RESEARCH NOTE

The association between the rs4987105 of 5-lipoxygenase (ALOX5) gene and gestational glucose metabolism in Chinese population

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Abstract

Objective: The arachidonate 5-lipoxygenase (ALOX5) pathway has been investigated in diverse chronic inflammatory diseases including metabolic disorders. Recently, the ALOX5 polymorphism rs4987105 was identified to confer susceptibility to type 2 diabetes mellitus (T2DM), implicating its role in regulating glucose homeostasis. Gestational diabetes mellitus (GDM) shares similar pathogenic mechanism with T2DM. Thus, we aimed to evaluate the association between rs4987105 and gestational glucose metabolism in Chinese pregnant women.

Results: A total of 380 unrelated Chinese pregnant women including 241 GDM patients and 139 controls were included in this study. The genotypes of rs4987105 were examined by the Agena MassARRAY iPLEX platform, the association between rs4987105 and fasting plasma glucose (FPG) levels at 24–28 gestational weeks was evaluated using different statistical methods. We found that carriers of rs4987105 CT/TT genotypes exhibited significantly lower FPG levels ($P = 0.011$). In addition, we observed a significant association between rs4987105 and FPG levels after adjusting confounding variables in the linear regression analysis using dominant genetic model ($b = -0.218$; $P = 0.01$). The present study for the first time reported that the rs4987105 of 5-lipoxygenase (ALOX5) gene was associated with gestational glucose metabolism in Chinese pregnant women.

Keywords: Gestational diabetes mellitus, Genetic susceptibility, ALOX5, Polymorphism, Fasting plasma glucose

Introduction

Gestational diabetes mellitus (GDM) is defined as abnormal glucose intolerance with onset or first recognition during pregnancy. It is a prevalent and clinically significant disease threatening both the mother and the offspring [1–4]. The incidence of GDM was estimated to be 8.9–53.4% worldwide and is rapidly increasing each year [5]. GDM not only brings about adverse pregnant outcomes such as stillbirth, macrosomia, shoulder dystocia, neonatal hypoglycemia and neonatal respiratory distress syndrome [6, 7], but also has substantial long-term negative effects on the health of both the patients and their offspring including increased risk of developing type 2 diabetes mellitus (T2DM), obesity, metabolic syndromes and cardiovascular diseases in later life [8, 9]. Therefore, developing effective early detection and intervention strategies to protect pregnant women against maternal and fetal complications has become an urgent need.

Normal pregnancy is associated with an altered inflammatory profile compared to the non-pregnant state. Tightly controlled balance between pro- and anti-inflammatory cytokines is necessary for normal implantation, trophoblast invasion and placentation [10–12]. Dysregulation of the immune system favoring pro-inflammatory responses has been identified as a pivotal pathogenic

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Li *et al. BMC Res Notes*   (2020) 13:102

Page 2 of 5

factor in GDM [13]. Growing evidence suggests that this enhanced degree of low-grade systemic inflammation is an important factor leading to the insulin secretion defects and uncompensated insulin resistance underlying impaired gestational glucose metabolism [13–15].

ALOX5 encodes the central enzyme in proinflammatory leukotriene biosynthesis [16]. The lipid mediator leukotriene with increased production during inflammation plays an essential role in the development of insulin resistance and mediates β-cell destruction resulting in reduced insulin production [14, 17, 18]. Stimulated 5-lipoxygenase pathway in activated macrophages of obese mice contributed to the insulin resistant state [19]. Additionally, ALOX5 deficient mice had decreased insulin secretion and significantly increased fasting glucose levels and fat mass [20]. siRNA-mediated knockdown of *ALOX5* in isolated human islets reduced both insulin gene expression and secretion [20]. These studies underline the important role of ALOX5 in regulating glucose homeostasis.

ALOX5 and its polymorphisms have been explored as first-line candidates in a wide variety of inflammatory diseases including metabolic disorders, asthma, cardiovascular diseases, neurodegeneration, and cancer [16, 17, 20–28]. A very recent study by Nejatian et al. for the first time identified a significant association between the ALOX5 polymorphism rs4987105 and T2DM susceptibility in German population [29]. Additionally, Tsekmekidou et al. demonstrated that another ALOX5 polymorphism rs11239524 was also associated with T2DM in Greek population [30]. Since T2DM and GDM have similar pathogenic mechanism [31, 32], we speculate that polymorphisms of *ALOX5* may also have an impact on gestational glucose control. Thus, in this study we aimed to investigate the association between the more frequently studied *ALOX5* polymorphism rs4987105 and gestational glucose metabolism in Chinese pregnant women.

