvl.org), PolyPhen2 (http://genetics.bwh.harvard.edu/pph2) and Mutation T@sting (http://www.muatationtaster.org) software were used to predict the pathogenicity.

Clinical and laboratory examinations

Clinical parameters including age at diagnosis of diabetes, height, weight, family history of diabetes, past medical history, treatment before referring to PUMCH and physical examination were recorded for each participant. Body mass index (BMI) was calculated as weight (kg) divided by the square of height (m^2). Routine biochemical examinations included measurement of live and renal function, fasting and postprandial glucose, serum lipid profiles and hsCRP with an automatic biochemical analyzer (AU5800; Beckman Coulter, USA). The serum insulin

and C-peptide levels were measured by direct chemiluminescence immunoassay (SIMENS ADVIA Centaur XP, Germany). The high performance liquid chromatography method was used to determine glycated hemoglobin (HbA1c) levels. GAD and IA2 were determined by ELISA (SIMENS ADVIA Centaur XP, Germany), and ICA were measured using indirect immunofluorescence (SIMENS ADVIA Centaur XP, Germany).

Statistical analysis

All the statistical analyses were performed using SPSS version 16.0 and Graphpad Prism 6.0. The normal distribution of continuous variables was evaluated by Kolmogorov–Smirnov test. Comparisons between two groups (MODY group vs MODY negative group or MODY group vs T2DM group) were conducted using Student's *t* test for normally distributed continuous variables and nonparametric test for skewed continuous variables expressed as median and range. A Chi-square test was used for categorical variables expressed as percentage. Receiver operating characteristic (ROC) curve analysis was applied to evaluate the performance of hsCRP in distinguishing MODY3 from T2DM, and also used to identify cutoffs of hsCRP with optimal sensitivity and specificity. $P < 0.05$ was considered significant.

Results

Genetic screening of HNF1A and HNF4A mutation

HNF1A gene mutation analysis was conducted in all subjects diagnosed clinically as MODY. In total, seven different mutations in 17 patients from 8 families were identified, with a mutation pick-up rate of 13.6%. Among these mutations, five were missense, one insertion and one deletion mutation. The insertion mutation (c.864_865insG) had not been reported previously. Five mutations (c.864_865insG, c.873delC, c.1135 C > A, c.1265 T > C and c.1556 C > T) were located in transactivation domain and two (c.608G > A and c.788G > A) in the DNA-binding domain. Each mutation was identified only in a single family except for c.873delC. Three out of seven mutations (42.9%) were resided in exon 4. The detailed information of *HNF1A* mutations were shown in Table 1 and Fig. 1.

For MODY individuals with no *HNF1A* gene mutation, *HNF4A* mutation testing was then performed. Only one *HNF4A* mutation was detected in two patients from one family (Fig. 2). The MODY1 subjects were heterozygous for a novel G–A transversion at position 992 (c.992 G > A) resulting in a replacement of arginine by histidine at amino acid 331 (p.R331H). The Polyphen-2 bioinformatics tool predicted the R331H substitution to be probably damaging, "Disease causing" and "Intolerant" was showed by Mutation Taster and SIFT, respectively. Furthermore, R331 residue was highly conserved across ten different species

Table 1 Molecular and clinical findings of MODY3 patients

No.	Age at diagnosis of diabetes	Age at diagnosis of MODY3	Treatment at diagnosis	Nucleotide change	Aminoacid change	Region	Consequence
F1-1	18	23	Metformin	c.608G > A	p.R203H	Exon 3	Missense
F1-2	51	53	Metformin	c.608G > A	p.R203H	Exon 3	Missense
F2	24	25	Insulin + metformin	c.873delC	p.P291Pfs*49	Exon 4	Deletion
F3-1	8	9	Insulin + metformin	c.864_865insG	p.G288Gfs*28	Exon 4	Insertion
F3-2	20	39	Insulin	c.864_865insG	p.G288Gfs*28	Exon 4	Insertion
F3-3	19	34	Insulin	c.864_865insG	p.G288Gfs*28	Exon 4	Insertion
F4-1	15	15	Insulin	c.873delC	p.P291Pfs*49	Exon 4	Deletion
F4-2	35	37	Insulin	c.873delC	p.P291Pfs*49	Exon 4	Deletion
F4-3	42	58	Insulin	c.873delC	p.P291Pfs*49	Exon 4	Deletion
F5-1	12	12	glimepiride	c.788G > A	p.R263H	Exon 4	Missense
F5-2	23	45	Insulin	c.788G > A	p.R263H	Exon 4	Missense
F6-1	16	19	Metformin	c.1135 C > A	p.P379T	Exon 6	Missense
F6-2	--	40	Insulin	c.1135 C > A	p.P379T	Exon 6	Missense
F6-3	14	15	Metformin	c.1135 C > A	p.P379T	Exon 6	Missense
F7	45	50	Acarbose	c.1556 C > T	p.L422P	Exon 6	Missense
F8-1	24	24	glimepiride	c.1556 C > T	p.P519L	Exon 8	Missense
F8-2	43	50	Insulin	c.1556 C > T	p.P519L	Exon 8	Missense

