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The association between serum resistin and PPARγ Pro12Ala polymorphism in patients with gestational diabetes mellitus

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Abstract: PPARγ Pro12Ala polymorphism is associated with the expression level of resistin and can induce insulin resistance. However, the possible effects of PPARγ Pro12Ala polymorphism for the origin of gestational diabetes mellitus are still unclear. A total of 156 patients with GDM and 160 normal pregnancy women were recruited. The serum parameters, resistin and genomic DNA were detected. We collected the patients with gestational diabetes mellitus and found that genotype Pro/Pro and Pro/Ala existed in the patients and controls. Moreover, the genotype Pro/Ala group had a higher level of serum parameters and resistin. Our observations suggest that PPARγ Pro12Ala polymorphism may elevate the expression of resistin and it may be involved in the pathogenesis of gestational diabetes mellitus.

Keywords: PPARγ Pro12Ala polymorphism, gestational diabetes mellitus, resisitin.

INTRODUCTION

Gestational diabetes mellitus (GDM) is defined as glucose metabolism disorders which are first diagnosed during pregnancy. The frequency of GDM is about 2-5% and may increase in the future because of the new life styles (Ashwal and Hod *et al*., 2015). The main cause of GDM is absolute or relative deficiency of insulin secretion or insulin resistance, which is similar to the type 2 diabetes (Barbour *et al*., 2007). The etiology and pathogenesis of GDM is still not clear. Several studies have explained the GDM etiology from the perspectives of genetic susceptibility. Resistin is an adipocytokine involved in the process of insulin resistance and can be synthesized in the placenta during gestation period (Kusminski *et al*., 2005). The level of resistin expression was usually higher at the end of the gestation period (Megia *et al*., 2008). Previous study demonstrated that the level of resistin in patients with GDM significantly elevated and positively correlated with insulin resistance (Vitoratos *et al*., 2011). However, the roles of the factors associated with elevated resistin in the pathophysiology of GDM were still poorly understood.

Peroxisome proliferator activated receptors (PPARs) are ligand-regulated transcriptional factors, which belong to the nuclear receptor superfamily. PPARs include three genotypes (α, β and γ) and the PPARγ gene is located on chromosome 3p25. PPARγ is widely distributed throughout the body and is involved in the adipocyte differentiation and metabolism by regulating the transcription of adipocyte specific genes (Meirhaeghe *et al*., 1998). In addition, PPARγ can directly control the expression of resistin in the macrophages (Patel *et al*., 2003). The down regulation of PPARγ may induce the release of resistin, which leads to the decrease of insulin sensitivity and the development of type 2 diabetes mellitus (Koika *et al*., 2009). Moreover, researchers have found that PPARγ Pro12Ala polymorphism synergistically correlated with the expression of the resistin in Japanese population (Osawa *et al*., 2008).

In the previous studies, the associations between several gene polymorphisms such as inflammation factors (TNF-α rs1800629, IL-6 rs1800795) and insulin resistance-related genes (ADIPOQ rs2241766) and etiology of GDM have been validated, which showed that genetic background might contribute to the development of GDM (Feng *et al*., 2018). However, the relationship between PPARγ Pro12Ala polymorphism and GDM was still needed to be investigated.

In this study, we detected the distributions of the PPARγ Pro12Ala polymorphism and several serum parameters in both patients with GDM and controls. Our aim was to identify the association between serum resistin and PPARγ Pro12Ala polymorphism in patients with GDM.

MATERIALS AND METHODS

Patients and specimens

A total of 156 patients with GDM and 160 normal pregnancy women were recruited. The study protocol was approved by the hospital Ethics Committees and the informed consents were obtained from all GDM patients and healthy subjects. The details of medical history and physical examination were obtained in accordance with

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the standard formats. Height and weight were measured and the body mass index (BMI) = body weight (kg) / height (m) 2 was calculated. Serum total cholesterol (TC), triglyceride (TG), fasting blood glucose (FBS), fasting insulin level (FINS) and resistin were detected. Insulin resistance index (HOMA-IR) = fasting insulin (U/ml) X fasting blood glucose (mmol/L) /22.5.

