A Prospective Case–Control Study Analyzes 12 Thrombophilic Gene Mutations in Turkish Couples with Recurrent Pregnancy Loss

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Introduction
Recurrent pregnancy loss (RPL) represents a significant clinical problem affecting 2% of women.¹ The pathophysiology of RPL is complex and poorly understood. Genetic, infective, anatomical, endocrine, and immune defects have been postulated as causes for RPL.² However, even after detailed investigation, as many as 80% of all cases remain unexplained.³ Thrombophilias have been postulated as a cause of RPL.⁴ Five studies in the recent years have examined the incidence of specific thrombo-

Keywords
Couples, mutation, recurrent pregnancy loss, thrombophilic genes

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Submitted April 24, 2009; accepted October 1, 2009.

Citation
doi:10.1111/j.1600-0897.2009.00770.x

Problem
Recurrent pregnancy loss (RPL) is a heterogeneous disorder. The contribution of specific thrombophilic genes to the pathophysiology of RPL has remained controversial. We evaluated the prevalences of 12 thrombophilic gene mutations among homogenous Caucasian couples with RPL and fertiles.

Method of study
This was a prospective case–control study evaluating 272 women with RPL and 152 of their male partners, and a control group of 56 fertile couples. We investigated mutations including FV Leiden, factor V H1299R, factor II prothrombin G20210A, F XIII V34L, β-fibrinogen ~455G>A, plasminogen activator inhibitor-1, GPIIIa L33P (HPA-1 a/b L33P), MTHFR C677T, MTHFR A1298C, ACE I/D, Apo B R3500Q, and Apo E.

Results
Overall, heterozygous mutations of FV Leiden, FXIII V34L, GPIIIa L33P, Apo E4, and prothrombin G20210A and homozygous mutations of PAI-1 and MTHFR C677T were associated with RPL. There was no meaningful association between RPL and other studied genes.

Conclusion
In contrast to the other mutations and polymorphisms, FV Leiden, FXIII V34L, GPIIIa L33P, Apo E, prothrombin G20210A, PAI-1 and MTHFR C677T gene mutations may help to identify the couples at risk for recurrent pregnancy loss.
TEN GENE MUTATIONS IN COUPLES WITH RECURRENT PREGNANCY LOSS

The present study aimed (i) to compare the prevalence of 12 thrombophilic gene mutations in couples with a history of recurrent miscarriage with fertile control couples with no history of miscarriage and (ii) to identify which mutations were necessary to identify the risk of RPL.

Materials and methods

Patients

This was a prospective case-control study conducted in the gynecology clinic at Cumhuriyet University Hospital. The study was approved by the local ethics committee of Cumhuriyet University and informed consent was obtained from all of the subjects. During the study period, between May 2006 and December 2008, 272 women with a history of RPL, defined as two or more consecutive early RPL at 5–12 weeks of gestation (determined either by the ultrasound or last menstrual period) by a single spouse with no history of full term pregnancies before or after the RPLs during the study period, were recruited as RPL study group. And also 152 male partners of women in RPL study group, who were available for blood withdrawn, were included. A control group of 56 parous, age-matched couples with at least two successful pregnancies and with no history of miscarriage was recruited from post-natal wards.

Chromosome analyses were performed on both female and male subjects of all groups in the study. In particular, all women with RPL were examined by ultrasonography or hysterosalpingogram for detection of anatomic abnormalities of the genital tract and had blood drawn for testing for immunologic risk factors including antiphospholipid antibodies, antinuclear antibodies, antithyroid antibodies, and lupus anticoagulant. In 272 female and 152 male participants, peripheral blood karyotyping was performed. Women who had anatomic abnormalities, endocrinologic dysfunction, autoimmune disease, and urogenital infections were excluded.

