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Relationship between a lipoprotein lipase gene polymorphism in placental tissue and insulin resistance in patients with gestational diabetes mellitus

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ABSTRACT. The aim of this study was to investigate the relationship between a lipoprotein lipase (*LPL*) gene polymorphism in placental tissue and insulin resistance (IR) in patients with gestational diabetes mellitus. Using polymerase chain reaction-restriction enzyme fragment length polymorphism (PCR-RFLP) analysis, the *LPL Hin*dIII RFLP was examined in the placental tissue of 110 patients with gestational diabetes mellitus (observation group) and 110 women with normal gestation (control group). The relationships between fasting plasma glucose (FPG), postprandial plasma glucose (PPG), fasting insulin (FINS), cholesterol (TC), triglyceride (TG), high density lipoprotein (HDL), low density lipoprotein (LDL), body mass index (BMI), and IR indices and the *LPL* polymorphism in the two study groups and their offspring were determined. The frequency of the H+ allele was significantly higher in the observation group than in the controls ($P < 0.05$). There were statistically significant differences in the observation

group between the FPG, PPG, LDL, TC, TG, HDL, BMI, FINS, and IR indices of the H+H+ group and those of the non H+H+ type patients ($P < 0.05$). Correlation analysis showed that the *LPL* gene polymorphism was positively related to IR. There were statistically significant differences between HDL, BMI, and IR indices between the two study groups ($P < 0.05$). In conclusion, the *LPL* gene polymorphism was determined to be the main factor related to IR in women with gestational diabetes, and was also found to be related to the IR of their offspring.

Key words: Gestational diabetes mellitus; Insulin resistance; Lipoprotein lipase gene polymorphism

INTRODUCTION

Gestational diabetes mellitus refers to diabetes mellitus or abnormalities in glucose tolerance that first appears in pregnant woman during the gestational period (Han et al., 2012). Numerous recent studies have shown that insulin resistance (IR) and disorders of glucose and lipid metabolism occur in patients with gestational diabetes mellitus (Buchanan et al., 1998; Golden et al., 2009). In addition, there has been a clinical focus on whether IR or glucose and lipid metabolism disorder appear in the offspring of patients with gestational diabetes mellitus. Lipid metabolism disorder is a primary pathophysiological process of type 2 diabetes mellitus (T2DM) (Goodarzi et al., 2005). Lipoprotein lipase (LPL) is the main rate-limiting enzyme in the lipid metabolism pathway. It primarily catalyzes the hydrolysis of triglycerides in chylomicrons and very low density lipoprotein into free fatty acid and monoacylglycerol (Kersten, 2014; Liu et al., 2014). In recent years, research from many countries has demonstrated a close relationship between a *Hin*dIII restriction enzyme fragment length polymorphism (RFLP) in the eighth intron of the *LPL* gene and diabetes, obesity, lipid metabolism disorders, coronary heart disease, etc. (Su et al., 2002; Süsleyici Duman et al., 2005; Huang et al., 2010; Qi et al., 2011). It has also been shown that treatment of rats with high saturated or unsaturated fatty acids could lead to IR and increased blood sugar levels (Estrany et al., 2011). These results suggested that the *LPL Hin*dIII RFLP might be associated with IR and lipid metabolism disorders. This polymorphism might also predict the metabolic status of the offspring of patients with gestational T2DM.

MATERIAL AND METHODS

Subjects

In this study, 110 patients with gestational T2DM who delivered in our hospital from June 2012 to June 2014 were selected as research subjects. Healthy woman with normal deliveries in our hospital during the same period were recruited as the control group. The patients in the observation group were classified in accordance with the standard of clinical diagnosis of T2DM (Yang, 2007). The exclusion criteria for the two study groups were as follows: pre-gestational diabetes, hypertension, abnormal liver and kidney function, thyroid and other endocrine system disorders, cardiac insufficiency, acromegaly or dwarfism, recent blood transfusion, or hormone use. The average age of patients in the observation group was 28.1 ± 2.5 years, and the gestational ages were 38.9 ± 3.1 weeks. The mean age in the control group was 29 ± 2.1 years, and the gestational age was 38.5 ± 2.8 weeks. There were no statistically significant differences in

age, gestational weeks, or other indicators in the two study groups, and they were comparable overall ($P > 0.05$). This study was conducted in accordance with the Declaration of Helsinki, and with approval from the Ethics Committee of Henan University, Huaihe Hospital. Written informed consent was obtained from all participants.

