

High leptin level and leptin receptor Lys656Asn variant are risk factors for preeclampsia

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ABSTRACT. The aim of this study was to investigate the relationship between the leptin receptor (LEPR) polymorphism/serum leptin level and preeclampsia. The prevalence of a single nucleotide polymorphism in the *LEPR* gene exon 14 at -656 and the serum leptin concentrations in 97 preeclamptic pregnant mothers were compared to those of 110 healthy controls. The Lys656Asn genotype and Lys656Asn + Asn656Asn frequencies in the *LEPR* gene were significantly more prevalent in preeclampsia mothers than in controls ($P < 0.05$). The serum leptin levels of preeclampsia cases were significantly higher than those of controls. In addition, there were higher serum leptin levels in individuals with the GC + CC genotype both in the total cohort and in women with preeclampsia than in those with the GG genotype. Our findings suggest that the Lys656Asn polymorphism is a functional variant in the LEPR, which can affect the interaction of leptin and its receptor. Furthermore, high leptin level and the LEPR variant are risk factors for preeclampsia in Chinese women.

Key words: Leptin; Leptin receptor; Polymorphism; Preeclampsia

INTRODUCTION

Leptin, an anti-obesity hormone, was originally discovered as a product of adipose cells responsible for regulating food intake and body weight (Scheller et al., 2010). Accumulating evidence suggests that leptin also functions as a crucial cytokine in diverse physiological processes such as immunity and angiogenesis, as well as in reproduction and regulation of arterial blood pressure (do Carmo et al., 2011). In addition, leptin abundance or deficiency has been implicated in the pathogenesis of a variety of diseases, including essential hypertension and preeclampsia (Dalamaga et al., 2011). Preeclampsia is a common and serious complication of human pregnancy, affecting 5-7% of all primigravid women. It is the foremost cause of maternal and fetal morbidity and mortality in industrialized countries. Preeclampsia may lead to kidney failure, liver damage, intracranial hemorrhage, delayed fetal intrauterine growth, and fetal death. It is a multisystem disorder characterized by new-onset hypertension and proteinuria in the second half of gestation. Although abnormal placentation such as incomplete trophoblast invasion of the spiral arteries seems to play a crucial role in the pathogenesis and pathophysiology of the disorder, its etiology remains unknown (Romundstad et al., 2010). Leptin is a mitogenic and pro-angiogenic factor in various cells; it acts synergistically with vascular endothelial growth factor and fibroblast growth factor 2 to promote angiogenesis. Leptin also affects the expression of several genes involved in angiogenesis such as matrix metalloproteinase (MMP)-2 and -9 (Cao et al., 2001). Leptin was implicated in the pathogenesis of several diseases, including essential hypertension and preeclampsia; recently, several groups reported increased serum and placental leptin levels in preeclamptic patients (Jarvenpaa et al., 2009). However, it is unknown whether elevated leptin levels are the causes or consequences of preeclampsia. In addition, the effects of leptin might also be influenced by variations of the leptin receptor (*LEPR*) gene. In the present study, we evaluated the association between *LEPR* Lys656Asn (G656C) genetic polymorphisms and preeclampsia.

MATERIAL AND METHODS

Participants

Ninety-seven pregnant women with preeclampsia and 110 pregnant healthy women were included in this study from the Department of Obstetrics and Gynecology at Tongji Hospital. Preeclampsia was defined as new-onset, persistent hypertension ($\geq 140/90$ mmHg occurring on at least 2 occasions ≥ 4 h apart) and new-onset proteinuria (≥ 300 mg/24 h) after the 20th gestational week, in the absence of urinary tract infection. Patients with multiple pregnancies, chronic hypertension, gestational diabetes mellitus, autoimmune disease, and renal diseases were excluded from the study. All participants were of the same ethnic origin and this study was approved by the Institutional Ethics Committee of Tongji Hospital, Wuhan. The clinical characteristics of the preeclamptic and control women are summarized in Table 1.

Polymorphism detection

Peripheral blood samples were collected from patients and controls in 1-mL ethylenediaminetetraacetic acid vacutainer tubes, and genomic DNA was extracted using standard

chloroform/phenol extraction and stored at -80°C . Information regarding the single nucleotide polymorphisms (SNPs) of the *LEPR* gene was derived from the SNP database (dbSNP) established by the National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov/SNP/>). Polymorphisms of the *LEPR* gene have been described in detail by Gotoda et al. (1997).

Enzyme-linked immunosorbent assay (ELISA) detection

Serum leptin levels were measured by ELISA (Beijing North Institute of Biological Technology), with a sensitivity of 0.05 ng/mL and a normal range of 10-100 ng/mL.

Statistical analysis

Continuous parametric variables were compared between preeclamptic and control patients using the *t*-test, whereas continuous non-parametric variables were compared using the Mann-Whitney U-test. The Pearson χ^2 test was used to compare categorical variables between groups. Logistic regression analysis was performed using SPSS version 19.0 for Windows (SPSS Inc., IBM Corporation, Endicott, NY, USA). The Pearson χ^2 test was also applied to evaluate whether the observed genotype frequencies were in Hardy-Weinberg equilibrium. For haplotype estimation, we used the Arlequin version 3.5 software (Excoffier and Lischer, 2010). For all statistical analyses, $P < 0.05$ was considered to be statistically significant.

