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Identification and management of GCK-MODY complicating pregnancy in Chinese patients with gestational diabetes

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Abstract

Precise differentiation of glucokinase (GCK) monogenic diabetes from gestational diabetes mellitus (GDM) is critical for accurate management of the pregnancy outcome. We screened GCK-MODY complicating pregnancies in Chinese GDM patients, explored the pathogenesis of novel *GCK* mutations, and evaluated the patients' pregnancy outcome and management. The *GCK* gene from 411 GDM patients was screened with PCR–direct sequencing and multiplex ligation-dependent probe amplification (MLPA) and 15 *GCK* mutations were identified. We also retrospectively analyzed a total of 65 pregnancies from 21 GCK-MODY families, wherein 41 were from 15 maternal families and 24 were from six paternal families. Bioinformatic analysis and biochemical functional study were conducted to identify novel *GCK* mutations. In total, we identified 21 *GCK* mutations: 15 from the 411 GDM patients and six from 24 fathers. Of th Asp78Asn (GAC → AAC), Met87Arg (ATG → AGG), Leu451Val (CTT → GTT), Leu451Pro (CTG → CCG) and 1019 + 20G > A e mutations, five, i.e., were novel and deleterious, with markedly decreased enzyme activity and thermal stability. The unaffected offspring of *GCK* mutation-affected mothers were heavier than affected offspring ($p < 0.001$). Of 21 insulin-treated affected mothers, 10 had maternal hypoglycemia (47.6%) and seven had perinatal complications (33.3%), and the affected offspring of the insulin-treated affected mothers had significantly lower birth weights than that of the 20 diet-control affected mothers ($p = 0.031$). In this study, the prevalence of GCK-MODY complicating pregnancy in Chinese GDM patients was 3.6% (15/411). The defective GCK may contribute to the hyperglycemia in GCK-MODY. Insulin therapy is not beneficial for GCK-MODY complicating pregnancy and therefore should not be recommended.

Keywords GCK-MODY complicating pregnancy · Gestational diabetes mellitus (GDM) · Identification · Management · Pregnancy outcome

Abbreviations

FPG	Fasting plasma glucose
FPR	False-positive rate
G-6-P	Glucose-6-phosphate
GCK-WT	GCK-wild type
GCK:	Glucokinase
GDM	Gestational diabetes mellitus
2hPG	2-H postprandial glucose
mGCKs	GCK mutants
OGTT	Oral glucose tolerance test
TG	Triglyceride

Introduction

Sixteen million babies are born in China annually [1], and the prevalence of gestational diabetes mellitus (GDM) is as high as 17.5% [2]. Although GDM is defined by the results of the oral glucose tolerance test (OGTT) at 24–28 weeks of pregnancy, the diagnosis also encompasses previously undiagnosed hyperglycemia, especially asymptomatic hyperglycemia maturity-onset diabetes of the young (MODY) type 2 [3, 4]. MODY2 caused by heterozygous inactivating mutations in the GCK gene, which located to 7p15.3-p15.1 and contains 12 exons and encodes 465-amino-acid protein

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[5]. In pancreatic beta cells, GCK acts as a glucose sensor to determine glucose utilization, which is essential for glucose-stimulated insulin secretion [6]. In liver cells, GCK determines the rate of glucose uptake and glycogen synthesis and is critical for the regulation of various glucose-responsive genes [7]. The ethnic prevalence of GCK mutations were reported varies from < 1 to 63% of the patient samples [8–11]. However, based on varied screening criteria, the prevalence of MODY2 complicating pregnancy varies from 0 to 6% in GDM populations of different ethnicities [12–16]. According to the diagnostic criteria used for diabetes outside of pregnancy, women found to have diabetes should be categorized as having diabetes complicating pregnancy, which includes monogenic diabetes, and should be managed accordingly [3].

GCK-MODY complicating pregnancy tends to affect the fetus, and includes macrosomia, related perinatal complications, and fetal birth weight loss resulting from maternal insulin treatment during pregnancy [17, 18]. Fetal birth weight is influenced by maternal and fetal *GCK* genotypes [17] Moreover, the mode of inheritance of GCK-MODY is autosomal dominant; therefore, the offspring of MODY2 pregnant women have a 50% chance of inheriting the same *GCK* mutation from their mothers [19]. Fetal insulin secretion occurs in response to maternal glycemia, which plays a crucial role in fetal growth and development [17]. Low birth weight or macrosomia results from the fetal response to the intrauterine environment [20]. Therefore, identifying MODY2 pregnant women from GDM women is critical for pregnancy management and genetic counseling, and could reduce the frequencies of being misdiagnosed as type 1 and type 2 diabetes in the offspring [21].

Universal clinical and laboratory criteria for screening *GCK* mutations in GDM have not been fully established. Recent pregnancy-specific screening criteria [13], i.e., pre-pregnancy body mass index (BMI) < 25 kg/m^2 and fasting plasma glucose (FPG) ≥ 5.5 mmol/l during pregnancy used for screening GCK mutations in Italian (Caucasian) women with GDM yielded a positive predictive value of only 43% [22]. In addition, in cases that were not initially included in the screening cohort according to the Ellard criteria [4] for *GCK* mutations in multiethnic GDM groups, the actual prevalence of *GCK* mutations was up to 12% [23]. Consequently, applying these screening criteria will result in misdiagnosis in a proportion of MODY2 pregnant women, which might seriously affect blood glucose management and pregnancy outcome. However, the prevalence of GCK-MODY complicating pregnancy in GDM patients has not been reported in Asians, including Chinese.

Several clinical approaches have been proposed for managing MODY2 pregnant women, and include insulin therapy for preventing macrosomia [24], or close monitoring of the pregnancy with minimal insulin therapy and early labor if necessary [25]; however, its management remains controversial. Studies have indicated that GCK-MODY patients with mild hyperglycemia and few complications should not receive hypoglycemic therapy, as it is ineffective [10, 26]. Patients with MODY3 caused by mutations in HNF1A should be treated with sulfonylurea hypoglycemic agents since metformin is ineffective [27]. While T2DM associated with MODY gene polymorphisms [28, 29], the metformin is the preferred initial pharmacologic agent (unless contraindicated or not tolerated). Once started, metformin should be continued and other medications, including insulin, should be added to metformin for ongoing glycemic and metabolic benefits [30]. Thus, we hypothesize that applying the current screening criteria [4, 13] may misdiagnose MODY2 complicating pregnancy and insulin therapy may not be appropriate for these patients. In the present study, we screened GCK-MODY complicating pregnancy in Chinese GDM patients, explored the pathogenesis of novel mutations, and evaluated the patients' pregnancy outcome and management.