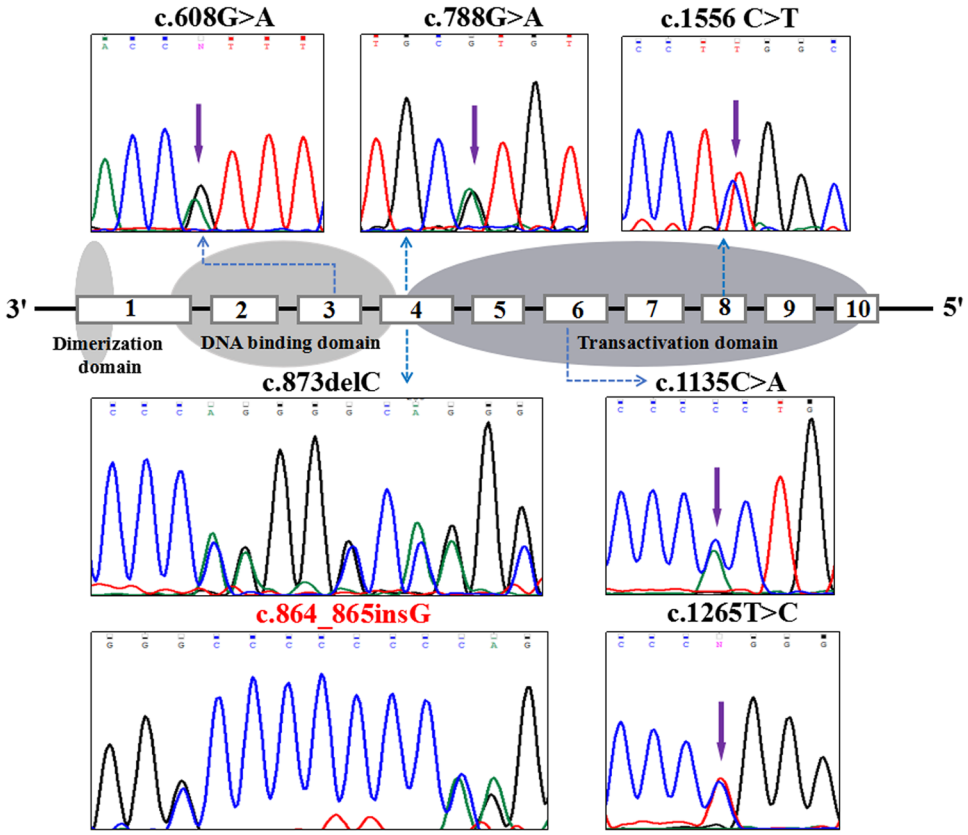

Fig. 1 Distribution and the sequencing chromatogram of *HNF1A* mutations. The functional domains of the hnf1a protein are shown. Mutations occurring in more than one subject are shown only once. Novel mutation is showed in red. Arrows indicate the changed nucleotide base

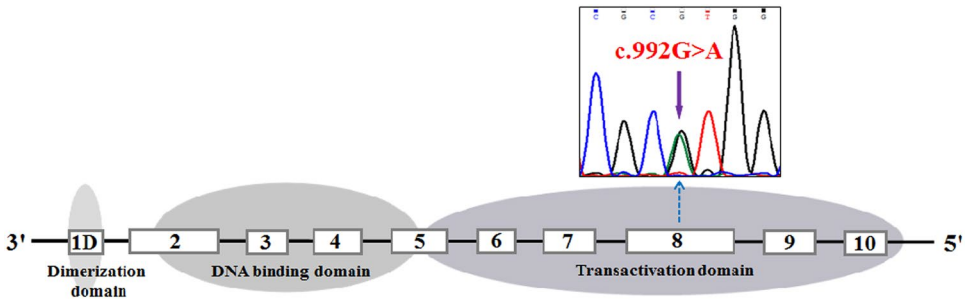

Fig. 2 The sequencing chromatogram and position of the novel *HNF4A* mutation. Arrows indicate the changed nucleotide base

(Figure S1). These results indicated R331H mutation was pathogenic.

