



# Using Clinical Indices to Distinguish MODY2 (*GCK* Mutation) and MODY3 (*HNF1A* Mutation) from Type 1 Diabetes in a Young Chinese Population

Junling Fu · Tong Wang · Jieying Liu · Xiaojing Wang ·  
Qian Zhang · Ming Li · Xinhua Xiao

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## ABSTRACT

**Introduction:** Accurate diagnosis of maturity-onset diabetes of the young (MODY) is required in order to select appropriate treatment options and to assess prognosis. The aim of this study was to explore potential clinical indicators that could be used to differentiate MODY2, MODY3, and type 1 diabetes (T1D) in young subjects.

**Methods:** Twelve patients with MODY3 and 29 patients with MODY2 were characterized and compared to 26 patients with T1D. These three groups were matched for age and gender. Clinical profiles of the 67 patients were collected. Receiver operating characteristic (ROC) curves were used to identify the optimal cutoff values of clinical indicators.

**Results:** Compared to patients with T1D, subjects with MODY3 had higher fasting C-peptide levels ( $1.34 \pm 1.51$  vs.  $0.29 \pm 0.22$  ng/mL;

$P < 0.001$ ) and lower high-sensitivity C-reactive protein (hsCRP) levels ( $0.18 \pm 0.15$  vs.  $1.22 \pm 1.49$  mg/L,  $P = 0.004$ ); patients with MODY2 had lower hsCRP ( $0.37 \pm 0.39$  vs.  $1.22 \pm 1.49$  mg/L;  $P = 0.003$ ), total cholesterol ( $4.12 \pm 0.68$  vs.  $4.61 \pm 0.81$  mmol/L,  $P = 0.034$ ), and low-density lipoprotein cholesterol (LDL-C) ( $2.24 \pm 0.68$  vs.  $2.67 \pm 0.79$  ng/L,  $P = 0.002$ ) levels and higher fasting C-peptide levels ( $0.96 \pm 0.42$  vs.  $0.29 \pm 0.22$  ng/mL,  $P = 0.002$ ). The ROC-derived hsCRP values for discriminating MODY2 from T1D, MODY3 from T1D, and MODY3 from MODY2 were 0.675, 0.833, and 0.763, respectively. The ROC-derived fasting C-peptide levels for discriminating MODY2 from T1D and MODY3 from T1D were 0.951 and 0.975, respectively. The ROC-derived total cholesterol and LDL-C values for discriminating MODY2 from T1D were 0.670 and 0.662, respectively; the ROC-derived triglyceride value for discriminating MODY3 from MODY2 was 0.756. Additionally, a combination of indicators permitted better discrimination of MODY subtypes than any single parameter.

**Conclusion:** Our findings suggest that fasting C-peptide, hsCRP, and lipid levels permit good discrimination among MODY2, MODY3, and T1D. These clinical indicators could be used as markers of MODY2 and MODY3 in young patients with diabetes.

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J. Fu · T. Wang · J. Liu · X. Wang · Q. Zhang · M. Li ·  
X. Xiao (✉)  
Peking Union Medical College Hospital, Beijing,  
China  
e-mail: xiaoxh2014@vip.163.com

