Genetic polymorphisms on the factor V gene in women with recurrent miscarriage and acquired APCR

Feroza Dawood1,4, Roger Mountford2, Roy Farquharson3 and Siobhan Quenby1

1Department of Reproductive and Developmental Health, Liverpool Women’s Hospital, University of Liverpool, Crown Street, Liverpool L8 7SS, UK; 2Department of Molecular Genetics, Liverpool Women’s Hospital, Crown Street, Liverpool L8 7SS, UK; 3Department of Obstetrics and Gynaecology, Liverpool Women’s Hospital, Crown Street, Liverpool L8 7SS, UK
4Correspondence address. Tel: +44 151 702 4112; Fax: +44 151 702 4024; E-mail: fdawood@liv.ac.uk

BACKGROUND: Recurrent miscarriage (RM) has been associated with the thrombophilia, activated protein C resistance (APCR). The factor V Leiden mutation located on the B domain of the factor V gene, causes 95% of APCR and since the B domain is pivotal to APCR, it seemed plausible that other mutations or polymorphisms affecting this active domain may instigate acquired APCR. The objective of this study was to determine whether other polymorphisms exist on the parts of the gene encoding the B domain of the factor V in women with acquired APCR and RM.

METHODS: There were 51 women with RM and acquired APCR, 24 parous women (with no history of miscarriage and at least one normal full-term delivery) and 15 women with a history of idiopathic RM, who formed the study and two control groups, respectively. Six exons of the B domain of the factor V gene were intensely analysed using polymerase chain reactions, single-strand conformation polymorphism, genetic sequencing and restriction enzyme digestion analysis to identify single-nucleotide polymorphisms (SNPs).

RESULTS: A significantly increased frequency of some SNPs on the factor V gene were observed in the women with acquired APCR and RM when compared with the control groups.

CONCLUSIONS: The presence of some of these SNPs may predispose these women to acquired APCR and RM.

Keywords: acquired APCR; polymorphisms; recurrent miscarriage; factor V

Introduction

Recurrent miscarriage (RM) is defined as the loss of three or more consecutive pregnancy losses and afflicts 1–2% of couples (Stirrat, 1990). Despite burgeoning areas of research in this perplexing field, in ~50% of cases the clinical situation remains inexplicable.

The emerging role of the thrombophilia-activated protein C resistance (APCR) has generated considerable interest regarding the pathogenesis of RM. APCR induces a functional clotting abnormality generating a hypercoagulable state and hence is an important factor in the development of thrombosis. It has been postulated that thrombophilia may be associated with RM as a result of decreased uteroplacental perfusion (Dizon-Townson et al., 1997; Kupferminc et al., 1999; Gharavi et al., 2001). APCR has been categorized as hereditary or acquired. Hereditary APCR is due to the factor V Leiden (FVL) mutation, while the true cause of acquired or non-Leiden APCR remains unknown.

While no consensus has been reached regarding the relationship between FVL APCR and RM (Balasch et al., 1997; Brenner et al., 1997; Dizon-Townson et al., 1997; Tal et al., 1999), a more definitive association has been described between acquired (non-Leiden) APCR and RM (Rai et al., 2001; Sarig et al., 2002). More specifically, in the context of RM, there is mounting evidence that acquired APCR has a strong association with recurrent fetal loss (Younis et al., 2000; Dawood et al., 2003; Lindqvist et al., 2006).

The aim of this study was to search for mutations or polymorphisms on the factor V gene (other than the FVL mutation), in an attempt to explain the presence of acquired APCR. Moreover, the aim was to focus on a group of women who suffered RM (with a preponderance of fetal losses), and who demonstrated acquired APCR.

We know that the FVL mutation arises due to the Arg506→Gln mutation on the B domain of the factor V gene (Bertina et al., 1994). This mutation accounts for ~95% of cases of APCR. However, a number of patients who do not display the FVL mutation have demonstrated a pathological resistance to activated protein C (APC) as determined by functional tests. The existence of APCR in the absence of this mutation and the variability of the APCR phenotype in heterozygotes for the R506Q mutation suggested the possibility that alternative gene variations may be responsible for, or contribute to, APCR (Bernardi et al., 1997).
Materials and Methods

The complete complementary DNA and derived amino acid sequence of the factor V gene have already been determined. The gene has been localized to chromosome 1q21-25 and spans ~80 kb of DNA and consists of 25 exons and 24 introns (Jenny et al., 1997). The factor V gene encodes a large glycoprotein synthesized by the liver hepatocytes (Wilson et al., 1984; Mazzorana et al., 1989) and megakaryocytes (Gewertz et al., 1992). It has a molecular weight of 330 kD and circulates in plasma as an asymmetrical single chain. Factor V is also partially stored in platelets (Tracey et al., 1982). Factor V plays an integral role in the coagulation pathway.

Analysis of factor V cDNA has demonstrated that the protein is multidomain and contains two types of internal repeats with the following domain structure: A1-A2-B-C1-C2 (Chuch et al., 1984; Toole et al., 1984; Vehar et al., 1984). The protein is composed of three homologous A-type domains, two smaller homologous C-type domains and a heavily glycosylated B domain that connects the N-terminal A1-A2 region with the light chain and the C-terminal A3-C1-C2 region (Rosing and Tans, 1997; Ajzner et al., 1999). Most changes are located in the heavily glycosylated B domain (Pittman et al., 1994). B domain fragments derived from the APC-mediated cleavage of intact factor V have been directly implicated in the protein C anticoagulant pathway (Lu et al., 1996).