Determination of clinical indices

Fasting venous blood (5 mL) was drawn from individuals in the two study groups in the early morning and treated with anticoagulant. Fasting blood glucose (FBG), serum total cholesterol (TC), triglyceride (TG), high density lipoprotein-cholesterol (HDL-C), and low density lipoprotein-cholesterol (LDL-C) were detected using an automatic blood detector (Beckman-Coulter, Miami, FL, USA). The homeostatic model assessment IR index (HOMA-IR) was defined as FBG x fasting insulin (FINS) / 22.5. Body mass index (BMI) was defined as weight/height $(m)^2$.

*Hin*dIII *LPL* gene polymorphism detection by polymerase chain (PCR)-RFLP

Placental tissue was obtained after childbirth for each subject. Total genomic DNA was extracted from 0.1 g placental tissue using a Kangwei Genome Extraction Kit (Beijing, China). The sequences of the primers designed to amplify a 700 bp amplicon including the *LPL Hin*dIII polymorphic locus from genomic DNA were as follows: LPL-F: 5'-TGA AGC TCA AAT GGA AGA GT-3'; LPL-R: 5'-TAC AAG CAA ATG' ACT AAA-3'. The following reaction system was prepared: 25 μL 2X Primer STAR Mix Premix (TaKaRa, Dalian, China), 1 μL LPL-F, 1 μL LPL-R, and 1 μL template, supplemented to 50 μL with deionized water. PCR was performed according to the following reaction conditions: denaturation at 98°C for 5 min, followed by 30 cycles of denaturation at 98°C for 30 s, annealing at 58°C for 30 s and extension at 72°C for 1 min, with a final extension at 72°C for 7 min. The PCR products (5 μL) were analyzed using agarose gel electrophoresis, and the specificity of the PCR amplicon band was observed. Restriction enzyme digestion was performed by preparing a 20 μL reaction mix containing 17 μL PCR product, 2 μL 10X *Hin*dIII restriction endonuclease buffer and 1 μL *Hin*dIII restriction endonuclease (TaKaRa) that was placed in a 37°C water bath for 4 h, followed by analysis with 2% agarose gel electrophoresis.

Subject follow-up and assessment

The infants of woman in the two groups were followed-up for assessment. In the observation and control groups, 29 and 33 cases, respectively, were lost to follow-up. Blood was extracted from the fingertips of infants after completion of their first month of life. FBG, TC, TG, HDL-C, and LDL-C were detected as previously described. HOMA-IR and BMI changes in the neonates were analyzed.

Statistical analysis

All data were analyzed with the SPSS17.0 statistical software (SPSS Inc., Chicago, IL, USA). The measurement data are shown as means ± SD. The measurement data were compared using a *t*-test between the two groups. The count data were compared with a chi square

test. The relationship between the *LPL Hin*dIII polymorphism and IR was analyzed with Pearson's correlation analysis. $P < 0.05$ was taken to indicate statistical significance.

