Body mass index and C-peptide are important for the promptly differential diagnosis of maturity-onset diabetes from familial type 2 diabetes in outpatient clinic

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Abstract. Type 2 diabetic patients are becoming younger and having a tendency to family aggregation, they are easily suspected as maturity-onset diabetes of young (MODY) in the outpatient clinic and send to genetic testing. 9 diabetic families were compared in our outpatient clinic who met the primary diagnosis criteria of MODY. Detailed clinical features and laboratory data including gene sequence were collected and analyzed. The patients met the primary clinical diagnostic criteria of MODY for genetic testing at the first look. However, members of families A1 to A3 had normal Body mass index (BMI) and a lower C-peptide level which indicated impaired pancreatic islet function. In contrast, the members with diabetes of families B1 to B6 had normal or increased C-peptide level which indicated insulin resistance and were overweight with BMI. Genetic testing showed that the mutations in HNF1A, INS, KCNJ11 and so on in families A were consistent with the diagnosis of MODY. No pathogenic mutation was found in the members of families B which were diagnosed with familial T2D. Before the clinical laboratory testing and the further gene test, BMI and the concentration of C-peptide are important for the promptly differential diagnosis of MODY from familial type 2 diabetes and medication instruction in the outpatient clinic which could help to alleviate the burden of genetic testing for them.

Key words: Body mass index, C-peptide, Type 2 diabetes, Maturity-onset-diabetes-of-young (MODY), Outpatient

DIABETES is a group of metabolic diseases characterized by hyperglycemia and hypoglycemia. Hyperglycemia results from defects of insulin secretion or insulin resistance. Diabetes is associated with chronic damage, dysfunction and failure of multiple organs, which can be classified into the following general categories: type 1 diabetes, type 2 diabetes, gestational diabetes mellitus (GDM), and specific types of diabetes due to other causes, e.g., monogenic diabetes syndromes [1]. In clinic, specific types of diabetes was misdiagnosed as type 1 or 2 diabetes, resulting in initial improper therapy on patients because of relatively rare condition and awareness of the clinical phenotype and availability of testing, especially Maturity-onset diabetes of the young (MODY), one of the most popular specific type of diabetes resulting from monogenic mutation [2].

MODY is a rare, young-onset, dominantly-inherited and non-insulin-dependent type of diabetes resulting from β-cell dysfunction. The term MODY was first mentioned in a case report published in 1974, before which MODY was often defined as type 2 diabetes, resulting in misdiagnosis [3, 4]. Previous studies agree that the diagnostic criteria of MODY are: autosomal dominant inheritance, insulin independence within two years of onset and at least one family member was diagnosed with diabetes before the age of 25 [4]. Genetic testing for MODY patients has been possible since the major genetic causes were identified in the 1990s [5]. Since then, 14 different genes have been identified as being responsible for MODY [6-8]. These genes encode the transcription factor HNF4α hepatocyte nuclear factor 4α (MODY1), GCK glycolytic enzyme glucokinase (MODY2), HNF1α...
Mutations in each of 14 genes lead to the development of different MODY subtypes, differing in population distribution, clinical features, prevalence and management strategies [9-11]. The prevalence rate of MODY subtypes varies in different countries. MODY3 and MODY2 account for more than 90% of MODY patients [12], mostly in Caucasians patients. In contrast, only 7–11% of Asian patients are diagnosed with MODY2, MODY3 and MODY12 [10, 13-16].

Further genes related to MODY are likely to be found since most MODY patients have unknown mutations. This group is defined as MODYX. MODYX is responsible for 80%–90% of MODY in China which is different from other populations from 30% to 45% [14, 15, 17, 18]. A lack of awareness of MODY, as well as similar clinical features to other types of diabetes, means that clinicians cannot often easily differentiate MODY from type 2 diabetes without genetic testing. This is often based on a single, dated gene which might easily cause misdiagnosis. Accurate diagnosis of MODY requires a detailed history as well as genetic screening, which is nearly 3,000–7,000 yuan ($431.7–1,007.4) equaling with a common family monthly profits in China. In conclusion, there are two dominantly reasons for a Chinese diabetic patient difficulty to make a decision on genetic testing. On one hand, more than 80% genes responsible for MODY patients in China were unclear and genetic screening only covered part of candidate genes. On the other hand, genetic testing was expensive for the average class and was not covered by Chinese medical insurance institution at present.