**Keywords:** C-peptide; hsCRP; Lipid; MODY; Type 1 diabetes

## INTRODUCTION

Maturity-onset diabetes of the young (MODY) is a group of heterogeneous monogenic disorders characterized by early-onset autosomal-dominant inheritance and dysfunction of pancreatic beta cells [1]. Currently, 14 causative genes involved in beta-cell development or insulin secretion have been identified for MODY [1, 2]. The two most common subtypes, MODY2 and MODY3, which are caused by mutations in a gene encoding glucokinase and mutations in a gene responsible for hepatocyte nuclear factor 1-alpha (*HNF1A*), respectively, account for approximately 90% of these patients [1]. Individuals with MODY2 present with mild fasting hyperglycemia, rarely suffer from chronic complications, and do not require antidiabetic agent treatment. Patients with *HNF1A* mutations manifest with progressive beta-cell dysfunction and a high risk of diabetes-related micro- and macrovascular complications, and these cases are particularly sensitive to sulfonylurea [1, 3]. Thus, precise diagnosis promotes optimal glycemic control. However, it is still challenging for clinicians to differentiate MODY2 and MODY3 from other forms of diabetes due to their overlapping clinical features. Additionally, molecular genetic testing is too expensive and is not available in many areas. Consequently, cost-effective and practical biomarkers that can help to identify patients with a high probability of *GCK/HNF1A* mutations are needed. Our previous study (as well as other studies) indicated that high-sensitivity C-reactive protein (hsCRP) is significantly lower in MODY3 patients than in those with young-onset type 2 diabetes (T2D) and other subtypes of diabetes [4, 5]. Laboratory characteristics that help to distinguish MODY2 from other subtypes of diabetes include a small increase in glucose levels according to a oral glucose tolerance test (OGTT) at 120 min, as 95% of MODY2 patients will show glucose levels < 83 mg/dL at 120 min [6]. MODY and type 1 diabetes (T1D) patients are relatively young at disease onset, and most are non-obese.

Therefore, some MODY2 and MODY3 patients are misdiagnosed with T1D. Nevertheless, studies defining the clinical spectrum are still scarce in comparison to those focused on T1D in Chinese patients. Hence, we aimed to analyze the clinical features of subjects with MODY2 and MODY3. In addition, the present study evaluated the feasibility of using clinical features as diagnostic markers for differentiating *HNF1A/GCK* mutation carriers from T1D patients.

## METHODS

This study included 26 subjects with T1D, 29 subjects with MODY2, and 12 subjects with MODY3 who were matched in age and gender and were of Chinese origin. T1D was diagnosed according to the criteria of the American Diabetes Association [7] and the positivity of at least two persistent [including glutamic acid decarboxylase antibody (GAD), islet cell antibody (ICA), and protein tyrosine phosphatase antibody (IA2)]. MODY2 and MODY3 were diagnosed based on the following criteria: (1) a family history of diabetes with an autosomal-dominant mode of inheritance in at least two generations; (2) an age at onset of diabetes of  $\leq 45$  years; (3) absence of autoantibodies; (4) non-obesity ( $BMI < 28 \text{ kg/m}^2$ ), and positive genetic testing results [8]. The patients included in our study were aged 6–45 and were consecutively recruited from the outpatient clinic of the endocrinology department at Peking Union Medical College Hospital (PUMCH), Beijing, China, between January 2017 and December 2018. All procedures followed were in accordance with the ethical standards of the Peking Union Medical College Hospital Ethics Committee and with the 1964 Declaration of Helsinki, as revised in 2013. Written consent was obtained from all participants or from responsible family members.

The exons of the genes associated with MODY2 (*GCK*) and MODY3 (*HNF1A*) were screened using primer sequences designed using Premier 5 software (see Table S1 in the Electronic supplementary material, ESM). The NCBI BLAST database was used to identify variants

through alignment with the reference sequences NM\_000162 (*GCK*) and NM\_000545.6 (*HNF1A*). All variants found in our study were considered pathogenic based on prediction analysis, and were cosegregated with clinical phenotypes of MODY within families.

Clinical indices including height and weight were recorded for each participant. Venous blood samples were collected after an overnight ( $\geq 10$  h) fast. None of the participants were suffering from an illness (fever, infection, etc.) in the days before the study. Fasting insulin and C-peptide as well as 2-h postprandial insulin and C-peptide were measured by chemiluminescent analysis. The patients were told to have regular meals without too much sugar and fat. Glycated hemoglobin A1c (HbA1c) was measured using a dedicated high-performance liquid chromatography system. GAD and IA2 were determined by ELISA (SIMENS ADVIA Centaur XP, Germany), and ICA was measured using indirect immunofluorescence. The plasma concentrations of high-sensitive C-reactive protein (hs-CRP), total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglyceride, and glycated albumin were assayed using an automatic biochemical analyzer (AU5800; Beckman Coulter, USA).

