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ORIGINAL ARTICLE

Preliminary screening of mutations in the glucokinase gene of Chinese patients with gestational diabetes

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ABSTRACT

Aims/Introduction: Mutations in the glucokinase gene (*GCK*) are a pathogenetic cause of maturity-onset diabetes of the young. Studies have found that female patients with GCK maturity-onset diabetes of the young often present with gestational diabetes during pregnancy. Our aim was to preliminarily assess the prevalence of mutations in the glucokinase gene in Chinese women with gestational diabetes.

Materials and Methods: Chinese gestational diabetes patients who underwent a 100-g oral glucose tolerance test in Peking Union Medical College Hospital from July 2005 to May 2008 were retrospectively analyzed. Participants were selected for direct sequencing of the *GCK* gene if they met the following criteria: (i) fasting plasma glucose between 5.5 and 10.0 mmol/L; and (ii) a small increment (<4.6 mmol/L) during a 2-h oral glucose tolerance test.

Results: Of the 501 participants with gestational diabetes, there were 38 participants who met the criteria for *GCK* analysis. In the 29 participants whose deoxyribonucleic acid samples were available, two mutations in coding regions were detected, c.626 C>T (p.T209M, NP_000153.1) mutation in exon 6 and c.824 G>A (p.R275H, NP_000153.1; rs767565869) mutation in exon 7. According to our results, the minimum prevalence of *GCK* mutations in Chinese women with gestational diabetes was estimated to be 0.4%, and the minimum prevalence of GCK maturity-onset diabetes of the young in the Chinese population might be one in 2,000.

Conclusions: Our screening criteria allowed for the identification of glucokinase-deficient patients who were diagnosed with gestational diabetes, and these mutations in the *GCK* gene were not common in Chinese women with gestational diabetes.

INTRODUCTION

As the glucose sensor of pancreatic β-cells, glucokinase plays a key role in the regulation of glucose-stimulated insulin secretion. Genetic studies have shown that mutations in the glucokinase gene (*GCK*) are responsible for various disorders of glucose regulation[1]. The most frequent mutations are heterozygous inactivating *GCK* mutations that are the pathogenetic causes of maturity-onset diabetes of the young (GCK-MODY). Although patients with GCK-MODY have persistent hyperglycemia after giving birth, it is not easy to make an early diagnosis because of the lack of typical symptoms of diabetes and diabetic complications[2]. Studies have found that female patients with GCK-MODY often present with gestational diabetes (GDM)[3–12], as their asymptomatic hyperglycemia is detected by routine testing during pregnancy. Therefore, this represents a feasible way to screen for *GCK* mutations in patients with GDM.

It is important to distinguish these patients with GCK-MODY from other common patients with GDM, because they have a predictable clinical course and a definitive autosomal dominant inheritance mode, which is useful for genetic counseling. A newborn has a 50% chance of inheriting the same mutation as its mother, and identification of the mutation

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could help to avoid the anxiety associated with an incidental finding of hyperglycemia during childhood and a misdiagnosis of type 1 diabetes. More importantly, the method of glycemic control during pregnancy can vary depending on whether the mother and her fetus have a *GCK* mutation. Tight glycemic control is often required to prevent macrosomia in mothers with GDM, but for a fetus that has the same *GCK* mutation as its mother, strict glycemic control could result in intrauterine growth restriction and a low birthweight[13]. Insulin treatment of the mother is only appropriate when an increase in fetal abdominal growth suggests that the fetus is unaffected[14].

Several studies have suggested that the prevalence of *GCK* mutations in patients with GDM is 1–6%[4–12]. China has both the largest population and the largest population of citizens with diabetes in the world. However, research on MODY, especially in terms of epidemiology, is rare. The aim of the present study was to screen for mutations in the glucokinase gene in Chinese women with GDM and to estimate the population prevalence of GCK-MODY in China.

METHODS

Study population

The study population comprised of Chinese participants from an earlier study of GDM that was carried out from July 2005 to May 2008. The study was approved by the ethics committee of Peking Union Medical College Hospital.