Materials and methods

Participants and identification of *GCK* mutations

This study was approved by the Shanghai Jiaotong University Affiliated Sixth People's Hospital Institutional Review Board, and written informed consent, medical and family history questionnaires were obtained from the participants or their guardians. Schematic representation of the study is shown in Fig. 1. From 2009 to 2019, pre-existing diabetes patients (FPG ≥ 7.0 mmol/l and/or glycated hemoglobin [HbA1c] ≥ 48 mmol/mol [6.5%]) were excluded at 12 weeks of pregnancy at the Shanghai Jiaotong University Affiliated Sixth People's Hospital obstetric clinic. GDM patients (n = 411) were diagnosed and identified based on the results of the 75 g OGTT at 24–28 weeks of pregnancy according to the criteria established by the American Diabetes Association (ADA) in 2011 [31]. Pregnant women were diagnosed with GDM when any of the following values was met or exceeded: FPG ≥ 5.1 mmol/l; 1 h PG ≥ 10.0 mmol/l; 2 h PG ≥ 8.5 mmol/l. Multiple pregnancies and preterm births before 28 weeks were excluded.

Blood or saliva samples from the participants and from 400 unrelated non-diabetic controls (age ≥ 60 years, normal glucose tolerance, HbA1c < 38 mmol/mol [5.6%], no family history of diabetes) were collected, and the genomic DNA was extracted [10]. In the GDM patients, 12 exons and the surrounding regions of the *GCK* gene (GenBank accession no. AH005826), expressed in the pancreatic β cells and hepatocytes, were screened by PCR–direct sequencing with specific primers with some modifications and with multiplex ligation-dependent probe amplification (MLPA) [10].

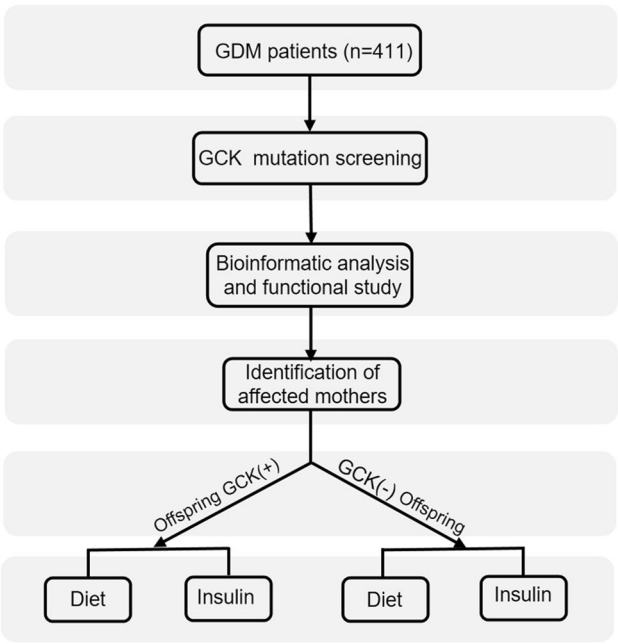

Fig. 1 Schematic representation of the study

The mutations were numbered according to the nomenclature of the Human Genome Variation Society (http://www.hgvs.org/). The novelty of the identified *GCK* mutations was checked against the gnomAD database (https://gnomad.broadinstitute.org/). The pedigrees of GCK-MODY complicating pregnancy were screened for *GCK* mutations, and the maternal nuclear families were then retrospectively investigated. We also confirmed the absence of the identified mutations in the 400 unrelated non-diabetic controls. Further, we also analyzed 24 paternal nuclear families with *GCK* mutations from our Monogenic Diabetes Database. We collected the laboratory data and clinical information such as the gestational age, fetal birth weight, details of prenatal insulin treatment, perinatal complications, and maternal hypoglycemia of all members of the nuclear families.

Clinical study

The mothers were asked the details of their pregnancies. Fifty-three of 65 maternal reports were checked against the medical records. All but four of the 53 women reported birth weight differences of < 90 g, so the maternal report was used when no medical records were available ($n = 12$). Centile growth was evaluated using standard centile growth charts according to World Health Organization growth standards [32]. Forty-one affected mothers were grouped based on the existence of *GCK* mutation in the offspring, and the effects of insulin therapy and diet control were analyzed by retrospective investigation. For the affected mothers, insulin (median dose, 0.6 U/kg/day [range, 0.3–1.0 U/kg/day]) was commenced at 24–28 weeks of gestation.

Functional characterization of GCK mutations

To assess the biochemical effects of missense mutations on GCK function, the complementary DNA (cDNA) of His-tagged GCK-WT (wild-type) and *GCK* mutants (mGCKs) generated by the QuikChange Site-Directed Mutagenesis Kit (Stratagene, La Jolla, CA, USA) were cloned into pET28a expression vectors to express recombinant GCK proteins as described previously [10]. The enzymatic activity and thermostability of GCK-WT and mGCKs were determined as previously described [10]. The primers for introducing the mutations are listed in Supplementary Table 1. Intron mutations were evaluated by two splicing site prediction tools, i.e., regSNP-intron and dbscSNV [33, 34].

Statistical analysis

All clinical and assay values are expressed as the mean ± SD or the median (interquartile range) as appropriate. Comparisons among groups were conducted using one-way ANOVA. The data of skewed distribution were logarithmically transformed; categorical variables were compared by the χ^2 test or Fisher's exact test. Difference in birth weight (or birth weight centile) was corrected for multiple comparisons using the Bonferroni method, and was further adjusted for pre-pregnancy BMI, gestational age, and fetal gender by multiple linear regression. The statistical analysis was performed using SPSS 21.0. $p < 0.05$ was considered significant.

Results

Comparison of the clinical characteristics between GDM and GCK-MODY complicating pregnancy patients

We identified 15 *GCK* mutations from the 411 GDM patients (Fig. 2). Then, we further extended 26 maternal nuclear families with *GCK* mutations from 7 GCK-MODY pregnant women. Including another six *GCK* mutations from 24 affected fathers, a total of 21 GCK mutations were identified in this study (Supplementary Table 2), wherein five mutations, i.e., D78N, M87R, c.1019 + 20G > A, L451V, and L451P, were novel and were not present in the 400 non-diabetic controls or in the gnomAD database, suggesting that they are not polymorphisms but mutations. In addition, seven known mutations, i.e., Arg36Gln (CGA → CAA), Ser212Phe (TCC → TTC), Thr228Met (ACG → ATG), Gly246Arg (GGG → CGG), Cys252Arg (TGC → CGC), Leu315Pro (CTT → CCT), and c.863 + 1G > A were identified for the