Genes

Twelve thrombophilic gene mutations, identified from the existing literature to be associated with RPL were investigated. The thrombophilic markers are: factor V G1691A (FV Leiden), factor V H1299R, factor II prothrombin G20210A (prothrombin G20210A), factor XIII V34L, factor XIII V34L (FXIII V34L), β-fibrinogen –455G>A (β-fibrinogen), plasminogen activator inhibitor-1 (PAI-1), GPIIa L33P (HPA-1 a/b L33P), methylenetetrahydrofolate reductase C677T (MTHFR C677T), methylenetetrahydrofolate reductase C677T (MTHFR C677T), ACE I/D, Apo B R3500Q, and Apo E (E2, E3, E4). The thrombophilic mutations, which were considered in this study, are listed in Table 1. Apo E mutation was referring to E4 genotype of Apo E. For the ACE gene, D allele mutation was evaluated as the suggested risk factor.

Mutation Analysis

 Blood was drawn from the antecubital vein from all participants and analyzed for 12 thrombophilic gene mutations. Peripheral blood tissues containing EDTA from patients and healthy donors (1 mL) were used for genomic DNA isolation. Total genomic DNA was extracted from peripheral blood samples from each couple by the Invitrek kit extraction technique (Invitrek).
tek®; Invisorb spin blood, Berlin, Germany) and remained stored at −20°C until genetic analysis was performed. Twelve thrombophilic genes (FV Leiden, FV H1299R, prothrombin G20210A, FXIII V34L, β-fibrinogen -455G>A, PAI-1, GPIIa L33P, MTHFR C677T, MTHFR A1298C, ACE I/D, Apo B, and Apo E) were simultaneously amplified in a biotin-labeled single multiplex amplification reaction (Viennalab®; PGX-HIV StripAssay, Vienna, Austria) which is based on the reverse-hybridization principle automatically and evaluated for possible mutations. The multiple polymerase chain reaction (PCR) was performed in a Perkin Elmer 9600 and the profile consisted of an initial melting step of 2 min at 94°C; followed by 35 cycles of 30 s at 94°C, 30 s at 61°C, and 30 s at 72°C; and a final elongation step of 7 min at 72°C. The normal, heterozygous and homozygous mutant genotype profiles of each of the genes were determined using the enclosed Collector™ sheet.

During evaluation of heterozygosity and homozygosity of female and male subjects, we used a classification as no risk (no mutation in the couple), mild-risk (couples having at least one heterozygous mutation but not any homozygous mutation) and high-risk (couples having at least one homozygous mutation).

### Statistical Analysis

Statistical analysis was performed using SPSS version 16 (SPSS, Chicago, IL, USA). The frequencies of homozygous and heterozygous thrombophilic gene mutations, the frequencies of allelic mutations in female and male participants and the mild- or high-risk couples experiencing RPL and controls were compared using chi-square analysis. In calculating the allelic mutations, a heterozygous mutation was considered as one gene mutation and a homozygous mutation as two gene mutations.

