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ARTICLE

Matrix metalloproteinases 1, 2, 3 and 9 functional single-nucleotide polymorphisms in idiopathic recurrent spontaneous abortion

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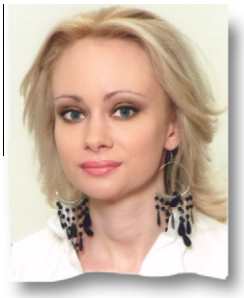

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Abstract Idiopathic recurrent spontaneous abortion (IRSA) has been associated with abnormalities in the remodelling of endometrial extracellular matrix, as well as aberrant matrix metalloproteinase (MMP) gene expression in endometrium of IRSA women and chorionic villi of IRSA concepti. This study investigated the association of five functional MMP gene promoter polymorphisms (MMP1 −1607 1G/2G, MMP2 −735 C/T, MMP2 −1306 C/T, MMP3 −1612 5A/6A and MMP9 −1562 C/T) with IRSA. A total of 149 couples with at least three consecutive IRSA and 149 fertile couples were included in a case–control study. Genotype analysis was performed using PCR restriction fragment length polymorphism. Statistically significant differences were found in distributions of MMP2 −735 CT (chi-squared 10.21, $P = 0.006$; OR 2.15, 95% CI 1.34–3.45, $P = 0.001$), and MMP9 −1562 CC (chi-squared 9.06, $P = 0.010$; OR 2.21, 95% CI 1.30–3.80, $P = 0.004$) between IRSA women and controls. Combined analysis of MMP gene polymorphisms did not increase their predictive value. There were no statistically significant differences in genotype and allele frequencies of any polymorphism between IRSA men and controls. MMP2 −735 C/T and MMP9 −1562 C/T functional gene polymorphisms might be associated with an increased risk of IRSA in women.

KEYWORDS: endometrium, extracellular matrix, genetic association study, pregnancy, proteolysis

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Introduction

Recurrent spontaneous abortion is the loss of three or more consecutive pregnancies before the 24th week of gestation, affecting approximately 3% of couples trying to conceive (Carrington et al., 2005). Although there are several identifiable non-genetic and genetic causes, the aetiology remains unknown in almost 50% of couples (Toth et al., 2010). Numerous potential causative mechanisms have been proposed for this group of idiopathic recurrent spontaneous abortion (IRSA); however, to date none of them have provided a clear answer about its aetiology. Considering the insufficient knowledge on genetic contribution to pregnancy loss, studies on genetic causes of IRSA are of great importance.

Pregnancy loss in IRSA occurs mostly prior to the 12th week of gestation, a period of early pregnancy marked by intensive formation of the fetal–maternal interface (Salker et al., 2010; Teklenburg et al., 2010). Development of a histologically and functionally normal endometrium is critical for subsequent endometrial decidualization, receptivity and implantation (Cartwright et al., 2010; Jokimaa et al., 2002). After fertilization, the proper communication and interaction between maternal decidual cells and the embryo is essential for the two to induce changes in each other and promote the establishment of a functional fetal–maternal interface.

Degradation and remodelling of the extracellular matrix (ECM) is a key event in all processes involved in normal human reproduction (Hulboy et al., 1997). Remodelling of the ECM, which is essential for endometrial decidualization, as well as trophoblast implantation and placentation, is primarily enabled by the enzymes matrix metalloproteinases (MMP). The MMP comprise a large group of zinc-dependent endopeptidases which includes collagenases (MMP1, MMP8 and MMP13), stromelysins (MMP3, MMP7 and MMP10) and gelatinases (MMP2 and MMP9). Balanced with their natural inhibitors, MMP are essential regulators of cell migration, proliferation, apoptosis and angiogenesis (Sternlicht and Werb, 2001).