Glucose tolerance testing was carried out between gestational weeks 24 and 28 on all women with glucose values that were ≥7.8 mmol/L 1 h after a 50-g glucose load. Diagnosis was made on the basis of a 100-g oral glucose tolerance test (OGTT) according to the American Diabetes Association criteria (2000)[15]. GDM was diagnosed if two or more plasma glucose levels met or exceeded the following thresholds: fasting glucose concentration of 5.3 mmol/L; 1-h glucose concentration of 10 mmol/L; 2-h glucose concentration of 8.6 mmol/L; or 3-h glucose concentration of 7.8 mmol/L. When there was only one glucose level that met or exceeded the threshold, gestational impaired glucose tolerance (GIGT) was diagnosed. All participants were classified as normal glucose tolerance, GIGT or GDM. Clinical and anthropometric data were obtained from all participants, and glycosylated hemoglobin (HbA1c) was measured by high-performance liquid chromatography (Varian II; Bio-Rad, Hercules, California, USA).

The participants were selected for analysis of the *GCK* gene if they met the following two criteria[16,17]: (i) a fasting plasma glucose between 5.5 and 10.0 mmol/L; and (ii) an increment between the fasting and 2-h plasma glucose concentrations in a 100-g OGTT of <4.6 mmol/L.

Genetic analysis

Genomic deoxyribonucleic acid (DNA) from the participants of our earlier study was isolated from peripheral blood lymphocytes using the QIAamp® DNA Blood Kit (Qiagen, Hilden, Germany) and was stored at −80°C. DNA from the participants who met both criteria was amplified by polymerase chain reaction (PCR). The PCR was carried out in a volume of 30 μL containing 20–100 ng of DNA template, 10 pmol/L of each primer and 2 × Taq PCR MasterMix (Biomed, Beijing, China). The reaction cycle included the following: 94°C for 3 min, 35 cycles of 94°C for 30 s, melting temperature for 40 s, extension (72°C) for 1 min and an additional 3 min at 72°C for the final extension. Primers for amplifying the exons, the intron–exon boundaries and the promoter sequences[18] of the *GCK* gene are shown in Table S1. The PCR products were verified by 2% agarose gel electrophoresis in 1X TBE buffer and then examined by direct sequencing with an ABI 3730XL DNA Analyzer (Applied Biosystems, Foster City, California, USA). The sequences were compared with the published sequences (NM_000162.3) using the human BLAT search software (http://genome.ucsc.edu/cgi-bin/hgBlat) from the University of California Santa Cruz. The Single Nucleotide Polymorphism Database (release 146) and the Human Gene Mutation Database (HGMD Professional, trial version June 2013) were used to determine whether the identified variant is novel. The novel missense mutations were analyzed *in silico* using SIFT and PolyPhen-2 (http://sift.jcvi.org/ and http://genetics.bwh.harvard.edu/pph/ respectively).

Statistical analysis

Stata software (version 10.0; StataCorp LP, College Station, Texas, USA) was used to analyze the variables. Data are expressed as the mean ± SD.

RESULTS

A total of 854 participants were enrolled, of which 353 were diagnosed with normal glucose tolerance, 179 with GIGT and 322 with GDM. There were 52 participants who met the criterion of a fasting plasma glucose between 5.5 and 10.0 mmol/L, and 306 participants who met the criterion of an increment <4.6 mmol/L. There were 38 participants who met both criteria simultaneously, including five participants who were diagnosed with GIGT and 33 who were diagnosed with GDM. All the HbA1c values were <7.5% in the 38 participants who fulfilled *GCK* analysis. Their average level of HbA1c was 5.8%. The average age at conception of the Chinese participants was 31.5 years. It was the first pregnancy for more than 90% of the participants. Of the 38 participants identified, there were 15 participants who had a history of type 2 diabetes in a first-degree relative. The clinical and anthropometric data of the participants are shown in Table 1.

Because the previous studies depleted the DNA samples from some participants, we only had 29 samples from the 38 participants who fulfilled the criteria for *GCK* analysis. In the 29 samples, two heterozygous mutations in the coding regions were detected, c.626 C>T (p.T209M, NP_000153.1) in exon 6 and c.824 G>A (p.R275H, NP_000153.1) in exon 7 (both shown in Figure S1). In addition, an unreported variation c.1020-64 G>T (NM_000162.3) in intron 8 was also identified (shown in