#### Table I The Studied Genes Which Were Considered Genetic Risk Factor for Thrombosis in the Study

<table>
<thead>
<tr>
<th>Name</th>
<th>Mutation</th>
<th>Pathologic mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>FV Leiden</td>
<td>1691 G→A substitution in the gene of coagulation factor V</td>
<td>Blocked inactivation of factor Va by activated protein C, resulting in reduced clearance of factor Va</td>
</tr>
<tr>
<td>FVH 1299R</td>
<td>4070 A→G transition</td>
<td>Carriership of the FV H1299R allele is associated with mild APC resistance and with a relative excess of the more thrombogenic FV isoform (FV1) in plasma.</td>
</tr>
<tr>
<td>Prothrombin G20210A</td>
<td>20210 G→A substitution in the 3′-untranslated region of the prothrombin gene</td>
<td>Elevated prothrombin levels in plasma</td>
</tr>
<tr>
<td>FXIII V34L</td>
<td>G→T transition in exon 2 coding for a substitution of valine with leucine at residue 34.</td>
<td>Activated factor XIII (FXIII) crosslinks fibrin to enhance the mechanical strength of a blood clot and increase its resistance to fibrinolysis.</td>
</tr>
<tr>
<td>β-Fibrinogen – 455G&gt;A</td>
<td>−455 G→A substitution in the promoter of the gene for the β-chain</td>
<td>A/A genotype causes higher plasma fibrinogen levels</td>
</tr>
<tr>
<td>PAI-1</td>
<td>4G polymorphism in the PAI-1 promoter, 675 bp upstream from the start site of transcription</td>
<td>Key regulating element in the fibrinolysis cascade</td>
</tr>
<tr>
<td>GPIIIa L33P</td>
<td>1565 C→T substitution in the gene for platelet GPIIIa</td>
<td>Platelet GPIIIa is essential for aggregation and thrombus formation</td>
</tr>
<tr>
<td>MTHFR C677T</td>
<td>Cytosine-to-thymine substitution at base 677 that causes a substitution of valine for alanine</td>
<td>Thermolabile variant the enzyme with reduced catalytic activity and elevated plasma levels of homocysteine</td>
</tr>
<tr>
<td>MTHFR A1298C</td>
<td>Glutamate-to-alanine A1298C</td>
<td>Combined heterozygosity with MTHFR C677T mutation is associated with hyper-homocysteinemia, which is as a risk factor for venous and arterial thrombosis</td>
</tr>
<tr>
<td>ACE I/D</td>
<td>Deletion (D) polymorphism in intron 16 of the gene</td>
<td>D allele leads to an increased PAI-1 expression, resulting in reduced fibrinolysis</td>
</tr>
<tr>
<td>Apo B</td>
<td>Apolipoprotein B R3500Q mutation</td>
<td>Elevated APO B level is significantly associated with platelet-dependent thrombosis</td>
</tr>
<tr>
<td>Apo E</td>
<td>The Apo E gene has three alleles – epsilon 2 (E2), epsilon 3 (E3), and epsilon 4 (E4) – on the long (q) arm of chromosome 19 at position 13.2</td>
<td>Individuals carrying the E4 allele have a higher total cholesterol level whereas those harboring the E2 allele have a lower total cholesterol level than those with E3/E3</td>
</tr>
</tbody>
</table>
mutation was considered as two gene mutations. A value of \( P < 0.05 \) was considered as statistically significant.

Results

Patients

The demographic details and outcomes of previous pregnancies of the study subjects in the RPL and control groups were summarized in Table II. The mean age of the female partners experiencing RPL was \( 27.2 \pm 5.6 \) years and the number of previous miscarriages were ranged from 2 to 8.

Thrombophilic Gene Mutations

Fig. 1 presents the frequencies of heterozygous mutations of the studied genes in the RPL and control women. The frequencies of heterozygous mutations for FXIII V34L and Apo E (Apo E4/Apo E3) were significantly higher in RPL women compared with control women (\( P < 0.05 \)). The frequency of heterozygous mutation for prothrombin G20210A was 7\% in RPL women, however there was no heterozygous mutation in control women (\( P < 0.05 \)). For FV Leiden, FV H1299R, and GPIIIa L33P genes, the frequencies of heterozygous mutations were higher but not significantly different in RPL women compared with the control women (\( P > 0.05 \)). The frequencies of heterozygous mutations for \( \beta \)-fibrinogen \( 455G \mapsto A \), PAI-1, MTHFR C677T, MTHFR A1298C, and ACE I/D genes were similar in the RPL and control women.

