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ARTICLE

Genetic association of adipokine and *UCP2* polymorphism with recurrent miscarriage among non-obese women

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Abstract The adipokines produced from adipose tissues influence energy homeostasis, resulting in alterations of the adipokine concentrations. This process may be associated with fertility impairment, resulting in recurrent miscarriage. The present study investigated whether there was any association between the *UCP2* 45-bp indel polymorphism and the adipokine gene polymorphisms, namely leptin 2549 (C/A), adeponectin 276 (G/T) and 45 (T/G) and resistin 420 (C/G) in 200 non-obese recurrent miscarriage patients and 300 ethnically matched negative controls. These markers were studied using gene-specific PCR single specific primer and restriction fragment length polymorphism. For leptin 2549 and adeponectin 276, the A allele and G allele showed 3.42-fold ($P = 0.0001$) and 1.36-fold ($P = 0.036$) increased risk of recurrent miscarriage, respectively. Combined analysis of *UCP2* 45-bp indel and leptin 2549 showed U0-L0 and U1-L0 variants to be at 2- and 3-fold increased associative risk, respectively. Combined analysis of leptin 2549 and adeponectin 276 showed L0-D0 and L0-D1 variants to be at 2- and 4-fold increased associative risk, respectively. The combination U1-L0-D1-A1-R1 was 4.39-fold higher ($P = 0.0007$) among recurrent miscarriage patients. In conclusion, the results highlight the role of the studied adipokine and *UCP2* polymorphisms in recurrent miscarriage among the North Indian non-obese population.

KEYWORDS: adipokine, non-obesity, recurrent miscarriage, single-nucleotide polymorphism, UCP2, homeostasis

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Introduction

White adipose tissue secretes biologically active substances. It provides a repertoire of triacylglycerols and is a source of various hormones, namely leptin, adiponectin, resistin and resistin-like molecules. Normal concentrations of adipokines are required to maintain integrity of the hypothalamus–pituitary–gonadal axis, regular ovulatory processes and successful embryo implantation, resulting in physiological pregnancy (Slavov and Dzhelebov, 2010). Hence alterations in the adipokine concentrations or their mechanism of action may be associated with fertility impairment and recurrent miscarriage of unknown aetiology.

Most of the adipokine-related studies done previously have taken into consideration metabolic function and obesity. However, there are very few studies to evaluate the role of adipokines in non-obese recurrent miscarriages with no known cause of repeated pregnancy losses. The present study evaluated whether there was any genetic association between the *UCP2* 45-bp indel polymorphism and the adipokine gene polymorphisms, namely leptin 2549 (C/A), adeponectin 276 (G/T), adeponectin 45 (T/G) and resistin 420 (C/G), with recurrent miscarriage.

Leptin, located on 31.3 region of chromosome 17, is a product of the obese (ob) gene expression. Leptin plays an essential role in reproduction as it regulates gonadotrophin-releasing hormone secretion from the hypothalamus. It has a remarkable role in the embryo development, functioning of the ovary and the endometrium. In humans, plasma leptin concentrations are higher in females than in males and are significantly higher in the luteal phase of the menstrual cycle (Hickey et al., 1996). Low concentrations of maternal plasma leptin are associated with suboptimal pregnancy outcomes (Lea et al., 2000). Plasma leptin concentrations in normal fertile women are reported to be increased in the first trimester of pregnancy compared with pre-pregnancy (Lage et al., 1999).

The adiponectin gene, located on chromosome 3q27, is encoded by *ADIPOQ* (adipocyte C1q and collagen domain). A genome-wide scan and linkage studies of the chromosome 3q27 region showed susceptibility for obesity (Vionnet et al., 1997). Single-nucleotide polymorphisms (SNP) namely C45G15G (T/G) in exon 2 and C276 (G/T) in intron 2 of *ADIPOQ* showed a striking association with obesity and insulin resistance (Fumeron et al., 2004).