### Statistical Analysis

All statistical analyses were performed using the Statistical Package for Social Sciences (SPSS 21.0 for Windows, Chicago, IL, USA). Skewed distributions were natural-logarithmically transformed prior to analysis. ANOVA with Bonferroni's post hoc test was utilized for continuous outcomes, and parameter values were expressed as the mean  $\pm$  standard deviation (SD). Logistic regression models were applied to analyze the associations of metabolic traits with different diabetic subtypes after controlling for gender and age. Odds ratios (ORs) and 95% confidence intervals (CIs) were evaluated.  $P < 0.05$  was considered to indicate a statistically significant difference. Receiver operating characteristic (ROC) curves were used to determine the optimal cutoff values of metabolic traits to

apply in order to distinguish among T1D, MODY2, and MODY3 patients. The optimal cutoff points were defined based on the maximum Youden index calculated as sensitivity + specificity – 1 [9].

## RESULTS

### Clinical and Laboratory Characteristics of Patients with MODY2 and MODY3

Among the 67 diabetic participants in this study, there were 26 T1D, 29 MODY2, and 12 MODY3 patients. The basic features of the subjects are summarized in Table 1. The mean ages of the T1D, MODY2, and MODY3 patients were  $22.88 \pm 5.85$ ,  $23.29 \pm 11.54$ , and  $21.75 \pm 8.74$  years, respectively. The three groups were matched in age and gender, and showed comparable heights and weights.

Compared to subjects with T1D, those with MODY3 had higher fasting C-peptide levels ( $1.34 \pm 1.51$  vs.  $0.29 \pm 0.22$  ng/mL;  $P < 0.001$ ) and 2-h C-peptide ( $2.42 \pm 1.35$  vs.  $0.62 \pm 0.57$  ng/mL;  $P = 0.002$ ) and lower hsCRP levels ( $0.18 \pm 0.15$  vs.  $1.22 \pm 1.49$  mg/L,  $P = 0.004$ ).

Similarly, patients with MODY2 showed different clinical features to subjects with T1D; they had lower HbA1c levels ( $6.42 \pm 0.35\%$  vs.  $9.52 \pm 2.99\%$ ;  $P < 0.001$ ), fasting glucose ( $6.92 \pm 0.58$  vs.  $9.58 \pm 4.42$  mmol/L;  $P = 0.003$ ), 2-h glucose ( $9.22 \pm 1.78$  vs.  $15.57 \pm 5.64$  mmol/L,  $P < 0.001$ ), hsCRP ( $0.37 \pm 0.39$  vs.  $1.22 \pm 1.49$  mg/L;  $P = 0.003$ ), total cholesterol ( $4.12 \pm 0.68$  vs.  $4.61 \pm 0.81$  mmol/L,  $P = 0.034$ ), and LDL-C levels ( $2.24 \pm 0.68$  vs.  $2.67 \pm 0.79$  mmol/L,  $P = 0.050$ ), but they had higher levels of fasting C-peptide ( $0.96 \pm 0.42$  vs.  $0.29 \pm 0.22$  ng/mL,  $P = 0.002$ ) and 2-h C-peptide ( $3.93 \pm 2.02$  vs.  $0.62 \pm 0.57$  ng/mL,  $P < 0.001$ ).