The proper expression and local activation of MMP in both the decidua and extravillous trophoblast is a critical component of early human pregnancy (Goldman and Shalev, 2003; Salamonsen et al., 2000, 2003). Almost all MMP are expressed by the uterine NK cells and fibroblasts in decidua where they control implantation (Anacker et al., 2011; Naruse et al., 2009). MMP2 and MMP9 have crucial roles in terminal differentiation of human endometrial stomal cells into decidual cells (Seval et al., 2004; Xu et al., 2000) and as they are detected in decidual tissues throughout pregnancy, they have been assigned key roles in regulation of trophoblast invasion and angiogenesis (Niu et al., 2000; Staun-Ram et al., 2004). However, other MMP, such as MMP1 and MMP3, are also detected in the extravillous trophoblast where they contribute to blastocyst implantation (Huppertz et al., 1998; Husslein et al., 2009).

Abnormalities in the remodelling of endometrial ECM, in terms of altered ECM turnover and aberrant MMP2 and MMP9 gene expression prior to implantation as well as aberrant MMP2 gene expression in chorionic villi of IRSA concepti, have been previously associated with IRSA (Jokimaa et al., 2002; Serle et al., 1994). Women with IRSA show retarded endometrial development in the peri-implantation period (Li et al., 2002; Serle et al., 1994) and a longer receptivity window which might affect the normal process of embryo selection (Salker et al., 2010; Teklenburg et al., 2010). These women also have higher levels of MMP2 mRNA and lower MMP9 mRNA in mid-secretory endometrium, especially during the window of implantation, indicating an aberrant ECM turnover and an altered decidual phenotype (Inagaki et al., 2003; Jokimaa et al., 2002; Skrzypczak et al., 2007a,b; Wu and Zhou, 2003). Also, significantly lower levels of MMP2 gene expression are found in chorionic villi of IRSA concepti (Choi et al., 2003). All of these results indicate that there is a dysregulation of MMP gene expression in IRSA in both the mother and the embryo.

The promoter regions of several MMP genes contain biallelic single-nucleotide polymorphisms (SNP) that affect the level of their gene expression (Sternlicht and Werb, 2001; Ye, 2000). The present study selected five SNP of the MMP genes crucial in early pregnancy: MMP1 −1607 1G/2G, MMP2 −735 C/T, MMP2 −1306 C/T, MMP3 −1612 5A/6A and MMP9 −1562 C/T. The effect of these polymorphisms on MMP gene expression has been extensively characterized in previous studies (Blankenberg et al., 2003; Price et al., 2001; Rutter et al., 1998; Ye et al., 1996; Yu et al., 2004). In view of the important roles MMP have during normal pregnancy and the fact that the functional genetic variations might affect their gene expression, the aim of this study was to investigate the association of functional promoter polymorphisms in MMP1, MMP2, MMP3 and MMP9 genes with IRSA.

Materials and methods

Patient and control samples

A case–control study was performed to examine the association of functionally important gene polymorphisms of MMP1, MMP2, MMP3 and MMP9 with IRSA. All samples were collected at the Institute of Medical Genetics, Department of Obstetrics and Gynaecology, UMC Ljubljana, Slovenia from 2005 to 2011. All subjects included in the study were of Slovenian origin with predecessors living in Slovenia for at least three generations, reflecting the ethnicity of most patients attending the Institute of Medical Genetics. Data collection procedures were the same for patients and control subjects. Written informed consent to participate in this study was obtained from all the individuals. The study was approved by Slovenian and Croatian National Ethics' Committees.

A total of 149 women with a history of three or more consecutive spontaneous abortions of unknown aetiology before the 24th week of gestation and 149 of their male partners were included in the study. Characteristics of IRSA couples and the control subjects are presented in **Table 1**. Inclusion criteria were normal karyotypes of couples with IRSA and no history of endocrine or metabolic disorders (including diabetes mellitus), autoimmune disease or other systemic diseases, previous venous or arterial thrombosis or uterine anatomical abnormalities. A possible infectious aetiology was also ruled out by assessing the reports of the microbiological cultures of the samples obtained from