Vascular endothelial growth factor gene polymorphisms and idiopathic recurrent pregnancy loss

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Objective: To investigate whether four common polymorphisms (-2578C/A, -1154G/A, -634G/C, and 936C/T) of the gene encoding for vascular endothelial growth factor (VEGF) are associated with idiopathic recurrent miscarriage.
Design: Prospective case–control study.
Setting: University teaching hospital.
Patient(s): Fifty-two patients with a history of three or more unexplained consecutive pregnancy losses and 82 healthy, postmenopausal controls with at least two live births and no history of pregnancy loss.
Intervention(s): None.
Main Outcome Measure(s): Polymerase chain reaction and restriction fragment length polymorphism analysis were performed to identify the different VEGF alleles.
Result(s): There was a significant difference in the -1154G/A genotype and allele frequency between women with recurrent pregnancy loss and controls. The risk of recurrent pregnancy loss was lower in the carriers of the G allele than in women carrying the A allele (odds ratio = 1.91, 95% confidence interval, 0.12–3.28). No significant association between recurrent spontaneous abortions and -2578C/A, -634G/C, and 936C/T genotypes was found. Between women with primary and secondary idiopathic recurrent miscarriage, no statistically significant differences with respect to allele frequencies were observed.
Conclusion(s): This is the first report on VEGF gene polymorphisms in women with recurrent miscarriage, demonstrating that the -1154G/A VEGF gene polymorphism is associated with idiopathic recurrent abortions. (Fertil Steril® 2005;83:959–63. ©2005 by American Society for Reproductive Medicine.)

Key Words: Recurrent abortion, vascular endothelial growth factor, polymorphism

Recurrent spontaneous abortions (RSA) are a frequent reproductive problem, with three or more affecting 1% to 2% and two or more affecting up to 5% of women of reproductive age (1). A series of etiologic factors, including parental chromosomal abnormalities, uterine abnormalities, hereditary thrombophilias, endocrinologic disorders, immunologic factors, infections, and nutritional and environmental factors have been identified for this condition (2). In up to 50% of cases, however, the exact underlying pathophysiologic mechanisms remain undetermined (2, 3).

There is strong evidence supporting a close relationship between embryonic development and the state of vascularization of the chorionic villi. Normal chorionic villous vascularization is essential for the undisturbed development of pregnancy (4, 5). It is not known, however, whether abnormal changes in utero–placental vascular development predispose to recurrent miscarriages. Diminished immunoreactivity of placental trophoblastic vascular endothelial growth factor (VEGF) has been described in decidual endothelium of spontaneous miscarriages (6), and increased blood vessel density in decidua parietalis has been associated with spontaneous human first-trimester abortion (7).

The VEGF/VEGF receptor system is the best characterized regulator of angiogenesis. The VEGF family of proteins includes VEGF-A (VEGF), VEGF-B, VEGF-C, VEGF-D, the placenta growth factor, and their receptors VEGFR-1/Flt-1, VEGFR-2/KDR, and VEGFR-3/Flt-4 (8, 9). Polymorphisms of the VEGF gene have been identified and have been correlated with variation in VEGF protein production

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TABLE 1

Genotype frequencies of −2578A/C, −1154G/A, −634G/C, and 936C/T polymorphisms among women with RSA and controls.

Genotypes	RSA (n = 52)	Controls (n = 82)	*P*
−2578A/C			
CC	15 (28.9)	27 (33)	CC vs. AA: .65
AC	21 (40.4)	34 (41.4)	AC vs. AA: .78
AA	16 (30.7)	21 (25.6)	AA vs. CC, AC: .65
−1154G/A			
AA	15 (28.8)	12 (14.6)	AA vs. GG: .04
GA	19 (36.5)	28 (34.1)	GA vs. GG: .35
GG	18 (34.6)	42 (51.2)	GG vs. AA, GA: .09
−634G/C			
CC	16 (30.7)	18 (21)	CC vs. GG: .29
GC	22 (42.3)	35 (42.7)	GC vs. GG: .68
GG	14 (26.9)	29 (35.3)	GG vs. CC, GC: .4
936C/T			
TT	1 (1.9)	1 (1.2)	TT vs. CC: —
CT	16 (30.7)	17 (20.7)	CT vs. CC: —
CC	35 (67.3)	64 (78)	CC vs. TT, CT: .24

Note: Data are presented as n (%). RSA = recurrent spontaneous abortion.

Papazoglou. VEGF and recurrent abortion. Fertil Steril 2005.

(10–14). In the present study, we sought to establish an association between four common functional VEGF single nucleotide polymorphisms, -2578C/A and -1154G/A in the promoter region (10, 11), -634G/C in the 5′-untranslated region (13), and 936C/T in the 3′-untranslated region (14), and the occurrence of RSA.

MATERIALS AND METHODS

The study was approved by the local ethics committee, and informed consent from all subjects was obtained. Institutional review board approval was also obtained. The study group consisted of 52 women (age range, 23–45 years; median, 33 years) who had a documented history of at least three consecutive spontaneous abortions occurring before 20 weeks' gestation, with the same partner, and in whom anatomic, hormonal, chromosomal, infectious, or autoimmune causes had been excluded. The median number of miscarriages was 3 (range, 3–5), whereas the mean number of live births was 0.3 (range, 0–3). Thirty-eight of the women had never had a pregnancy carried to term, whereas fourteen had. The control group consisted of 82 women, each of whom had had at least two live births and no history of miscarriage. All control women were postmenopausal to exclude possible future miscarriages after inclusion in the study.

Deoxyribonucleic acid was isolated from 200 μL of anticoagulated peripheral blood with a commercially available kit, according to the manufacturer's instructions (QIAamp DNA Blood Mini Kit; QIAGEN, Valencia, CA). Amplification of the four regions of the VEGF gene containing the polymorphisms -2578C/A, -1154, -634G/C, and 936C/T were carried out in a thermal cycler (Mastercycler gradient; Eppendorf, Hamburg, Germany).

For the -2578C/A polymorphism the following specific/common primers were used, generating a polymerase chain reaction (PCR) product of 77 base pairs (bp): 5′-TAGGCCAGACCCTGGCAC-3′ or 5′-TAGGCCAGACCCTGGCAA-3′ with 5′-TGCCCCAGGGAACAAAGT-3′, whereas for the -1154G/A polymorphisms the following specific/common primers were used, generating a PCR product of 130 bp: 5′-GCCCGAGCCGCGTGTGGAG-3′ or 5′-GCCCGAGCCGCGTGTGGAA-3′ with 5′-CCCCGCTACCAGCCGACTT-3′. For the -634G/C the following primers amplified a fragment of 304 bp: forward 5′-ATTTATTTTTGCTTGCCA-3′, reverse 5′-GTCTGTCTGTCTGTCCGTCA-3′; and for the 936C/T the following primers amplified a fragment of 208 bp: forward 5′-AAGGAAGAGGAGACTCTGCGCAGAGC-3′, reverse 5′-TAAATGTATGTATGTGGGTGGGTGTGTCTACAGG-3′. The VEGF -634G/C polymorphism was analyzed by digestion of the PCR product with restriction endonuclease *BsmF*I (New England Biolabs, Beverly, MA). The -634G allele was cut into two fragments of 193 bp and 111 bp, whereas the -634C allele remained uncut (304 bp). The VEGF 936C/T polymorphism was analyzed by digestion of the PCR product with restriction endonuclease *Nla*III (New England Biolabs). The 936C allele remained uncut (208 bp), whereas the 936T