RESULTS

*LPL Hin*dIII polymorphism analysis

Using genomic DNA extracted via a commercial kit from subject blood samples as a template, we successfully generated amplicons using primers flanking the *LDL Hin*dIII polymorphism consistent with expected results (Figure 1A). RFLP analysis of PCR products was performed using the *Hin*dIII restriction endonuclease, resulting in the detection of three polymorphic genotypes: H+H+ (540 and 240 bp), H+H- (780, 540, and 240 bp), and H-H- (780 bp) genotypes (Figure 1B). Allele frequency analysis in the two study groups showed that the frequencies of the H+ allele in the observation and control groups were 79.55 and 65.91%, respectively. The frequency of the H+ allele in the observation group was significantly higher than that in the control group ($P < 0.05$) (Table 1). A chi-square test for the distribution of *Hin*dIII genotypes in the two study groups demonstrated they were in accordance with Hardy-Weinberg equilibrium.

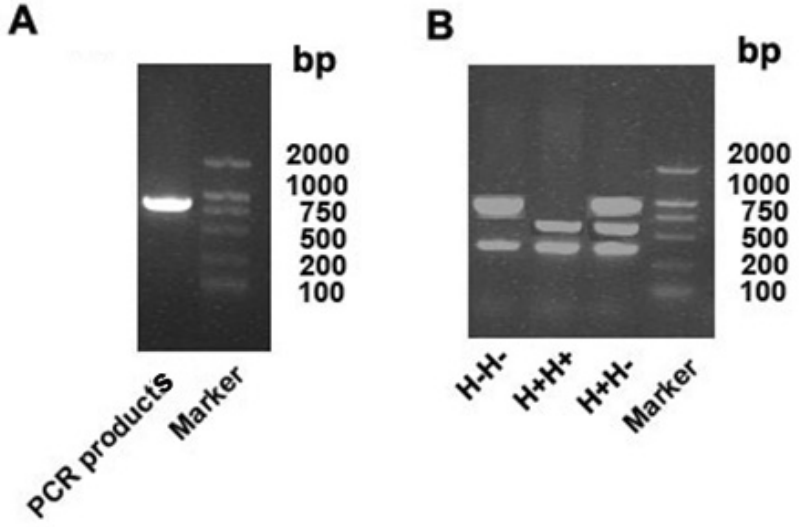

Figure 1. Analysis of the lipoprotein lipase *Hin*dIII polymorphism. **A.** *LPL* PCR product; **B.** *Hin*dIII enzyme digestion results of different *LPL* genotypes. LPL = lipoprotein lipase; PCR = polymerase chain reaction.

Table 1. Population distribution of the lipoprotein lipase *Hin*dIII polymorphism.

Groups	Patients	Genotypes			Alleles	
		H+H+	H+H-	H-H-	H+	H-
Observation group	110	72	31	7	175 (79.55)	45 (20.45)
Control group	110	50	45	15	145 (65.91)	75 (34.09)

Comparison of the clinical indices of subjects with different placental genotypes in the two groups

This study further analyzed the clinical indices associated with different genotypes in the two groups; the results are shown in Table 2. There were significant differences in TC, TG, HDL-C, FINS, BMI, and HOMA-IR indices between individuals with the H+H+ genotype in the observation group compared with those of the non-H+H+ genotype population ($P < 0.05$). In contrast, there was no statistically significant differences in TC, TG, HDL-C, FINS, BMI, or HOMA-IR indices between the H+H+ genotype population in the control group and those with non-H+H+ genotypes ($P > 0.05$).

Table 2. Comparison of the clinical indices of subjects with different genotypes in each group.