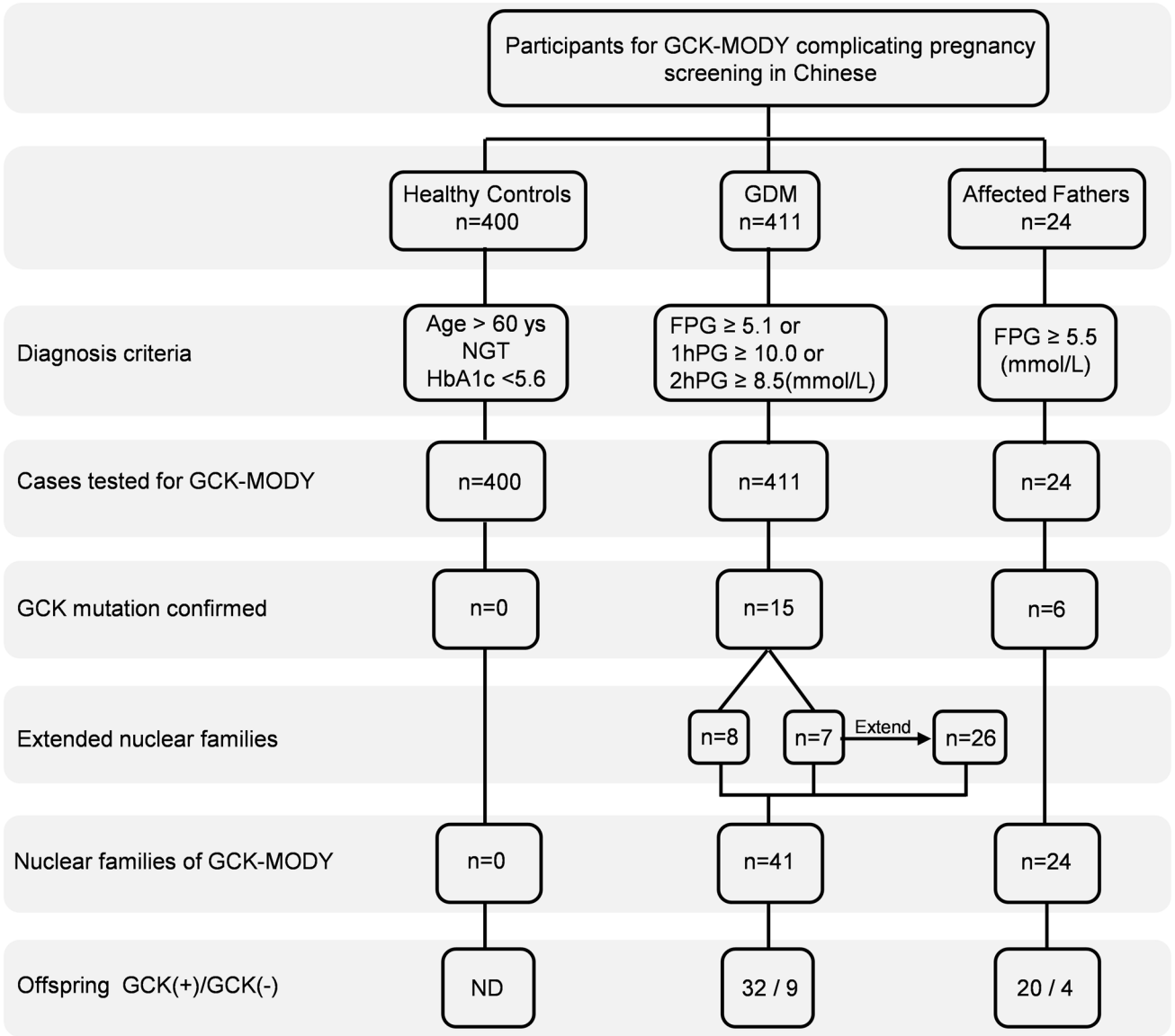

Fig. 2 Flow diagram of the study population. Breakdown of the number of cases from the GDM cohort screened for GCK-MODY complicating pregnancy in this study. The last row identifies the number of cases in offspring in each group. For further details of the cases, please refer to Supplementary Table 1. *NGT* Normal glucose tolerance

first time in Chinese population [35–37], and other nine of the 21 mutations were reported by our previous study [10].

Four of the five novel mutations are missense mutations located in the coding region, and these amino acids are highly conserved across mammalian species, suggesting that they may be critical for GCK structure and/or function. c.1019 + 20G > A, an intron 8 mutation, may generate a novel disease-causing splicing site, as predicted by two splicing site prediction algorithms, i.e., regSNP-intron and dbscSNV (Table 1) [33, 34].

c.863 + 1G > A is located on the splicing sites, but c.1019 + 20G > A is located off them. dbscSNV was used to predict only for the splicing sites, and closer the ADA or RF values (separated by semicolons) are to 1, the greater the likelihood of alternative splicing [34]. As the ADA; RF values were 1; 0.938, c.863 + 1G > A was verified as a classical splicing site (Table 1). The 12 new mutations, i.e., the five novel mutations and seven known mutations reported for the first time in a Chinese population, were predicted to be deleterious by SIFT, PolyPhen-2, LRT, MutationTaster, FATHMM, and Radial SVM (Table 1).

The constructed active holo closed conformation (3VEY) structure model [10] illustrates the positions of 10 missense mutations: four were novel (Fig. 3a). Furthermore, the 21 mutations were scattered across the *GCK* gene (Fig. 3b). In addition, compared with the unaffected GDM group

Table 1 The effect prediction of 12 *GCK* mutations on the glucokinase protein

No	Mutation		Previously reported	SIFT	PolyPhen2	LRT	Mutation taster	FATHMM	Radial SVM	Func.refGene	RegSNP	dbscSNV (ADA; RF)
	Nucleotide	Amino acid										
1	c.1351C > G	L451V	No	D	D:	D	D	D	D	Exonic		
2	c.683C > T	T228M	Yes	D	D	D	D	D	D	Exonic		
3	c.107G > A	R36Q	Yes	T	D	D	D	D	D	Exonic		
4	c.944 T > C	L315P	Yes	D	D	D	D	D	D	Exonic		
5	c.863 + 1G > A	–	Yes	–	–	–	–	–	–	Splicing	D:0.00269; on	1;0.938
6	c.635 C > T	S212F	Yes	D	D	D	D	D	D	Exonic		
7	c.1019 + 20G > A	–	No	–	–	–	–	–	–	Intronic	D:0; off	
8	c.232G > A	D78N	No	D	D	D	D	D	D	Exonic		
9	c.736G > C	G246R	Yes	D	B	D	D	D	D	Exonic		
10	c.1352 T > C	L451P	No	D	D	D	D	D	D	Exonic		
11	c.260 T > G	M87R	No	D	D	D	D	D	D	Exonic		
12	c.754 T > C	C252R	Yes	D	D	D	D	D	D	Exonic		

