Association of polymorphisms in STRA6 gene with gestational diabetes mellitus in a Chinese Han population

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Abstract

Cell and animal experiments have found that in addition to being a retinol transporter, Stimulated by Retinoic Acid 6 (STRA6) also functions as a surface signaling receptor by which retinol regulates insulin responses. Several studies revealed that the STRA6 gene may contribute to the pathogenesis of type 2 diabetes mellitus (T2DM). Gestational diabetes mellitus (GDM) and T2DM have some risk factors in common. The present study was directed to investigate whether the 3 single nucleotide polymorphism (SNPs) (rs11633768, rs351219, and rs736118) of STRA6 correlate with the development of GDM in Chinese pregnant women. We aimed to estimate the relationship between SNPs with fasting blood glucose level, 1-hour and 2-hour glucose levels after 75 g oral glucose intake, fasting insulin and insulin resistance levels to better study the relationship between STRA6 and glucose metabolism.

Case–control studies were conducted to compare the GDM and control groups. A total of 334 cases and 367 controls were recruited. Three tagSNPs of STRA6, rs11633768, rs351219, and rs736118, were selected. A chi-square test, logistic regression, and linear regression were used to estimate the relationship between SNPs with GDM risk and oral glucose tolerance test (OGTT), fasting insulin and homeostasis model assessment of insulin resistance (HOMA-IR) levels. Regression analyses were all adjusted by maternal age, pre-pregnancy BMI, and weekly BMI growth. The Bonferroni correction was applied for multiple comparisons.

After adjusting the maternal age, pre-pregnancy BMI and weekly BMI growth, STRA6 rs736118 was associated with fasting insulin level (β = −1.468, 95% CI: −2.163 to −0.772, P = 0.002), and the association between rs736118 and HOMA-IR was of borderline significance (β = −0.290, 95% CI: −0.552 to 0.072, P = 0.093) under the dominant model.

This study found that there is a significant association between STRA6 polymorphism and GDM.

Abbreviations: GDM = gestational diabetes mellitus, holo-RBP = retinol-RBP4 complex, HOMA-IR = homeostasis model assessment of insulin resistance, JAK2 = Janus Kinases 2, OGTT = oral glucose tolerance test, PPARY = peroxisome proliferator-activated receptor γ, RBP4 = retinol binding protein 4, SNP = single nucleotide polymorphism, SOCS3 = cytokine signaling 3, STAT5 = Activators of Transcription 5, STRA6 = Stimulated by Retinoic Acid 6, T2DM = type 2 diabetes mellitus.

Keywords: gestational diabetes mellitus, single nucleotide polymorphisms, stimulated by retinoic acid 6

1. Introduction

Gestational diabetes mellitus (GDM) is defined as glucose intolerance first detected during pregnancy. The prevalence of GDM has been increasing in recent decades, moving from 1.7% to 11.6% among populations. During pregnancy, insulin resistance is enhanced physiologically and can be further strengthened by some factors such as obesity, leading to a high risk of GDM.

Retinol-binding protein 4 (RBP4) has been implicated as a driver of insulin resistance in rodents and humans. RBP4, an adipocytokine, is mainly synthesized by hepatocytes and adipose tissues. An increase in serum RBP4 levels can induce hepatic expression of phosphoenolpyruvate carboxykinase, a gluconeogenic enzyme, to increase gluconeogenesis, and impair insulin signaling in muscles through decreasing the expression of phosphoinositide-3 kinase. Further studies on the mechanism by which RBP4 can lead to insulin resistance showed that Stimulated by Retinoic Acid 6 (STRA6) played an important role. STRA6 is a membrane protein acting as a receptor for retinol-RBP4 complex (holo-RBP) to remove the retinol from the complex and transport it across the cell membrane. Berry and colleagues found that the binding of holo-RBP to STRA6 results in recruitment of Janus Kinases 2 (JAK2) that, in turn, catalyze...
phosphorylation of a tyrosine residue in the cytosolic domain of STRA6, leading to recruitment and activation of the transcription factor Signal Transducers and Activators of Transcription 5 (STAT5). \[9\] The expression levels of cytokine signaling 3 (SOCS3) and peroxisome proliferator-activated receptor γ (PPARγ), 2 endogenous STAT target genes, can be upregulated by holo-RBP. PPARγ is a key regulator of adipose lipid storage. SOCS3 is a potent inhibitor of insulin receptor-mediated signaling. Treatment of adipocytes with holo-RBP inhibited insulin-induced activation of insulin receptor and insulin-induced mobilization of the glucose transporter GluT4 to plasma membranes. Berry et al found that holo-RBP induces the expression of STAT target genes and inhibits insulin signaling only in STRA6-expressing tissues in Vivo. These abovementioned findings establish that, in addition to being a retinol transporter, STRA6 also functions as a surface signaling receptor by which retinol regulates insulin responses. \[9\]

