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ORIGINAL RESEARCH

Replication of previous genome-wide association studies of *HKDC1*, *BACE2*, *SLC16A11* and *TMEM163* SNPs in a gestational diabetes mellitus case–control sample from Han Chinese population

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Background: Four novel glucose metabolism risk loci (*HKDC*1 rs4746822, *BACE2* rs6517656, *SLC16A11* rs13342232 and *TMEM163* rs998451) were identified in recent genome-wide association studies (GWAS) of Afro-Caribbean, European, Hispanic, Thai, Mexican, Latin American and Indian populations. None of the abovementioned SNPs has been reported in a Han Chinese population.

Aim: To replicate the relationships between *HKDC*1 rs4746822, *BACE2* rs6517656, *SLC16A11* rs13342232 and *TMEM163* rs998451 with gestational diabetes mellitus (GDM) in a Han Chinese population.

Methods: This was a case–control study which enrolled 334 pregnant women with GDM and 367 pregnant women with normal glucose tolerance. The linear regression and logistic regression were used to estimate the association between SNPs with the risk of GDM, HOMA-IR and fasting insulin levels. The fasting insulin concentration and HOMA-IR were log10 transformed before analysis.

Results: No significant differences in the alleles and genotypes of *SLC16A11* rs13342232, *HKDC*1 rs4746822 and *BACE2* rs6517656 were observed between cases and controls. After adjusting the weekly BMI growth, pre-pregnancy BMI and maternal age, under the additive model, *SLC16A11* rs13342232 was associated with $\log_{10}$fasting serum insulin (Beta=0.046, p=0.016), $\log_{10}$HOMA-IR level (Beta=0.061, p=0.003) and fasting plasma glucose level (Beta=0.164, p=0.011); *HKDC*1 rs4746822 was associated with OGTT 2-hr plasma glucose level (Beta=0.239, p=0.016); and *BACE2* rs6517656 was associated with $\log_{10}$fasting serum insulin (Beta=−0.053, p=0.044) and $\log_{10}$HOMA-IR level (Beta=−0.060, p=0.048). After correction for multiple testing, the associations of *SLC16A11* and *HKDC*1 with glucose metabolism remained statistically significant. The A allele of *TMEM163* rs998451 was not detected in this population.

Conclusion: *HKDC*1 rs4746822, *BACE2* rs6517656 and *SLC16A11* rs13342232 are associated with glucose metabolism in pregnant women of Han Chinese.

Keywords: gestational diabetes mellitus, SLC16A11, HKDC1, BACE2, TMEM163

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Introduction

Gestational diabetes mellitus (GDM) is defined as glucose intolerance first detected during pregnancy.[1] It is estimated by the International Diabetes Federation that 21.3 million women (16.2% of live births) in 2017 had some form of hyperglycemia in pregnancy, and of these, 86.4% were due to GDM.[2] During pregnancy, insulin

resistance is enhanced physiologically and can be further promoted by other factors such as obesity, leading to a high risk of GDM.[3–5] Genome-wide association studies (GWAS) have led to the identification of 8 common-penetrance loci (*CDKAL1, GCKR, G6PC2, PCSK1, PPP1R3B, MTNR1B, HKDC1* and *BACE2*) for GDM, principally through 2 GWAS carried out among women of Afro-Caribbean, European, Hispanic, Thai and Korean descent.[6,7] In replication studies, most (but not all) risk alleles have been shown to confer similar effects in different ethnic populations.[8–16]

The study by Hayes et al identified two novel significant genome-wide associations: 2-hr plasma glucose with *HKDC1* rs5030937 and rs4746822 (top associated SNP) and fasting C-peptide with *BACE2* rs6517656 in women with hyperglycemia and adverse pregnancy outcomes (HAPO).[6] *HKDC1* encodes hexokinase domain containing 1 and is a member of the hexokinase family.[17] A follow-up study to the GWAS demonstrated that hexokinase domain containing 1 has hexokinase activity and that lower levels of HKDC1 expression are associated with higher levels of maternal 2-hr glucose.[18] In the only replication study of this locus, the T allele of *HKDC1* rs4746822 showed a significant association with GDM in a south Indian population.[8] *BACE2*, which encodes β-site amyloid polypeptide cleaving enzyme 2, is expressed in human pancreatic beta cells, where its protein product is located in endocytic vesicles and has been shown to both augment and inhibit insulin secretion and/or production in human islets.[19,20] *BACE2* rs6517656 has not been studied in different ethnic GDM populations.