SIFT score: D = Deleterious variant, *T* Tolerated variant. PolyPhen-2: D = Probably damaging, *P* Possibly damaging, *B* Benign. LRT: D = Deleterious, *N* Neutral, *U* Unknown. MutationTaster: A = Disease-causing automatic, *D* Disease-causing, *N* Polymorphism, *P* Polymorphism automatic. FATHMM: D = Deleterious, *T* Tolerated. Radial SVM: D = Deleterious, *T* Tolerated. Higher scores denote more deleterious variants. regSNP-intron: the columns are FPR (false positive rate), disease (disease category): *B* Benign, *PD* Possibly damaging, *D* Damaging, splicing site (on/off). dbscSNV (ADA; RF): splice site prediction by AdaBoost and Random Forest

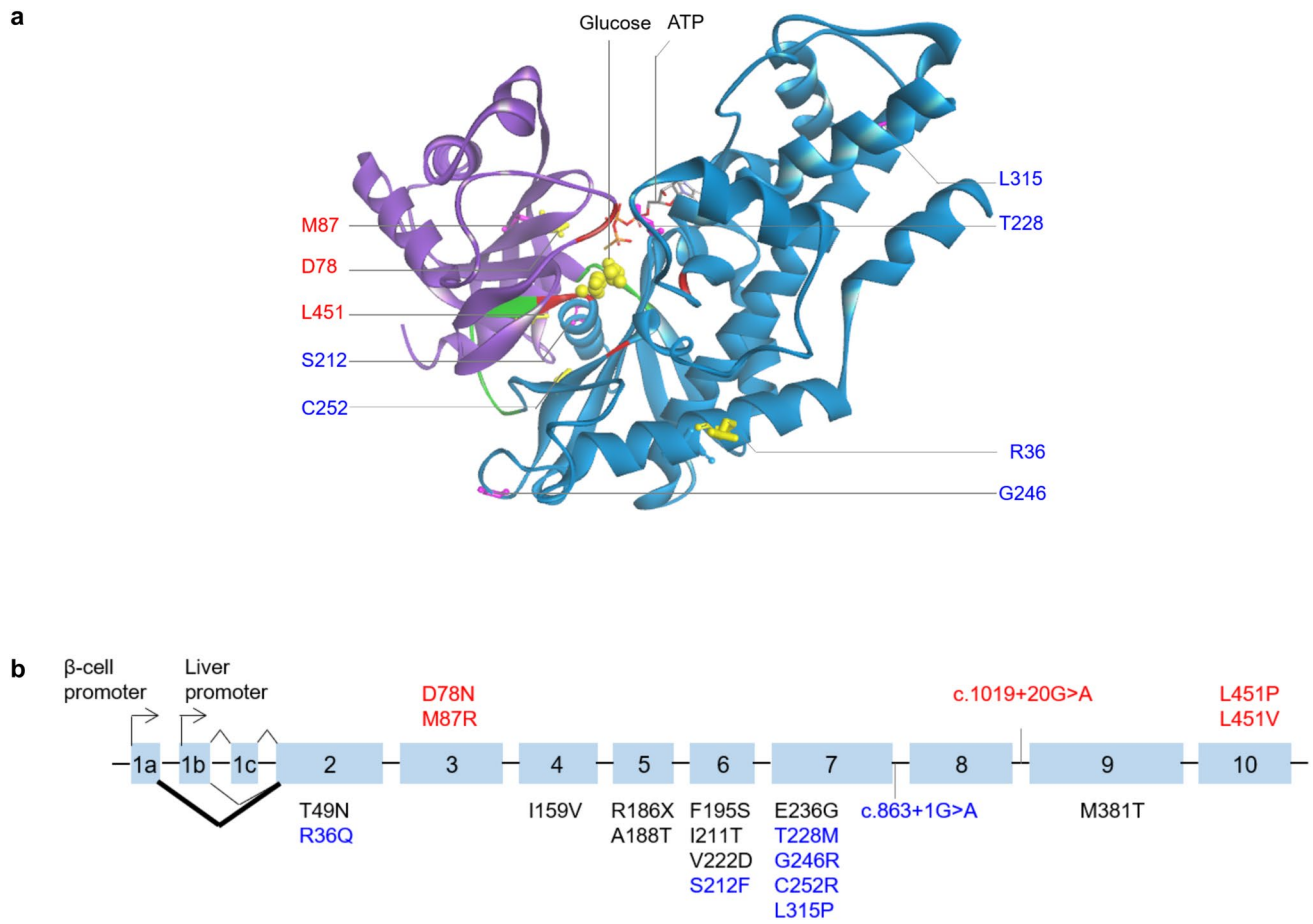

Fig. 3 Locations of mutations in a 3D model and schematic structure of the human *GCK* gene. **a** Locations of the nine mutated amino acids in the constructed GCK model. The locations of 10 mutations (actually involving nine sites) are shown in the 3D model of human β cell GCK. The GCK structure in the active closed form (3VEY) is shown: purple, small domain; blue, large domains; grey, connecting region; yellow spheres, glucose; multi-colored chain, ATP. Six glucose binding sites, i.e., T168, K169, E256, E290, N204, and D205, are marked with red in the GCK molecular chain. Deep pink or yellow chains, mutation sites. **b** Schematic structure of the human *GCK* gene and localization of mutations identified in GDM patients and in affected fathers. The β cell isoform of GCK is encoded by exons la and 2–10; the hepatocyte isoform is encoded by exons lb, 1c, and 2–10. Amino acids are designated relative to the human β cell GCK sequence

($n = 396$), the GCK-MODY pregnant women had significantly lower BMI and triglycerides (TG), and elevated HbA1c, fasting plasma glucose (FPG), and 2-h postprandial glucose (2hPG) (each, $p < 0.001$, Table 2).

mGCK kinetic analysis and thermostability

We found that all mutants were expressed well in vitro (Figs. 4a, 5); however, their enzymatic activities and thermostability were lower than that of the WT. The enzymatic activities of two mutants, T228M and L315P, were completely inactive (Fig. 4b). This was consistent with their catalytic efficiency for glucose (GCK catalytic constant/affinity constant for glucose [$K_{cat}/S_{0.5}$]) (Table 3). Compared with GCK-WT, all eight mGCKs showed decreased thermostability at 48.5 °C, all mGCKs lost > 50% activity, whereas GCK-WT was at 50% (Fig. 4c). Compared with the half-life of GCK-WT with 7.8 min at 52.5 °C, the mGCKs had shorter half-lives (Fig. 4d). Notably, because the T228M and L315P mutants were inactive, dynamic parameter and thermostability analyses were not applicable.

