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ORIGINAL ARTICLE

Recognition of maturity-onset diabetes of the young in China

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Keywords
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ABSTRACT

Aims/Introduction: Given that mutations related to maturity-onset diabetes of the young (MODY) are rarely found in Chinese populations, we aim to characterize the mutation spectrum of MODY pedigrees.

Materials and Methods: Maturity-onset diabetes of the young candidate gene- or exome-targeted capture sequencing was carried out in 76 probands from unrelated families fulfilling the clinical diagnostic criteria for MODY. MAF <0.01 in the GnomAD or ExAC database was used to filter significant variants. Sanger sequencing was then carried out to validate findings. Function prediction by SIFT, PolyPhen-2 and PROVEAN or CADD was carried out in missense mutations.

Results: A total of 32 mutations in six genes were identified in 31 families, accounting for 40.79% of the potential MODY families. The MODY subtype detection rate was 18.42% for *GCK*, 15.79% for *HNF1A*, 2.63% for *HNF4A*, and 1.32% for *KLF11*, *PAX4* and *NEUROG3*. Seven nonsense/frameshift mutations and four missense mutations with damaging prediction were newly identified novel mutations. The clinical features of MODY2, MODY3/1 and MODYX are similar to previous reports. Clinical phenotype of *NEUROG3* p.Arg55Glufs*23 is characterized by hyperglycemia and mild intermittent abdominal pain.

Conclusions: This study adds to the emerging pattern of MODY epidemiology that the proportion of MODY explained by known pathogenic genes is higher than that previously reported, and found *NEUROG3* as a new causative gene for MODY.

INTRODUCTION

Maturity-onset diabetes of the young (MODY) is a monogenetic disease with the characteristics of autosomal dominant inheritance, early onset (<25 years) and non-insulin dependence, accounting for 1–4% of patients with diabetes[1,2]. Mutations in at least 13 different genes (*HNF4A*, *GCK*, *HNF1A*, *PDX1*, *HNF1B*, *NEUROD1*, *KLF11*, *CEL*, *PAX4*, *INS*, *BLK*, *ABCC8* and *KCNJ11*) have been shown to cause MODY subtypes 1–13[3–5]. Previous studies have shown that mutations in the *GCK* gene (MODY2), *HNF1A* (MODY3) and *HNF4A* gene (MODY1) represent the most common causes of MODY in the Western population, accounting for 80–90% of all MODY cases, but mutations in these genes account for just 10–20% of MODY cases in Asia (including China, Japan and Korea)[6]. Therefore, genetic causes of >80% of Asian patients with MODY remain as yet unidentified[7,8]. Accordingly, there is a clear unmet need to reassess the mutational spectrum of MODY in the Chinese population. In the current study, 76 families with possible MODY were first screened for mutations using a next-generation sequencing strategy, and the results were then validated using Sanger sequencing.

METHODS

Participants

A total of 76 unrelated families who participated in the "National Registration Project for Monogenetic Diabetes" initiated by the Chinese Diabetes Society from 2013 to 2016 were

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recruited from 35 hospitals (Table S1). All the families met the generally accepted clinical diagnostic criteria for MODY as follows: a positive familial history of diabetes, with at least two successive generations affected; early-onset hyperglycemia (at least one family member diagnosed aged <25 years); and non-insulin dependence (lack of a need for insulin treatment or a serum C-peptide level of >0.60 ng/mL, even after 3 years of insulin treatment)[9]. The mean age at diagnosis of the probands was 22.30 ± 9.33 years, and the mean body mass index (BMI) was 22.31 ± 5.64 kg/m^2. A total of 74 pedigrees were of Chinese Han ethnicity, and two were of Tibetan and Manchu ethnicity. The clinical data of all probands are shown in Table S2.

The study conforms to the provisions of the Declaration of Helsinki, and was approved by the Clinical Medical Research Ethics Committee of the Third Affiliated Hospital of Sun Yat-sen University, Approval No. [2013]2-106. All informed consent was obtained from all the participants(s) and/or guardian(s).

Target gene and whole-exome capture sequencing

Genomic deoxyribonucleic acid (DNA) was extracted from peripheral blood leukocytes using the QIAamp DNA Blood Mini Kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's manual. The DNA concentration and purity were measured using a NanoDrop 2000 (Thermo Fisher Scientific, Wilmington, DE, USA). A 260 : 280 ratio of ~1.8 is accepted to indicate purity of DNA.