### Potential of Clinical Indices to Discriminate Among MODY Subtypes and T1D

A separate multivariable logistic regression analysis was performed to examine the feasibility of using each variable as a MODY subtype

**Table 1** General characteristics of the study subjects

	T1D ( <i>n</i> = 26)	MODY 2 ( <i>n</i> = 29)	MODY 3 ( <i>n</i> = 12)	<i>P</i>
Age (years)	22.88 ± 5.85	23.29 ± 11.54	21.75 ± 8.74	0.889
BMI (kg/m <sup>2</sup> )	19.83 ± 3.18	19.47 ± 4.37	20.70 ± 1.65	0.623
HbA1c%	9.52 ± 2.99	6.42 ± 0.35*	8.31 ± 2.62 <sup>#</sup>	<b>1.18 × 10<sup>-5</sup></b>
Fasting glucose (mmol/L)	9.58 ± 4.42	6.92 ± 0.58*	8.26 ± 3.17	<b>0.010</b>
2-h glucose (mmol/L)	15.57 ± 5.64	9.22 ± 1.78*	13.46 ± 6.34 <sup>#</sup>	<b>3.52 × 10<sup>-5</sup></b>
Fasting C-peptide (ng/mL)	0.29 ± 0.22	0.96 ± 0.42*	1.34 ± 1.51*	<b>1.88 × 10<sup>-4</sup></b>
2-h C-peptide (ng/mL)	0.62 ± 0.57	3.93 ± 2.02*	2.42 ± 1.35 <sup>#</sup>	<b>1.91 × 10<sup>-9</sup></b>
hsCRP (mg/dL)	1.22 ± 1.49	0.37 ± 0.39*	0.18 ± 0.15*	<b>0.004</b>
Total cholesterol (mmol/L)	4.61 ± 0.81	4.12 ± 0.68*	4.41 ± 0.93	0.098
Triglyceride (mmol/L)	0.74 ± 0.32	0.65 ± 0.38	0.97 ± 0.43	0.051
HDL-C (mmol/L)	1.28 ± 0.53	1.47 ± 0.25	1.32 ± 0.39	0.227
LDL-C (mmol/L)	2.67 ± 0.79	2.24 ± 0.68	2.57 ± 0.86	0.125

Results are from one-way ANOVA; significant differences ( $P < 0.05$ ) versus T1D are indicated by \*, and significant differences ( $P < 0.05$ ) versus MODY2 individuals are indicated by <sup>#</sup>

Values in bold are significant ( $P < 0.05$ ) after Bonferroni correction

T1D type 1 diabetes, MODY 2 maturity-onset diabetes of the young type 2, MODY 3 maturity-onset diabetes of the young type 3, hs-CRP high-sensitivity C-reactive protein, LDL-C low-density lipoprotein cholesterol, HDL-C high-density lipoprotein cholesterol

predictor. As shown in Table 2, after adjusting for age and gender, decreased HbA1c (OR 0.163; 95% CI 0.04–0.61), fasting glucose (OR 0.69; 95% CI 0.52–0.91), 2-h glucose (OR 0.58; 95% CI 0.42–0.80), lnCRP (OR 0.48; 95% CI 0.25–0.94), total cholesterol (OR 0.37; 95% CI 0.16–0.89), and LDL-C (OR 0.36; 95% CI 0.14–0.92) levels and increased fasting C-peptide (OR 870; 95% CI 10.6–(7.2 × 10<sup>4</sup>)) were independent predictors of MODY2 compared to T1D. Additionally, decreased lnCPR levels (OR 0.19; 95% CI 0.04–0.95) and increased fasting C-peptide (OR 5.7 × 10<sup>4</sup>; 95% CI 1.9–(1.7 × 10<sup>9</sup>)) and 2-h C-peptide (OR 17.6; 95% CI 2.0–152) levels were clinical markers of MODY3 versus T1D. Further, HbA1c (OR 2.94; 95% CI 1.15–7.56), 2-h glucose (OR 1.47; 95% CI 1.02–2.12), and triglyceride (OR 12.47; 95% CI 1.64–94.80) levels were positive predictors whereas hsCRP level (OR 0.21, 95% CI 0.05–0.86) was a negative predictor of MODY3 versus MODY2.

### ROC Curve Analysis

ROC curve analysis was used to evaluate the performance of the indicators as diagnostic markers for discriminating among different diabetic subtypes in young patients.