Pregnancy outcome of GCK-MODY complicating pregnancy and GDM patients

Both the birth weights and birth weight centiles of the affected offspring of affected mothers were significantly lower than that of the unaffected offspring, and were statistically significant after Bonferroni correction for multiple comparisons ($p < 0.008$, 0.05/6) (Table 4). Among the offspring of affected fathers, although the birth weights and birth weight centiles of the affected offspring were significantly lower than that of the unaffected offspring ($p = 0.017$ for birth weights; $p = 0.015$ for birth weight centiles), the

Table 2 Comparison of clinical characteristics between GDM and GCK-MODY complicating pregnancy patients

Clinical characteristics	GDM ($n=396$)	GCK-MODY complicating pregnancy ($n=41$)	p value
Age at pregnancy, years	31.8 ± 4.8	28.1 ± 4.9	< 0.001
Gestational age, weeks	39.0 ± 1.4	39.8 ± 0.7	< 0.001
Pregestational BMI, kg/m^2	22.7 ± 3.4	20.2 ± 2.1	< 0.001
Cholesterol, mmol/l	4.9 (4.3–5.5)	4.5 (4.0–5.1)	0.285
Triglycerides, mmol/l	1.5 (1.1–2.1)	1.0 (0.8–1.1)	< 0.001
HbA1c, mmol/mol	35.0 ± 4.7	40.2 ± 3.5	< 0.001
HbA1c, %	5.4 ± 0.4	5.8 ± 0.3	< 0.001
FPG, mmol/l	5.0 ± 0.7	5.9 ± 0.4	< 0.001
2hPG, mmol/l	8.4 ± 1.7	9.0 ± 1.3	0.036
Insulin treatment, *n* (%)	9 (2.3)	21 (51.2)	< 0.001
Fetal gender, M/F	206/190	23/18	0.619

Data are the mean ± SD, median (interquartile range), or *n* (%)

M male, *F* female

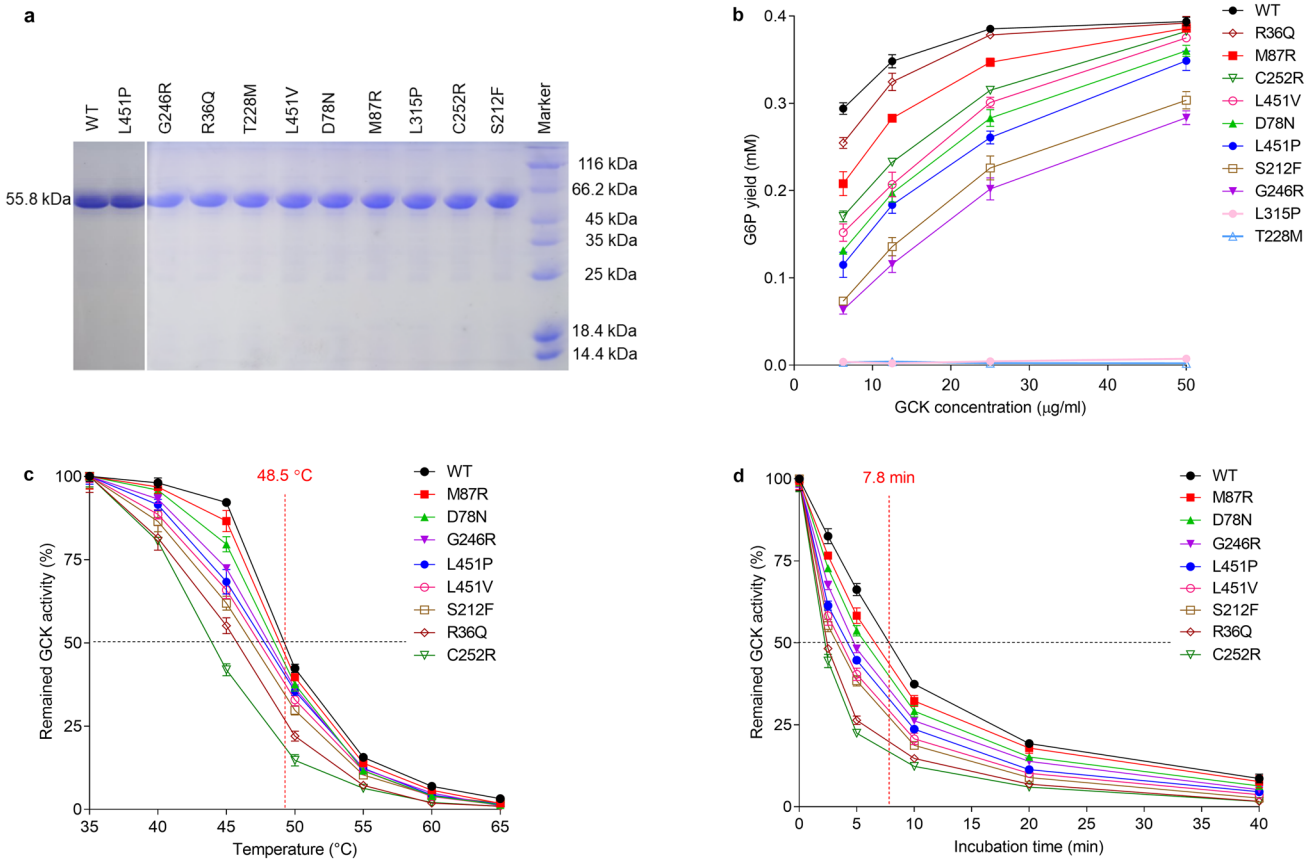

Fig. 4 Purification, activity, and thermostability of mGCKs. **a** Sodium dodecyl sulfate—polyacrylamide gel electrophoresis (SDS-PAGE) of purified WT-GCK and mGCK proteins. Proteins (2 μg per lane) were loaded onto 15% SDS-PAGE to confirm their purity. **b** GCK activity. G6P yield was analyzed under different enzyme concentrations. **c** Remaining GCK activity after incubation at different temperatures. The eight proteins were pre-incubated at various temperatures for 30 min in reaction buffer without substrates, then ATP (5 mM) and glucose (200 mM) were added to the reaction mixtures and incubated for 10 min at 37 °C; the product G6P was quantified. The G6P yields of the heated enzymes were divided by that of unheated enzymes and used as the remaining relative activity. **d** Time course of GCK heat inactivation at 52.5 °C. According to the protocol in **c**, the GCKs were preheated at 52.5 °C for increasing durations, and the remaining relative activity was quantified. As T228M and L315P activity was too low to generate a clear product under the assay conditions, thermostability analysis for the two was not possible