Precise judgment for an outpatient clinician plays an important role in initial differential diagnosis and therapy. However, Chinese diabetes practitioners faced hundreds of patients every day and were challenging to make a quick first differential diagnosis in a very limited time. They tried to figure out patients who needed genetic testing at first sight out in order to minimized expenses. Therefore, new criteria relied on family history, physical signs and simple routine blood testing were essential for outpatient physicians. This study showed that BMI and the concentration of C-peptide might be important indexes except for three classical diagnostic indexes in considering the genetic testing of MODY, which help to differentiate MODY from familial type 2 diabetes more precisely before genetic testing.

Materials and Methods

Subjects
Nine probands were recruited from unrelated families admitted to the Second Hospital of Xiangya, the Second People’s Hospital of Shenzhen and the First People’s hospital of Huai-hua outpatient clinic. There were 19 subjects in all who indicated their willingness to participate. All these 9 families with a suspected MODY diagnosis fulfilled the following criteria: age of onset up to 25 years, family history of diabetes indicating autosomal dominant inheritance, negative autoimmune antibody and at least two years of insulin independence [4]. The study was approved by the Ethics Committee of the National Clinical Research Center for Metabolic Disease, Second Xiangya Hospital of Central South University and registered at Chinese clinical trial registry with registration number of ChiCTR1800019030. Informed consent was obtained from all patients for being included in the study.

Family history
Clinical data were collected by professional physicians and included: 1) gestational age and age of onset; 2) height and weight at the time of reporting; 3) family history of diabetes; 4) medication; and 5) complications.

Laboratory testing
The blood samples were centrifuged for serum isolation, frozen, and the fasting C-peptide and blood glucose were analyzed by the chemiluminescent method. The isolated serum was sent to the Diabetes Center of Central South University for further screening of ZnT8A, GADA, and IA-2A [19]. Three biochemical measurements were taken. The concentration of pancreatic autoimmune antibody was detected by radioligand binding assay (RLA). The detailed process of antibody assay was described previously [20]. The concentration of C-peptide, tested by a Bayer Centaur Immunoassay System; HbA1c, tested by ion-exchange HPLC with the normal range of 3.9–6.1% [20].

Genetic testing
Genomic DNA was extracted from peripheral blood samples taken from two probands. DNA samples were amplified using PCR with primers covering all exons and exon-intron junctions of the HNF1A, HNF4A, and
HNF1B because of the drug history and hypoglycemia symptoms tending to MODY1, 3 and 5. The forward and reverse primers are listed in Table 1. PCR was conducted using 50 μL of amplification reaction mixture which contained 1.5 μL of 100 ng genomic DNA, 1.0 μL of 1.0 μM of each primer and 25 μL of 2 × GreenMaster Mix. Cycling conditions were as follows: 95°C for 5 min followed by 35 cycles of amplification (94°C denaturation for 30 s, 60°C annealing for 30 s, 68°C elongation for 30 s) and a final extension at 68°C for 5 min, then a hold at 4°C. The cycling program took place in an Applied Biosystems thermocycler and the PCR products were determined using an ABI 700 (Applied BioSystems) sequencer extraction kit. Probands with a negative result for MODY1, 3, 5 were further screened with 45 genes known to be associated with monogenic forms of diabetes. The target genes are shown in Table 2. The screening was carried out via next-generation sequencing by BGI Dx in Shenzhen, China.