The library was prepared, and the genes of interest or exome were captured using the BGI MGISEQ-2000 (BGI, Shenzhen, Guangdong, China) platform. Briefly, genomic deoxyribonucleic acid was broken into 100–500 bp fragments by an enzyme kit (Segmentase; BGI), and the "A" base was then added at 3′ overhangs of 280–320 bp fragments, which were collected by magnetic beads. After adaptor ligation, the library was amplified by ligation-mediated polymerase chain reaction. Capture enrichment was carried out by array hybridization of the library with specific capture probes designed to target genes of interest or exon regions (Roche NimbleGen, Madison, WI, USA), followed by elution and post-capture amplification. Agilent 2100 bioanalyzer and BMG were used to determine the concentration of the products. Qualified single-chain library products were circularized into DNA nanoballs and finally sequenced on the MGISEQ-2000 platform of BGI.

Mutation screening and bioinformatics analysis

The flow chart of mutation screening is shown in Figure S1. Briefly, 76 probands from unrelated families with MODY were screened with the target gene panel of monogenic diabetes-related disorders (DX0270; BGI-Shenzhen Huada Gene Technology Co Ltd., Shenzhen, China), covering 13 genes previously associated with MODY (*HNF4A*, *GCK*, *HNF1A*, *PDX1*, *HNF1B*, *NEUROD1*, *KLF11*, *CEL*, *INS*, *BLK*, *ABCC8*, *KCNJ11*). All variants with a minor allele frequency <0.01 in the Genome Aggregation Database (GnomAD) v2.1.1 or ExAC (v1.0) database found by panel detection were further validated using Sanger sequencing for both probands and family members. For families without identifying genetic causes in the MODY-target gene panel sequencing, whole-exome sequencing was carried out to broaden the scope of genetic screening.

Functional prediction of new missense variants was carried out by SIFT, PolyPhen-2, Protein Variation Effect Analyzer (PROVEAN) or combined annotation-dependent depletion (CADD). A variant was predicted to be deleterious, with SIFT score <0.05, PolyPhen-2 HumVar score >0.95 and PROVEAN score <−2.5 or CADD Phred score >20.

Missense variants with a positive phyloP score and/or with a PhastCons value closer to 1 were predicted to be conserved[10].

For pathogenic variants, the criteria for pathogenic or likely pathogenic in the American College of Medical Genetics/Association for Molecular Pathology Variant Interpretation Standards and Guidelines[11] were applied.

Clinical assessment and laboratory measurement

The following clinical data were obtained from patients and their family members: age, sex, nationality, native place, diagnostic approach, age at diagnosis, BMI, glycated hemoglobin (HbA1c), serum glucose, insulin and C-peptide (CP), lipid profile (total cholesterol, low-density lipoprotein, high-density lipoprotein and triglycerides), past medical history (e.g., hypertension, diabetic microvascular and macrovascular complications), and treatment.

A 75-g oral glucose tolerance test (OGTT) was carried out in probands and their family members without a prior history of diabetes to determine glucose tolerance and evaluate islet function. A steamed bread meal test (SBMT) was carried out for those with previously diagnosed diabetes. The steamed bread was made of 100 g flour containing carbohydrates equivalent to approximately 75 g glucose. Patients began fasting after dinner the day before the test. On the test day, after collecting fasting blood samples, patients ate the steamed bread completely within 15 min, and venous blood samples were collected at 30 and 120 min. SBMT- and OGTT- stimulated blood glucose, serum insulin and C-peptide are equivalent[12]. In China, SBMT is often used as a simulation to replace OGTT to observe the postprandial changes in blood glucose, and islet function in patients with diabetes for safety and comfort reasons.

Glucose-related indices were calculated, including incremental 2-h glucose (Δ2h-G) during the OGTT or SBMT, the area under the curve (AUC) of glucose (AUC_{Glu}), the AUC of CP (AUC_{CP}), AUC_{CP}/AUC_{Glu} ($AUC_{CP/G}$) and CP30' increment/glucose30' increment (ΔCP30'/ΔGlu30'). The AUC was calculated using the trapezoidal principle

Homeostatic model assessment of insulin resistance (HOMA-IR) and homeostatic model assessment of β-cell function (HOMA-B) were calculated using the following formulas: fasting insulin (μIU/mL) × fasting plasma glucose (FPG; mmol/L) / 22.5 and 20 × fasting insulin (μIU/mL) / (fasting plasma glucose; mmol/L − 3.5) (%), respectively.