Resistin plays a vital role in the regulation of insulin resistance (Steppan and Lazar, 2002). It is expressed and secreted by the placenta (Sagawa et al., 2002) and its concentration increases by the third trimester in humans (Chen et al., 2005). Resistin, along with adiponectin, modulates glucose and fat homeostasis, influences insulin action and thus may mechanistically link insulin resistance and fertility.

The mitochondrial uncoupling protein 2 (UCP2) is present in the mitochondrial inner membrane and is the member of the anion carrier protein family. *UCP2* is located on chromosome 11q13, spanning across 6.3 kb and is expressed in adipose tissue, skeletal muscle and pancreatic islets (Fleury et al., 1997). Tissue-dependent UCP2 has a vital role in direct and indirect fat metabolism, e.g. by effecting insulin secretion (Chan and Kashemsant, 2006). The current study examined the polymorphism involving the 45-bp indel variant of *UCP2* located on exon 8 of the 3′ untranslated region, which has been shown to have a relationship with susceptibility for obesity (Cassell et al., 1999).

The risk of spontaneous abortion is higher in obese women (Lashen et al., 2004) and the above-mentioned polymorphisms have been shown to be associated with obesity (Dolinková et al., 2006; Duarte et al., 2003; Krízová et al., 2008). However, the present study evaluated the independent effect of these SNP in non-obese women undergoing recurrent miscarriages.

Materials and methods

Genomic DNA samples

All patient and control samples were collected from the Out Patients Department of Sanjay Gandhi Post Graduate Institute of Medical Sciences (SGPGIMS), Lucknow, and Queen Mary Hospital of CSM Medical University, Lucknow, Uttar Pradesh, India. All patients had a history of at least three spontaneous miscarriages (mean 4, range 3–7) and no previous successful pregnancy. All the patients were screened for factors relating to various known causes of miscarriages, including parental chromosomes, day-2 FSH (3–11 U/l), LH (3–12 U/l) and testosterone (0.5–3 nmol/l), antiphospholipid antibodies including lupus anticoagulant (positive likelihood ratio, 0.8–1.05) and anticardiolipin antibodies (IgG 0–12 GPL units, IgM 0–5 MPL units) and prothrombotic risk factors including activated protein-C resistance (2.6–4.36 ratio), factor V Leiden and prothrombin mutations, luteal-phase insufficiency, prolactin dosage, glycaemic curve, thyroid hormone concentrations, toxoplasmosis, cytomegalovirus, rubella, HIV, group B streptococci, *Chlamydia trachomatis*, hepatitis B and C and bacterial vaginosis. The uterine cavity was investigated for cervical incompetence by hysteroscopy, hysterosalpingography and serial ultrasound. Initially, 1000 cases were screened and recurrent miscarriage cases with no known cause of repeated miscarriage and also not obese were selected randomly. The total number of cases selected was 200. Height and weight was taken to calculate the body mass index (BMI) by dividing weight in kilograms by the square of the height in metres (kg/m^2). All recurrent miscarriage patients were primary aborters, not having any live children and belonged to four different caste groups: upper caste Hindus (Brahmin, Vaishya, Kayastha and Kshatriya), backward class (OBC and scheduled caste/ scheduled tribes (SC/ST)), Muslims (Shiya and Sunni) and others (Sindhi, Sikh, Jain and Christians). Patients' detailed clinical information was recorded prior to inclusion (Parveen et al., 2010). Healthy controls from the same ethnic distribution had at least two live births with no history of miscarriage, pre-eclampsia, ectopic pregnancy or preterm delivery and were non-obese (as defined by WHO guidelines; World Health Organization, 2000).

From both controls and recurrent miscarriage women, 5 ml blood was collected in EDTA-coated collection vials and DNA was extracted using Qiagen kits. This investigation was approved by the Ethics Committees of SGPGIMS and Queen Mary Hospital, CSM Medical University, Lucknow, Uttar Pradesh, India (AA01:PGI/SRF/IEC/52/19/11/2010,