**Table 1** Nucleotide sequences of DNA primers for PCR

| Exon-fragments | Forward primer (5’-3’) | Reverse primer (5’-3’) |
|----------------|------------------------|-----------------------|
| HNF4A_e1a      | GGGCAGCTGGGAGGCCAGTC   | GCTGTAGGACCAACCTACC   |
| HNF4A_e1b      | TCTGTTGTGACAGCTGGC    | CTGGAGCTGGCAGCCCTAC   |
| HNF4A_e1c      | CACAGGTTTGGCAAGTGAAGC | CACCCGAAATGGCGTTATGTC |
| HNF4A_e2       | AAGGCTCCTTAGTGCCCTG   | CCAGTGCCGAGAACAGCAGCT |
| HNF4A_e3       | CCTAGTCCTGTCCTAAAGAG | GTCTAAAAGTTGGCTACAG   |
| HNF4A_e4       | CCACCCCTACCTCCATCCCTG | CCCTCCGTCAGCTGCCTCA  |
| HNF4A_e5       | GTCAGGGGACACAGAATGCG  | AATCAAGCCGCTCCAGGGCTAT |
| HNF4A_e6       | GCCCAAGCTGCTAGGTTGCTA | TGGCTGGGAGTGTCCTACAC |
| HNF4A_e7       | GCACCAGCTACTTGGCAAC  | AGGAGAAGTCTGGGACAGCG |
| HNF4A_e8       | GGCAACTCTGCGTCTGTACCC | TCACCTGTTGAGGCTCTGCTCC |
| HNF4A_e9       | TGGTTGATGGCCAGCAGCCTG | ATCTGTTGTACCTCGCTTC   |
| HNF4A_e10      | CATTTACTCCACAAAGGCT  | GACACAGTGATCAGCAGGCT |
| HNF1A_e1       | GCCAGGAAAGCGCAACCAAGC | GAAGGGGGGTGGTGTAGAGCC |
| HNF1A_e2       | CATGACGATTCCTCCACACTA | CTTCCACACCCCCACATAGG |
| HNF1A_e3       | GGCAAGTGTCAGGGGAAATGGA | CAGCCCAAGCACAACAGCAC |
| HNF1A_e4       | CAGAACCTCTTCCGCCATGCC | GCTTCCCTAGGGACCTGTCCT |
| HNF1A_e5       | GGCGAGACAGGCAATGCGCTA | GCTTCCCTAGGGACCTGTCCT |
| HNF1A_e6       | TGGGACGATGCTTGGGAGGC  | GTTGGCCACTGAGCCCTAC |
| HNF1A_e7       | GGTCTGGGCAAGGGTGAGGAT | CCTCTGCACTCCATGGCAGCC |
| HNF1A_e8       | GAGGCTGGAGGACTAGGGTGT | CTCTGTAAGCCAGGGAGAG |
| HNF1A_e9       | CAGACGGCTACCCCTACCATAC | CAGCCCAAGCAGGCAGTCACA |
| HNF1B_e1       | GGCTGAGGAGGGTCTGCTGAG | CGGGCCAGTCTGAGCAGTACA |
| HNF1B_e2       | CTTCCACATCTACCCCTAAC | GAGAGGGCAGGCTGCTCAGC |
| HNF1B_e3       | AGTGAAGGCTACAGACCTATC | TCTTGGGCTTGTGACTTCAT |
| HNF1B_e4       | CCCCCATCTACTCCCAAACCAA | AAACCTGAAGATGCGTGCC |
| HNF1B_e5       | TGGCGGATCTGGTGTCAGAGG | CTTCTTATCTAAGGCTCCAG |
| HNF1B_e6       | CAGTGCCGACTTAAATTGCCCA | GGTGAGTGGAGGAGAGCAGTA |
| HNF1B_e7       | ATCCACCTCTCTCTATCCAG | ACTTCCGAGAAAGTCTGACG |
| HNF1B_e8       | TTTGCGTGTTATGCTGTCCCT | GAGGTCGTCAGGTCGTAAGC |
| HNF1B_e9       | GGCGCATCATTCCCTAGAGAAA | ACGTGTCGTCAGGTAAGC |

**Statistics**

Statistical analysis was performed using Statistical Systems thermocycler and the PCR products were determined using an ABI 700 (Applied BioSystems) sequencer extraction kit. Probands with a negative result for MODY1, 3, 5 were further screened with 45 genes known to be associated with monogenic forms of diabetes. The target genes are shown in Table 2. The screening was carried out via next-generation sequencing by BGI Dx in Shenzhen, China.
package for Social Sciences (SPSS for Windows, version 22.0), Levene’s test and Independent sample t test were used for comparison between MODY and T2D groups. Data are presented as mean ± SD and p values <0.05 were considered statistically significant.