Clinical characteristics of MODY3 subjects

For MODY3 patients, the mean age of diagnosis of diabetes was 25 years, but the onset of diabetes above 35 years old was found in five subjects. Fourteen patients were misdiagnosed other types of diabetes more than one year, and the longest length was 16 years. BMI of all MODY3 patients was below 24 kg/m^2. Compared to non-*HNF1A* mutation carriers, no significantly statistical differences in glucose and lipid metabolism traits were observed except for lower postprandial C-peptide ($p=0.029$) (Table 2).

Besides, MODY3 patients were younger, leaner and exhibited with lower fasting and postprandial C-peptide compared to T2DM ($p<0.001$). Serum concentrations of triglycerides (TG) were lower ($p=0.006$), whereas high-density lipoprotein cholesterol (HDL-c) levels were higher in MODY3 than T2DM subjects ($p=0.004$). Particularly, the levels of hsCRP were significantly decreased in MODY3 patients [0.17(0.07,0.73)] in comparison with T2DM [2.27(0.68,4.38); ($p<0.001$)] (Table 2; Fig. 3A).

Clinical presentation of MODY1 patients

The two identified MODY1 patients were from one family, that is proband and his mother. The proband, a 10-year-old boy, was born at term with a birth weight of 4.4 kg. He was

Table 2 Comparison with clinical features and biochemical parameters between MODY3, T2DM and non-MODY3/1 subjects

	MODY3 ($n=17$)	Non-MODY3/1 ($n=55$)	T2DM ($n=33$)	P1	P2
Age (years)	25.56 ± 13.32	24.74 ± 12.52	32.27 ± 6.98	0.817	**0.023**
F/M (%)	12/5 (70.6%)	27/28 (49.1%)	11/22 (33.3%)	0.12	**0.012**
BMI(Kg/m^2)	20.91 ± 2.23	22.71 ± 5.19	27.43 ± 5.41	0.256	**< 0.001**
HbA1c (%)	8.36 ± 2.14	7.93 ± 1.79	8.62 ± 1.99	0.464	0.682
FBG (mmol/L)	8.75 ± 2.93	8.58 ± 2.41	9.40 ± 4.08	0.833	0.582
PBG (mmol/L)	16.19 ± 6.08	13.41 ± 4.38	12.25 ± 6.41	0.123	0.09
C-p (ng/mL)	0.91 ± 0.40	1.15 ± 0.68	1.82 ± 0.89	0.261	**< 0.001**
2 h-Cp (ng/mL)	1.77 ± 0.93	3.07 ± 1.65	3.75 ± 1.82	**0.029**	**< 0.001**
TC (mmol/L)	4.53 ± 1.02	4.42 ± 1.01	4.48 ± 1.30	0.717	0.889
TG (mmol/L)	1.00 (0.67,1.64)	0.97 (0.58,2.27)	1.93 (1.00,2.96)	0.77	**0.006**
HDL-c (mmol/L)	1.26 (0.96,1.49)	1.15 (1.05,1.38)	0.90 (0.82,1.11)	0.565	**0.004**
LDL-c (mmol/L)	2.75 ± 0.86	2.40 ± 0.75	2.74 ± 0.84	0.139	0.951
hsCRP(mg/L)	0.17 (0.07,0.73)	0.62 (0.22,0.94)	2.27 (0.68,4.38)	0.08	**< 0.001**

F female, *M* male, *BMI* body mass index, *HbA1c* glycated hemoglobin, *FBG* fasting blood glucose, *PBG* postprandial blood glucose, *C-p* C-peptide, *TG* triglycerides, *TC* total cholesterol, *HDL-c* high-density lipoprotein cholesterol, *LDL-c* low-density lipoprotein cholesterol, *hsCRP* high sensitivity C reaction protein

Normal range: C-p 0.8–4.2 ng/mL; TC 2.85–5.70 mmol/L; TG 0.45–1.70 mmol/L; HDL-c 0.93–1.81 mmol/L; hsCRP 0–3 mg/L

