Article

Multiple thrombophilic gene mutations are risk factors for implantation failure

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Abstract

While the role of inherited thrombophilia has been accepted as a cause of recurrent late pregnancy complications, the contribution of mutated thrombophilic genes to implantation failure has not been studied. Proteins involved in fibrinolysis are necessary for trophoblast invasion into the endometrium. This study compared the prevalence of 10 thrombophilic gene mutations among 42 women with a history of recurrent implantation failure after IVF–embryo transfer with 20 fertile control women. Buccal swabs were taken from all of the women for DNA analyses. Women with a history of implantation failure after IVF–embryo transfer displayed a higher prevalence of PAI-1 4G/5G mutations than controls ($P = 0.007$). No differences in the frequency of the other specific gene mutations were detected. However, the prevalence of total gene mutations among patients with implantation failure was significantly higher than among controls. More than three gene mutations among the 10 genes studied were observed in 74% of women with implantation failure and 20% of controls ($P = 0.0004$). It is concluded that inherited thrombophilias are associated with implantation failure. This association is manifest by total number of mutations as well as with PAI-1 mutations.

Keywords: *embryo transfer, implantation failure, inherited thrombophilia, IVF, thrombophilic genes*

Introduction

Implantation is the rate-limiting factor for the establishment of pregnancy after IVF and embryo transfer. Successful implantation requires a blastocyst to interact with the endometrium. This interaction includes a variety of molecules secreted by human trophoblastic as well as endometrial cells. The cross-talk between the implanting blastocyst and the endometrium involves either cell-to-cell or cell-to-extracellular matrix interactions, which are mediated by matrix metalloproteinases, cytokines and growth factors (Polan *et al.*, 1995; Klemtzeris, 1997; Salamonsen *et al.*, 2000; Herrler *et al.*, 2003; Nardo *et al.*, 2003). Molecular interactions involving the coagulation and fibrinolytic systems at the embryo–maternal interface during the times of adhesion and invasion have been shown to play an important role in these communications (Axelrod, 1985; Feng *et al.*, 2000, 2001; Chung *et al.*, 2001; Whiteside *et al.*, 2001; Solberg *et al.*, 2003; Aflalo *et al.*, 2004). These findings lead to the question of what influence, if any, inherited thrombophilic gene mutations would have on implantation. While the role of inherited thrombophilic genes has been accepted as a risk factor for difficulties in maintaining pregnancy (Ridker *et al.*, 1998; Foka *et al.*, 2000; Younis *et al.*, 2000; Pihusch *et al.*, 2001; Reznikoff-Etievan *et al.*, 2001; Finan *et al.*, 2002), their contribution to problems in establishing pregnancy is not known. The present study was undertaken to compare the prevalence of 10 thrombophilic gene mutations among women with a history of recurrent implantation failure after IVF–embryo transfer and fertile control women.

Materials and methods

Ten thrombophilic gene mutations, identified from the existing literature to be associated with adverse pregnancy outcomes,

were investigated. The thrombophilic markers were: factor V [G1691A; Leiden] (Casroldi *et al*., 2000; Ozcan *et al*., 2001; Buchholz and Thaler, 2003); factor V [H1299R (R2)] (Castoldi *et al*., 2000; Buchholz and Thaler, 2003); factor V [Y1702C] (Castoldi *et al*., 2000; Buchholz and Thaler, 2003); factor II prothrombin G20210A] (Castoldi *et al*., 2000; Ozcan *et al*. 2001; Buchholz and Thaler, 2003); factor XIII [V34L] (Pasrinen *et al*., 1998; Kohler *et al*., 1999; Kakko *et al*., 2002; Buchholz and Thaler, 2003); β-fibrinogen [–455G→A] (Behague, 1996; Kohler *et al*., 1999); plasminogen activator inhibitor-1 (PAI-1) [4G/5G] (Eriksson, 1995; Pasrinen *et al*., 1998; Sartori *et al*. 1998; Gluek *et al*., 2000b; Buchholz and Thaler, 2003); human platelet antigen 1 (HPA1) [a/b9L33P)] (Pasrinen *et al*., 1998; Feng *et al*. 1999); methylenetetrahydrofolate reductase (MTHFR) [C677T] (Pasrinen *et al*., 1998; Ozcan *et al*. 2001; Bojesen *et al*., 2003; Buchholz and Thaler, 2003); MTHFR [A1298C] (Weisberg *et al*., 1998) (see **Table 1**).