Indices	Observation group				Control group			
	H+H+	Non H+H+	*t*	P	H+H+	Non H+H+	*t*	P
FBG (mM)	4.56 ± 0.46	4.76 ± 0.58	0.891	0.146	5.24 ± 0.29	5.98 ± 0.57	1.209	0.220
2hBG (mM)	6.87 ± 0.91	6.71 ± 0.86	0.799	0.201	6.59 ± 1.01	6.67 ± 1.23	0.919	0.468
TC (mM)	6.17 ± 1.09	5.38 ± 1.13	2.014	0.028*	5.08 ± 0.99	5.46 ± 0.79	1.921	0.201
TG (mM)	2.54 ± 0.74	2.10 ± 0.80	2.029	0.032*	2.12 ± 1.01	2.49 ± 0.98	2.151	0.098
HDL-C (mM)	0.86 ± 0.51	1.15 ± 0.58	2.309	0.041*	1.25 ± 0.59	1.29 ± 0.45	1.092	0.165
LDL-C (mM)	3.01 ± 0.81	2.49 ± 0.99	1.994	0.030*	2.61 ± 1.12	2.50 ± 1.00	0.601	0.591
FINS (mIU/L)	22.71 ± 5.41	14.68 ± 6.01	5.001	<0.001*	14.55 ± 5.04	13.78 ± 4.68	1.351	0.138
BMI (kg/m^2)	27.89 ± 3.98	24.14 ± 4.10	3.980	<0.001*	24.68 ± 2.62	24.19 ± 3.26	1.581	0.101
HOMA-IR	1.78 ± 0.61	1.19 ± 0.69	4.012	<0.001	1.12 ± 0.39	1.09 ± 0.44	1.284	0.142

*P < 0.05. FBG = fasting blood glucose; 2hBG = 2-h postprandial blood glucose; TC = serum total cholesterol; TG = triglyderide; HDL-C = high density lipoprotein-cholesterol; LDL-C = low density lipoprotein-cholesterol; FINS = fasting serum insulin; BMI = body mass index; HOMA-IR = homeostasis model assessment-estimated insulin resistance.

Relationship between IR and the *LPL* gene *Hin*dIII polymorphism

The above studies showed that there were significant differences in TC, TG, HDL-C, FINS, BMI, and HOMA-IR indices between different genotype populations within each group ($P < 0.05$). Therefore, this study analyzed the relationship between TC, TG, HDL-C, FINS, BMI, and HOMA-IR and the *LPL Hin*dIII polymorphism using Pearson's correlation analysis. The results demonstrated that the *LPL Hin*dIII polymorphism in the placental tissue of women with T2DM was positively correlated with TC ($r = 0.619$, $P = 0.038$), TG ($r = 0.406$, $P = 0.0317$, HDL-C ($r = 0.508$, $P = 0.001$), FINS ($r = 0.418$, $P = 0.001$), BMI ($r = 0.301$, $P = 0.018$), and HOMA-IR ($r = 0.599$, $P = 0.003$).

Comparison of the clinical indices of infants delivered by women with different placental genotypes in the observation group

The infants delivered by women in the observation group were followed-up; of the original participants, 29 cases were lost follow-up, including 13 cases with the H+H+ placental genotype and 16 cases with the non-H+H+ genotype. Routine examinations of the infants with the two different genotypes showed that the BMI, HOMA-IR, FINS, and other indices of neonates delivered by women with the H+H+ placental genotype were significantly higher than those of the non-H+H+ genotype women ($P < 0.05$) (Table 3).

Table 3. Clinical indices comparison of infants delivered by mothers with different placental genotypes in the observation group.

Indices	H+H+	Non H+H+	*t*	P
FBG (mM)	5.37 ± 0.78	5.43 ± 0.98	0.917	0.258
2hBG (mM)	6.92 ± 1.09	6.79 ± 0.98	0.512	0.313
TC (mM)	4.37 ± 0.76	4.41 ± 0.69	1.012	0.091
TG (mM)	1.32 ± 0.18	1.10 ± 0.17	0.618	0.129
HDL-C (mM)	0.68 ± 0.61	0.81 ± 0.67	0.701	0.110
LDL-C (mM)	2.67 ± 0.55	2.43 ± 0.89	0.521	0.212
FINS (mIU/L)	19.32 ± 3.19	10.33 ± 3.89	3.189	<0.001*
BMI (kg/m^2)	22.19 ± 4.18	16.42 ± 5.02	2.927	<0.001*
HOMA-IR	2.01 ± 1.17	1.29 ± 0.63	2.103	0.001*

*P < 0.05. FBG = fasting blood glucose; 2hBG = 2-h postprandial blood glucose; TC = serum total cholesterol; TG = triglyderide; HDL-C = high density lipoprotein-cholesterol; LDL-C = low density lipoprotein-cholesterol; FINS = fasting serum insulin; BMI = body mass index; HOMA-IR = homeostasis model assessment-estimated insulin resistance.