Main text
Methods
Subjects
The unrelated Chinese pregnant women enrolled in this study were recruited from Buji people's hospital and Songgang people's hospital, Shenzhen, China, between July 2012 and May 2013. The diagnosis of GDM was made based on the one-step GDM screening: a 2 h 75 g oral glucose tolerance test (OGTT) at 24–28 gestational weeks, and we adopted the International Association of the Diabetes and Pregnancy Study Groups (IADPSG) criteria: fasting plasma glucose (FPG) $\geq$ 5.1 mmol/L, or 1 h plasma glucose (1 h-PG) $\geq$ 10.0 mmol/L, or 2 h plasma glucose (2 h-PG) $\geq$ 8.5 mmol/L [33]. We firstly recruited

241 subjects diagnosed with GDM into the patient group, and then later we randomly collected another 139 pregnant women with normal glucose tolerance admitted to the hospitals at the same time period as the control group. A fasting plasma glucose level and 1-, 2-h OGTT plasma glucose level less than 5.1, 10.0 and 8.5 mmol/L, respectively, were defined as normal glucose tolerance. Subjects meeting the following criteria were excluded in our study: (1) the pregnant women had a past medical history of diabetes; (2) the pregnant women had a family history of diabetes or hypertension; (3) the pregnant women did not have a past medical history of diabetes but in their first antenatal examination they were diagnosed as pre-gestational diabetes mellitus (PGDM).

Clinical and biochemical measurements
Anthropometric variables including height, weight before pregnancy were recorded at 24–28 weeks of gestation. Pre-pregnancy body mass index (pre-BMI) (kg/m^2) was calculated from these data. Other clinical information, such as age, educational background and family history of hypertension and diabetes, was also collected. The plasma glucose concentrations were measured with the glucose oxidase–peroxidase method using an automated biochemical instrument (Beckman Coulter, AU5800, Indianapolis, Indiana, USA).

Genotype analysis
Genomic DNA was isolated from peripheral blood leukocytes using QIAamp DNA Blood Mini Kit (Qiagen, Germany). The polymorphism was genotyped using the Agena MassARRAY iPLEX platform (Agena Inc., CA). For quality control we randomly tested a 5% sample of cases and controls using the same sets of primers, and the results were 100% consistent.

Statistical analysis
Hardy–Weinberg equilibrium (HWE) for each SNP was examined by Chi square test and there were no deviations for all genotyped SNPs in both cases and controls. Differences in clinical characteristics between GDM and control group were tested using independent sample *t*-test, Chi square test or Fisher's exact test. The association between rs4987105 and FPG levels was analyzed using independent sample *t*-test and multivariate linear regression in the dominant genetic model with confounding factors adjusted and regression coefficients (*b*) with 95% CI presented. Pre-pregnancy BMI (kg/m^2), maternal age at delivery (years), parity (nulliparous, multiparous), educational levels (high school or lower, college or higher), history of delivering infants with respiratory distress syndrome (yes, no), and history of macrosomia delivery (yes, no) were selected as the confounding factors adjusted

Li *et al. BMC Res Notes*   (2020) 13:102

Page 3 of 5

Table 1 Clinical and Biomedical Characteristics of the Study Population

Characteristics	All participants ($n = 380$)	Cases ($n = 241$)	Controls ($n = 139$)	*P* value
Gestational Age (year)	28.55 ± 4.96	28.52 ± 5.06	28.59 ± 4.80	0.899
Pre-pregnancy BMI (kg/m^2)	21.98 ± 1.95	22.15 ± 1.56	21.69 ± 2.47	*0.047*
FPG (mmol/L)	4.86 ± 0.80	5.18 ± 0.72	4.31 ± 0.62	*$< 10^{-6}$*
History of delivering infants with RDS				0.366
Yes	1 (0.003%)	0 (0%)	1 (0.01%)	
No	379 (0.997%)	241 (100%)	138 (0.99%)	
History of macrosomia delivery				0.531
Yes	5 (1.3%)	2 (0.01%)	3 (0.02%)	
No	375 (98.7%)	239 (0.99%)	136 (0.98%)	
Parity				0.594
0	178 (46.84%)	110 (45.64%)	68 (48.92%)	
≥ 1	202 (53.16%)	131 (54.36%)	71 (51.08%)	
Educational level				0.363
High school or lower	344 (90.53%)	221 (91.70%)	123 (88.49%)	
College or higher	36 (9.47%)	20 (8.30%)	16 (11.51%)	

Data were presented as *n* (%) and Mean $\pm$ SD

BMI body mass index, *FPG* fasting plasma glucose, *RDS* respiratory distress syndrome

The significant *P* values (*P* < 0.05) are highlighted in italic

in the final models based on their biologic plausibility reported in previous studies. All of the statistical tests were performed with SPSS 16.0 software and were two-sided. *P* < 0.05 was considered statistically significant.