Many studies have focused on the association between RBP4 and GDM. A meta-analysis showed that high serum RBP4 levels represented a risk factor for GDM. The GDM diagnostic criteria affected the strength of association between RBP4 level and GDM risk. Adopting a higher threshold of oral glucose tolerance test (OGTT) would result in a larger difference of serum RBP4 level between GDM women and controls. \[10\] To date, several genetic variants that affect RBP4 expression levels (e.g., rs3758539 and rs12915846) have been investigated for their potential association with the risk of GDM. \[11\]-\[13\] However, to date, no studies have focused on the association between GDM and polymorphisms in STRA6, the only identified high-affinity receptor for RBP4. Nair et al found that the STRA6 rs974456 T allele, rs736118 A allele, and rs4886578 A allele were associated with a lower risk of type 2 diabetes mellitus (T2DM) in a south Indian population. \[14\] Huang et al analyzed the association of STRA6 rs974456, rs736118, rs4886578 and rs17173617 with T2DM in southern Han Chinese and verified the results of Nair et al on rs974456 and rs736118. \[15\] STRA6 may not only be associated with T2DM but also may be associated with the risk of GDM. The present study investigated whether the 3 SNPs (rs11637678, rs351219, and rs736118) of STRA6 correlate with the development of GDM in Chinese pregnant women. We also aimed to estimate the relationship between SNPs with fasting blood glucose level, 1-hour and 2-hour blood glucose levels after 75 g oral glucose intake, fasting insulin and insulin resistance levels to better study the relationship between STRA6 and glucose metabolism.

2. Methods

2.1. Ethics statement

The study protocol was reviewed and approved by the Central-South University’s Ethical and Confidentiality Committee. All participants provided written informed consent. The authors assert that all procedures/methods were carried out in accordance with the approved guidelines.

2.2. Study design

The research population and most parts of the statistical methods of this study were consistent with one of our previous articles, \[16\] therefore, the same content was not repeated here. Briefly, this was a case-control study which enrolled pregnant women with GDM and pregnant women with normal glucose tolerance who visited prenatal clinics regularly and underwent OGTT from 24 to 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 hours after 75 g oral glucose intake. When 1 or more OGTT indicators reached or exceeded the abovementioned boundaries, the pregnant woman was diagnosed with GDM. The following information was collected on the OGTT morning: maternal age, gestational age, parity, height, weight, fasting insulin levels, systolic blood pressure, and diastolic blood pressure. Homeostasis model assessment of insulin resistance (HOMA-IR) = fasting insulin (mIU/L) x fasting blood glucose (mmol/L)/22.5.

Weekly body mass index (BMI) growth = (BMI on the OGTT morning-pre-pregnancy BMI)/gestational age (weeks). A chi-square test, logistic regression, and linear regression were used to estimate the relationship between SNPs with GDM risk and OGTT, fasting insulin and HOMA-IR levels. Regression analyses were all adjusted by maternal age, pre-pregnancy BMI and weekly BMI growth. Three SNPs were included in the analysis; therefore, \( \alpha \) was equal to 0.017 (0.017 = 0.05/3). The alleles, minor allele frequency (MAF) and SNPs covered by tagSNP are shown in Table 1. The primers for each SNP are shown in Table 2.

### Table 1

| dbSNP ID   | Functional consequence | Alleles | HWE \( P \) value | MAF   | SNPs covered by tagSNP |
|------------|------------------------|---------|-------------------|-------|------------------------|
| rs11637678 | intron variant         | GT      | 0.713             | 0.31  | rs12915846             |
| rs351219   | intron variant         | TC      | 0.409             | 0.49  | rs351222, rs351223, rs351224 |
| rs736118   | downstream variant 500B, missense | CT | 0.158 | 0.41 | rs351229, rs351230, rs351237, rs351238, rs11635868 |

* These SNPs have been found to be associated with GDM/T2DM risk in previous studies.
* These SNPs were TagSNPs.
* The second allele was the minor allele.

### Table 2

| SNP          | 5’utr region primers | 3’utr region primers |
|--------------|----------------------|----------------------|
| rs11637678   | ACCTTTGCGATGATGTGCTGCTGGCAAAAGTACC | AGCGTTGATGAGACACACAGAGCGCCACCAAG |
| rs351219     | AGCGTTGCGATGATGTGCTGCTGGCAAAAGTACC | AGCGTTGATGAGACACACAGAGCGCCACCAAG |
| rs736118     | ACCTTTGCGATGATGTGCTGCTGGCAAAAGTACC | AGCGTTGATGAGACACACAGAGCGCCACCAAG |
3. Results

3.1. Demographic and clinical characteristics

A total of 334 cases and 367 controls were analyzed. The clinical characteristics of cases and controls are summarized in Table 3. Compared with the control group, the case group had higher pre-pregnancy BMI (\(P < .001\)), larger weekly BMI growth (\(P < .001\)), higher systolic blood pressure (\(P < .001\)) and higher diastolic blood pressure (\(P < .001\)).