Results

Family history

Family A consisted of 3 families (from A1–A3) and B was 6 (from B1–B6). There are 6 and 13 diabetic patients in families A and families B respectively, and at least two of them in each family were diagnosed with diabetes and at least one of their age of onset was before 25. Genetic analysis indicated that they were autosomal dominant inheritance (See Figs. 1 and 2).

Medications, complications and laboratory tests

The detailed clinical characteristics of the families are listed in Tables 3 and 4. In family A1, the proband was a 27-year-old female diagnosed with diabetes at the age of 18. Her brother was diagnosed at the age of 21. Their mother was found to have diabetes at the age of 49. BMI and C-peptide values in this family ranged from 16.45–19.96 kg/m² and 114.9–341.3 pmol/L, respectively, and had no significant differences with the initial values while diabetes occurred. The families A1 proband has been receiving glipizide, a kind of oral sulfonylureas, and dimethylbiguanide treatment. The proband has been receiving long-acting insulin glargine injection (10 units per night) with occasional blurred sight. Her brother has been receiving gliclazide treatment without any complications. In family A2, the proband was a 41-year-old male, his daughter was 20 years old, that he and his daughter were diagnosed with diabetes at age of 36 and 16 respectively. And they have not suffered any complication. Family A3 proband was a 22-year-old female diagnosed with diabetes at her 15 whom mother and grandma also suffered from hyperglycemia without complication at age of 35 and 55.
In family B1, the proband was a 22-year-old male diagnosed with diabetes at the age of 21. His cousin was diagnosed at 12. BMI and C-peptide values ranged from 23.4–32.2 kg/m$^2$ and 309.2–1,076.2 pmol/L, respectively. All patients with diabetes in this family were untreated and did not suffer any complications. In family B2, the proband was a 52-year-old male suffered from diabetes and gouty arthritis for nearly 13 years. His parents, sisters, daughter had diabetes and his daughter was diagnosed with diabetes at age of 22. The proband has received remedy of glibenclamide and dimethylbiguanide for 12 years without any chronic complication including diabetic nephropathy, retinopathy and so on. Family B3 proband was a 42 year-old female suffered from diabetes and then received glibenclamide for 8 years. Her father died of diabetes and 2 little sisters and a brother all had diabetes with insulin treatment after at least 4 years diabetic diagnosis, and her sisters and brother were diagnosed with diabetes at the age no more than 25. The proband of family B4 was a 19 year-old male with 4 years hyperglycemia and received glibenclamide for 2 years. His mother and grandparents also suffered from diabetes. In family B5, the proband was a 16 year-old female diagnosed with diabetes recently. His grandpa died from diabetes and his father and father’s brother also suffered diabetes several years. The proband of family B6 was 37 years old and had been diagnosed with diabetes 5 years ago without any treatment and complication. His mother and 2 little sisters were found hyperglycemia before 25 years old.

All members were negative for pancreatic autoimmune antibodies, including GADA-Ab, ZnT8-Ab, and...
Strongly associated with B.

tive autoimmune antibody and insulin independence of at
or mutation and includes MODY, neonatal diabetes, con-
Tables 3 and 4, we concluded and analyzed the age of
A and B, including retinopathy, nephropathy and so on,
Families A and Families B
Comparison of Clinical characters in probands of
A and B.

Diagnosis of monogenic diabetes could help to under-
stand the pathogenesis of diabetes, insulin resistance and
β-cell function [1]. Single gene mutations that primarily affect pancreatic
β-cell function account for approximately 1–2% of all cases of diabetes. MODY is the most common form of
monogenic diabetes, accounting for about 2–5% of
patients. The 14 genes identified as being responsible for
MODY include HNF4A, GCK, HNF1A, PDX1, HNF1B,
NEUROD1, KLF11, CEL, PAX4, INS, BLK, ABCC8,
KCNJ11 and APPL1 [6, 7, 21]. However, most MODY
patients in China have unknown mutations, meaning that
only 5–20% of patients with MODY are correctly diag-
nosed [2, 21, 22]. Several clinical features of MODY
overlap with common forms of diabetes, making a diag-
nosis of MODY a challenge [2]. Genetic testing plays a
significant role in the prediction, prognosis, progression
and treatment of the disease as well as implications for
other family members. Effective genetic testing for
monogenic diabetes usually involves high-throughput
nucleotide sequencing, which improves the sensitivity
and accuracy of results by greatly increasing the number
of nucleotides sequenced. However, this technique is
expensive, leading to a heavy economic burden when
diagnosing diabetes type from many know or unknown
genes. Hence, the new standard before genetic testing
was 1/6 (16.7%) and 2/13 (15.4%), respectively.
From Tables 3 and 4, we concluded and analyzed the age of
diagnosis, fasting blood glucose, 0-minute C-peptide
concentration, body mass index and HbA1c through
SPSS 22 with Levene’s test and Independent-Samples T
test. The results (Table 5) showed that BMI and concen-
tration of C-peptide had homogeneity variance and
significance (p < 0.05) of which BMI was divided from
20.5 to 28.6 kg/m² as well as C-peptide from 154.4 to
738.6 pmol/L.