In this replication study, we not only analyzed the association between *HKDC1* rs4746822 and *BACE2* rs6517656 with GDM risk but also included two genes which were revealed to be significantly genome-wide associated with Type 2 diabetes mellitus (T2DM). The SIGMA Type 2 Diabetes Consortium analyzed 9.2 million SNPs in Mexican and Latin American populations and identified a novel locus associated with T2DM at genome-wide significance spanning the solute carriers SLC16A11 and SLC16A13.[21] The top associated variant is *SLC16A11* rs13342232. The SLC16A11 protein is localized in the endoplasmic reticulum and plays an important role in lipid metabolism in the liver, salivary gland and thyroid.[21] *SLC16A11* rs13342232 has been studied in pregnant Mexican women and showed no association with GDM risk.[22] Tabassum et al performed a GWAS in an Indian population and identified a new signal for T2DM on chromosome 2q21. The strongest signal on the newly identified locus 2q21 mapped to *TMEM163*, which encodes a probable vesicular transporter in nerve terminals. The association of *TMEM163* variants (rs6723108 and rs998451) with reduced fasting plasma insulin levels and Homeostasis model assessment of insulin resistance (HOMA-IR) indicates that it might modulate susceptibility to T2DM by affecting insulin secretion.[23]

The association between specific gene with the risk of the same disease may vary among different races. Therefore, the results of GWAS need to be verified in different ethnic groups, especially when GWAS did not include these ethnic groups. To the best of our knowledge, there have been no association studies on the association between *HKDC1* rs4746822, *BACE2* rs6517656, *SLC16A11* rs13342232 and *TMEM163* rs998451 with GDM risk in a Chinese population to date. Therefore, we aimed to study the associations between the 4 newly identified glucose metabolism GWAS SNPs with GDM risk in a Han Chinese population.

Materials and methods

Ethics statement

The "Central-South University's Ethical and Confidentiality Committee" reviewed and approved the study protocol (document reference number: CTXY-13003). We got written informed consent from all participants. This study was conducted in accordance with the Declaration of Helsinki.

Study design and statistical analysis

The research population and most part of the statistical methods of this study were completely consistent with our previous articles,[24,25] so the same content is not repeated here (Full version of the materials and methods are available as supplementary file). Briefly, this was a case–control study which enrolled 334 pregnant women with GDM and 367 pregnant women with normal glucose tolerance who visited prenatal clinics regularly and underwent oral glucose tolerance test (OGTT) during 24–28 weeks. The boundaries of OGTT were 5.1 mmol/L, 10.0 mmol/L and 8.5 mmol/L for fasting glucose and 1 and 2 hrs after 75 g oral glucose intake. When one or more OGTT indicators reached or exceeded the above-mentioned boundaries, the pregnant woman was diagnosed with GDM. The following information were collected on the OGTT morning: maternal age, gestational age, parity, height, weight, fasting insulin levels, systolic blood pressure and diastolic blood pressure. We also collected the information of family diabetes history. Relatives included the paternal grandfather, paternal grandmother, maternal grandfather, maternal grandmother, father, mother, brothers, sisters, and brothers and

sisters of the father and mother. One or more relatives were diagnosed with diabetes and the pregnant woman was judged to have a family history of diabetes. Positive family diabetes history rate=number of cases (controls) with family diabetes history/number of cases (controls), we calculated the positive rate in cases and controls, respectively.

Four SNPs (*HKDC*1 rs4746822, *BACE2* rs6517656, *SLC16A11* rs13342232 and *TMEM163* rs998451) were genotyped in this study. Genomic DNA was extracted from whole blood using a TIANamp Blood DNA Kit (DP318-03, TIANGEN, Beijing), which is based on silica membrane technology and uses a special buffer system for DNA extraction from fresh or frozen whole blood. SNPs were genotyped with the SEQUENOM MassARRAY iPLEX platform. The assay consists of an initial locus-specific PCR reaction, followed by single-base extension and matrix-assisted laser desorption/ionization–time of flight mass spectrometry to identify the SNP allele.[26] Table 1 shows the functional consequence, alleles, HWE *p*-value of controls and the minor allele frequency (MAF) of Han Chinese from HapMap project of *SLC16A11* rs13342232, *HKDC*1 rs4746822, *BACE2* rs6517656 and *TMEM163* rs998451. The primers for each SNP in PCR reaction are shown in Table 2.