Patients

Forty-two women with a history of recurrent implantation failure after IVF–embryo transfer were included in the study. Recurrent implantation failure was defined in this study as a total of eight cleaved embryos transferred or four blastocysts transferred with human chorionic gonadotrophin (HCG) serum concentrations <5 mIU/ml 14 days after embryo transfer (Coulam, 1995). Both male and female partners of couples experiencing recurrent implantation failure were evaluated. Semen analysis and sperm DNA integrity assay were performed on all male partners. All women were examined by hysterosonography or hysterosalpingogram for detection of anatomic abnormalities of the uterine cavity. Records of previous IVF–embryo transfer cycles were reviewed.

Twenty fertile women served as controls. Buccal swabs were obtained from all women and analysed for 10 thrombophilic gene mutations. All women entered into the study were Caucasian.

Thrombophilia panel

DNA was extracted from the buccal swab samples using the Qiagen DNA Mini Kit (Qiagen, Crawley, UK), and followed by multiplex polymerase chain reaction (PCR) amplification according to established protocols (Sambrook and Russell, 2001). PCR products were analysed for the 10 respective genetic markers using standard methods, including reverse hybridization and agarose gel electrophoresis (with ethidium bromide staining).

Statistical analysis

The frequencies of homozygous and total number of thrombophilic gene mutations were compared between women experiencing recurrent implantation failures and controls using a 2 × 2 contingency table with Fisher's exact test. In calculating the total number of gene mutations, a heterozygous mutation was considered as one gene mutation and a homozygous mutation was considered as two gene mutations. A two-tailed P-value < 0.05 was considered significant.

Results

Patients

The mean age of female partners experiencing recurrent implantation failure was 36.7 years and the number of previously failed IVF cycles was 4.3. All of the women had normal hysterosonographic findings and all had endometrial linings measuring >8 mm during previous IVF cycles. One of the study patients and none of the controls had a diagnosis of polycystic ovarian syndrome. All of the male partners had normal semen parameters on standard semen analysis, as well as normal DNA fragmentation indexes determined by the sperm DNA integrity assay. All couples had a total of at least eight cleaved embryos judged by the attending embryologist as 'good quality' or four viable blastocysts previously transferred.

Thrombophilia gene mutations

The frequencies of heterozygous mutations among 10 thrombophilic genes tested are shown in **Figure 1**. No differences in specific gene mutations were observed when patients experiencing recurrent implantation failure were compared with control women. **Figure 2** illustrates the prevalence of homozygous mutations. PAI-1 was the only specific gene to show a significant difference between women with a history of recurrent implantation failure and controls. Of the 42 women with implantation failure, 16 (38%) were homozygous for PAI-1 4G/4G compared with two of 20 (10%) of control women ($P = 0.03$). In addition, women experiencing recurrent implantation failure displayed significantly more total homozygous mutations (31/42 or 74%) than control women (4/20 or 20%) ($P = 0.007$).

When the total number of mutations was compared counting a heterozygous mutation as one gene mutation and a homozygous mutation as two mutations, women experiencing recurrent implantation failure demonstrated significantly more total mutations than control women (74 versus 20%, $P = 0.0004$) (**Figure 3**).

Table 1. Summary of specific gene mutations and polymorphisms affecting haemostasis included in the study.

Thrombophilic marker	*Specific gene*
Coagulation	Factor V
	Factor V Y1702C
	Factor V G1691A (Leiden)
	Factor V H1299R (R2)
	Factor II
	Prothrombin G20210A
	β-Fibrinogen 0455G/A
	Factor XIII V34L
Fibrinolysis	PAI 1 4G/5G
Thrombosis	HPA 1
	MTHFR
	C677T
	A1298C