Table 1 | Clinical and anthropometric data of the participants

	Participants with NGT (n = 353)	Participants with GIGT (n = 179)	Participants with GDM (n = 322)	Participants fulfilling the criteria for *GCK* analysis (n = 38)
FBG (mmol/L)	4.5 ± 0.3	4.6 ± 0.4	4.9 ± 0.6	5.9 ± 0.5
2-h BG (mmol/L)	7.1 ± 0.9	7.9 ± 1.1	9.6 ± 1.3	8.7 ± 1.3
Increment of BG (mmol/L)	2.6 ± 1.0	3.3 ± 1.2	4.7 ± 1.3	2.8 ± 1.1
HbA1c (%)	5.2 ± 0.4	5.3 ± 0.4	5.5 ± 0.5	5.8 ± 0.6
Age at conception (years)	31.0 ± 3.8	31.5 ± 3.6	32.1 ± 3.8	32.7 ± 4.0
First parity (n)	337	170	299	32
BMI (kg/m^2)	20.1 ± 2.5	21.3 ± 2.7	22.2 ± 3.5	24.1 ± 4.6
History of type 2 diabetes	54 (n = 292)	50 (n = 169)	85 (n = 312)	15

Data are expressed as the mean ± SD. 2-h BG, 2-h blood glucose in 100-g oral glucose tolerance test; BMI, body mass index before pregnancy; FBG, fasting blood glucose; GDM, gestational diabetes mellitus; GIGT, gestational impaired glucose tolerance; NGT, normal glucose tolerance.

Figure S1). Based on the results of previous studies[19,20], the T209M mutation was the cause of GDM in one of the participants. In contrast, the R275H mutation has not been reported in GDM patients. The c.824 G>A mutation is registered as rs767565869 in the Single Nucleotide Polymorphism Database release 146, and it is not a common single nucleotide polymorphism. In the annotations of rs767565869, the allele frequency of T (A) was only 0.001% (1/121410). The bioinformatics tools PolyPhen-2 and SIFT were used to predict the effect of the R275H mutation, and the effect was predicted to be benign by PolyPhen-2 (score 0.011) and damaging by SIFT (score 0.03).

The patient who was heterozygous for the T209M mutation was in her first pregnancy at the age of 29 years, and had a body mass index (BMI) of 22.9 kg/m^2. GDM was diagnosed on the basis of an OGTT at gestational week 26 (0 h = 6.1 mmol/L, 1 h = 11.1 mmol/L, 2 h = 8.9 mmol/L, 3 h = 8.4 mmol/L). Her HbA1c was 6.1%. Although the use of insulin had been recommended because of her persistent fasting hyperglycemia during pregnancy, she refused and instead chose dietary therapy to control her blood glucose levels. She eventually delivered a full-term female infant weighing 3.3 kg. The participant's mother was diagnosed with type 2 diabetes because of the obvious fasting hyperglycemia (7–8 mmol/L) in her late 30 s. She was treated with metformin and her blood glucose levels were stable.

The patient who was heterozygous for the R275H mutation was also in her first pregnancy. She was aged 30 years when she conceived, and her BMI before pregnancy was 20.1 kg/m^2. She denied a history of abnormal blood glucose, and GDM was diagnosed on the basis of an OGTT at gestational week 28 (0 h = 5.94 mmol/L, 1 h = 11.61 mmol/L, 2 h = 7.94 mmol/L, 3 h = 4.72 mmol/L). She used diet to control her blood glucose levels during pregnancy, and she eventually delivered a full-term male infant weighing 4.5 kg by cesarean section. She had no apparent history of diabetes in her family.

DISCUSSION

The present study shows that mutations in the *GCK* gene might be a rare cause of GDM in Chinese women. Because of the absence of some samples, this conclusion might need to be confirmed in a large-scale study. Based on the assumption that the missing samples were all negative and extrapolating from the results of this study, the minimum prevalence of *GCK* mutations was approximately 0.4% (2/501) in Chinese women with GIGT and GDM. Previous studies have shown that the prevalence of *GCK* mutations in Caucasians with GDM was 1–6%[6,9,10,12], but the data of other ethnicities[4,5,8,11], such as Asians, Africans and Latinos, were rare. The differences in the prevalence of *GCK* mutations in the GDM population might be related to many factors, such as the ethnicity, the screening method for *GCK* analysis and the diagnostic criteria for GDM. The study of Carmody[21] suggested that the prevalence of GCK-MODY might be consistent across different ethnicities, but there were not enough cases to support this view.