P1: MODY3 vs non-MODY1/3; P2: MODY3 vs T2DM; P < 0.05 are highlighted in bold

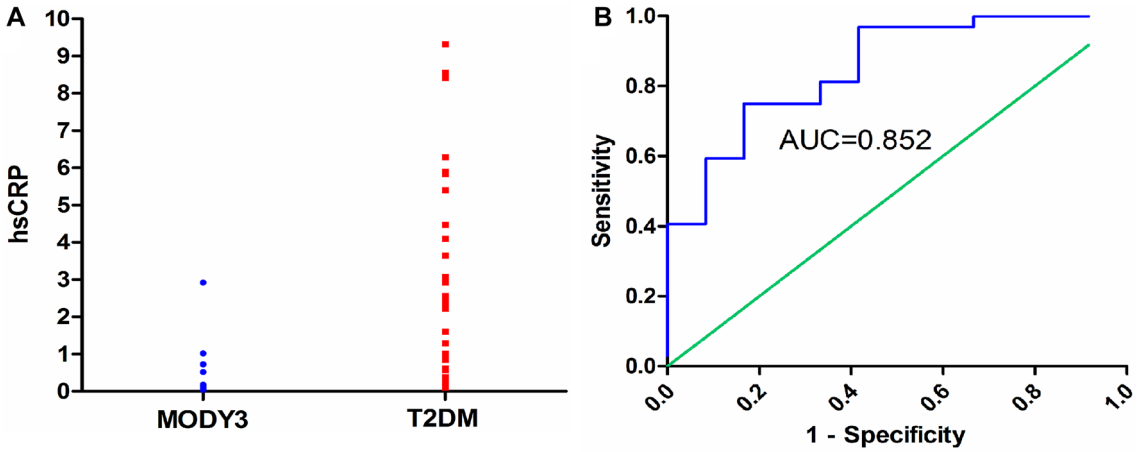

Fig. 3 **a** Distribution of hsCRP in 17 MODY3 and 33 newly diagnosed early-onset T2DM subjects. **b** The ROC curve shows the capacity of hsCRP to discriminate MODY3 from T2DM. The area under the curve is 0.852

the only child of a non-consanguineous couple. At 8 years, he presented with polyuria, but paid no attention. High postprandial serum glucose (13.1 mmol/L) was ever observed unintentionally at home by detection capillary blood glucose, but no treatment was received. Two years later, he was referred to our hospital for the first time for further examination and management. His height was 147.5 cm (+1 to +2SD) and weight was 46 kg. Liver and renal function was normal. HbA1c was 8.6% (70 mmol/mol). Relatively decreased pancreatic islet function with fasting C-peptide of 0.88 μg/L and postprandial C-peptide of 2.15ug/L was found. Elevated LDL-c (2.89 mmol/L) was measured. The levels of TG and hsCRP were within normal range and the value was 0.65 mmol/L and 0.95 mg/L, respectively. His mother had a history of gestational diabetes mellitus. Four years after delivery, hyperglycemia was detected because of ketosis and then insulin treatment was initiated. Unfortunately, her laboratory examination was not available.

Diagnostic value of hsCRP

ROC curve analysis was used to further evaluate the performance of hsCRP as a diagnostic marker to discriminate MODY3 patients from newly diagnosed early onset

T2DM. The results showed good discrimination with an area under the curve (AUC) of 0.852 and identified a cut-off hsCRP < 0.79 mg/L with 75% sensitivity and 83% specificity (Fig. 3b).

Efficacy of sulfonylureas treatment

Among the 17 MODY3 patients, only two patients received correct treatment with sulfonylureas at the onset of diabetes because of identification *HNF1A* mutation in our hospital. Insulin treatment was initially given in more than half of MODY3 patients. 11.8% patients were treated with insulin and oral hypoglycemic agents, 29.4% other oral hypoglycemic medicine only. After molecular diagnosis with MODY3, insulin treatment as well as other oral hypoglycemic agents was gradually off and switched to glimepiride. HbA1c levels decreased markedly to below 7% (53 mmol/mol) in most patients (Fig. 4) following 3 months of glimepiride treatment and no adverse effect was observed. The MODY1 proband was also transferred from insulin to glimepiride successfully. HbA1c was dropped to 6.2% (44 mmol/mol) after 6 months of this transition. During the follow-up period, no deterioration in glycemic control was observed in all MODY1/3 patients.