3.2. Test for Hardy–Weinberg equilibrium and LD analysis

The SNP genotyping detection rate was 99.5%. For all SNPs, the Hardy–Weinberg equilibrium (HWE) was observed in the control group (Table 1). Pairwise linkage disequilibrium parameters (\(D'\) and \(r^2\)) were estimated for STRA6 rs11633768, rs351219, and rs736118 (Table 4).

3.3. Association between alleles and genotypes with GDM

As shown in Table 5, no significant differences in the alleles and genotypes of STRA6 rs11633768, rs351219, and rs736118 were observed between cases and controls.

3.4. Association between genetic models with GDM

As shown in Table 6, after adjusting the maternal age, pre-pregnancy BMI and weekly BMI growth, the results of the logistic regression analysis revealed that comparing cases with controls, STRA6 rs11633768, rs351219, and rs736118 were not associated with GDM, regardless of additive model, dominant model, or recessive model comparisons.

3.5. Association analysis of genetic variants in STRA6 with OGTT, fasting insulin and HOMA-IR levels

In addition to fasting blood glucose level, OGTT 1-hour blood glucose level and 2-hour blood glucose level, which constitute the diagnostic criteria for GDM, fasting insulin level and HOMA-IR

Table 3
Demographic and clinical characteristics of the study subjects.

|                        | Controls (N = 367) | Cases (N = 334) | \(P\)  |
|------------------------|------------------|----------------|--------|
| Maternal age, yr       | 29 (28,32)       | 29 (27,32)     | .672   |
| Gestational age at sampling, weeks | 25.11 ± 2.724 | 25.35 ± 2.948 | .458   |
| Pre-pregnancy BMI, kg/m² | 20.55 (19.14,22.64) | 22.31 (20.29,24.14) | .000*  |
| Weekly BMI growth*, kg/m² | 0.114 ± 0.054 | 0.131 ± 0.056 | .000** |
| SBP*, mmHg             | 111 ± 10.30      | 116 ± 11.12    | .000** |
| DBP*, mmHg             | 70 ± 8.38        | 74 ± 8.09      | .000** |
| Parity                 |                  |                |        |
| 0                      | 230 (62.7%)      | 216 (64.7%)    | .312***|
| 1                      | 123 (33.5%)      | 93 (27.8%)     |        |
| 2                      | 5 (1.4%)         | 7 (2.1%)       |        |

*Wilcoxon rank sum test due to a non-normal distribution of the tested characteristics, median and quartiles were used for the statistical description;
**Student’s test due to a normal distribution of the tested characteristics, mean and SD were used for the statistical description;
***Chi-square test, constituent ratio was used for the statistical description.

\*BMI measured on the morning of the oral glucose tolerance test minus the pre-pregnancy BMI and then divided by the gestational age (weeks) was defined as “Weekly BMI growth”;
\*SBP (systolic blood pressure) and DBP (diastolic blood pressure) were the blood pressures measured on the morning of the oral glucose tolerance test.

Table 4
Pair-wise linkage disequilibrium analyses of STRA6 rs11633768, rs351219 and rs736118.

| Group | SNP       | \(D'\) | \(r^2\) | \(D'\) | \(r^2\)   |
|-------|-----------|--------|---------|--------|---------|
| Controls | rs11633768 | 0.987  | 0.382   | 0.436  | 0.136   |
|        | rs351219  | –      | –       | 0.710  | 0.278   |
| Cases  | rs11633768 | 0.986  | 0.368   | 0.338  | 0.080   |
|        | rs351219  | –      | –       | 0.758  | 0.307   |

Table 5
The distribution of alleles and genotypes of STRA6 rs11633768, rs351219 and rs736118.