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Statistical analysis

Statistical analysis was carried out using Statistical Package for Social Sciences (SPSS for Windows, version 25.0, SPSS Inc., Chicago, IL, USA). Clinical data and laboratory parameters are expressed as the mean ± standard deviation. Comparisons of numerical variables between or among groups were carried out by Student's *t*-test or the Kruskal–Wallis test; the χ^2-test was used for categorical data. The *P*-value was corrected by the Bonferroni correction. All tests were two-sided. In all statistical analyses, $P < 0.05$ was considered to show statistical significance.

RESULTS

Mutation detection rate

Among 76 pedigrees, 32 mutations in six genes (Table 1) that were either previously described or novel likely pathogenic were identified in 31 pedigrees (Figure S2), accounting for 40.79% of MODY cases. The mutations in each proband, including 15 *GCK* mutations (MODY2), 12 *HNF1A* mutations (MODY3), two *HNF4A* mutations (MODY1), one *KLF11* mutation (MODY7), one *PAX4* mutation (MODY9) and one *NEUROG3* mutation, are listed in Table 1. All mutations were inherited genetic alterations, except for two de novo mutations (*HNF4A*-MODY1 p.Cys808Ser and *HNF1A*-MODY3 p.Lys120Glu). Proband 16 carried compound *GCK* mutations inherited from her father. Thus, the frequency of MODY subtype was 18.42% for MODY2, 15.79% for *HNF1A*, 2.63% for *HNF4A*, and 1.32% for *PAX4*, *KLF11* and *NEUROG3*. In addition, an *ABCC8* variant is suspected to be causal of MODY12 (Table 1). All of these mutations are absent from or have a minor allele frequency <0.01 in the GnomAD and ExAC databases, and all missense mutations except HNF 1A p.Ile618Met affect a highly conserved residue with a positive PhyloP score and PhastCons score equal to or close to 1 (Table 1).

MODY2 mutations

A total of 12 different *GCK* mutations were identified (Table 1), including six previously described missense mutations. Four of the six new *GCK* mutations found in the present study are inactivating, as they caused truncation of proteins due to the introduction of a premature stop codon (p.Tyr289-Metfs*5 and p.Tyr413Ter) or caused nonsense proteins due to the modification of the reading frame (p.Asp341-Ser418del and p.Leu415Alafs*44). Two new missense mutations (p.Asp160Glu and p.Lys169Glu) were predicted to be deleterious by SIFT, PolyPhen-2 and PROVEAN, with a CADD Phred score >20 (Table S3). The Arg191Trp, a previously reported and functionally verified mutation in white and Asian populations, was identified in four families (Table 1), making it the most common *GCK* mutation in the present study.

MODY3 and MODY1 mutations

Nine different *HNF1A* mutations were identified, all of which have been reported previously. Three known mutations (p.Arg54Ter, p.Arg271Trp, p.Pro291fs*26) were detected in two probands each. In addition, three mutations (p.Arg54Ter, p.Pro291fs*26, p.Val380Cysfs*39) are truncation mutations that represented 41.67% of the *HNF1A* mutations identified in our MODY3 population.

Two new *HNF4A* mutations were identified. p.Ser296Thrfs*31 is a truncation mutation, and p.Cys80Ser is a de novo mutation and suspected to be a harmful variant according to SIFT, PROVEAN and PolyPhen-2, with a CADD Phred score of 26.4 (Table S3).

Other MODY mutations

Other MODY mutations (MODY-others) included those in *KLF11* (MODY7), *PAX4* (MODY9) and a novel mutation in *NEUROG3* (Table 1). None of these mutations have been described previously. *KLF11* p.Lys453del causes a single amino acid deletion, which might have a deleterious effect on the function of the protein, and has damaging predictions by PROVEAN (Table S3). *PAX4* p.Pro111Leu is predicted to be harmful by SIFT, PolyPhen-2 and PROVEAN, with a high CADD (Phred score = 28.7; Table S3). *NEUROG3* p.Arg55Glufs*23 changes the reading frame, causes premature termination of translation and is considered a disease-causing mutation.

Suspected causal variant

ABCC8 p.Thr1115Met was found in a pedigree. This variant is absent from the GnomAD and ExAC databases of East Asia, is evolutionally conserved (Table 1), and is predicted to be damaging by SIFT, PolyPhen-2 and PROVEAN, and has a CADD phred score >30 (Table S3). Therefore, although the co-segregation between mutation and phenotypes could not be clearly defined (Figure S2), we treat it as a suspected causal variant of MODY in our study.