The chi-square test was used to compare the distribution of genotypes between the case and control groups. In the analysis of the association between SNPs and disease risk, GWAS used additive models to assign the SNPs (wild-type homozygotes=0, heterozygous assignments=1, and mutant homozygotes=2, continuity variable). Therefore, this study also used the additive model to analyze the association between SNPs and GDM risk and glucose metabolism levels. Logistic regression was used to estimate the odds ratio (OR) and 95% confidence interval (CI) of each SNP adjusted by maternal age, pre-pregnancy BMI and weekly BMI growth. Linear regression was used to estimate the relationship between SNPs and the OGTT, fasting insulin and HOMA-IR levels, adjusted by maternal age, pre-pregnancy BMI and weekly BMI growth. HOMA-IR=Fasting insulin (mIU/L)*fasting blood glucose (mmol/L)/22.5. The fasting insulin concentration and HOMA-IR were $\log_{10}$ transformed before analysis. Weekly BMI growth=(BMI on the OGTT morning-pre-pregnancy BMI)/gestational age (weeks). The fasting insulin concentration and HOMA-IR were log10 transformed before analysis. Three SNPs were finally included in the analysis; therefore, α was equal to 0.017 (0.017=0.05/3).

Results

Base information

Table 3 shows the cases' and controls' clinical characteristics. Positive family diabetes history rate ($p<0.001$), weekly BMI growth ($p<0.001$), pre-pregnancy BMI ($p<0.001$), diastolic blood pressure ($p<0.001$) and systolic blood pressure ($p<0.001$) were higher in case group than control group.

Hardy–Weinberg equilibrium

The SNP genotyping detection rate was 99.5%. Hardy–Weinberg equilibrium (HWE) was observed in the control group for all SNPs (Table 1).

Table 1 The functional consequence, alleles, HWE *p*-value of controls and the MAF of Han Chinese from HapMap project of the SNPs

Gene	dbSNP ID	Functional consequence	Alleles *	HWE *p*-value of controls	HapMap MAF of Han Chinese
SLC16A11	rs13342232	Synonymous	AG	0.051	0.18
HKDC1	rs4746822	Intron variant	CT	0.750	0.23
BACE2	rs6517656	Intron variant	GA	0.308	0.06
TMEM163	rs998451	Intron variant	GA	-	0

Note: *The second allele was the minor allele.

Table 2 The 5′ UTR and 3′ UTR region primers of the SNPs in PCR reaction

Gene	SNP	5′ UTR region primers	3′ UTR region primers
SLC16A11	rs13342232	ACGTTGGATGTGAGGTGGAGGGTGATCGC	ACGTTGGATGACCCTCTCGCGTTACTTCTC
HKDC1	rs4746822	ACGTTGGATGACCTATTGATAGCACCAGGC	ACGTTGGATGGTTGACATACAGAGCCATGA
BACE2	rs6517656	ACGTTGGATGATCTCTGGTTGATGCACTGG	ACGTTGGATGTCAGGCTGCTAATCACTAAC
TMEM163	rs998451	ACGTTGGATGTGGAAAGACAGTGGTCACAG	ACGTTGGATGTCTAAAGCCTGTGTCAAGCC

Table 3 Demographic and clinical characteristics of the cases and controls

	Controls (N=367)	Cases (N=334)	*p*
Maternal age, years	29 (28,32)	29 (27,32)	0.672*
Gestational age at sampling, weeks	25.11±2.724	25.35±2.948	0.458**
Pre-pregnancy BMI, kg/m^2	20.55 (19.14,22.64)	22.31 (20.29,24.14)	<0.001*
Weekly BMI growth, kg/m^2	0.114±0.054	0.131±0.056	<0.001**
SBP, mmHg	111±10.30	116±11.12	<0.001**
DBP, mmHg	70±8.38	74±8.09	<0.001**
Parity			
0	230 (62.7%)	216 (64.7%)	0.312***
1	123 (33.5%)	93 (27.8%)	
2	5 (1.4%)	7 (2.1%)	
Family history of diabetes			
Yes	62 (17.4%)	94 (29.3%)	<0.001***
No	295 (82.6%)	227 (70.7%)	

Notes: *Wilcoxon rank sum test (median, quartiles); **Student's *t*-test (mean±SD); ***Chi-square test (number, constituent ratio; in controls column, constituent ratio in the cell=number of subjects in the cell/number of controls; in cases column, constituent ratio in the cell=number of subjects in the cell/number of cases.
Abbreviations: SBP, systolic blood pressure; DBP, diastolic blood pressure.

Association between alleles and genotypes with GDM

As shown in Table 4, no significant differences in the alleles and genotypes of *SLC16A11* rs13342232, *HKDC*1 rs4746822 and *BACE2* rs6517656 were observed between cases and controls; after adjusting for the weekly BMI growth, pre-pregnancy BMI and maternal age, the results were the same (Table 5). The A allele of *TMEM163* rs998451 was not detected in this population.