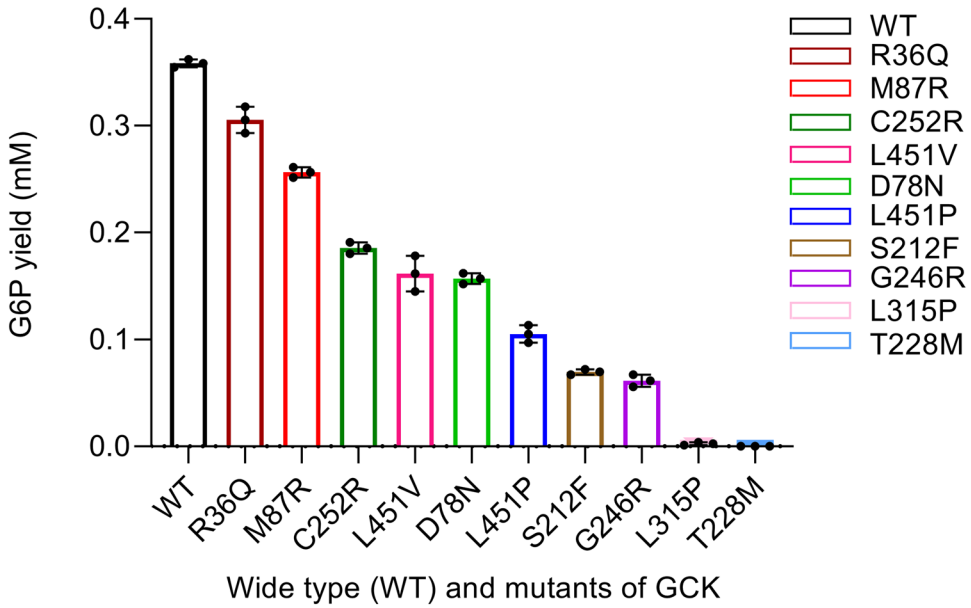

Fig. 5 Comparison of G6P yield at 6.25 μg/ml

significant difference disappeared after Bonferroni correction, that is, $p > 0.008$ (0.05/6) (Table 4). In the affected mother group, 33.3% (3/9) of unaffected offspring were macrosomic, with birth weights ≥ 4.0 kg. Affected offspring born to affected mothers had significantly heavier birth weights than those born to affected fathers, with $p < 0.008$ (0.05/6) (i.e., $p = 0.007$) after Bonferroni correction (Table 4). However, the significant difference disappeared after Bonferroni correction for birth weight percentiles.

The effects of insulin therapy for GCK-MODY pregnant women

Compared with the diet-control group, insulin treatment resulted in a significant decrease in both birth weights and birth weight centiles in affected offspring born to affected mothers, which was statistically significant after multiple linear regression adjustment for pre-pregnancy BMI, gestational age, and fetal gender ($p = 0.031$ for birth weights; $p = 0.035$ for birth weight centiles). However, although the unaffected offspring of insulin-treated GCK-MODY mothers tended to have lower birth weights and birth weight centiles, they were not significantly different from that of the diet-control group ($p = 0.470$ for birth weights; $p = 0.519$ for birth weight centiles) (Table 5).

Of the affected offspring ($n = 32$), six offspring from 17 insulin-treated affected mothers (35.3%) had perinatal complications, and 47.1% (8/17) of the mothers had hypoglycemia (Table 5). Of the unaffected offspring ($n = 9$), one offspring from four insulin-treated affected mothers (25%) had perinatal complications, and 50% (2/4) of the mothers had hypoglycemia. Ten insulin-treated affected mothers presented with hypoglycemia symptoms, i.e., hunger, sweating, palpitations, tremor, accompanied by plasma glucose concentration < 3.9 mmol/l [38]. However, neither perinatal complications nor hypoglycemia were observed among the 20 diet-control mothers (Table 5).

Discussion

In the present study, 15 cases of gestational GCK-MODY were identified from 411 GDM patients, and another six *GCK* mutations were identified from 24 fathers, totaling 21 *GCK* mutations. Five mutations were novel (i.e., D78N, M87R, c.1019 + 20G > A, L451V, and L451P), and of the 16 known mutations, nine have been reported previously [10], while the other seven have been identified for the first time in a Chinese population [35–37]. To date, this is the first and largest screening for gestational GCK-MODY in Chinese GDM patients since the recent finding that MODY2 accounted for 10.4% of the classic MODY population in Chinese [10], rather than being rare [39]. The present study suggests that gestational GCK-MODY may account for at least 3.6% (15/411) of GDM women without being related to family history of diabetes. As GDM cases account for 17.5% of pregnant women in China [2], therefore gestational GCK-MODY may account for at least 6.3% of pregnant women.

Based on 2014 screening criteria [13], the prevalence of GCK-MODY complicating pregnancy in GDM women in the present study would be reduced to 2.4% (6/411); therefore, the rate of misdiagnosis would be 33.3% (5/15), suggesting that these criteria are not applicable to Chinese patients. With the decreasing cost of DNA sequencing [19] and increasing awareness among health care professionals of GCK-MODY complicating pregnancy, whole-*GCK* gene

Table 3 Kinetic parameters of WT-GCK and mGCKs

Mutation	Yield (mg/L)	$S_{0.5}$ (glucose)	Hill coefficient (*h*)	Inflection point (mM)	K_m (ATP)	K_{cat1} (glucose)	K_{cat2} (ATP)	Activity index	Relative activity index	$K_{cat1}/S_{0.5}$
WT	25.6 ± 0.3	8.17 ± 0.23	1.71 ± 0.03	3.73 ± 0.18	0.45 ± 0.03	11.47 ± 0.32	15.62 ± 0.22	0.26 ± 0.02	1.00 ± 0.08	1.40 ± 0.06
R36Q	18.2 ± 0.2***	9.94 ± 0.85*	1.58 ± 0.02**	3.86 ± 0.22	0.77 ± 0.03***	10.16 ± 0.32**	23.51 ± 0.32***	0.19 ± 0.01**	0.75 ± 0.01**	1.02 ± 0.03***
M87R	17.1 ± 0.1***	14.65 ± 0.39***	1.59 ± 0.02**	5.78 ± 0.37***	0.55 ± 0.03**	11.12 ± 0.22	13.51 ± 0.23***	0.12 ± 0.00***	0.47 ± 0.00***	0.76 ± 0.03***
C252R	15.2 ± 0.2***	13.04 ± 0.68***	1.68 ± 0.01	5.76 ± 0.36***	0.95 ± 0.04***	8.12 ± 0.34***	14.71 ± 0.23**	0.07 ± 0.01***	0.29 ± 0.01***	0.62 ± 0.05***
L451V	21.0 ± 0.1***	12.35 ± 0.31***	1.67 ± 0.03	5.39 ± 0.43**	0.49 ± 0.02	7.51 ± 0.21***	13.36 ± 0.16***	0.09 ± 0.00***	0.35 ± 0.00***	0.61 ± 0.02***
D78N	22.6 ± 0.2***	16.21 ± 0.34***	1.51 ± 0.01***	5.64 ± 0.39**	1.48 ± 0.10***	9.49 ± 0.38**	1.34 ± 0.12***	0.08 ± 0.00***	0.31 ± 0.00***	0.59 ± 0.02***
L451P	17.8 ± 0.1***	14.13 ± 0.70***	1.65 ± 0.04	6.02 ± 0.31***	1.00 ± 0.05***	7.81 ± 0.11***	14.48 ± 0.25**	0.07 ± 0.00***	0.26 ± 0.00***	0.55 ± 0.02***
S212F	23.2 ± 0.2***	14.56 ± 0.25***	1.64 ± 0.02*	6.14 ± 0.25***	0.48 ± 0.01	6.13 ± 0.18***	16.31 ± 0.28*	0.06 ± 0.00***	0.24 ± 0.00***	0.42 ± 0.03***
G246R	21.9 ± 0.1***	18.85 ± 1.39***	1.57 ± 0.02**	7.22 ± 0.35***	0.33 ± 0.02**	6.35 ± 0.26***	6.84 ± 0.16***	0.05 ± 0.01***	0.21 ± 0.00***	0.34 ± 0.02***
L315P	1.5 ± 0.1***	34.56 ± 1.02***	1.63 ± 0.02**	14.38 ± 1.02***	0.44 ± 0.02	0.11 ± 0.02***	12.53 ± 0.22***	0.00***	0.00***	0.00***
T228M	23.1 ± 0.2***	nd	nd	nd	nd	nd	nd	nd	nd	nd