### Diagnostic Value of hsCRP

The results showed that the hsCRP level allowed good discrimination between MODY2 and T1D, with an AUC of 0.675. A cutoff hsCRP value of < 0.280 mg/L was identified which gave 66.7% sensitivity and 67.9% specificity. When discriminating MODY3 from T1D, hsCRP exhibited an AUC of 0.833, and a cutoff of < 0.200 mg/L gave 80.0% sensitivity and 80.0% specificity. For MODY3 vs. MODY2, the hsCRP level gave an AUC of 0.763, and a cutoff value of < 0.185 mg/L yielded 75.0% sensitivity and 80.0% specificity (Fig. 1).

**Table 2** Potentials of different variables to discriminate between the MODY subtypes and T1D

Variables	T1D vs. MODY 2		T1D vs. MODY 3		MODY 2 vs. MODY 3	
	OR (95% CI)	<i>P</i>	OR (95% CI)	<i>P</i>	OR (95% CI)	<i>P</i>
HbA1c%	0.16 (0.04–0.61)	<b>0.007</b>	0.81 (0.60–1.09)	0.169	2.94 (1.15–7.56)	<b>0.025</b>
Fasting glucose (mmol/L)	0.69 (0.52–0.91)	<b>0.009</b>	0.94 (0.76–1.15)	0.526	1.57 (0.97–2.54)	0.069
2-h glucose (mmol/L)	0.58 (0.42–0.80)	<b>0.001</b>	0.94 (0.82–1.08)	0.375	1.47 (1.02–2.12)	<b>0.039</b>
ln(fasting C-peptide) (ng/mL)	870 (10.6–(7.2×10 <sup>4</sup> ))	<b>0.002</b>	5.7×10 <sup>4</sup> (1.9–(1.7×10 <sup>9</sup> ))	<b>0.037</b>	2.31 (0.50–10.80)	0.287
ln(2-h C-peptide) (ng/mL)	2.7×10 <sup>4</sup> (0.5–(1.4×10 <sup>9</sup> ))	0.065	17.6 (2.0–152)	<b>0.009</b>	0.19 (0.03–1.13)	0.067
lnCRP (mg/dL)	0.48 (0.25–0.94)	<b>0.033</b>	0.19 (0.04–0.95)	<b>0.043</b>	0.21 (0.05–0.86)	<b>0.031</b>
Total cholesterol (mmol/L)	0.37 (0.16–0.89)	<b>0.025</b>	0.81 (0.32–2.04)	0.650	1.70 (0.65–4.45)	0.277
Triglyceride (mmol/L)	0.30 (0.04–2.08)	0.224	8.14 (0.86–77.50)	0.068	12.47 (1.64–94.80)	<b>0.015</b>
HDL-C (mmol/L)	3.85 (0.73–20.48)	0.113	1.48 (0.28–7.71)	0.641	0.14 (0.01–2.04)	0.149
LDL-C (mmol/L)	0.36 (0.14–0.92)	<b>0.032</b>	0.91 (0.36–2.35)	0.852	1.98 (0.70–5.64)	0.200

The OR (95% CI) and *P* values shown are from logistic regression and have been adjusted for age and gender. Values in bold are significant at *P* < 0.05.

T1D type 1 diabetes, MODY 2 maturity-onset diabetes of the young type 2, MODY 3 maturity-onset diabetes of the young type 3, hs-CRP high-sensitivity C-reactive protein, LDL-C low-density lipoprotein cholesterol, HDL-C high-density lipoprotein cholesterol.