Fig. 2 shows the frequencies of homozygous mutations of the studied genes in the RPL and control women. The frequencies of homozygous mutations for \( \beta \)-fibrinogen \( 455G \mapsto A \), PAI-1 4G/4G and MTHFR C677T were 6.2\%, 21.7\% and 10.8\% respectively in RPL women, whereas in control women they were 1.2\%, 9.2\% and 8.2\% respectively. The frequencies of homozygous mutations for FXIII V34L and Apo E were significantly higher in RPL women compared with control women (\( P < 0.05 \)).
respectively in the RPL women, but there was no homozygous mutation for those genes in the control women ($P < 0.05$). For MTHFR A1298C, the frequency of homozygous mutation was higher but not significantly different in RPL women compared with the control women ($P > 0.05$). The frequencies of homozygous mutations for prothrombin G20210A was significantly lower in RPL men compared with control men ($P < 0.05$).

Fig. 3 demonstrates the frequencies of heterozygous mutations of the studied genes in the RPL and control men. The frequencies of heterozygous mutations for prothrombin G20210A was significantly lower in RPL men compared with control men ($P < 0.05$). The frequency of heterozygous mutation for FV Leiden was 19.1% in the RPL women, but there was no heterozygous mutation in the control women ($P < 0.05$). For FXIII V34L, $\beta$-fibrinogen -455G>A, PAI-1, GPIIa L33P, MTHFR C677T, MTHFR A1298C, ACE I/D, Apo E, the frequency of heterozygous mutation for FV Leiden was significantly higher in RPL men compared with control men ($P < 0.05$). The frequencies of heterozygous mutations for prothrombin G20210A was significantly lower in RPL men compared with control men ($P < 0.05$). For ACE I/D gene, the frequency of homozygous mutation was higher but not significantly different in RPL men compared with the control men ($P > 0.05$). The frequencies of homozygous mutations for FV H1299R and ACE I/D genes were similar in the RPL and control women.

Fig. 4 presents the frequencies of homozygous mutations of the studied genes in the RPL and control men. The frequency of homozygous mutation for PAI-1 was significantly higher in RPL men compared with control men ($P < 0.05$). The frequency of homozygous mutation for MTHFR C677T was 13.8% in the RPL men, but there was no homozygous mutation in the control men ($P < 0.05$). For ACE I/D gene, the frequency of homozygous mutation was higher but not significantly different in RPL men compared with the control men ($P > 0.05$). The frequencies of homozygous mutations for FV H1299R, prothrombin G20210A, $\beta$-fibrinogen -455G>A, PAI-1, GPIIa L33P, MTHFR C677T, MTHFR A1298C, ACE I/D, and Apo E were similar in the RPL and control men.

The total number of mutated alleles was calculated by doubling the number of homozygous recessives and adding the heterozygotes. This was compared with the total number of non-mutated alleles, which was computed by doubling the number of homozygous wild-type individuals and adding the heterozygotes. The results were the frequencies of allelic mutated genes in both RPL and control subjects. The data of the comparison between RPL subjects and controls are shown in Table III. The frequency of allelic mutation in women for Apo E was significantly higher in the RPL women compared with the control women ($P < 0.05$). The frequencies of allelic mutations in women for FV Leiden, FV H1299R, FXIII V34L, $\beta$-fibrinogen -455G>A, PAI-1, GPIIa L33P, MTHFR C677T in the RPL women (7.2%, 6.8%, 19.9%, 55.7%, 12.5%, and 30.9%, respectively) were higher (3.6%, 3.6%, 10.7%, 39.3%, 7.1%, and 21.4%) but not significantly different compared to the control women. The frequencies of allelic mutations for $\beta$-fibrinogen -455G>A, MTHFR A1298C and ACE I/D were similar in the RPL and control women.
The frequencies of allelic mutations in men for FXIII V34L, PAI 1, GPIIIa L33P, MTHFR C677T and ACE I/D in the RPL men (21.4%, 60.9%, 10.9%, 33.7%, and 58.2%, respectively) were higher (11.5%, 50%, 7.1%, 30.8%, and 50%) but not significantly different compared to the control men. The frequency of allelic mutation in women for FV Leiden was 11.5% in the RPL men, but there was no mutation in the control men (P < 0.05). The frequencies of allelic mutations for FV H1299R, prothrombin G20210A, β-fibrinogen 455G>A, MTHFR A1298C and Apo E were similar in the RPL and control men.