GCK-WT = GCK-wild type, mGCKs = *GCK* mutants. As the activity of T228M was so low such that it did not generate clear products in the presence of 1 mg/mL protein, the dynamics parameters could not be calculated. Data are the means ± SD of three separate enzyme expressions

h Hill coefficient; *nd* not detected. vs. wild-type

*$p < 0.05$

**$p < 0.01$

***$p < 0.001$

Table 4 The comparison of pregnancy outcome among unaffected and affected offspring of affected mothers or fathers

Pregnancy outcome	Affected mothers			Affected fathers		
	GCK(+) offspring ($n=32$)	GCK(−) offspring ($n=9$)	p value	GCK(+) offspring ($n=20$)	GCK(−) offspring ($n=4$)	p value
Gestational age, weeks	39.8 ± 0.7	39.8 ± 0.8	0.878	40.0 ± 0.6	40.0 ± 0.2	0.842
Birth weight, g	3195.5 ± 196.0	3734.4 ± 534.0	< 0.001**	2962.5 ± 288.8	3353.8 ± 168.5	0.017
Birth weight centile	42.2 ± 14.5	72.7 ± 29.3	< 0.001**	29.9 ± 19.0	55.1 ± 9.7	0.015
Macrosomia *n* (%)	0	3 (33.3%)	0.008*	0	0	–
Insulin treatment (%)	53.1 (17/32)	44.4 (4/9)	0.719	–	–	–
Fetal gender, M/F	18/14	5/4	1.000	4/16	2/2	0.251

Data are the means ± SD or % (*n*). $p < 0.008$ (0.05/6) was considered statistically significant following Bonferroni correction for multiple comparisons

*Represent that of borderline significant for macrosomia

**Represent statistically significant, such as birth weight and birth weight centile

GCK(+) with GCK mutation, *GCK(−)*without GCK mutation

Table 5 The effects of insulin therapy on 41 GCK-MODY complicating pregnancy mothers grouped by affected and unaffected offspring

Pregnancy outcome	GCK(+) offspring		p value	GCK(−) offspring		p value
	Diet ($n=15$)	Insulin ($n=17$)		Diet ($n=5$)	Insulin ($n=4$)	
Age, years	27.7 ± 5.4	29.2 ± 4.9	0.414	25.8 ± 1.9	27.5 ± 5.3	0.575
Gestational age, weeks	39.9 ± 0.5	39.8 ± 0.8	0.663	40.0 ± 0.9	39.5 ± 0.6	0.374
Pregestational BMI, kg/m^2	21.1 ± 1.9	19.5 ± 1.9	0.022	19.8 ± 2.6	20.7 ± 2.0	0.600
Birth weight, g	3295.7 ± 182.0	3176.1 ± 166.4	0.031	3868.0 ± 399.2	3567.5 ± 693.5	0.470
Birth weight centile	49.4 ± 13.7	35.9 ± 12.3	0.035	81.2 ± 16.7	61.9 ± 40.5	0.519
Fetal gender, M/F	9/6	9/8	0.688	3/2	2/2	0.764
Perinatal complications (%)	0	35.3 (6/17)	0.011	0	25.0 (1/4)	0.444
Hypoglycemia (%)	0	47.1 (8/17)	0.003	0	50.0 (2/4)	0.167

Data are the means ± SD or % (*n*). *p* values of birth weight and body weight percentile were adjusted for pre-pregnancy BMI, gestational age, and fetal gender by multiple linear regression

*GCK(+)*with GCK mutation, *GCK(−)*without GCK mutation

mutation screening for all GDM patients, but not the proposed selective clinical screening criteria [4, 13, 40], will become the most reasonable and accurate diagnostic method for preventing misdiagnosis in such patients.

GCK is highly expressed in pancreatic β cells and hepatocytes, catalyzing the first rate-limiting reactions of glycolysis and glycogen synthesis in the islets and the liver, respectively, by converting glucose to glucose-6-phosphate (G6P) [41]. Accordingly, GCK maintains glucose homeostasis by regulating pancreatic β cell insulin release and liver glycogen synthesis. Not all *GCK* missense mutations are disease-causing, such as p.Thr354Met [42]; therefore, functional analysis of mutant GCK enzymes is necessary. Of the 21 *GCK* mutations in the present study, 19 were missense mutations (4 novel, 15 known) localizing on exons 2–10, and two intronic mutations, i.e., c.1019 + 20G > A (novel) and c.863 + 1G > A are localized on intron 8 and 7, respectively, suggesting that these mutations occur on the common exons and introns for both the pancreatic and hepatic GCK isoforms. All these mutations were co-segregated with hyperglycemia in their MODY nuclear pedigrees. Therefore, they may cause defective GCK function in the islet β cells and hepatocytes. In the present study, four novel mutations and six known mutations (i.e., R36Q, S212F, T228M, G246R, C252R, L315P) resulted in varying degrees of decreased GCK enzyme activity and thermostability (Fig. 4, Table 3).

The location of the novel L451V or L451P mutation is adjacent to an allosteric site, V452, and is also close to another allosteric site, V455, in steric conformation [43]. Therefore, L451 mutations may result in allosteric disorders and substantial decreases in GCK enzyme activity (Fig. 4, Table 3). The novel mutations D78N and M87R are located in the highly conserved region of amino acid residues 74–99 of GCK in β cells [44]. The K_m (affinity constant for ATP) of D78N was 3.3 times higher and the $S_{0.5}$ was 2.0 times higher than that of GCK-WT (Table 3), suggesting that

steric disruption of the ATP-binding hairpin caused by this mutation results in obviously decreased ATP affinity. For the M87R mutation, Met87, which is non-polar hydrophobic and uncharged, is substituted by Arg87, which is polar hydrophilic and positive-charge, which may alter the spatial conformation of glucose binding. Kinetic analysis showed that the K_{cat1} of M87R was identical to that of GCK-WT, but its $S_{0.5}$ was 1.8 times higher, indicating that the mutation can reduce GCK glucose binding (Table 3).