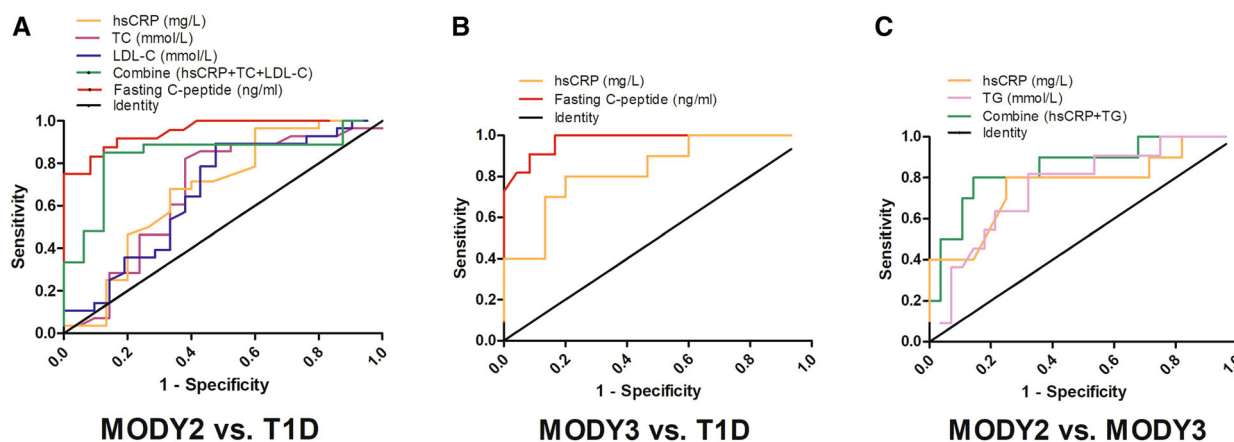
### Diagnostic Value of Fasting C-Peptide

The results showed that the fasting C-peptide level permitted good discrimination between MODY2 and T1D, with an AUC of 0.951; a cutoff fasting C-peptide value of > 0.580 ng/mL yielded 87.5% sensitivity and 87.5% specificity. When this clinical parameter was used to discriminate MODY3 from T1D, we obtained an AUC of 0.975 and a cutoff fasting C-peptide value of > 0.485 ng/mL, which provided 100% sensitivity and 83.3% specificity. However, the fasting C-peptide level could not be used to distinguish between the two subtypes of MODY (Fig. 1).

### Diagnostic Value of Lipids

The results showed good discrimination between MODY2 and T1D using total cholesterol, with an AUC of 0.670 and a cutoff value of < 4.525 mmol/L, which allowed 61.9% sensitivity and 82.1% specificity. When discriminating between MODY2 and T1D using LDL-C, the AUC was 0.662 and the identified cutoff value was < 2.65 mmol/L, yielding 52.4% sensitivity and 89.3% specificity. Additionally, discriminating between MODY3 and MODY2 using the triglyceride level led to an AUC of 0.756 and a cutoff value of > 0.65 mmol/L, which permitted 81.8% sensitivity and 67.9% specificity (Fig. 1).





**Fig. 1** Use of ROC curves to examine the abilities of various clinical indices to discriminate between different subtypes of diabetes. **a** MODY2 vs. T1D; the area under the curve is 0.951 for fasting C-peptide, 0.675 for hsCRP, 0.670 for total cholesterol, 0.662 for LDL-C, and 0.833 for the combination of hsCRP, total cholesterol, and LDL-C.

### Diagnostic Value of Combining Metabolic Indices

Since hsCRP, fasting C-peptide, total cholesterol, and LDL-C were all independent predictors of the MODY2 phenotype, we used ROC analysis to investigate whether a combination of those indicators could be used to predict MODY subtypes more effectively than the individual indices. The ROC curve for the combination of hsCRP with fasting C-peptide, total cholesterol, and LDL-C resulted in the highest AUC (1.0) for discriminating MODY2, whereas the combination of hsCRP, total cholesterol, and LDL-C yielded an AUC of 0.833. The combination of hsCRP with fasting C-peptide provided an AUC of 1.0 for distinguishing MODY3 from T1D. The combination of hsCRP and triglyceride gave an AUC of 0.85 for differentiating MODY3 from MODY2. Thus, we found that combinations of biochemical indicators can be used as markers to distinguish MODY subtypes.