Table III The Frequencies of Allelic Mutated Genes in Both RPL and Control Subjects (Counting a Heterozygous Mutation as One Mutation and a Homozygous Mutation as Two Mutations)

<table>
<thead>
<tr>
<th>Thrombophilic genes</th>
<th>RPL women % (n = 272)</th>
<th>Control women % (n = 56)</th>
<th>RPL men % (n = 152)</th>
<th>Control men % (n = 56)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FV Leiden</td>
<td>7.2</td>
<td>3.6</td>
<td>11.5\†</td>
<td>0</td>
</tr>
<tr>
<td>FV H1299R</td>
<td>6.8</td>
<td>3.6</td>
<td>6.9</td>
<td>7.1</td>
</tr>
<tr>
<td>Prothrombin G20210A</td>
<td>4.2</td>
<td>0</td>
<td>2.3</td>
<td>7.1</td>
</tr>
<tr>
<td>FXIII V34L</td>
<td>19.9</td>
<td>10.7</td>
<td>21.4</td>
<td>11.5</td>
</tr>
<tr>
<td>β-fibrinogen −455G&gt;A</td>
<td>22.4</td>
<td>25</td>
<td>23.7</td>
<td>28.6</td>
</tr>
<tr>
<td>PAI-1</td>
<td>55.7</td>
<td>39.3</td>
<td>60.9</td>
<td>50</td>
</tr>
<tr>
<td>GPIIa L33P</td>
<td>12.5</td>
<td>7.1</td>
<td>10.9</td>
<td>7.1</td>
</tr>
<tr>
<td>MTHFR C677T</td>
<td>30.9</td>
<td>21.4</td>
<td>33.7</td>
<td>30.8</td>
</tr>
<tr>
<td>MTHFR A1298C</td>
<td>42.4</td>
<td>39.3</td>
<td>35.1</td>
<td>35.7</td>
</tr>
<tr>
<td>ACE I/D</td>
<td>54</td>
<td>53.6</td>
<td>58.2</td>
<td>50</td>
</tr>
<tr>
<td>Apo E</td>
<td>20*</td>
<td>2.9</td>
<td>28</td>
<td>48.2</td>
</tr>
</tbody>
</table>

The frequency of allelic mutation in women for Apo E was significantly higher in RPL women compared with control women and the frequency of allelic mutation in men for FV Leiden was significantly higher in RPL men compared with control men (P < 0.05).