Clinical characteristics of MODY subtypes and MODYX

The participants with MODY subtypes included probands and their mutation-positive relatives, whereas participants with MODYX included only probands. As shown in Table 2, the carriers of MODY mutations had a mean age of 34.46 ± 17.91 years, a mean age at diagnosis of 28.79 ± 16.21 years, a mean BMI of 20.7 ± 3.58 kg/m^2 and a mean HbA1c of 6.65 ± 1.20%.

MODY2 showed mild fasting hyperglycemia (6.76 ± 0.90 mmol/L) and markedly elevated Glu120' levels in OGTT or SBMT (11.76 ± 2.99 mmol/L). The mean 2-h glucose increment of OGTT or SBMT (Δ2h-G) for MODY2 was 5.05 ± 2.99 mmol/L. A small increase in mean HbA1c was found in MODY2 (6.35 ± 0.44%). After dividing MODY2 participants into two groups on the basis of median Δ2h-G (5.61 mmol/L), those with Δ2h-G <5.61 mmol/L were significantly younger, younger at diagnosis, and had a shorter diabetes duration, lower BMI and higher ΔCP30'/ΔG30' than those with Δ2h-G ≥5.61 mmol/L (Table S4). Among MODY2 participants, 62.07% were treated with diet alone, 37.93% with oral hypoglycemic agents (OHA) and 10.34% with insulin (Table 2). The

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Table 1 | List of mutations selected to be pathogenic and suspected for maturity-onset diabetes of the young

Probands	Gene	Sex	Age at diagnosis (year)	BMI (kg/m^2)	Mutation source	cDNA changes	AA changes	Reported	GnomAD (v2.1.1) East Asian	ExAC (v1.0) East Asian	PhyloP	PhastCons
Pathogenic mutation												
1	*HNF4A*	Female	16	20.00	*De novo*	c.238T>A	p.Cys80Ser	No	/	/	5.046	1
2	*HNF4A*	Female	27	21.56	M	c.885_894delG AGCTTGGAG	p.Ser296Thrfs*31	No	/	/		
3	*GCK*	Female	25	17.22	P	c.480T>G	p.Asp160Glu	No	/	/	3.277	1
4	*GCK*	Female	24	24.58	M	c.505A>G	p.Lys169Glu	No	/	/	5.034	1
5	*GCK*	Female	9	10.30	M	c.571 C>T	p.Arg191Trp	Yes[18,43]	5.437e-5 (1/18,392)	/	0.54	0.974
6	*GCK*	Male	38	18.52	P	c.571 C>T	p.Arg191Trp	Yes[18,43]	5.437e-5 (1/18,392)	/	0.54	0.974
7	*GCK*	Male	36	23.89	P	c.571 C>T	p.Arg191Trp	Yes[18,43]	5.437e-5 (1/18,392)	/	0.54	0.974
8	*GCK*	Male	10	15.73	P	c.571 C>T	p.Arg191Trp	Yes[18,43]	5.437e-5 (1/18,392)	/	0.54	0.974
9	*GCK*	Female	17	15.06	P	c.622 G>A	p.Ala208Thr	Yes[43]	0 (0/1,560)	/	6.234	1
10	*GCK*	Female	24	16.61	M	c.683 C>T	p.Thr228Met	Yes[44]	0 (0/18,372)	0 (0/8,582)	5.915	1
11	*GCK*	Female	6	13.77	M	c.781 G>A	p.Gly261Arg	Yes[44]	0 (0/18,388)	0 (0/8,614)	5.864	1
12	*GCK*	Male	12	17.19	M	c.865delT	p.Tyr289Metfs*5	No	/	/		
13	*GCK*	Male	5	14.86	P	c.971T>C	p.Leu324Pro	Yes[45]	/	/	3.107	1
14	*GCK*	Female	15	17.44	M	c.1020-7_c.1035del23	p.Asp341-Ser418 del	No	/	/		
15	*GCK*	Male	7	16.91	M	c.1166T>A	p.Val389Asp	Yes[19]	/	/	4.991	1
16	*GCK*	Female	7	17.36	P	c.1239C>A	p.Tyr413Ter	No	/	/	6.034	1
	GCK	Female	7	17.36	P	c.1241_1242insA	p.Leu415Alafs*44	No	/	/		
17	*HNF1A*	Male	24	-	M	c.139G>A	p.Gly47Arg	No	0 (0/19,720)	0 (0/7,460)	0.459	1
18	*HNF1A*	Female	13	20.54	M	c.160C>T	p.Arg54Ter	Yes[29,46]	/	/	0.383	0.297
19	*HNF1A*	Female	13	17.30	P	c.160C>T	p.Arg54Ter	Yes[29,46]	/	/	0.383	0.297
20	*HNF1A*	Male	13	18.90	M?	c.358A>G	p.Lys120Glu	Yes[28]	/	/	4.669	1
21	*HNF1A*	Male	17	19.88	M	c.476 G>A	p.Arg159Gln	Yes[27]	/	/	5.569	0.999
22	*HNF1A*	Female	30	21.00	M	c.599G>A	p.Arg200Gln	Yes[27]	0 (0/18,394)	/	4.974	0.991
23	*HNF1A*	Female	12	20.20	M	c.811 C>T	p.Arg271Trp	Yes[47]	0 (0/18,342)	/	1.277	1
24	*HNF1A*	Female	12	19.65	M	c.811 C>T	p.Arg271Trp	Yes[47]	0 (0/18,342)	/	1.277	1
25	*HNF1A*	Female	24	18.07	M	c.872dupC	p.Pro291fs*26	Yes[26,48]	4.850e-4 (9/18,558)	1.838e-3 (11/5,984)		
26	*HNF1A*	Female	15	29.14	M	c.872dupC	p.Pro291fs*26	Yes[26,48]	4.850e-4 (9/18,558)	1.838e-3 (11/5,984)		
27	*HNF1A*	Female	22	19	P	c.1136_1137insC	p.Val380Cysfs*39	Yes[49]	0 (0/18,356)	/		
28	*HNF1A*	Male	24	29.74	M	c.1854C>G	p.Ile618Met	Yes[50]	3.509e-4 (7/19,950)	/	−2.829	0.012
29	*KLF11*	Male	15	27.78	M	c. 1357_1359 delAAG	p.Lys453del	No	1.631e-4 (3/18,394)	1.156e-4 (1/8,654)		
30	*PAX4*	Male	12	18.83	M	c.332 C> T	p.Pro111Leu	No	6.524e-4 (13/19,926)	6.096e-4 (5/8,202)	5.625	1
31	*NEUROG3*	Female	14	22.22	P	c.163delC	p.Arg55Glufs*23	No	/	/		
Suspected mutation												
32	*ABCC8*	Male	5	19.44	M	c.3344C>T	p.Thr1115Met	No	0 (0/18,394)	0 (0/8,644)	6.424	1

