ASSOCIATION OF LEPTIN GENETIC POLYMORPHISM -2548 G/A WITH GESTATIONAL DIABETES MELLITUS

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ABSTRACT: The aim of this study was to investigate possible associations of -2548 G/A polymorphism in leptin gene promoter and pregnancy-associated diseases with abnormal fetal growth such as preeclampsia and gestational diabetes. The study was also focused on whether it is rather maternal or fetal variants that determines the pathological growth status. Peripheral or cord blood samples obtained from 49 preeclamptic women and their 39 newborns, 53 healthy controls and their 53 healthy newborns and 48 patients with gestational diabetes mellitus were evaluated for leptin gene (LEP) locus -2548 genotypes. The significantly higher risk for gestational diabetes mellitus was observed in the presence of an allele (AA and AG genotypes) against carriers of GG genotype (OR=2.84, 95%CI 1.14-7.07, p=0.02). There is a significant risk of diabetes mellitus associated to A allele (OR=1.79, 95%CI 1.02-3.14, p=0.03). Furthermore, evaluations of preeclamptic patients’ data revealed a significant association of genotype distribution and delivery and spontaneous abortion rate, where the GG carriers performed the highest pregnancy rate while the AG carriers performed the lowest spontaneous abortion rate. Our results support the hypothesis for -2548 G/A leptin gene polymorphism involvement in ethiopathogenesis of pregnancy-associated diseases with abnormal fetal growth, especially gestational diabetes mellitus.

KEY WORDS: Gestational Diabetes Mellitus, Leptin, Polymorphism, and Preeclampsia

INTRODUCTION

Leptin, a small peptide produced by adipocytes, is implicated in a great number of endocrine regulations, including obesity, satiety regulation and fertility. This 167 amino acid protein transcribed from the ob gene, was originally cloned in the mouse during research directed at identifying the molecular defect in an obesity-prone strain, the ob/ob mouse (Zhang Y et al, 1994). Human leptin gene is located to 7q31 and contains three exons. At first, leptin was considered to be a signaling molecule limiting food intake and increasing energy expenditure (Zhang et al, 1994). This was essentially supported by the fact that rodents with genetic (Campfield et al, 1995; Halaas et al, 1995; Pelleymounter et al, 1995; Stephens et al, 1995; Weigle et al, 1995) or diet induced (Campfield et al, 1995) obesity that were injected with leptin had manifested with decrease in bodyweight and improvement of metabolic parameters. Furthermore, hypothalamus has been identified as the most probable critical target for the satiety effect of leptin that can be transported through the blood/brain barrier via a saturable transport system (Baumann et al, 1996; Golden et al, 1997).

Recently, human placentas have been identified as a major source of leptin and the existence of placenta specific upstream enhancer indicates that placenta leptin may be regulated differently to that of adipose origin (Green et al, 1995; Bi et al, 1997; Masuzaki et al, 1997). Furthermore, the placenta leptin localization suggests it could be released into both maternal and fetal blood. The localization of the leptin receptor on the maternal side of the placenta supports the hypothesis that placenta leptin may have an autocrine role on the placenta itself as well as an endocrine role in the mother (Lea et al, 2000). The recent reports suggest that leptin may exert physiological effects on the placenta and conception, including fetal and placenta angiogenesis, fetal growth and development, embryonic hematopoiesis (Holness et al, 1999; Henson and Castracane, 2000).

Taking into account that hyperinsulinemia and hypoxia induce partially overlapping pathophysiological disturbances during pregnancy and both of them are known to induce leptin secretion, we may ask what elements of the leptin promoter are responsible for these effects. Recently, evidence was provided that insulin...
and hypoxia act as agonists on the human leptin transcription but on two different regulatory elements. It has been published that hypoxia induces leptin transcription by a hypoxia-inducible-factor-1 (HIF-1) dependent mechanism (Meissner et al, 2003) by identifying at least one hypoxia-responsive element, located -120 bp to -116 bp in the leptin promoter being involved in this HIF-1-mediated effect on the transcriptional regulation. Moreover, it was reported (Grosfeld et al, 2001) that the human leptin promoter carries a potential insulin response element, located in the region from -720 bp to -150 bp. Therefore, it could be suggested that placenta leptin synthesis can be stimulated by the combination of local (e.g., hypoxia) and generalized factors (e.g., hyperinsulinism). This is in agreement with recent finding that leptin gene expression and production are markedly elevated in placenta of diabetic women treated with insulin. The previous findings provide strong evidence that leptin production can be regulated in utero and emphasize the role of placenta leptin in human pregnancy (Lepercq et al, 1998).