RESULTS

Table 1 shows the demographic and clinical characteristics of all subjects included in the present study. There were no significant differences in maternal age, pre-pregnancy body mass index (BMI), and gestational age at delivery between preeclamptic cases and controls. The preeclamptic case group had lower fetal birth weight and fetal BMI than those of controls ($P < 0.05$). Table 2 shows the relationship between the mothers' serum leptin levels and their clinical characteristics. The concentrations of the mothers' leptin and insulin were higher in preeclamptic cases than in controls, and the differences were statistically significant. We also found that the fasting blood glucose level was higher in the preeclampsia group than in controls, although this finding was not statistically significant. Table 3 shows the relationship between the mothers' serum leptin levels and the newborns' clinical characteristics. There were differences between mothers' serum leptin levels, umbilical cord blood leptin level, and insulin level, and differences were also observed in the fetal birth weight and fetal BMI. The genotype and allele frequencies of *LEPR* polymorphisms between preeclampsia cases and controls are shown in Table 4. The genotype frequencies of the *LEPR* were in Hardy-Weinberg equilibrium. There were no significant differences in the Asn656Asn genotype distribution and C allele frequency of Lys656Asn in the *LEPR* gene between preeclampsia cases and controls ($P > 0.05$). However, the Lys656Asn and Lys656Asn + Asn656Asn genotypes of the 656G>C in the *LEPR* gene were significantly more prevalent in preeclampsia cases than in controls (odds ratio = 4.27 and 4.16, respectively; $P = 0.04$). We also studied the association between the SNPs and serum leptin values; we observed statistically significant differences between CC+GC subjects vs GG subjects in the total population and in the preeclampsia group ($P < 0.05$; Figure 1).

Table 1. Clinical characteristics of the preeclamptic patients and controls.

	Preeclamptic patients (N = 97)	Controls (N = 110)	P value
Maternal age (years)	29.53 ± 5.19	29.52 ± 4.07	NS
Pre-pregnancy BMI (kg/m ²)	20.97 ± 2.47	20.15 ± 2.6	NS
Gestational age at delivery (weeks)	36.2 ± 2.0	38.4 ± 1.6	NS
Fetal birth weight (kg)	2.620 ± 0.670	3.154 ± 0.501	<0.05
Fetal BMI (kg/m ²)	11.92 ± 0.93	10.38 ± 1.44	<0.05

BMI = body mass index; NS = not significant. Data are reported as means ± standard deviation for continuous variables.

Table 2. Relationship between mothers' serum leptin levels and the clinical characteristics.

	N	Mothers' leptin level (µg/L)	BMI (kg/m ²)	Total cholesterol (mM)	Fasting blood glucose (mM)	Insulin (U)
Controls	110	13.16 ± 6.61	27.35 ± 3.21	6.69 ± 1.57	4.91 ± 0.84	18.59 ± 2.81
Preeclampsia	97	19.48 ± 5.87*	27.37 ± 2.26	7.06 ± 1.33	5.21 ± 0.73	27.56 ± 2.07*

BMI = body mass index. *P < 0.05 significant difference, compared with controls.

Table 3. Relationship between mothers' serum leptin levels and the newborn clinical characteristics.

	N	Mothers' leptin level (µg/L)	Umbilical cord blood leptin level (µg/L)	Insulin (U)
Controls	110	13.16 ± 6.61	4.91 ± 0.84	18.59 ± 2.81
Preeclampsia	97	19.48 ± 5.87*	5.21 ± 0.73*	27.56 ± 2.07*

*P < 0.05 significant difference, compared with controls.

Table 4. Distribution of allele frequencies in preeclamptic patients and control pregnant mothers.

Genotype	Preeclamptic patients	Control mothers	OR	(95%CI)	P
Lys656Lys (GG)	49	71 (65%)			Reference
Lys656Asn (GC)	42	33 (30%)	4.27	0.30-0.97	0.04
Asn656Asn (CC)	6	6 (5%)	0.38	0.21-2.27	0.54
Lys656Asn + Asn656Asn	48	39	4.16	0.32-0.98	0.04
G	140	175			Reference
C	54	78	0.47	0.77-1.74	0.49

OR = odds ratio; 95%CI = 95% confidence interval.