The proband's mother died, neither his father nor his mother's sister carried the mutation. AA change, amino acid change; EX, exon; Het, heterozygote; Hom, homozygote; IN, intron; M, maternal; OHA, oral hypoglycemic agents; P, paternal.

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Table 2 | Clinical characteristics of maturity-onset diabetes of the young subtypes and maturity-onset diabetes of the young X

	MODY total	MODY2	MODY3/1	MODY-others	MODYX	$P_{T\ vs\ X}$*	$P_{2\ vs\ 3/1\ vs\ O}$*
Sex: M/F	36/51	20/25	10/22	6/4	25/19	0.094	0.228
Age (years)	34.46 ± 17.91	33.43 ± 19.25	37.81 ± 15.29	28.60 ± 19.02	24.09 ± 7.89	0.003	0.268
Age at diagnosis (years)	28.79 ± 16.21	29.91 ± 18.28	28.29 ± 12.87	25.40 ± 16.86	19.70 ± 5.40	0.004	0.695
Duration of diabetes (years)	5.67 ± 6.48	3.52 ± 3.90	9.52 ± 8.25	3.20 ± 3.55	4.39 ± 4.75	0.483	0.003
BMI (kg/m^2)	20.70 ± 3.58	19.82 ± 3.17	21.37 ± 3.13	22.60 ± 5.39	24.35 ± 5.59	<0.001	<0.001
HbA1c (%)	6.65 ± 1.20	6.35 ± 0.44	7.11 ± 1.64	6.58 ± 1.57	8.35 ± 2.12	<0.001	0.051
Glu0′ (mmol/L)	7.34 ± 2.09	6.76 ± 0.90	8.23 ± 2.91	7.45 ± 2.41	8.44 ± 3.19	0.065	0.066
CP0′ (ng/mL)	1.08 ± 0.86	1.04 ± 0.94	1.02 ± 0.85	1.38 ± 0.36	2.45 ± 2.65	0.002	0.355
AUC_{Glu} (mmol/L min)	1442 ± 417	1289 ± 232	1696 ± 533	1426 ± 398	1489 ± 460	0.001	<0.001
AUC_{CP} (ng/mL min)	388 ± 315	423 ± 329	324 ± 331	400 ± 182	601 ± 682	0.032	0.214
$AUC_{CP/G}$ (μg/mmoL min)	0.29 ± 0.25	0.33 ± 0.23	0.22 ± 0.31	0.31 ± 0.17	0.45 ± 0.56	0.095	0.552
Δ2h-G (mmol/L)	6.63 ± 3.93	5.05 ± 2.99	8.87 ± 4.28	7.49 ± 3.66	6.22 ± 4.74	0.008	<0.001
ΔCP30′/ΔG30′ (μg/mmol)	0.40 ± 0.35	0.49 ± 0.32	0.22 ± 0.19	0.50 ± 0.57	0.68 ± 1.05	0.032	0.011
HOMA-IR	1.75 ± 1.01	1.48 ± 0.85	1.78 ± 1.21	2.65 ± 0.72	2.51 ± 1.73	0.068	0.006
HOMA-B	36.95 ± 28.18	30.86 ± 14.70	26.69 ± 20.23	74.83 ± 44.61	62.84 ± 73.61	0.027	<0.001
CHOL (mmol/L)	3.84 ± 1.20,	3.75 ± 0.96	4.45 ± 1.12	2.38 ± 1.14	3.99 ± 1.22	0.218	<0.001
TRIG (mmol/L)	0.90 ± 0.71	0.81 ± 0.59	1.09 ± 0.91	0.76 ± 0.30	1.94 ± 2.65	0.015	0.169
HDL (mmol/L)	1.22 ± 0.37	1.24 ± 0.28	1.38 ± 0.38	0.68 ± 0.19	1.05 ± 0.35	0.032	<0.001
LDL (mmol/L)	2.20 ± 0.85	2.15 ± 0.79	2.53 ± 0.82	1.46 ± 0.77	2.31 ± 0.81	0.426	0.011