Table IV The Risk Classification of RPL and Control Couples

<table>
<thead>
<tr>
<th>Thrombophilic genes</th>
<th>Risk</th>
<th>RPL % (n = 152)</th>
<th>Control % (n = 56)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FV Leiden</td>
<td>Mild risk</td>
<td>26.9\†</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>High risk</td>
<td>1.4</td>
<td>0</td>
</tr>
<tr>
<td>FV H1299R</td>
<td>Mild risk</td>
<td>23.9</td>
<td>19.6</td>
</tr>
<tr>
<td></td>
<td>High risk</td>
<td>0.6</td>
<td>0</td>
</tr>
<tr>
<td>Prothrombin G20210A</td>
<td>Mild risk</td>
<td>11.4</td>
<td>15.4</td>
</tr>
<tr>
<td></td>
<td>High risk</td>
<td>0.6</td>
<td>0</td>
</tr>
<tr>
<td>FXIII V34L</td>
<td>Mild risk</td>
<td>54.3</td>
<td>41.7</td>
</tr>
<tr>
<td></td>
<td>High risk</td>
<td>4.6</td>
<td>0</td>
</tr>
<tr>
<td>β-fibrinogen −455G&gt;A</td>
<td>Mild risk</td>
<td>53.4</td>
<td>53.8</td>
</tr>
<tr>
<td></td>
<td>High risk</td>
<td>8.5</td>
<td>15.4</td>
</tr>
<tr>
<td>PAI-1</td>
<td>Mild risk</td>
<td>15.3</td>
<td>23.1</td>
</tr>
<tr>
<td></td>
<td>High risk</td>
<td>44.3*</td>
<td>7.7</td>
</tr>
<tr>
<td>GPIIa L33P</td>
<td>Mild risk</td>
<td>36.7</td>
<td>23.1</td>
</tr>
<tr>
<td></td>
<td>High risk</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>MTHFR C677T</td>
<td>Mild risk</td>
<td>58</td>
<td>81.8</td>
</tr>
<tr>
<td></td>
<td>High risk</td>
<td>18.1\†</td>
<td>0</td>
</tr>
<tr>
<td>MTHFR A1298C</td>
<td>Mild risk</td>
<td>59.1</td>
<td>69.2</td>
</tr>
<tr>
<td></td>
<td>High risk</td>
<td>25.6</td>
<td>30.8</td>
</tr>
<tr>
<td>ACE I/D</td>
<td>Mild risk</td>
<td>25</td>
<td>23.1</td>
</tr>
<tr>
<td></td>
<td>High risk</td>
<td>52.8</td>
<td>46.2</td>
</tr>
<tr>
<td>Apo E</td>
<td>Mild risk</td>
<td>31.1</td>
<td>42.5</td>
</tr>
<tr>
<td></td>
<td>High risk</td>
<td>4.9</td>
<td>0</td>
</tr>
</tbody>
</table>

Mild-risk ratio for FV Leiden and high-risk ratio for PAI-1 and MTHFR C677T were significantly higher in RPL couples compared with control couples (P < 0.05).\†P < 0.0001 versus control women group.\*P < 0.0001 versus control men group.
There was no mutation in the Apo B gene in patients and controls, thus, this gene was removed for further calculations.

Discussion

This is the first study to report the prevalence of 12 thrombophilic gene mutations in both the female and male partners of couples with recurrent pregnancy loss. We found that the heterozygous mutations in FXIII V34L, FV Leiden, GPIIIa L33P and the homozygous mutations of PAI 1 and MTHFR C677T genes were higher in couples with RPL. Also the heterozygosity of Apo E, prothrombin G20210A, and FV H1299R and the homozygosity of fibrinogen were higher in only females with RPL. The heterozygosity of fibrinogen and the homozygosity of ACE I/D was higher in only males with RPL. This conclusion was supported by the data that the allele frequencies of PAI-1, MTHFR C677T, FXIII V34L, GPIIIa L33P, and FV Leiden were more often in couples with RPL. The allele frequency of Apo E was higher in only females with RPL. The mild-risk ratios, mainly heterozygosity, for FV Leiden, FXIII V34L, and GPIIIa L33P, and FV Leiden were more often in couples with RPL. The allele frequency of Apo E was higher in only females with RPL. The high-risk ratios, mainly homozogosity, for FV H1299R and the homozygosity of fibrinogen and the homozygosity of ACE I/D were higher in only females with RPL. This conclusion was supported by the data that the allele frequencies of PAI-1, MTHFR C677T, and Apo E and were higher in couples with RPL. The prevalences of some of the genes were significant but not meaningful because the yield was very low especially in homozygosity.

The prevalence studies on FV Leiden and FII prothrombin G20210A mutations in women with recurrent miscarriages found that both FV Leiden and FII G20210A mutations are major inherited risk factors associated with RPL. Some other studies, however, suggested that there were no statistical differences in the prevalence of FV Leiden and/or prothrombin G20210A between the patient and control groups. In a Turkish study by Altintas et al., the frequencies of carriers of FV Leiden in patients with RPL and controls were 7.9% and 7%, respectively. We found a mild to moderate association between the prevalence of carriers of FV Leiden and prothrombin G20210A and homozygous MTHFR C677T mutated RPL patients and recurrent miscarriage.