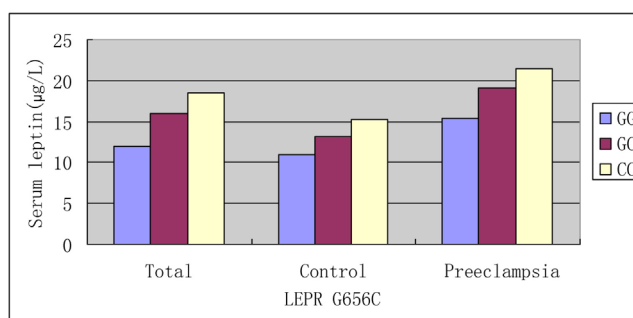


Figure 1. Correlation between serum leptin values and LEPR Lys656Asn genotypes. Serum leptin values are reported as means ± standard error, and shown according to the different genotypes for the polymorphism under investigation. Comparisons were performed in the total population (preeclampsia + controls) and in the preeclampsia and control groups separately. LEPR Lys656Asn: a statistically significant difference between CC+GC subjects vs GG subjects in the total population and in the preeclampsia group was observed. *P < 0.05.

DISCUSSION

Leptin is a hormone that is mainly produced by adipose tissue and binds to receptors in the hypothalamus. Leptin circulates in both free and receptor-bound forms (Scheller et al., 2010). It acts via the receptors located in adipose tissue, stomach, endometrium, liver, spleen, lungs, heart, ovaries, and placenta (Kielar et al., 1998; Gauster et al., 2011). Leptin was initially associated with preeclampsia when significantly higher plasma leptin levels were detected in preeclamptic patients compared with normotensive pregnant women (Mise et al., 1998). Poston's (2002) study showed that augmented leptin levels could regulate a variety of processes, including increasing sympathetic activity and mitochondrial superoxide synthesis, inducing expansion of Th1 cells secreting pro-inflammatory cytokines and activating expression of MMPs and tissue MMP inhibitors, thus modulating vascular structure. All of these mechanisms contribute to generate oxidative stress and endothelial and placental dysfunction, eventually leading to preeclampsia (Evans et al., 2011). On the other hand, elevated leptin levels could also have beneficial effects by stimulating placental growth and angiogenesis, reducing apoptotic stimuli (e.g., hypoxia), promoting endothelial nitric oxide synthesis, and shielding the effects of pro-inflammatory cytokines, thus acting to prevent preeclampsia (Ng et al., 2005). It appears that interplay of the contrasting consequences produced by increased leptin levels determines its multifunctional effects, although the potential mechanisms suggested by Poston (2002) have not yet been confirmed in preeclamptic patients. In addition, it is still debated whether increased leptin levels are a cause or a consequence of preeclampsia.

Leptin acts via its cognate receptor, LEPR. The *LEPR* gene is located on chromosome 1 and is a member of the class 1 cytokine receptor family. There are 6 LEPR isoforms. Many studies confirmed that high leptin level and its receptor polymorphism are associated with preeclampsia (Nizard et al., 2012). Interestingly, *LEPR* gene expression failed to parallel increased leptin concentrations in preeclampsia, suggesting that other factors such as *LEPR* gene polymorphisms may modify leptin function (Wang et al., 2011). King (2006) reported that a decrease in LEPR signaling was caused by LEPR mutations. This phenomenon was demonstrated in some severely obese subjects who are homozygous for a mutation in the *LEPR* and have very high leptin levels (Lahlou et al., 2002).

Different polymorphisms in the *LEPR* (G2548A, G1019A, and A223G) were studied, with unclear results (Skibola et al., 2004; Rigo et al., 2006; Liu and Liu, 2011). The polymorphism at codon 656 produces a change that might be functional. The G656C polymorphism (rs8179183) has been identified in exon 14 of the *LEPR* gene, which results in a lysine-to-methionine substitution and appears to decrease the LEPR function due to its decreased ability to effectively interact with leptin (Phillips et al., 2010). The present study assessed the rate of the *LEPR* G656C gene variant in preeclamptic patients. We found that the concentrations of the mothers' leptin and insulin were higher in preeclamptic cases than controls. Higher frequencies of the *LEPR* 656G/C and 656GC+656CC genotype were detected among our preeclamptic patients, while the presence of the C allele was not found to increase the risk of preeclampsia. The presence of the 656C allele of *LEPR* has been reported to be associated with increased insulin resistance in healthy women under the age of 30 years (Phillips et al., 2010). A previous study demonstrated that in carriers of the 656C allele, 120-min glucose and area under the curve values after a 75-g oral glucose tolerance test were higher in premenopausal women with impaired glucose tolerance (Phillips et al., 2010), and we obtained similar results. In the

case of 656C allele carriers, insulin levels were higher than those of patients with the 656GG genotype (data not shown), suggesting that the presence of the C allele in the polymorphism may increase the risk of insulin resistance. Insulin resistance is considered to be a risk factor for preeclampsia (Asvold et al., 2011). Hyperinsulinemia may directly result in hypertension by stimulating renal sodium reabsorption and the sympathetic nervous system, while hyperglycemia may promote endothelial dysfunction (Carty et al., 2010). Based on the previously mentioned studies, it was plausible that the *LEPR* gene variant could result in the secretion of leptin, causing an increase in reactivity and altered insulin metabolism; these mechanisms could lead to the development of preeclampsia. In conclusion, this study provided evidence for the role of the *LEPR* Lys656Asn polymorphism in the predisposition to preeclampsia. However, further studies are needed to reveal the molecular details of this association.

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