Overweight, *n* (%)	13, 15.66%	6, 13.64%	3, 10.34%	4, 40%	18, 42.86%	0.001	0.073
Hypertension, *n* (%)	11, 14.86%	4, 9.76%	5, 21.74%	2, 20%	7, 18.42%	0.628	0.384
Retinopathy, *n* (%)	4, 4.60%	0	4, 12.50%	0	2, 4.55%	1.000	0.027
Nephropathy, *n* (%)	2, 2.30%	0	2, 6.25%	0	3, 6.82%	0.334	0.172
Neuropathy, *n* (%)	2, 2.30%	0	2, 6.25%	0	2, 4.55%	0.602	0.172
Macrovascular, *n* (%)	2, 2.30%	0	2, 6.25%	0	0	0.550	0.172
Treatment at time of visit (*n*)	66	29	28	9	43		
Intensive insulin, *n* (%)	2,3.03%	0	2, 7.14%	0	7, 16.28%	0.027	0.247
Insulin, *n* (%)	19,28.79%	3, 10.34%	14, 50.00%	2, 22.22%	24, 55.81%	0.005	0.004
OHA, *n* (%)	30,45.45%	11, 37.93%	17, 60.71%	2, 22.22%	21, 48.84%	0.729	0.072
Sulfonylurea, *n* (%)	12,18.18%	5, 17.24%	7, 25.00%	0	5, 11.63%	0.357	0.235
Metformin, *n* (%)	20,30.30%	8, 27.59%	11, 39.29%	1, 11,11%	18, 41.86%	0.216	0.254
Others, *n* (%)	13,19.70%	3, 10.34%	9, 32.14%	1, 11,11%	11, 25.58%	0.469	0.092
Diet only, *n* (%)	26,39.39%	18, 62.07%	3, 10.71%	5, 55.56%	10, 23.26%	0.080	<0.001

Continuous values are expressed as mean ± standard deviation. *The *P*-value has been adjusted for age, duration and sex. The data of homeostatic model assessment of insulin resistance (HOMA-IR) and homeostatic model assessment of β-cell function (HOMA-B) included only the patients who were not treated with insulin. Δ2h-G, 2-h glucose increment of oral glucose tolerance test and steamed bread meal test; ΔCP30′/ΔG30′, CP30′ increment/glucose30′ increment; AUC_{CP}, area under the curve of C-peptide; AUC_{Glu}, area under the curve of 2-h glucose; BMI, body mass index; CHOL, cholesterol; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MODY, maturity-onset diabetes of the young; OHA, oral hypoglycemic agents; T versus X, MODY total versus MODYX; 2 versus 3/1 versus O, MODY2 versus MODY3/1 versus MODY-others; TRIG, triglycerides.

mean duration of MODY2 was 3.52 ± 3.90 years, and no diabetic vascular complications were reported (Table 2). Further analysis showed that MODY2 patients treated with OHA were significantly older, older at diagnosis and had a longer duration with a relatively lower HOMA-B value than patients treated with diet alone (Table S5).