The screening methods were diverse in previous studies. We sought to ascertain the appropriate clinical criteria for *GCK* mutation screening in the Chinese GDM population. Heterozygous loss-of-function mutations in the *GCK* gene cause GCK-MODY. Despite the various mutations described, the clinical phenotype is relatively constant. The following features usually suggest the diagnosis of a *GCK* mutation[17]: (i) persistent fasting hyperglycemia ≥5.5 mmol/L; (ii) an HbA1c that is typically just above the upper limit of normal and rarely exceeds 7.5%; (iii) in an OGTT, a small increment ([2-h glucose] − [fasting glucose] ≤4.6 mmol/L); and (iv) parents who have type 2 diabetes with no complications or who might not be diabetic. On the basis of the aforementioned features, some researchers have also included other indicators to screen for *GCK* mutations in the GDM population[7,8,10,12], such as age at conception, treatment history of GDM, BMI and a family history of diabetes. However, because of the lack of sufficient positive cases, it is difficult to evaluate the efficiency of their screening methods. A study from Chakera[12] showed that the combined criteria of a BMI <25 kg/m^2 and a fasting glucose ≥5.5 mmol/L has a sensitivity of 68% and specificity of 96% for differentiating GCK-MODY from GDM.

China has a one-child policy, and encourages late marriage and childbearing. Therefore, it is difficult to use treatment

history of GDM or a young age at conception as screening indicators. The stable persistent hyperglycemia of GCK-MODY also makes it unnecessary to set limits based on the age of conception. In addition, because the blood glucose in GDM and GCK-MODY both increase slightly, there is an overlap in the value of HbA1c between the two populations. In the present study, we also found that the HbA1c level of the patient with the T209M mutation was similar to some GDM patients without *GCK* mutations, so we infer that HbA1c is probably not a good indicator for distinguishing GCK-MODY from common GDM.

Compared with the standards used in previous studies, the glucose cut-off values used by the American Diabetes Association (2000) for GDM are stricter. Therefore, the increase in the target population also decreased the prevalence of *GCK* mutations in the present study. On the basis of the new evidence from the Hyperglycemia and Adverse Pregnancy Outcome study, the International Association of the Diabetes and Pregnancy Study Groups defined new diagnostic values for GDM in 2010[22]. The American Diabetes Association accepted the new criteria in 2011[23], and the World Health Organization accepted it in 2013[24]. The new criteria cancel the definition of GIGT, and the diagnosis of GDM is made when any of the following plasma glucose values are exceeded: fasting ≥5.1 mmol/L, 1-h ≥ 10.0 mmol/L or 2-h ≥ 8.5 mmol/L. Although the new criteria might further decrease the prevalence of GCK-MODY in the GDM population by increasing the denominator, it is also possible to increase the total number of GCK-MODY identified in this population.

Two heterozygous mutations were identified in the present study – T209M and R275H. The T209M mutation would result in a significant decrease in the affinity of glucokinase for adenosine triphosphate and a slight decrease in the affinity for glucose[20], and it has also been reported in GCK-MODY[19]. The R275H mutation has not been reported in families with GCK-MODY. In the medical record in 2006, the R275H mutation carrier in the present study denied a history of fasting hyperglycemia of herself and her family members. However, because of her loss to follow up, the blood glucose status of her family members could not be further checked. Therefore, whether the mutation co-segregates within her family or whether the baby had the genetic change was still unclear. Because the bioinformatics tools, PolyPhen-2 and SIFT, had different predictions regarding the effects of the R275H mutation, whether it is pathogenic is still unknown, and further functional research is required. A recent study of R275C[25], which was identified in a Pakistani family, reported that the R275C mutation breaks a hydrogen bond between the R275 side-chain and the carbonyl oxygen of D267, which destabilizes the F260-L271 loop structure and the protein. This promotes the formation of dimers/aggregates, and suggests that increased cellular degradation is the molecular mechanism by which R275C causes GCK-MODY. On this basis, R275H is highly suspected to be pathogenic. Once confirmed, the minimum prevalence of GCK-MODY in China might be approximately 1 in 2,000 (2 out of 501 [2 mutations in 501 GDM patients] × 14.7 [the frequency of GDM][26] ≈ 1 of 2,000; the prevalence of *GCK* mutations is expected to be similar among women and men). This result was similar to the observations of other researchers[4,21]. Although the prevalence is low, there are still a great number of patients with GCK-MODY considering the large population of China. However, most of these patients need to be identified and diagnosed.

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DISCLOSURE

The authors declare no conflict of interest.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1| Primers for amplification of the glucokinase gene.
Figure S1| Direct sequencing of the wild-type and mutant *GCK* genes.