**Discussion**

Monogenic diabetes is caused by a single gene defect
or mutation and includes MODY, neonatal diabetes, con-
genital hyperinsulinism and Wolcott-Rallison syndrome.
patients of all these families who had been diagnosed with MODY. Families A1 showed transmission of diabetes over three generations due to a c.779 C>T (ACG 260 ATG, p.T260M) missense mutation in the exon 4 of HNF-1A gene which was first time to be detected by our research team in the Chinese population [23] causing a substitution in position 487 from serine to asparagine [24]. Family A2 showed a variation on ABCC8 (TNDM2) c.1423T>C (Tyr475His) heterozygous mutation which was a novel mutation. Family A3 had a c.212dupG heterozygous mutation in INS (MODY10). Families B was also clinically suspected to have MODY according to the criteria. However, no mutation was found in 45 genes known to be associated with monogenic forms of diabetes. Interestingly, we found a Medical Examination Report of the B1 proband’s grandfather which showed that his fasting blood glucose was normal at the age of 65, and consequently families B was diagnosed as familial T2D.

From Table 5, we found differences in terms of BMI and C-peptide (Tables 3, 4): patients with T2D in families B had higher BMI and C-peptide, which indicated higher pancreatic islet secretion and insulin resistance at present.

Due to the existence of MODYX and overwhelming expenses for genetic testing, precise and quick judgment for an outpatient clinician plays a role in initial differential diagnosis and therapy. The diabetes practitioners try to figure out patients who need genetic testing at first sight in order to minimized expenses. Therefore, we recommend that physicians who use genetics to make a diagnosis should also take BMI and C-peptide into consideration. As to OGTT (oral glucose tolerance test) curve, it depended upon a group of digestive, hepatic, hormonal and nervous processes. It is complicated on preparing and testing in outpatient clinic despite partly assistance on distinguishing MODY from T2D [25]. Comparing to OGTT curve, BMI and C-peptide cost less time and suffering.

As well as plasma glucose and clinical features of the proband, detailed family history and lifestyle were also crucial to determine the type of diabetes. By analyzing

| Subject | Gender | Age at diagnosis (yrs) | Age at reporting (yrs) | 0 minute blood glucose (mmol/L) | 120 minute blood glucose (mmol/L) | 0 minute C-peptide (pmol/L) | BMI (kg/m²) | Hb1Ac (%) |
|---------|--------|-----------------------|-----------------------|--------------------------------|----------------------------------|-----------------------------|-------------|-----------|
| B1      | F      | 41                    | 46                    | 8.74                           | N                                | 578.2                       | 27.3        | 7.3       |
| B1      | M      | 38                    | 41                    | 6.92                           | N                                | 891.7                       | 32.2        | 6.0       |
| B1      | M      | 21                    | 22                    | 3.93                           | N                                | 791.9                       | 30.1        | 5.8       |
| B1      | M      | 12                    | 13                    | 7.34                           | N                                | 1,076.2                     | 27.8        | 7.3       |
| B2      | F      | 44                    | 44                    | 7.87                           | N                                | 697.3                       | 30.9        | 7.8       |
| B2      | M      | 39                    | 52                    | 7.63                           | N                                | 763.6                       | 29.4        | 6.9       |
| B2      | F      | 44                    | 44                    | 7.87                           | N                                | 697.3                       | 30.9        | 7.8       |
| B3      | F      | 34                    | 42                    | 8.42                           | N                                | 659.2                       | 25.8        | 10.1      |
| B4      | M      | 15                    | 19                    | 7.65                           | N                                | 666                         | 26.9        | 7.2       |
| B5      | M      | 40                    | 40                    | 6.97                           | N                                | 722.6                       | 26.8        | 7.3       |
| B5      | M      | 39                    | 39                    | 7.86                           | N                                | 682.6                       | 27.9        | 7.7       |
| B5      | F      | 16                    | 16                    | 7.14                           | N                                | 629.4                       | 28.7        | 6.9       |
| B6      | M      | 32                    | 37                    | 10                             | N                                | 798.6                       | 28.7        | 7.6       |