## DISCUSSION

MODY is a relatively rare monogenic form of diabetes and is often misdiagnosed as T1D or

**b** MODY3 vs. T1D; the area under the curve is 0.833 for hsCRP and 0.975 for fasting C-peptide. **c** MODY2 vs. MODY3; the area under the curve is 0.763 for hsCRP, 0.756 for triglyceride, and 0.85 for the combination of hsCRP and triglyceride

T2D [1, 10]. Obtaining a definite genetic diagnosis of MODY is very important for predicting the clinical course of diabetes, guiding the optimization of treatment, and evaluating the risk to relatives. Therefore, it is crucial to identify novel and inexpensive biomarkers that could be used to identify these patients. To better define the phenotypic characteristics of Chinese patients with MODY2 and MODY3, we compared the clinical indicators of patients carrying mutations in *GCK/HNF1A* with those of T1D subjects and found that fasting C-peptide, hsCRP, and lipid levels could be used as biomarkers to distinguish MODY2 and MODY3 from T1D. This study therefore provides useful information for clinical workers who wish to identify the most common subtypes of MODY (MODY2 and MODY3) in patients with early-onset diabetes (< 45 years old).

### Serum C-Peptide Levels

Fasting C-peptide levels were significantly lower in T1D patients than in those diagnosed with MODY subtypes—the cutoffs were 0.580 ng/mL and 0.485 ng/mL, respectively, for MODY2 and MODY3. Decreased beta-cell function in T1D individuals can be explained; see [7]. MODY2 is

caused by an inactivating mutation of *GCK*, which serves as a key regulating enzyme in glucose-stimulated insulin secretion and acts as a glucose sensor in pancreatic beta cells; however, this mutation does not impair the function of beta cells [11]. Thus, *MODY2* is characterized by asymptomatic fasting hyperglycemia, which is present from birth and remains stable throughout life. Unlike *MODY2*, *MODY3* is caused by mutations of *HNF1A*, a transcription factor, and leads to decreased insulin secretion and progressive beta-cell damage [12]. The lack of a difference in fasting C-peptide levels between the two subtypes of *MODY* in our study may be due to the young age of the subjects included.

### Serum hsCRP Levels

The binding sites of the transcription factor *HNF1A* are harbored in the promoter of the *hsCRP* gene [13]. The inactivating mutations in *HNF1A* that are responsible for *MODY3* have been shown to result in a significant reduction in hsCRP [14]. Our previous study [4] and a study from the UK [5] confirmed that the level of serum hsCRP was significantly lower in *MODY3* patients than in T2D patients, and identified hsCRP cutoffs of 0.79 mg/L and 0.75 mg/L, respectively. In our study, we found that hsCRP levels were significantly lower in *MODY3* than in T1D and *MODY2* patients, in line with studies by McDonald [5] and Owen [15], and the relevant cutoffs were 0.200 mg/L and 0.185 mg/L, respectively. Additionally, we demonstrated that hsCRP levels were dramatically lower in *MODY2* subjects than in T1D subjects, in accord with a study by McDonald [5], and the cutoff was 0.280 mg/L. However, Owen et al. [15] reached a different conclusion—that hsCRP levels were higher in *MODY2* patients than in T1D patients, which may be due to racial/ethnic differences between the studies. Further large multicenter sample research may help to explain the results. Unlike *MODY3*, the mechanism for the association between *MODY2* and hsCRP is still unclear, although the low inflammatory response in

patients with *MODY2* may partly explain this clinical manifestation.