MODY 3/1 participants had the longest duration (9.52 ± 8.25 years), the highest AUC_{Glu} and the lowest ΔCP30′/ΔG30′ after OGTT or SBMT among MODY subtypes. Microvascular and macrovascular complications of diabetes, including diabetic retinopathy, nephropathy, neuropathy and stroke, were only reported in the MODY3/1 group. A total of 25% of MODY3/1 patients were treated with sulfonylureas, and 50% were treated with insulin.

MODY-others had the highest BMI, HOMA-IR and HOMA-B values among the MODY subtypes, and the frequency of overweight in MODY-others tended to be higher than MODY2 and MODY3/1 (Table 2). MODY7 and MODY9 showed a high proportion of overweight or obesity with a mean HOMA-IR of 2.86 ± 0.90 and a mean HOMA-B of 74.24 ± 41.33 (Table S6). The clinical phenotype of *NEUROG3* p.Arg55Glufs*23 is characterized by hyperglycemia with mild

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intermittent abdominal pain (the proband complained of abdominal pain, and diabetes was detected during her visit; her sister also showed intermittent mild abdominal pain and hyperglycemia). There was a lack of abdominal pressure pain, lack of rebound tenderness and no diarrhea in the *NEUROG3* mutation carriers, and no abnormality was found on laboratory examination of the abdominal gastrointestinal tract. A total of 55.56% of patients with MODY-others were treated with diet alone, and those treated with OHA and insulin accounted for 22.22% each.

MODYX patients had a significantly younger age and age at diagnosis; higher BMI, HbA1c and triglycerides; and lower high-density lipoprotein than MODY patients, and also showed significantly higher AUC_{Glu}, AUC_{CP}, ΔCP30'/ΔGlu30' and HOMA-B (Table 2). A significantly higher percentage of overweight was found in MODYX than in MODY patients. The proportions of MODY subtypes and MODYX patients treated with insulin were 28.79 and 55.81%, respectively ($P = 0.005$). A total of 16.28% of MODYX patients received initial intensive insulin treatment, whereas just 3.03% of MODY patients did. No statistically significant difference in other clinical features was found between MODY and MODYX patients.

DISCUSSION

In the current study, the molecular diagnosis of 31 out of 76 (40.79%) suspected MODY pedigrees was confirmed. MODY2 and MODY3 are common MODY subtypes, accounting for 18.42 and 15.79% of MODY cases, respectively, which was higher than that reported in Hong Kong (1% for MODY2 and 9% for MODY3)[14] and Shanghai (10.42% for MODY2 and 1.95% for MODY3)[15,16].

Among MODY2 mutations, some have been reported to affect the enzyme affinity for adenosine triphosphate (ATP) (p.Val389Asp, p.Thr228Met) or glucose (p.Gly261Arg), or to decrease the thermal stability of the protein (p.Arg191Trp)[17,18]. The new missense mutations, p.Lys169Glu and p.Asp160Glu, are located in the glucose- and ATP-binding sites of *GCK*[19,20], respectively, and a different missense change at both sites had been reported to be pathogenic in *in vitro* kinetic assays[21]. With regard to compound mutations, two different loss-of-function mutations (p.Tyr413Ter and p. Leu415Alafs*44) on the same allele were identified, and the nonsense mutation is likely to be the causative variant, because it precedes the frameshift. The p.Arg191Trp, the most common mutation (28.57%, 4/14) in the present study, was also found to be common (15.79%, 9/57) in the study reported by Yorifuji *et al.*[22] in Japanese populations and another Chinese study reported by Wang *et al.*[18] (16.67%, 2/12), but was rare in Norwegian (0.82%, 1/122) and Italian (0%, 0/66) populations[23,24].

Most MODY3 mutations found in the present study are located in the conserved domain of the gene[25] and lead to loss of function by inhibiting the activity of wild-type *HNF1A* through dominant negative effects (p.Arg159Gln, p.Pro291fs*26)[26,27] or by reducing DNA binding (p.Lys120Glu, p.Arg271Trp)[27,28]. The p.Arg54Ter nonsense mutation results in a severely truncated *HNF1A* protein that lacks DNA binding and transcriptional activation domains based on analysis of the 3-D structure[29].