Genetic analysis

Whole blood was collected for genomic DNA extraction and analysis from GDM patients and normal controls. The sequences of the primers for PPARγ Pro12Ala polymorphism detection were: forward: 5'-TCTGGGAGATTCTCCTATTGGC-3', reverse: 5'-CTGGAAGACAACTACAAGAG-3'. PCR conditions were: denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 45 sec, annealing at 55°C for 45 sec, extension at 72°C for 45 sec, and a final extension at 72°C for 8 min. The PCR products with a length 154 bps were digested by restriction endonuclease AluI and separated by gel electrophoresis. Afterwards, the digested fragments were stained by silver dye.

STATISTICAL ANALYSIS

Data are shown as the mean ± standard deviation (SD) from the mean; data were analyzed by a Student *t* test. Statistical analysis was performed using IBM SPSS Statistics Version 19 software.

RESULTS

A total of 156 GDM patients and 160 controls were included for our investigations. Genomic DNAs were extracted for PCR amplifications and the PCR products were digested by restriction endonuclease AluI. The restriction endonuclease cleavage site for AluI did not exist in the homozygous genotype Pro/Pro. Therefore, the PCR products could not be digested and showed one band in the gel. However, the PCR products of heterozygous genotype Pro/Ala and homozygous genotype Ala/Ala could be digested to three and two fragments, respectively (fig. 1). In our study, we only found homozygous genotype Pro/Pro and heterozygous genotype Pro/Ala in all samples and these two genotypes were distributed in both two groups. In addition, no statistical differences were found in genotypes distribution.

Serum parameters detection showed that serum total cholesterol (2.51±1.28 vs 2.29±1.05 mmol/L, $p<0.05$), fasting blood glucose (6.54±1.63 vs 3.71±0.58 mmol/L, $p<0.05$), fasting insulin level (13.08±7.29 vs 10.54±3.33 mIU/L, $p<0.05$) and insulin resistance index (2.30±1.08 vs 1.04±0.88, $p<0.05$) were significantly higher in GDM patients than in controls. Moreover, serum total cholesterol (2.64±0.95 vs 2.31±1.48 mmol/L, $p<0.05$), fasting blood glucose (6.84±1.74 vs 5.94±1.08 mmol/L, $p<0.05$), fasting insulin level (13.65±2.76 vs 12.98±9.30 mIU/L, $p<0.05$) and insulin resistance index (2.35±1.20 vs 2.12±0.57, $p<0.05$) were higher in the genotype Pro/Ala subgroup comparing to the genotype Pro/Pro subgroup in GDM patients. However, no significant difference was found between two genotypes in controls (table 1).

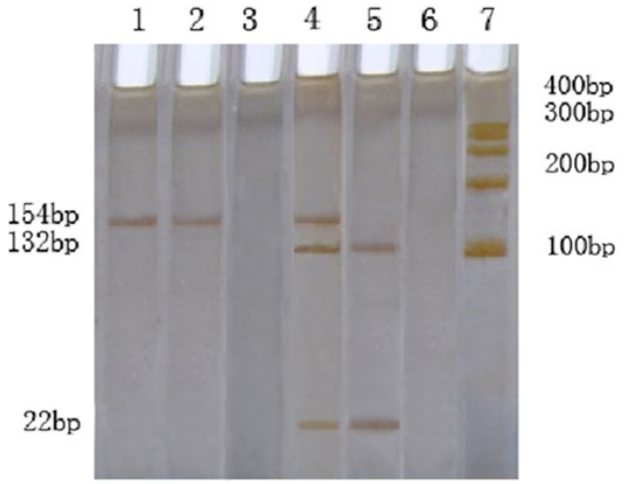

Fig. 1: The representative digested PCR bands of genotype Pro/Pro (lane 1 and 2), Pro/Ala (lane 4) and Ala/Ala(lane 5).

Next, we detected the serum resistin level in GDM patients and controls. As a result, we identified that serum resistin level was higher in GDM patients than in controls. Furthermore, the serum resistin level was higher in the genotype Pro/Ala subgroup comparing to the genotype Pro/Pro subgroup in patients with GDM. However, the serum resistin levels showed no significant difference between two genotypes in controls (table 2).