As previously described, DNA polymorphisms in leptin gene (LEP) are linked to extreme obesity (Jaquet et al, 1998). Unlike the other polymorphic sites, the G-2548A polymorphism in the 5′ region of the LEP gene was reported not only to be associated with overweight (Clement et al, 1996; Mammes et al, 2000) but also to have a strong influence on leptin gene expression and adipose tissue secretion (Hoffstedt et al, 2002). Thus, we suppose it might also influence leptin levels during pregnancy, especially when taking into account that the polymorphic site is located approximately 1800 bp from the insulin response element within the leptin promoter.

From the personal history of patients whose fetuses suffer from IUGR we knot that some women are prone to have IUGR pregnancy while in others pregnancies with IUGR can alternate with normal birth weight pregnancies. As IUGR can in these cases be considered a maladaptive maternal-fetal genotype, attention was focused on investigating possible genetic background of intrauterine growth restriction in preeclampsia not only on mothers but also on the newborns from these pregnancies.

Based on these observations and the fact the G-2548A polymorphism has proved to influence leptin gene expression, possible associations between the -2548 G/A leptin gene polymorphism variants and pregnancy associated diseases implicating abnormal leptin status such as preeclampsia and gestational diabetes mellitus were set out to be identified.

**MATERIALS AND METHODS**

**Subjects**

Forty-nine women (group A; median age 29, age range 19-46 years) with preeclampsia, thirty-nine newborns of these preeclamptic women (group B, median of birth weight 1950 g, birth weight range 700-2300 g), 23 of these newborns were diagnosed with gestational diabetes mellitus (group E; median age 30, age range 24-39 years) were enrolled in study. To ensure homogeneity of the genetic background, the healthy controls, originating from a regional Czech population, were enrolled by random selection. Women with polycystic ovary syndrome, hirsutism or menstrual cycle disturbances and women previously treated for infertility were excluded from both the study and control groups.

In nine cases of children coming from preeclamptic pregnancies we did not succeed in obtaining the cord blood sample. All individuals in the study were Caucasians; the pregnant women were followed-up and their children delivered at Gynecology and Obstetrics Clinic, University Hospital Brno, Czech Republic.

All individuals in the study had given informed consent prior to their inclusion in the study expressing their agreement to the fact their blood samples and blood samples of their children would be included in the study. The study was approved by the Committee for Ethics of Medical Experiments on Human Subjects, Faculty of Medicine, Masaryk University Brno.

**Glucose and diagnostic criteria for GDM**

A standard OGTT protocol was used. After a 12-h overnight fasting, venous plasma samples were collected fasting, 1-h and 2-h post-oral 75-g glucose. The diagnosis of GDM was based on the criteria of the World Health Organization (plasma glucose thresholds mmol/l: 0h, 7.0; 2h, 7.8). This OGTT was performed routinely between 24 and 28 weeks gestation, but occasionally performed at other stages of gestations if clinically warranted. Women with type 1 or type 2 diabetes diagnosed before the pregnancy were excluded from the study.

**Diagnostic criteria for preeclampsia**

Preeclampsia was defined as the development of hypertension and new-onset proteinuria (>300 mg of urinary protein in 24 h) in women with no proteinuria at baseline. Hypertension was defined according to current guidelines that accept 140 and/or 90mmHg of systolic and diastolic pressure, respectively, or higher, as hypertension, when measured on two consecutive occasions at least 24 h apart. Women with chronic hypertension were excluded from the study.

**Intrauterine growth restriction diagnostic criteria**

IUGR was defined as infants whose birth weight is below the 10th percentile of birth weight adjusted for sex and