Association analysis of genetic variants in SLC16A11 rs13342232, HKDC1 rs4746822 and BACE2 rs6517656 with OGTT, HOMA-IR and fasting serum insulin levels

Besides the fasting plasma glucose level, 1 hr and 2 hrs plasma glucose levels after oral glucose intake, the fasting plasma insulin and HOMA-IR levels are also important to evaluate the level of glucose metabolism. The results of Hayes et al GWAS[6] that needed to be replicated were the relationships assessed with linear regressions between genotypes and glycemic traits. Therefore, in this study, we not only analyzed the association between the SNPs and GDM risk but also analyzed the association between the SNPs and glycemic traits (fasting plasma insulin levels, etc.).

As shown in Table 5, the results of the linear regression analysis revealed that under the additive model, *SLC16A11* rs13342232 was associated with fasting plasma glucose levels (Beta=0.164, p=0.011), $\log_{10}$fasting serum insulin levels (Beta=0.046, p=0.016) and $\log_{10}$HOMA-IR levels (Beta=0.061, p=0.003); *HKDC*1 rs4746822 was associated with OGTT 2-hr plasma glucose levels (Beta=0.239, p=0.016); and *BACE2* rs6517656 was associated with $\log_{10}$ fasting serum insulin levels (Beta=−0.053, p=0.044) and $\log_{10}$ HOMA-IR levels (Beta=−0.060, p=0.048), after adjusting for the weekly BMI growth, pre-pregnancy BMI and maternal age. The p-values of the associations between *SLC16A11* rs13342232 with fasting plasma glucose levels (p=0.011), $\log_{10}$fasting serum insulin levels (p=0.016) and $\log_{10}$HOMA-IR levels (p=0.003); and between *HKDC*1 rs4746822 with OGTT 2-hr plasma glucose levels (p=0.016) were lower than the α value (α=0.017) after correction for multiple testing. We inferred that the *SLC16A11* rs13342232 G allele, the *HKDC*1 rs4746822 T allele and the *BACE2* rs6517656 G allele may be risk alleles for glucose metabolism in pregnant Chinese women.

Discussion

In this study, we identified six significant associations: fasting plasma glucose with *SLC16A11* rs13342232, 2-hr plasma glucose with *HKDC1* rs4746822, fasting serum insulin and HOMA-IR levels with *SLC16A11* rs13342232 and *BACE2* rs6517656 in a pregnant Han Chinese population. The A allele of *TMEM163* rs998451 was not detected in this population.

In their GWAS, Hayes et al identified two novel genome-wide significant associations: 2-hr plasma glucose with *HKDC1* rs4746822 and fasting C-peptide with *BACE2*

Table 4 The distribution of alleles and genotypes of rs13342232, rs4746822, rs6517656 and rs998451

Gene	SNP	Allele/genotype	Controls		Cases			
			n	%	n	%	χ^2	*p*
SLC16A11	rs13342232	A	657	90.0	583	88.3	1.000	0.317
		G	73	10.0	77	11.7		
		AA	299	81.9	258	78.2	2.102	0.350
		GG	7	1.9	5	1.5		
		AG	59	16.2	67	20.3		
HKDC1	rs4746822	C	552	75.4	488	73.3	0.836	0.361
		T	180	24.6	178	26.7		
		CC	207	56.6	176	52.9	0.968	0.616
		TT	21	5.7	21	6.3		
		CT	138	37.7	136	40.8		
BACE2	rs6517656	G	693	94.9	630	95.5	0.207	0.649
		A	37	5.1	30	4.5		
		GG	328	89.9	300	90.9	0.218	0.641
		AA	-	-	-	-		
		GA	37	10.1	30	9.1		
TMEM163	rs998451	G	734	100	668	100	-	-
		A	0	0	0	0		
		GG	367	100	334	100	-	-
		AA	0	0	0	0		
		GA	0	0	0	0		

Table 5 The association of rs13342262, rs4746822 and rs6517656 with GDM risk, OGTT, fasting insulin and HOMA-IR levels

Gene	SNP	OR	*p*	Fasting BG		1-hr BG		2-hr BG		log_{10} Fasting insulin		log_{10} HOMA-IR	
				Beta	*p*	Beta	*p*	Beta	*p*	Beta	*p*	Beta	*p*
SLC16A11	rs13342232	1.102 (0.772,1.572)	0.593	**0.164**	**0.011**	0.091	0.632	0.031	0.843	**0.046**	**0.016**	**0.061**	**0.003**
HKDC1	rs4746822	1.202 (0.925,1.563)	0.169	−0.020	0.675	−0.026	0.855	**0.239**	**0.016**	−0.006	0.679	−0.008	0.618
BACE2	rs6517656	0.862 (0.496,1.496)	0.597	−0.086	0.377	−0.207	0.476	−0.347	0.147	**−0.053**	**0.044**	**−0.060**	**0.048**

Note: Covariates in the logistic regression analyses and linear regression analyses were maternal age, pre-pregnancy BMI and weekly BMI growth. Bold font means the *p*-value was less than 0.05.