### Serum Lipid Levels

The hepatically expressed protein encoded by *HNF1A* is thought to bind to the genes responsible for lipid metabolism [16]. In humans, *HNF1A* defects are believed to increase the synthesis of bile acid [17] and influence the levels of total cholesterol, HDL-C, and LDL-C [18]. *HNF1A*-knockout mice (*HNF1A*<sup>-/-</sup>) were found to overexpress lipoprotein lipase (LPL) gene transcripts [19]. LPL encodes lipoprotein lipase, which is mostly found in the endothelial surfaces of capillaries in organs such as adipose tissues, liver, and skeletal muscle, where it hydrolyzes triglyceride-rich lipoprotein, including VLDL and chylomicrons [20]. This is why a previous study demonstrated that triglyceride levels were lower in *MODY3* than in T2D [21]. Lipid levels in *MODY3* patients were found to be no different from those in T1D patients in our study and in a previous study by Fendler et al. [22]; this may be explained by small sample sizes. Nevertheless, we found that subjects with *MODY2* had lower total cholesterol and LDL-C levels than T1D subjects, and we identified cutoffs of 4.525 mmol/L and 2.65 mmol/L, respectively. Previous studies have reported that overexpression of liver *GCK* could increase glycogen deposits, lower blood glucose levels, and increase triacylglycerol and free fatty acid levels [23]. Decreasing *GCK* activity may reduce the production of both glycogen and malonyl-CoA by lowering glycolytic flux [24]. Because it plays an important role in lipid metabolism, lowering the level of malonyl-CoA leads to weakened inhibition of carnitine palmitoyltransferase 1, thus increasing the oxidation of fatty acids [25]. This is why Spégel et al. [26] suggested that there were lower levels of free fatty acids and triglycerides in *MODY2* patients than in healthy control subjects. However, our results did not agree with those reported by Fendler et al. [27], who suggested that HDL-C could be used as a diagnostic tool for the clinical differentiation of *GCK-MODY* from *HNF1A-MODY* and T1D. These

conflicting results may be explained by small sample sizes and ethnic differences between the studies. Additionally, we found that triglyceride levels were lower (cutoff value: 0.65 mmol/L) in subjects with MODY2 than in those with MODY3, which was also reported by Mughal [28], indicating that metabolic control is more intact in MODY2 patients than in MODY3 subjects.

Importantly, we found that the combination of hsCRP, fasting C-peptide, and lipid levels yielded better discrimination of MODY2 and MODY3 from T1D than that obtained with any of those metabolic parameters individually. Our finding supports the hypothesis that one metabolic abnormality contribute only to an insufficient degree, while additive metabolic indicators have a more significant effect on the risk of diabetes.

Although we aimed to identify diagnostic markers that could be used to differentiate young *HNF1A/GCK* mutation carriers from young T1D patients for the first time in mainland China, some potential limitations should be noted. First, the small number of MODY2 and MODY3 subjects included in the study restricted our analysis of genotype and phenotype correlations, resulting in a large OR (95% CI) for some parameters. Second, our findings are based on data from Chinese diabetic patients, so they cannot be generalized to other racial populations without further replication. Additionally, information on patient lifestyle (e.g., diet and activity) was scarce. Finally, although we analyzed the most common biochemical indicators in our study, we are aware that many others have also been implicated as discriminatory indicators [29]. Nonetheless, the observations made in the present study should contribute to a better understanding of markers allowing MODY2 and MODY3 patients to be distinguished from T1D patients.

## CONCLUSION

In summary, we found that fasting C-peptide, hsCRP, total cholesterol, and LDL-C levels can be used to discriminate between MODY2 and T1D; fasting C-peptide and lnCRP levels can be

used to distinguish between MODY3 and T1D; and hsCRP and triglyceride levels can be used to discriminate MODY2 from MODY3. Combinations of multiple biochemical indicators can also be used as markers to better distinguish between MODY subtypes. Our study may help to identify individuals at high risk of MODY2 or MODY3 among young patients with diabetes in China.

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**Compliance with Ethics Guidelines.** All procedures followed were in accordance with the ethical standards of the Peking Union Medical College Hospital Ethics Committee and with the 1964 Declaration of Helsinki as revised in 2013. Informed consent was obtained from all individual participants included in the study.

**Data Availability.** The datasets obtained and/or analyzed during the current study are



available from the corresponding author on reasonable request.

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