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ARTICLE

Functional SNP −1562C/T in the promoter region of *MMP9* and recurrent early pregnancy loss

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Abstract Angiogenesis, invasion and decidualization play an important role in uterine preparation and embryo development. Matrix metalloproteinases (MMP) are crucial for the degradation/remodelling of the extracellular matrix and are involved in spiral artery formation and invasion of endometrium during implantation. A functional single-nucleotide polymorphism (SNP) in the *MMP9* promoter, −1562C/T, is known to influence expression in an allele-specific manner. The present study evaluated the association between maternal genotype of SNP −1562C/T of *MMP9* and recurrent early pregnancy loss (REPL) risk. This case–control study was comprised of REPL patients ($n = 106$) and women having one healthy child as controls ($n = 111$). Genotyping for SNP −1562C/T of *MMP9* was performed by PCR/restriction fragment length polymorphism followed by DNA sequencing. Allele and genotype distribution did not differ significantly between patients and controls (by allele, chi-squared 0.228, odds ratio 1.12, 95% confidence interval 0.695–1.816; by genotype, chi-squared 0.893). Thus SNP −1562C/T of *MMP9* was not associated with REPL risk in this population and further study in other populations will verify whether it is associated with REPL risk or not. REPL is a multifactorial pathology and other genetic or environmental factors may be contributing to the complex aetiology of REPL.

KEYWORDS: angiogenesis, −1562C/T SNP, decidualization, endometrium, matrix metalloproteinase, recurrent early pregnancy loss

Introduction

Recurrent early pregnancy loss (REPL) is defined as three or more consecutive pregnancy losses prior to the 10th week of gestation. The cause of REPL is multifactorial, but can be divided into embryological causes, mainly due to chromosomal abnormalities and maternal causes, which affect the endometrium and/or placental development. Known causes of maternal defects include coagulation disorders, autoimmune defects, endocrine disorders and endometrial defects (Laird et al., 2003). The aetiology in approximately 50% of cases of REPL is idiopathic. Decidualization, angiogenesis and invasion are the processes which take place during the early stages of pregnancy (Salamonsen et al., 2003; Smith, 2000; Xu et al., 2000) and they play a significant role in uterine preparation and embryo development; thus it is

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quite likely that aberrant gene expression of the genes implicated in these processes will affect the fetal development and survival.

Decidualization is the process in which stromal cells of the endometrium are differentiated into decidual cells (Kearns and Lala, 1983; Noyes et al., 1950). Decidualization involves the remodelling of stromal/decidua extracellular matrix (ECM): the interstitial-type ECM of the proliferative endometrium, which is enriched in fibronectin and collagen types I, III, V and VI before the process of decidualization, is replaced with a basal lamina composed primarily of residual interstitial proteins, laminin, heparin sulphate proteoglycan and collagen type IV after decidualization (Aplin et al., 1988; Kislaus et al., 1987). Matrix metalloproteinases (MMP) helps with decidualization by proteolysing the ECM. Regulation of angiogenesis largely depends on the balance of factors stimulating and inhibiting different steps of the vascular network formation (Khokha and Denhart, 1989). *MMP9* is known to be expressed in the endometrium during the implantation period and promotes the growth of blood vessels which results in the thickening of the functional layer of endometrium in preparation for a possible embryo. MMP also enable the penetration of cytotrophoblast in the maternal endometrium and the first third portion of myometrium by degrading maternal ECM (Curry and Osteen, 2003). Thus, REPL may be a consequence of impaired decidualization, angiogenesis and improper invasion during the early stages of pregnancy.

Genetic variation that influences *MMP9* expression may contribute to the REPL phenotype. Several polymorphisms have been reported in *MMP9*. One is a multi-allelic dinucleotide repeat (CA)n (rs3222264) that has lower transcription from a 14-repeat allele than from other alleles (Peters et al., 1999; Shimajiri et al., 1999). Another is a single-nucleotide polymorphism (SNP) with a C→T substitution at −1562 (rs3918242) that results in the loss of a putative transcriptional repressor-binding site and a subsequent increase in *MMP9* transcription (Van den Steen et al., 2003; Zhang et al., 1999). Other non-synonymous-coding SNP in *MMP9* include rs1805088 (A20V) in exon 1, rs17576 (R279Q) in exon 6, rs2250889 (P574R) in exon 10 and rs2274756 (R668Q) in exon 12. However, the (CA)n repeat and the C→T substitution at −1562 are in the gene promoter region and influence gene expression. Aberrant *MMP9* expression may result in abnormal implantation and pregnancy disorders (Malik et al., 2006; Skrzypczak et al., 2007). The −1562C/T polymorphism of *MMP9* has been studied in pregnancy disorders such as intrauterine growth retardation and pre-eclampsia (Coolman et al., 2007; Gremlich et al., 2007; Palei et al., 2010); thus it is quite likely that it may result in impaired decidualization, angiogenesis and improper invasion during the early stages of pregnancy and may lead to early pregnancy loss.

The present study evaluated the association between the maternal genotype of SNP −1562C/T of *MMP9* and REPL. It postulated that a genotype associated with a increase in *MMP9* expression could be linked with impaired/incomplete decidualization, angiogenesis and invasion which could result in the REPL phenotype.

Materials and methods

Subjects

This case−control study enrolled 123 REPL patients who had three or more first-trimester miscarriages and no births and thus had primary recurrent miscarriage. Patients were recruited from the outpatient department of University Hospital, Department of Obstetrics and Gynaecology, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India. Patients underwent a standardized clinical and laboratory evaluation. Patients who were positive for anticardiolipin antibodies (ACLA), lupus anticoagulant (LA) and toxoplasmosis other rubella cytomegalovirus and herpes (TORCH), and also those with thyroid dysfunction, chromosomal abnormalities and other uterine pathologies were excluded from the study. Patients with high activated partial thromboplastin time (APTT) were examined by LA-sensitive APTT reagent PTT-LA and STart 4 Hemostasis Analyser (Diagnostica Stago, France). The plasma clotting time increases in the presence of LA. A difference of 8 s between the two clotting times was accepted to be positive for LA. Solid-phase IgG enzyme-linked immunosorbent assay (Intec, Xiamen, China) was used for detecting ACLA and a value greater than 10 units IgG phospholipid was considered ACLA positive. A total of 17 patients during their clinical and laboratory investigations showed one or the other known anomalies and were excluded. Among them, four represented polycystic ovaries, three were ACLA positive, one was both LA and ACLA positive, four were TORCH positive, one had cervical incompetency, one had arcuate uterus, one had septate uterus and one had hypothyroidism. Karyotype analysis revealed two patients with abnormal numerical chromosome complements. Two patients had mosaic karyotypes (46,XX and 45,XO). The remaining 106 REPL patients were included for the present study. They all had regular cycles and none had pregnancy-related complications such as diabetes or hypertension. As controls, one hundred and eleven women with no history of previous miscarriage and at least one successful pregnancy were recruited as controls. All controls were recruited from the same outpatient department. A questionnaire was used with each patient to record details of their lifestyle, habits and family history. Informed consent was obtained from every participant in each group before their inclusion in study. Approval of the University's ethical committee for research on human material was obtained (no. Dean/2009−10/556).

Karyotyping

Blood lymphocyte culture was performed for each patient and patients showing abnormal chromosome complement were excluded.

Genotyping of SNP (−1562C/T) *MMP9* by PCR/restriction fragment length polymorphism

Genomic DNA was extracted from peripheral blood of 106 patients (with 46,XX karyotype) and 111 healthy controls using a standard salting-out procedure. PCR amplification of the −1562C/T mutation region in the *MMP9* promoter