DISCUSSION

T2DM is a kind of endocrine disease with resulting high blood sugar caused by IR and impaired pancreatic β cell insulin secretion. In patients, this disorder is often combined with lipid metabolism disorder as well (Nesto, 2005). A recent study has demonstrated that abnormal lipid metabolism is the primary pathological process of T2DM and its complications such as atherosclerosis, coronary heart disease, and hypertension (Adibhatla and Hatcher, 2008). T2DM is a complex genetic disorder that is also associated with environmental factors. In particular, epidemiologic studies have suggested that the prevalence rate of disease in relatives of patients with T2DM was 4-10 times that of non-relatives (Barnett et al., 1981), implying that genetic factors play an important role in T2DM.

For patients with gestational T2DM, current foci of clinical research are whether their offspring will inherit T2DM, and which factors are related to their IR. As lipid metabolism disorder is usually present in patients with T2DM, it has been concluded that LPL plays an important role in the process of lipid metabolism. Studies have identified a *Hin*dIII polymorphism in the *LPL* gene, and have shown that this polymorphism was associated with low levels of HDL-C in patients with T2DM (Ma et al., 2003). Another study has demonstrated that the *LPL Hin*dIII polymorphism was associated with lipid levels in patients with T2DM, and furthermore has shown that the frequency of the H+ allele in patients with hypertriglyceridemia was higher than that in those with non-hypertriglyceridemia (Long et al., 2006). Thus, the *LPL Hin*dIII polymorphism was suggested to be closely related with the lipid metabolism disorder in patients with T2DM. However, it is unknown whether this polymorphism has a specific relationship with IR in patients with T2DM and with the occurrence of diabetes mellitus in their offspring.

In the current study, genomic DNA was extracted from the placental tissue of pregnant women with T2DM for identification of the *Hin*dIII polymorphism using double enzyme digestion and RFLP analysis. The results showed that the frequency of the *LPL* H+ allele in the patients with T2DM was significantly higher than that in the normal pregnant population. The results further showed that the TC, TG, HDL-C, FINS, BMI, and HOMA-IR indices in members of the diabetic population with the H+H+ placental genotype were significantly higher than those in women with the non-H+H+ genotype. In addition, the results demon-

strated that abnormal blood lipid, BMI, FINS, and HOMA-IR indices were correlated with the *Hin*dIII gene polymorphism, which was consistent with previously published findings (Du et al., 2011). The correlation between TC, TG, HDL-C, FINS, BMI, and HOMA-IR indices and the *LPL Hin*dIII polymorphism was further analyzed, the results showed the above indices in LPL were positively related to the *Hin*dIII gene polymorphism. Some scholars have suggested that the *LPL Hin*dIII polymorphism might also associate with IR syndrome (Veerkamp et al., 2005; Ansar et al., 2011). The results from this study support this hypothesis.

In addition, to explore the change of lipid metabolism and IR of neonates potentially associated with gestational T2DM, this study analyzed the changes in indices of the neonates delivered by the patients with T2DM and different placental genotypes. The results showed that the BMI, HOMA-IR, FINS, and other indices of neonates delivered by the patients with the H+H+ placental genotype were significantly higher than were those born of non-H+H+ genotype mothers, suggesting that the genetic probability for T2DM in offspring of women with a H+H+ placental genotype and gestational diabetes might be increased.

In summary, the *LPL Hin*dIII polymorphism in gestational T2DM was closely related to pregnancy with IR, and to physical index and lipid metabolism abnormalities. The probabilities of IR, abnormal BMI, and lipid metabolism disorder in the offspring of women with T2DM and an H+H+ placental genotype were increased.

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