| Items | Family A (n = 6) | Family B (n = 13) | p value |
|-------|------------------|-------------------|---------|
| Age at diagnosis (Mean, yrs) | 25                | 30                | 0.441   |
| FBG (Mean, mmol/L) | 8.8               | 7.5               | 0.232   |
| Fasting C-peptide (Mean, pmol/L) | 154.4             | 738.6             | 0.000   |
| BMI (Mean, kg/m²) | 20.5              | 28.6              | 0.000   |
| Hb1Ac (Mean, %) | 8.8               | 7.3               | 0.124   |
the history of patients from families B and published articles from other clinical centers, we found that the age of onset of impaired glucose tolerance and T2D was younger in overweight patients, which was mainly related to unhealthy habits, such as sedentary lifestyle, overeating and lack of exercise [26]. Families may have similar eating and exercise habits, which might play an important role in the onset of familial T2D. They have higher C-peptide level and BMI. However, most types of MODY, loss function of genes gives rise to defects of insulin synthesis, transporting and secretion manner [6, 7, 21]. Hence, MODY patients show lower C-peptide level and BMI. Under the common condition of autosomal dominant inheritance, insulin independence after two years and at least one family member diagnosed before the age of 25, we recommended out-patient clinic physician that the range of value in BMI 25–26 kg/m² and C-peptide 550–600 pmol/L could help to fortify the incidence of MODY diagnosis before genetic testing. By virtue of the range of value we mentioned was the low limitation value of familial T2D which was interestingly still greater than the upper limitation of MODY from our research. And we chose the range from MODY (family A) patients’ maximum values to familial T2D (family B) patients’ minimum value for recommendation. This emphasizes the importance of BMI and insulin resistance when differentiating MODY from T2D. And we need larger sample size to validated the specific cut-off value.

In summary, the procedure of diagnosing MODY requires a detailed history and clinical characteristics of the proband and family members to be known subsequently undergoing genetic screening. As well as age at diagnosis, insulin independence and autosomal dominant inheritance, BMI and C-peptide might be used as inclusion criteria in the outpatient clinic when considering genetic testing. As the T2D patients become younger, it becomes more important to have specific criteria to diagnose MODY as distinguished from familial T2D.

Also, our study has several limitations. Firstly, more clinical data of the patients needed to be included. Secondly, the amount of sample is not enough to define the standard of differential diagnosis, but this study could be a pilot study for the future larger case studies. The last but not least, we need further follow-up study for these families for the purpose of deeply understanding the process of MODY progression to help differential diagnosis and provide instruction on treatment.

Acknowledgements

This work was supported by grants from the National Natural Scientific Foundation of China [grant numbers 81770880, 81370975, 81070278] and the Hunan Provincial Natural Science Foundation of China [grant numbers 2015JC3012] and the Shenzhen Municipal Science and Technology Innovation Committee [grant numbers JSGG 2016033103247408].

All authors have contributed significantly and in keeping with the latest guidelines of the International Committee of Medical Journal Editors. Hui-Xuan WU collected clinical information and wrote the article. Jun TANG and Long LI extracted DNA and sequenced it. Shi-Ping LIU, Zhi-Guang ZHOU, Hou-De ZHOU and De-Wen YAN provided patients information. Jian-Xing YANG helped to sequence. The work was conducted by Hou-De ZHOU. And all authors are in agreement with the content of the manuscript.

The datasets during and/or analyzed during the current study are available from the corresponding author on reasonable request. At last, we showed deep appreciation on probands and their families for their support.

Disclosure

No competing financial interests exist.

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