For the isolated occurrence of FXIII Val34Leu, no statistically significant difference between the cases of RPL and controls has been found. In the study by Goodman et al., however, the frequencies of mutations for FXIII Val34Leu were significantly increased among women experiencing RPL. Our data showed that heterozygous mutation of FXIII Val34Leu in RPL was significantly higher especially in RPL women. Finally, we demonstrated a moderate to severe association between FXIII Val34Leu and RPL.

Homozygosity for the deletion genotype (4G/4G) has been associated with PAI-1 concentrations higher than those associated with the insertion genotype (5G/5G), and hence with reduced fibrinolytic activity. In the previous study, the prevalence of the PAI-1 polymorphism in cases was similar for 4G/5G and homozygous 4G in controls as in the study by Wolf et al. In the current study, we found that, homozygosity and the high-risk-couples in the PAI-1 gene was significantly higher in RPL. So, we demonstrated a moderate association between homozygosity of PAI-1 and RPL.

Platelet GPIIIa is essential for thrombus formation. In the study by Pithus et al., the prevalence of GPIIIa mutations were similar in the recurrent pregnancy loss and fertiles. In the current study, in women, we found that the 1565 C/T (PIA1/A2) genotypes of platelet GPIIIa L33P in RPL patients and in controls were higher which means a
mild to moderate association between GPIIIa L33P mutation and RPL.

ACE interferes with hemostasis through different mechanisms, including an influence on fibrinolysis, platelet aggregation and blood clotting. Evidence exists for an association between the ACE D/D genotype and increased risk of thrombotic events. In the previous study by Vettriselvi et al., they found no significant difference in the frequency of the deletion allele between the patients and controls. Data by Fatini et al. showed the ACE I/D polymorphism to be a stronger risk factor for RPL. Buchholz et al. reported that the homozygosity for the D allele of ACE results in elevated PAI-1 concentration and thus associated with an elevated risk of RPL in Caucasians. In the current study, we could not find any association between ACE I/D mutation and RPL.

The -455G/A substitution of the β-fibrinogen gene appears to be consistently associated with higher plasma fibrinogen levels. Similar with the results reported by Goodman et al. and Coulam et al., we found no significant difference in the prevalence of β-fibrinogen -455G>A in RPL.

Recently other polymorphic markers of FV gene were described. The FV gene marked by the HR2 haplotype is able to contribute to determine a mild APC resistance phenotype. Similar with the results reported by Goodman et al. and Coulam et al., we found no association between the prevalence of the FV H1299R mutations and RPL. Individuals carrying the E4 allele have a higher total cholesterol level than people with the most common E3/E3 genotype, whereas those harboring the E2 allele have a lower total cholesterol level than those with E3/E3. In the previous study by Goodman et al., 21.7% of patients with RPL had Apo E4 genotypes, compared with the control women of 5.4% \( (P = 0.036) \). In our study, we found that the heterozygosity and allele frequency of E4 genotype of Apo E in women were frequent in RPL population. And also the high-risk of mutation of this genotype was also higher in RPL. So, we found a mild to moderate association in Apo E gene mutation in women with recurrent miscarriage. In our study, we also could not find any mutant alleles of Apo B gene either in the RPL and control couples.

Briefly, in women with recurrent pregnancy loss, the homozygosity of PAI-1 and MTHFR C677T was manifested, nevertheless, FV Leiden, FXIII V34L, Apo E and GPIIIa L33P only came into prominence if the partner was carrier. So, in our study, both male and female carriers were the risk for some of the genes, however, the risk was mainly the female. This is a case–control study among homogenous Caucasian couples of Turkish population in view of the conflicting evidence referred to above. There may be a major racial variation in gene polymorphisms between other studies from other countries in the literature. The mutations in thrombophilic genes could be associated with the disease and might be clinically useful as a marker to assess the couple’s risk for RPL. The combination of two or more thrombophilic genes might be discussed in the future to evaluate the high risk couples with recurrent pregnancy loss.

References