Discussion

To the best of our knowledge, this was the first study in Mainland China to screen the *HNF1A* and *HNF4A* mutation in a large cohort of Chinese MODY patients. We identified one novel and six recurrent *HNF1A* gene mutations, and one novel missense mutation in *HNF4A* gene. The prevalence of MODY3 and MODY1 was 13.6% and 1.7%, respectively. In addition, we confirmed that hsCRP levels can be used as a biomarker to help distinguish MODY3 from T2DM.

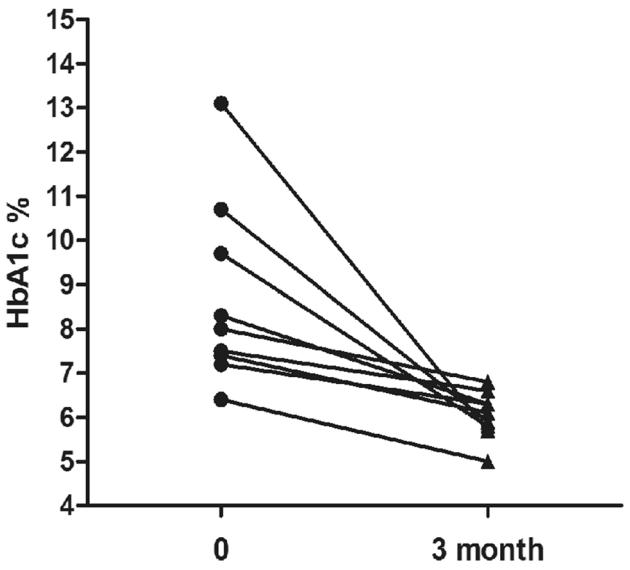

Fig. 4 The changes of HbA1c levels after treatment with glimepiride 3 months later

In Chinese population with an epidemic of diabetes, there are only three reports from HongKong focusing on the frequency of *HNF1A* and *HNF4A* mutation, in which prevalence of 5–10% for MODY3 and no MODY1 was reported [4–6]. In this study, extended criteria of age of diabetes onset to 45 increased pick up rate from 11.8 to 13.6%. The pick up rate was similar to Italy (14%) [14] but higher than US (4.4%) [15]. Unlikely MODY2, MODY3 patients usually developed diabetes after puberty, and the penetrance increased with age, which was almost complete by 55 years [16]. This was consistent with our findings that five MODY3 patients were diagnosed with diabetes after the age of 35 years, and even one onset at 51 years old. Thus, the higher age threshold for screening *HNF1A* mutation was necessary. On the other hand, excluding individuals with phenotype resembling MODY2 might also contribute to improving the positive mutation rates. However, the prevalence of MODY3 in Chinese population was strikingly lower than what had been reported in UK (52%) [7], Germany (31%) [17], Norway (29%) [18], Spain (35%) [19] and Denmark (36%) [20]. This discrepancy was more likely explained by different recruitment criteria for genetic test and ethnicity background.

In contrast with MODY2 and MODY3, MODY1 was relatively uncommon [2]. Till now, less than ten MODY1 patients were described in China. In the present study, we identified only one *HNF4A* mutation. The novel missense mutation c.992G > A occurred at amino acid position 331 of exon 8 in transactivation domain, where an arginine was replaced by histidine. The Arg331 code was highly conserved across various species, and the mutation was absent in 100 health controls. Furthermore, three bioinformatics tools we applied predicted R331H mutation to be pathogenic variants, and the mutation cosegregated with the phenotype in the pedigree. Accordingly, this mutation was responsible for the typical clinical features of the index case. In line with previous description, the proband showed macrosomia and increased LDL-c levels. It is worth mentioning that the birth weight of *GCK*-deficient fetuses varied depending on different *GCK* mutations and whether their mothers were affected. Consequently, a close fetal growth monitoring through frequent ultrasounds is valuable in the management of MODY2 pregnancies [21].

Although *HNF1A* mutations were widely distributed in different exons, of which 37.5% (3 out of 8 families) occurred in a polyC-tract of exon4, suggesting it was vulnerable to mutation and still a hotspot mutation in Chinese. This was consistent with previous observations in other population [22]. The novel insertion mutation c.864_865insG at

code 288 resulted in a frameshift generating an early termination signal at amino acid 316. The truncated protein lacking C-terminal transactivation domain was expected to severely impair transcriptional activity. With regard to subjects carrying different types or location of *HNF1A* mutation, no genotype and phenotype correlations were observed. Even the same *HNF1A* mutation carriers, the penetrance and expression varied. Other genetic or environmental factors might influence phenotype as well.