Abbreviations: BG, blood glucose; HOMA-IR, homeostasis model assessment of insulin resistance.

rs6517656 in pregnant women of Afro-Caribbean, European, Hispanic and Thai ancestry.[6] Our results show that the T allele of *HKDC1* rs4746822 is associated with higher 2-hr plasma glucose; the G allele of *BACE2* rs6517656 is associated with higher fasting serum insulin and higher HOMA-IR levels. We did not measure C-peptide; however, previous study revealed that peripheral insulin and C-peptide concentrations correlated well with each other.[27] Our results indicate that the T allele of *HKDC1* rs4746822 and the G allele of *BACE2* rs6517656 are not only associated with glucose metabolism in HAPO population but also associated with glucose metabolism in pregnant women of Han Chinese. Several studies have explored possible mechanisms by which *HKDC1* and *BACE2* affect glucose metabolism. In the follow-up study to the GWAS, the authors demonstrated that *HKDC1* is a novel human hexokinase gene.[18] *HKDC1* rs4746822 lies within a region of the genome exhibiting chromatin modifications consistent with active gene regulation across diverse tissues. *HKDC1* rs4746822 influences maternal glucose metabolism by altering the activity of regulatory elements that control *HKDC1* expression.[18] *BACE2* (Beta-Site APP-Cleaving Enzyme 2, 21q22.3) encodes an integral membrane glycoprotein cleaving the amyloid

precursor protein into the amyloid beta peptide.[28] In the normal human pancreas, *BACE2* expression was restricted to beta-cells, where it is located in endocytic vesicles. When BACE2 was pharmacologically inhibited, BACE2 content in clathrin-coated vesicles was increased. The insulin internalization rate was reduced, insulin receptor beta-subunit (IR beta) expression was decreased at the plasma membrane and increased in the Golgi apparatus, and a significant reduction in insulin gene expression was detected. Similar results were obtained after specific *BACE2* silencing in MIN6 cells. All these data point to a role for BACE2 in IR beta trafficking and insulin signaling.[19] Esterhazy et al identified BACE2 as the sheddase of the pro-proliferative plasma membrane protein Tmem27 in murine and human β cells. Mice with functionally inactive BACE2 exhibit reduced blood glucose levels, improved intraperitoneal glucose tolerance and increased β cell mass. Pharmacological inhibition of BACE2 increases β cell mass and improves glycemic control in mice with obesity-related insulin resistance.[20]

In the previous replication study of *SLC16A11* rs13342232, the authors did not analyze the association between *SLC16A11* rs13342232 and glucose metabolism indicators and indicated that *SLC16A11* rs13342232 is not associated with GDM in Mexican women.[22] Our study also showed negative results when we compared cases and controls with logistic regression analysis; however, we showed that the A allele of *SLC16A11* rs13342232 is associated with higher fasting plasma glucose, higher fasting serum insulin and higher HOMA-IR levels with linear regression analysis. SLC16A11 is a proton-coupled monocarboxylate transporter. The T2DM risk coding variants (including rs13342232) disrupt protein–protein interactions responsible for correct SLC16A11 function to affect the amount of SLC16A11 transport activity.[29]

In this study, the A allele of *TMEM163* rs998451 was not detected in Han Chinese population. According to the search results of the SNPedia website (https://www.snpedia.com/index.php/Rs998451), only the CEU European population from the HapMap project has been able to identify a population with the A allele present for the SNP rs998451; The A allele was not present in Han Chinese and other east Asian populations.

To the best of our knowledge, this is the first study that has focused on the associations between *SLC16A11* rs13342232, *HKDC1* rs4746822 and *BACE2* rs6517656 with glucose metabolism in a Han Chinese population. However, this study has certain limitations. First, we did not obtain any positive results in the logistic regression analysis comparing cases and controls, which may be due to an insufficient sample size. It is necessary to increase the sample size in subsequent studies to verify the results. Second, the association between these SNPs and other important glucose metabolism indicators, such as C-peptide, 1-hr serum insulin, 2-hr serum insulin, etc., needs further study in pregnant women of Han Chinese.

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Disclosure

The authors report no conflicts of interest in this work.

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