The clinical phenotypes of MODY2, MODY3/1 and MODYX reported herein are similar to those reported previously[30-32]. However, for MODY2, 57% had a high OGTT 2-h glucose increment (>4.6 mmol/L), which was different from the previous reports in a large European study[13] that showed 71% of MODY2 having a small OGTT 2-h increment (<3 mmol/L). Given that an increment of <4.6 mmol/L is often used as the criterion for priority screening of MODY2[9], this finding showed that a missed diagnosis might occur using this criterion to identify when *GCK* testing is appropriate in the Chinese population.

Sulfonylureas are considered the best first-line medications for patients with MODY3 and MODY1, and result in superior glycemic control than insulin[33] and metformin[34]. In the present study, approximately 25% of MODY3 and MODY1 patients were treated with sulfonylureas, indicating that most MODY3/1 patients did not receive the correct treatment. MODY2 is known to be a non-progressive form of diabetes, which usually can be controlled by diet alone without any medication, except during pregnancy[35]. Nevertheless, approximately 40% of MODY2 patients were treated with OHA in the present study. Patients treated with OHA were significantly older and had worse β-cell function than those treated with diet alone, suggesting that age-related decline in islet function might be related to administration of OHA for MODY2.

MODY7 and MODY9 are very rare causes of MODY. Their clinical features have not been fully established[36]. In the present study, MODY7 and MODY9 have a high proportion of overweight or obesity, and with relatively higher HOMA-IR than MODY2 and MODY3/1, suggesting a characteristic of metabolic syndrome, regardless, but no specific clinical characteristics other than common diabetes were found.

The newly reported gene, *NEUROG3*, is a key transcription factor in the differentiation of pancreatic and intestinal endocrine cells, and is responsible for transcriptional regulation of the *NEUROD1* (MODY6 pathogenic gene)[37]. Previous studies have shown that homozygous mutations in the *NEUROG3* gene lead to congenital malabsorptive diarrhea and neonatal diabetes[38], suggesting that the *NEUROG3* mutation has dual phenotypes regarding the gastrointestinal tract and blood glucose, which corresponds to the function of *NEUROG3*. This can also explain why the clinical phenotype of hyperglycemia and intermittent mild abdominal pain was observed in our patients with *NEUROG3* mutation.

Some limitations of the study were as follows: (i) because some genes have highly repetitive complex regions or pseudogenes, the high-throughput test cannot achieve 100% coverage, but the overall coverage can reach >95% (list link of low coverage regions http://db.bgidx.cn/); and (ii) MODY5 is a disease with low penetrant, and the most frequent mutations are

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monoallelic defects in all or some exons[39]. However, the NGS used in the present study cannot detect copy number variation in large genome fragments. Multiplex ligation-dependent probe amplification is a gold standard for copy number variation detection[40] and considered to be more advantageous than comparative genomic hybridization array in the genetic diagnostics of MODY5[41]. Therefore, estimation of MODY5 prevalence without multiplex ligation-dependent probe amplification analysis is a limitation of the present study. 17q12 deletion is the most common mutation of MODY5. As 70% of MODY5 caused by 17q12 deletion are sporadic[42], the criteria for MODY screening used in the present study, such as the presence of family history, might also have affected the assessment of the prevalence of MODY5.

Finally, there was still 60% of MODY patients with no confirmed genetic causes in the present study. The major genes for MODY in the Chinese population remain to be identified. Hence, more extensive genetic studies are required on Chinese patients with MODY.

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DISCLOSURE

The authors declare no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1 | List of participating centers.

Table S2 | Clinical characteristic of probands.

Table S3 | Prediction scores from SIFT, PolyPhen-2, Protein Variation Effect Analyzer and combined annotation-dependent depletion for the novel mutations.

Table S4 | Clinical features of maturity-onset diabetes of the young 2 stratified by the median of Δ2-h glucose.

Table S5 | Clinical features of maturity-onset diabetes of the young 2 patients treated with oral hypoglycemic agents and diet alone.

Table S6 | The clinical data of other maturity-onset diabetes of the young mutations patients.

Figure S1 | Flow chart of the procedures for screening of maturity-onset diabetes of the young mutations.

Figure S2 | Pedigrees of families with identified or suspected genetic causes of maturity-onset diabetes of the young.

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