For most included MODY patients, both *HNF1A* and *HNF4A* mutation were not detected. While *GCK* and *HNF1B* mutations were not analyzed in this study, we excluded subjects whose clinical features were strongly suggestive of MODY2 or MODY5. Consequently, common MODY subtypes were not the major causes of these MODY patients. Whole exome sequencing was needed to further investigate other rare or new MODY genes in future. To reflect the real-life situations encountered by clinicians, we compared MODY3 patients with non-MODY3/1 patients, and nonspecific clinical characteristic was observed. Thus, to select MODY patients for *HNF1A* mutation analysis only depending on clinical manifestations remained challenging for clinicians.

Despite an accurate molecular diagnosis of MODY3 was crucial for optimizing management, a vast majority of MODY3 patients were frequently misclassified as T2DM or other type diabetes [23]. In this study, 84.2% identified patients were misdiagnosed for more than 1 year. Therefore, a cheap and widely available biomarker was considerably valuable in assisting selection of individuals for *HNF1A* sequencing. The promoter of *CRP* gene harbored binding sites of transcription factors hnf1a which directly regulated *CRP* expression [24]. Loss of function mutations in *HNF1A* gene responsible for MODY3 had been shown to confer a substantial CRP reduction [12, 13]. Our study confirmed that the levels of serum hsCRP were significantly lower in MODY3 than T2DM patients, and ROC curve showed a good discrimination with a AUC of 0.852 and identified a cutoff hsCRP of 0.79 (75% sensitivity and 83% specificity). Interestingly, the results were consistent with reports from UK, which indicated that hsCRP distinguished MODY3 from T2DM with hsCRP < 0.75 mg/L showing 79% sensitivity and 70% specificity (AUC = 0.84) [25]. These evidence suggested that hsCRP was a clinically valid biomarker used to determine whether *HNF1A* mutation testing was initiated. Interestingly, Delvecchio M recently proposed to screen *HNF1A* gene first in patients with primarily fasting plasma glucose ≤ 150 mg/dl and HbA1c > 7.3%, irrespective of other clinical information. Large sample studies are warranted for further evaluation [26].

The clinical benefits of molecular diagnosis MODY3 and MODY1 were that these types of diabetes were classically sensitive to sulfonylurea treatment. A randomized crossover trial indicated that MODY3 patients had a 5.2-fold greater response to gliclazide than to metformin [27]. In this patient cohort, MODY3 and MODY1 patients were properly offered low-dose glimepiride therapy following genetic diagnosis. Insulin therapy was safely stopped and patients reported that their quality of life was improved. For all patients, blood glucose was controlled well throughout the follow-up period. Previous observational study also found most MODY3 patients transferred off insulin to sulfonylurea successfully and the efficacy appeared to be sustained over 3 years [28], similar response was also seen in MODY1 patients [29]. Importantly, due to the progressive nature of beta cell function, these patients should be closely monitored during long-term follow-up.

Some limitations should be stated. First, Sanger sequencing utilized in this study could not capture large deletions, which might underestimate the positive rate of *HNF1A* and *HNF4A* mutation. The method of multiplex-ligation dependent probe amplification (MLPA) should be applied in future. Second, a small number of MODY3 and MODY1 subjects restricted the analysis of genotype and phenotype correlations. Third, certain drugs such as statins were believed to reduce the CRP level. Nevertheless, the drug history of some cases with T2DM was not available, thus we could not evaluate its influence on our results.

Conclusions

The prevalence of *HNF1A* mutation was relatively lower in Mainland China and *HNF4A* mutation was rare, while the misdiagnosis rate was high. We identified one novel *HNF1A* and *HNF4A* mutation, respectively, which were probably pathogenic and expand the mutation spectrum of the two genes. The etiology of large number of non-MODY1/3 subjects required a broader range of investigation for possible pathogenic genes in future. Molecular diagnosis of MODY3 and MODY1 did transform management in clinical practice and facilitated the glycemic control. In addition, serum hsCRP concentrations were lower in MODY3 than T2DM and performed well in discriminating MODY3 from T2DM. hsCRP could be a useful biomarker in clinical practice to assist selecting patients for diagnostic *HNF1A* genetic testing.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical standard All procedures performed in this study involving human participants were in accordance with the ethical standards of the Peking Union Medical College Hospital Ethics Committee.

Human and animal rights The study was conducted in accordance with the principles of the Declaration of Helsinki of 1975, as revised in 2008.

Informed consent Informed consent was obtained from all patients for being included in the study.

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