Results

Clinical characteristics of the study population

The selected characteristics of 380 participants are presented in Table 1. GDM patients exhibited significantly higher pre-pregnancy BMI (22.15 vs. 21.69, kg/m^2) and fasting plasma glucose levels (5.18 vs. 4.31, mmol/L) than healthy controls. No statistically significant differences were observed in all other listed clinical characteristics between the two groups (Table 1).

Association between rs4987105 and fasting plasma glucose level

We found that carriers of rs4987105 CT/TT genotypes exhibited significantly lower FPG levels (*P* = 0.011, Table 2). We also examined the association between rs4987105 and FPG levels using multivariate linear regression analysis. In order to enhance the statistical power, we combined the rare homozygous genotype with heterozygous genotype to compare with the wild-type genotype in the dominant genetic model. We observed that rs4987105 was significantly associated with this trait after adjusting confounding variables [*b* = − 0.218 (95%CI − 0.384, − 0.053); *P* = 0.01] (Table 3).

Table 2 Correlations Between Genotypes of rs4987105 and FPG Levels

Genotypes	Fasting plasma glucose	
	Mean $\pm$ SD	*P* Value
rs4987105		
CC	4.94 ± 0.83	
CT/TT	4.73 ± 0.74	*0.011*

The significant *P* values (*P* < 0.05) are highlighted in italic

Table 3 The Association Between rs4987105 and FPG Levels

SNP ID	Minor allele		Fasting plasma glucose
rs4987105	T	*b* (95% CI)	− 0.218 (− 0.384, − 0.053)
		P Value	*0.01*

P values were adjusted for gestational age, prepregnancy BMI, parity, history of delivering infants with RDS, history of macrosomia delivery and educational levels in the linear regression model

The significant *P* values (*P* < 0.05) are highlighted in italic

Discussion

In the present study we for the first time investigated the association between the ALOX5 polymorphism rs4987105 and gestational glucose metabolism, and we observed a significant association between rs4987105 and FPG levels.

Li *et al. BMC Res Notes*   (2020) 13:102

Page 4 of 5

In a recent study conducted in a German cohort of 533 T2DM patients and 473 controls, Nejatian et al. reported that the minor T allele of rs4987105 was significantly less frequent in T2DM patients than in controls [OR (95% CI) = 0.7(0.54, 0.89); $P = 0.008$], indicating that the T allele was protective [29]. In agreement with their study, our analyses using both independent sample t-test ($P = 0.011$) and multivariate linear regression [b (95% CI) = $-0.218(-0.384, -0.053)$; $P = 0.01$] showed that the T allele of rs4987105 was significantly associated with lower FPG levels in the studied subjects, demonstrating that this allele may prevent the development of hyperglycemia during pregnancy.

The polymorphism rs4987105 residing in the exon 1 of *ALOX5* is a synonymous SNP close to the promoter region of this gene. Klotsman et al. showed that rs4987105 had functional impacts on the clinical responses to montelukast in asthma patients [22]. It is currently not clear how this nucleotide exchange influences the expression or activity of the enzyme. Future functional experiments are required to unravel the mechanism by which rs4987105 affects glucose metabolism.

The minor allele frequency (MAF) of rs4987105 displays ethnic variations. According to the 1000 genome data, it was lower in American (13%) and African (16%) populations, and higher in European (18%) and Asian (19%) populations. In the present study, it was 21%, which was close to the MAF in Southern Han Chinese (23%) and the MAF for the control groups reported in previous study such as Cai's study [34] in China (18%).

It is worth noticing that BMI thresholds for increased GDM risk displays racial/ethnic distinction [35, 36]. Among American and European women, typically GDM would occur primarily among women with a higher BMI of 30 kg/m^2 and above, whereas in Asian populations such as in our study subjects, lower BMI of only 22–23 kg/m^2 could also confer risk of the disease. Therefore for Asian pregnant women, BMI by itself should not be considered as a reliable indicator for GDM risk prediction. Aside from obesity, it has been shown that dietary patterns and physical activity significantly influence GDM risk [37–40]. Life-style changes such as more unhealthy fast food and more sedentary activities may underlie the globally escalating prevalence of the disease. Diets such as Mediterranean Diet (MedDiet) and Dietary Approaches to Stop Hypertension (DASH) diet, as well as less sedentary activities before or during early pregnancy may be effective strategies for preventing against the development of GDM nowadays.

As far as we know, this is the first report of the association between rs4987105 of *ALOX5* and gestational glucose metabolism.

Limitations

The main drawback of this study is the limited statistical power in only 241 patients and 139 controls. Besides, several confounding factors were not included and analyzed in this study, such as gestational weight gain, dietary and physical activity habits. Future multi-centered studies in different ethnic groups with larger sample sizes are needed to validate this finding.

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Li *et al. BMC Res Notes*   (2020) 13:102

Page 5 of 5

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