| SNP        | Allele/Genotype | Controls | Cases | \(\chi^2\) | \(P\)  |
|------------|-----------------|----------|------|-----------|--------|
| rs11633768 | G               | 531 (72.5) | 475 (71.8) | 0.108 | .743   |
|            | T               | 201 (27.5) | 187 (28.2) |       |        |
|            | GG              | 194 (53.0) | 169 (51.1) | 0.390 | .823   |
|            | TT              | 29 (7.9)   | 25 (7.6)   |       |        |
|            | GT              | 143 (39.1)| 137 (41.4)|       |        |
| rs351219   | T               | 357 (48.8) | 341 (51.2) | 0.824 | .364   |
|            | C               | 375 (51.2) | 325 (48.8) |       |        |
|            | TT              | 91 (24.9)  | 84 (25.2)  | 2.011 | .166   |
|            | CC              | 100 (27.3)| 76 (22.8)  |       |        |
|            | TC              | 175 (47.8)| 173 (52.0)|       |        |
| rs736118   | C               | 479 (65.4)| 427 (64.3)| 0.195 | .659   |
|            | T               | 253 (34.6)| 237 (35.7)|       |        |
|            | CC              | 155 (42.3)| 137 (41.3)| 0.249 | .883   |
|            | TT              | 42 (11.5) | 42 (12.7)  |       |        |
|            | CT              | 169 (46.2)| 153 (46.1)|       |        |
are also important indicators for evaluating the level of glucose metabolism. To better study the relationship between STRA6 and the level of glucose metabolism, we analyzed the relationship between fasting insulin level and other continuous indicators with STRA6 rs11633768, rs351219, and rs736118. As shown in Table 7, after adjusting the maternal age, pre-pregnancy BMI, and weekly BMI growth, the results of the linear regression analysis revealed that under the dominance model, STRA6 rs736118 was associated with fasting insulin level (Beta = 1.236, 95% CI: 0.747-2.045, P = .409), and the association between rs736118 and HOMA-IR was of borderline significance (Beta = −0.290, 95% CI: −0.606-0.026, P = .093). However, after correction for multiple testing, the association did not remain statistically significant. We inferred that the STRA6 rs736118 T allele might protect Chinese pregnant women from GDM. No significant results were observed in the association analysis of rs11633768, rs351219 with OGTT, fasting insulin, and HOMA-IR.

### 4. Discussion

STRA6 was identified as a specific membrane receptor for RBP in 2007 by Kawaguchi and colleagues. STRA6 binds to RBP with high affinity and has robust vitamin A uptake activity from the vitamin A-RBP complex. It is widely expressed in embryonic development and in the adult brain, spleen, kidney, female genital tract, and testis (and at lower quantities in heart and lung). In the present study, we analyzed the association between STRA6 rs11633768, rs351219, and rs736118 with GDM risk. We found STRA6 rs736118 was associated with fasting insulin level and inferred that the rs736118 T allele might protect Chinese pregnant women from GDM. The STRA6 gene is located on Chromosome 15q24.1. STRA6 rs736118 presents in exon 17. For the SNP of rs736118, the C→T variant will cause amino acid change from methionine to isoleucine. In addition, this SNP is located at the cytosolic C terminus of the protein, containing a docking site for the transcription factors STATs. As mentioned above, SOCS3, an endogenous STAT target gene, is a potent inhibitor of insulin receptor-mediated signaling. To date, this study is the first study concerning the association between STRA6 variants and GDM risk. STRA6 rs736118 has been studied twice in the T2DM region. Nair et al. analyzed 2002 unrelated South Indian individuals (1002 cases with T2DM and 1000 normoglycemic control subjects) and found the STRA6 rs736118 T allele was associated with a lower risk of T2DM, after adjustment for maternal age, sex, and BMI, the association still existed. Huang et al. analyzed 571 T2DM patients and 632 normal control subjects and found the allele T of SNP rs736118 on STRA6 was significantly associated with a lower risk of T2DM, after adjustment for sex, BMI, and triglycerides. To have better comparability with the previous results,
we also adjusted BMI, including pre-pregnancy BMI and weekly BMI growth. In fact, doing so may have underestimated the impact of STRA6 on GDM risk. The STAT target gene PPARG is a key regulator of adipose lipid storage. Treatment of adipocytes with holo-RBP increased triglyceride accumulation by the cells and did so in a STRA6-dependent manner. This evidence hinted that STRA6 may associate with BMI through its function in long-term lipid metabolism regulation. The association between BMI and GDM risk is obvious. We inferred that STRA6 may be a key regulator of adipose lipid storage. Treatment of adipocytes with holo-RBP increased triglyceride accumulation by the cells and did so in a STRA6-dependent manner. If BMI was not adjusted, BMI is one of the pathophysiological links between STRA6 and GDM. If BMI was not adjusted, STRA6 rs736118 was still associated with fasting insulin level (Beta = –1.533, P = .041), and the Beta value was greater than the value after adjustment. The results of HOMA-IR (Beta = –0.306) followed the same pattern.

To the best of our knowledge, this is the first study that has evaluated the relationship between genetic variants of STRA6 and GDM risk. However, the study has certain limitations. First, genetic susceptibility analysis only provides some hints about the association; the results need to be validated at other omics levels, such as proteomics. Second, the results need to be confirmed in other races and larger samples.

**Author contributions**

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