The novel mutation c.1019 + 20G > A is only 19 bp upstream of the classical *GCK* mutation (c. 1019 + 1G > A) [45] which may lead to the disruption of a potential splicing site in a highly conserved region predicted by regSNP-intron [33]. Therefore, the mutation may affect the splicing of *GCK* mRNA. However, the reliability of the prediction results requires further verification. In addition, at least five online prediction software predicted that the 12 mutations (five novel and seven known) are deleterious (Table 1), which is consistent with their reduced enzyme activity and thermal stability (Table 3).

We compared the clinical characteristics of GCK-MODY complicating pregnancy ($n = 41$, including 26 extended cases) and GDM patients ($n = 396$) at 24–28 weeks of gestation (Table 2). Compared with GDM patients, GCK-MODY complicating pregnancy patients had markedly elevated HbA1c, FPG, and 2hPG, and reduced TG (each, $p < 0.001$, Table 2). GCK-MODY patients have normal metabolic control, as their elevated blood glucose levels can be compensated by reductions in blood lipid levels [46], which is supported by the observations in the present study (Table 2). Elevated lipids in combination with elevated glucose lead to glucolipotoxicity of β cells, and therefore may cause β cell failure in type 2 diabetes mellitus [47], and insulin resistance [48]. Notably, the observation of lower TG levels in the GCK-MODY pregnant women as compared to the GDM patients implies that insulin sensitivity is reduced in the latter group or is normal in the GCK-MODY group. GDM increases the risk for developing type 2 diabetes, which is in line with the idea of a "type 2 diabetes-like" pathophysiology of GDM [49].

Our results show that the affected offspring had lighter birth weights than unaffected offspring born to GCK-MODY mothers (Table 4). Offspring inheriting maternal *GCK* mutations have the same elevated GSIS threshold as the mother, resulting in normal fetal insulin secretion and birth weight. In offspring without inherited maternal *GCK* mutations, maternal high glucose will cross the placenta and result in fetal hyperglycemia. The high fetal glucose level results in increased stimulation of fetal insulin secretion, birth weight, and birthweight centile. Here, macrosomia was prevalent in 33.3% (3/9) of unaffected offspring born to the affected mothers (Table 4). Based on the above data, the effect of maternal *GCK* mutations on birth weight depends on whether the fetus has inherited the mutation, which is consistent with findings from other ethnic groups [50].

In GCK-MODY patients, GCK function in the β cells is defective; therefore, GSIS thresholds are elevated and insulin secretion is decreased, which results in hyperglycemia [51]. Counter-regulation caused by counter-regulatory hormones such as glucagon occurs to maintain the higher glucose concentration resulting from defective glucose sensing during hyperinsulinemic hypoglycemic clamp [52]; therefore, *GCK* mutations may influence pregnant women's responses to exogenous insulin. In healthy individuals, when blood glucose is lowered with insulin to a threshold of 3.7 mM, glucagon secretion is initiated to maintain normoglycemia. By contrast, the GCK deficiency in the α cells [53] of GCK-MODY2 patients means that when blood glucose is lowered to an elevated threshold of 4.5 mM, glucagon secretion is initiated to maintain the hyperglycemia [52]. In addition, exogenous insulin therapy would be counteracted by the reduction in endogenous insulin secretion in GCK-MODY patients, suggesting that much larger doses of exogenous insulin would be required to overcome the counter-regulation, which may increase the hypoglycemia risk. A GCK-MODY complicating pregnancy patient in the UK required as high as 1 U/kg/day insulin to lower fasting hyperglycemia to euglycemic levels; finally, the patient presented with symptoms of hypoglycemia [54], which supports our speculation.

Our results show that birth weight was significantly reduced in the affected offspring of insulin-treated affected mothers when compared to that of the diet-control group (Table 5), which is supported by a recent Chinese study on GCK-MODY patients [55]. In addition, of the 21 affected insulin-treated mothers, 10 presented with maternal hypoglycemia (47.6%) and seven had fetal perinatal complications (33.3%) such as small-for-gestational age ($n = 2$), neonatal jaundice ($n = 2$), and one case each had patent ductus arteriosus, solitary kidney, and mild respiratory issues (Table 5). However, none of the above events were observed in the 20 diet-treated mothers (Table 5). These results suggest that Chinese GCK-MODY complicating pregnancy patients should be treated with diet control rather than insulin, regardless of whether the offspring inherits the mutation, which is similar to that in Japanese [18], albeit different from that in Caucasians [54]. In comparison with Caucasians [24, 25], insulin therapy is not recommended in Japanese GCK-MODY families, because of a lower risk of macrosomic birth injury and a higher risk of perinatal complications in affected offspring [18].

In the latter, insulin therapy of maternal hyperglycemia is only appropriate if the fetus has not inherited the *GCK* mutation, as these offspring are at increased risk of macrosomia and perinatal complications [54]. In view of the difficulty in lowering regulated maternal hyperglycemia

caused by *GCK* mutations, regular monitoring of fetal abdominal circumference and blood glucose of the affected mother during pregnancy is critical, as is early labor if necessary (e.g., at 38 weeks) [25, 54].

To our knowledge, this is by far the largest study to identify GCK-MODY complicating pregnancy in GDM patients. However, our study has its limitations. The prevalence of GCK-MODY complicating pregnancy in GDM patients, and the appropriateness of insulin therapy should be replicated and/or verified in larger samples from multiple centers. Not all birth weights were obtained from obstetric records; although the difference between them was < 90 g, that might have influenced the accuracy of the data.

In conclusion, we found that the prevalence of GCK-MODY complicating pregnancy was 3.6% in Chinese GDM patients. The 21 *GCK* mutations, which included five novel mutations, all with defective GCK, may contribute to the development in GCK-MODY. Insulin therapy appears not beneficial for GCK-MODY complicating pregnancy and may therefore not be recommended for such patients to avoid perinatal complications and maternal hypoglycemia.

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Data availability All data generated or analyzed during this study are included in this published article and its supporting materials.

Declarations

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

Ethical approval This study was approved by the Ethics Committee of the Shanghai Jiaotong University Affiliated Sixth People's Hospital, Shanghai, China (Approval Number: YS-2019-124), and conducted in accordance with the Declaration of Helsinki.

Informed consent Informed written consent was obtained from all study participants.

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