DISCUSSION

The occurrence of GDM is closely related to insulin resistance and the excessive weight gain during pregnancy is considered to be an important cause of insulin resistance (Moore Simas *et al*., 2017). PPARγ is a transcription factor involved in lipid utilization and storage, lipoprotein metabolism, gene regulation of obesity and insulin resistance. PPARγ can stimulate fibroblasts to differentiate into adipocytes as well as the growth of adipocytes (Rangwala *et al*., 2004). Previous studies indicated that the PPARγ Pro12Ala polymorphism was associated with obesity, type 2 diabetes mellitus and lipid disorders (Osawa *et al*., 2008).

Resistin was identified as a new polypeptide hormone secreted mainly by adipocytes in 2001 (Steppan *et al*., 2001). Many studies have confirmed that resistin can induce insulin resistance and impair glucose tolerance (de Luis *et al*., 2016, Santilli *et al*., 2016). Resistin could also

Table 1: The clinical and serum parameters for GDM patients and controls (PA: Pro/Ala genotype, PP: Pro/Pro genotype, GDM vs Control: $\Delta p<0.05$; PA vs PP, $^{*}p<0.05$)

Groups	N	BMI (kg/m^2)	TG (mmol/L)	TC (mmol/L)	FBS (mmol/L)	FINS (mIU/L)	HOMA-IR
GDM	156	25.23±1.72Δ	2.51±1.28Δ	5.08±2.01Δ	6.54±1.63Δ	13.08±7.29Δ	2.30±1.08Δ
PA	12	29.47±1.68*	2.64±0.95*	5.17±3.98	6.84±1.74*	13.65±2.76*	2.35±1.20*
PP	144	23.89±2.88	2.31±1.48	5.05±1.05	5.94±1.08	12.98±9.30	2.12±0.57
Control	160	23.51±2.12	2.29±1.05	4.99±1.19	3.71±0.58	10.54±3.33	1.04±0.88
PA	9	23.39±2.32	2.28±1.06	5.01±0.92	3.79±0.43	11.23±2.72	1.04±0.88
PP	151	23.72±1.84	2.30±0.99	4.96±1.30	3.67±0.71	10.47±4.33	0.98±0.47

Table 2: The expression level of resistin for GDM patients and controls (PA: Pro/Ala genotype, PP: Pro/Pro genotype, GDM vs Control: $\Delta p<0.05$; PA vs PP, $^{*}p<0.05$)

Group	N	Resistin (ng/ml)
GDM	156	30.86±1.98Δ
PA	12	36.54±3.21*
PP	144	28.52±1.57
Control	160	18.13±0.71
PA	9	17.04±0.88
PP	151	18.98±0.47

be expressed in trophoblast cells in placenta tissues (Yura *et al*., 2003). The expression of resistin in adipose tissue is relatively weak and there is no obvious change during pregnancy. Serum resistin level in GDM patients were significantly higher than normal pregnant women, suggesting placental resistin secretion may be the main resource of the serum resistin. It is speculated that the PPARγ gene mutation may directly regulate the expression of resistin in the placenta, while resistin further induces dyslipidemia and insulin resistance.

In this study, serum parameters including serum total cholesterol, fasting blood glucose, fasting insulin level and insulin resistance index were significantly higher in the GDM group than control group. However, we found that TG did not elevate significantly in the genotype Pro/Pro subgroup and the differences were mainly caused by the genotype Pro/Ala subgroup of the GDM patients. This result indicated that the PPAR gene Prol2Ala polymorphism could not affect the glucose and lipid metabolism in the normal pregnant woman. By contrast, GDM with PPAR gene Prol2Ala polymorphism might have a higher TG concentrations in the serum. At the same time, the serum resistin level in the genotype Pro/Ala subgroup was also significantly higher, which indicated that the PPAR gene Prol2Ala polymorphism might involve in the occurrence and development of GDM by regulating expression of resistin. Previous studies had shown that resistin could be involved in the lipid metabolism and controlled the deposition of Lipids in the cell (Rubio-Guerra *et al*., 2015; Wen *et al*., 2015). In addition, a positive correlation between the total expression of resistin and TG were also validated in patients with non-alcoholic fatty liver disease (Piotr *et al*., 2017). In sum, PPARγ Pro12Ala might lead to structural and functional changes of the protein, resulting in imbalance of adipogenesis.

CONCLUSION

We suggested that the PPARγ gene polymorphism Prol2Ala might not lead to GDM directly. On the contrary, this polymorphism could change the lipid metabolim by controlly the expression of resistin to aggravate the insulin resistence.

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