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ARTICLE

Association between *trefoil factor 3* gene variants and idiopathic recurrent spontaneous abortion

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Abstract *Trefoil factor 3* (*TFF3*) gene is an inflammatory mediator expressed in human endometrium during the window of implantation. The aim of this study was to evaluate the possible genetic association of *TFF3* variants in recurrent spontaneous abortion. Women with a history of recurrent spontaneous abortion ($n = 164$) and healthy pregnant women ($n = 143$) were genotyped for five *TFF3* polymorphisms (rs225439 G/A, rs533093 C/T, rs225361 A/G, rs11701143 T/C and rs77436142 G/C). In addition, haplotypes formed within the gene were analysed. Within the recurrent spontaneous abortion group, women who at some point had given birth and childless women had 4.19 ± 1.75 and 5.34 ± 3.42 consecutive spontaneous abortions, respectively. Women who had experience recurrent spontaneous abortions had a lower allele frequency of the rs11701143 promoter region minor C allele compared with fertile women (0.02 versus 0.05, $P = 0.015$). Patients with rs225361 AG genotype had significantly more successful pregnancies before spontaneous abortion than those with homozygous AA and GG genotypes ($P = 0.014$). No significant differences in haplotype frequencies

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between patients and controls were detected. Possible genetic risk factors identified that might contribute to the pathogenesis of idiopathic recurrent spontaneous abortion were *TFF3* gene variants.

KEYWORDS: haplotype, intestinal trefoil factor, polymorphism, recurrent miscarriage, trefoil factor

Introduction

Recurrent spontaneous abortion, defined as the occurrence of three or more consecutive pregnancy losses, affects 1–3% of women trying to conceive (Kolte et al., 2011; Teklenburg et al., 2010a), with a prevalence of 0.5–1.0% in fertile couples (Maybin et al., 2011), and an incidence that increases with rising maternal age (Brigham et al., 1999; Quenby and Farquharson, 1993). Recurrent spontaneous abortion is a heterogeneous condition of multifactorial causes (Kolte et al., 2011; Li et al., 2002a), including uterine abnormalities, endocrine disorders, paternal and fetal chromosomal abnormalities (Ljunger et al., 2011), autoantibodies, thrombophilia, as well as immunological and genetic disorders (Kolte et al., 2011; Li et al., 2002b). In up to 50% of recurrent spontaneous abortion cases, none of these factors are found in the couple, and is therefore considered idiopathic (Li et al., 2002c).

During the endometrial mid-secretory (receptive) phase, the limited time period of the implantation window ensures coordinated endometrial and embryonic development for successful implantation (Aghajanova et al., 2008; Altmäe et al., 2012). Increasing evidence shows that recurrent spontaneous abortion is a result of a selection failure in preventing 'poor quality' embryos from implanting, and being subsequently rejected as their development fails, leading to a later recurrent pregnancy loss (Quenby et al., 2002; Salker et al., 2010; Teklenburg et al., 2010b; Weimar et al., 2012). Furthermore, some endometrial factors can negatively affect the implantation process in women who have experienced recurrent spontaneous abortion (Coughlan et al., 2014; Maybin et al., 2011), causing alterations in factors essential for implantation and maintenance of full-term pregnancy (Lee et al., 2007). Recurrent spontaneous abortion might be a consequence of impaired decidualization, improper invasion or angiogenesis during the early establishment of pregnancy (Singh et al., 2012), thus being associated with aberrant expression of genes responsible for endometrial decidualization and inflammatory processes (Rull et al., 2012), ultimately leading to a prolonged endometrial receptive phase in women who have experienced recurrent spontaneous abortion (Salker et al., 2012).

Different association studies have searched for molecular markers for recurrent spontaneous abortion. Several polymorphisms within the genes involved in inflammation, thrombosis and cardiovascular system, detoxification system, immune response, hormonal regulation and placental function have been shown to be associated with recurrent spontaneous abortion, including tumour necrosis factor alpha gene, matrix metalloproteinase, tissue inhibitor of metalloproteinase, vascular endothelial growth factor, progesterone receptor, endocrine gland-derived vascular endothelial growth factor, p53, endothelial nitric oxide synthase, phosphodiesterase 8B, transforming growth factor beta 1, chorionic gonadotropin beta, annexin A5 gene, among other genes (Almawi et al., 2013; Eller et al., 2011; Granfors et al., 2012; Hayashi et al., 2013; Lee et al., 2013; Magdoud et al., 2013; Pereza et al., 2013; Rull et al., 2008, 2013; Schweikert et al., 2004; Singh et al., 2012; Su et al., 2010a, 2010b, 2011). Nevertheless, most of these findings are controversial, and have not been replicated in follow-up studies (Rull et al., 2012), so the search for molecular risk factors for recurrent spontaneous abortion continues.

In a previous genome expression analysis, we identified trefoil factor 3 (TFF3) as one factor that could have a role in the receptive endometrium at the time of embryo implantation (Altmäe et al., 2010). Trefoil factor 3, also known as intestinal trefoil factor, is a member of the trefoil factor mucin-associated peptides family that is produced in epithelial surfaces (Madsen et al., 2007; Samson et al., 2008), and is detected in the human endometrium (Borthwick et al., 2003; Kao et al., 2002). TFF3 possesses a mitogenic effect, which promotes epithelial cell migration during wound healing, and assists in the maintenance and restoration of the epithelial surface integrity (Thim, 1997; Williams and Wright, 1997). Furthermore, as TFF3 is expressed in almost all tissues containing mucus-secreting cells, an effect related to that of mucins is suggested (Kjellev, 2009; Wiede et al., 2001). It has previously been shown that women who have experienced spontaneous recrrent abortion have lower levels of endometrial mucins in the secretory phase compared with normal fertile women (Aplin et al., 1996). On the basis of these findings, the possible associations between recurrent spontaneous abortion susceptibility and genetic variation in the *TFF3* gene were analysed. Haplotype pattern within the *TFF3* gene and its association with recurrent spontaneous abortion were also analysed.

Materials and methods

Study participants

The present study was approved by the Ethical Review Boards at Uppsala University and Karolinska Institutet, Sweden on 1 May 2009 (International Review Board reference number: 2006/1545-31/4), and written informed consent was obtained from all participants. A total of 307 women participated in the study. Blood samples were collected from 164 patients with a mean age of 30.36 ± 5.71 years who had experienced three or more consecutive spontaneous abortions in the first or second trimester of pregnancy (5–21 weeks of gestation), and were recruited from the Departments of Obstetrics and Gynecology at the Uppsala University Hospital, Huddinge University Hospital, Karolinska University Hospital, and Danderyd University Hospital, Sweden (**Table 1**). Women with a history of spontaneous recurrent abortion diagnosed between the 1989 and 2009 were considered eligible, with the exclusion of patients with obvious risk factors