Cleavage of the internal B domain occurs via limited proteolysis by thrombin, the physiological activator of factor V (Dahlback, 1980). Although factor V and factor VIII share homologous A and C domains, the B domain of factor V is not homologous to that in factor VIII (Gitschier et al., 1984).

Cleavage of the B domain from factor V results in an inert coagulation factor V. This suggests that the B domain is of vital importance in APC cofactor activity in the clotting cascade, and that mutations in this domain may contribute to an impaired APC response (Kostka et al., 2000). The B domain of the factor V gene contains several repeats, which are of unknown function and of interest for the investigation of polymorphisms both at the DNA and at the protein level.

Thus, the B domain on the factor V gene of women who suffered RM, and also demonstrated APCR in the absence of the FVL mutation, was intensely scrutinized.

Materials and Methods

The Liverpool Research Ethics Committee granted ethical approval for the study. The study was conducted at the Recurrent Miscarriage Clinic and the Regional Molecular Genetics Laboratory based at the Liverpool Women’s Hospital NHS Foundation Trust.

Subjects

Study group

All the women attending the Recurrent Miscarriage Clinic had a history of at least three or more consecutive miscarriages at <24 weeks gestation. The initial consultation always took place pre-conceptually. As well as eliciting a detailed history, an investigative algorithm was augmented. This included testing for abnormal parental karyotypes, endocrinological abnormalities, pelvic ultrasonography and a thrombophilia screen. Women with a history of mid-trimester losses also underwent a hysteroscopy during which swabs were taken to exclude bacterial vaginosis. The thrombophilia screen included testing for antiphospholipid antibodies, lupus anticoagulant, protein C, protein S, antithrombin III levels and factor VIII levels, and was repeated within 6–12 weeks. Genetic testing also took place for the FVL mutation, prothrombin G20210A mutation and methylenetetrahydrofolate reductase deficiency. As these women were planning a pregnancy none of them were on any form of hormonal contraception. Neither were any of them pregnant at the time of testing and the interval between testing and their last pregnancy loss ranged between 7 and 52 weeks. Exclusion criteria included positive results for any of the above tests, personal or family history of thrombosis and the presence of concomitant antiphospholipid antibodies or lupus anticoagulant. In addition, only women with normal values of factor VIII (tested preconceptionally) were recruited. Inclusion criteria included positive functional tests for APCR and absence of the FVL mutation. There were 51 women who fulfilled these criteria and they comprised the study group. The age range of these women was 18–42 years.

Control groups

Parous controls. There were 24 healthy volunteers aged between 25 and 42 years, with a history of at least one full-term uncomplicated pregnancy enrolled to this control group. Exclusion criteria were a history of miscarriage, medical conditions that included a personal or family history of thrombosis and current usage of hormonal contraception or hormone replacement therapy. These control patients were also tested for APCR and all had values within the normal range. They also tested negative for the FVL mutation.

Idiopathic RM controls. There were 15 women with a history of RM and an age range similar to the study group who were also enlisted from the Liverpool Women’s Hospital Miscarriage Clinic. These women, therefore, also underwent the same preconceptional investigative protocol as the study group (including testing for antiphospholipid antibodies, factor VIII, protein C and protein S levels). Within this group, no discernible cause or association for their RM was identified. They also tested negative for APCR and the FVL mutation.

Measuring APCR sensitivity ratios

Venous blood samples were collected into 0.1 mmol/l sodium citrate (nine parts blood to one part anticoagulant). Fresh samples of plateletpoor plasma were then prepared by centrifugation at room temperature for 20 min, and analysed within 8 h of sampling. All reagents were used at room temperature. Resistance to APC was assessed on an automated coagulometer (MDA –180, Bio Merieux, and UK). Sensitivity to APC was expressed as a ratio of the activated partial thromboplastin time obtained in the presence and the absence of APC using the first-generation Coatest APC resistance kit (Chromogenix AB, Molndal, Sweden). Based on a pool of healthy volunteers in the local population and on local equipment calibration, a ratio <2.5 represented APCR. The normal ranges in the control groups were set at 3.0–5.6. This is also reflected in the range of APCR ratios in both of the tested control groups (n = 39) that we recruited (Fig. 1).

Polymerase chain reaction

DNA was extracted from whole blood using the Whatman Bioscience Genomic DNA Purification system (Whatman Biosciences, UK). Polymerase chain reactions (PCR) were performed on exons 5, 13 and 16 of the factor V gene on all samples. Exon 13 was analysed in four separate reactions (sections 13a–d), which covers the first 1200 bases of the exon. PCR was carried out in 10 ul reactions containing 5 ul of Reddy-Load master mix (Biogene UK), 0.5 uM exon-specific oligonucleotide primers (Invitrogen Life Technologies, UK) and 25 ng of patient DNA.
Thermocycling was performed on a Technne Genius (Technne, Cambridge, UK). Conditions for exon 13 (fragments a–d) were: initial denaturation at 95°C for 3 min was followed by 30 cycles of denaturation at 95°C for 1 min, annealing at 60°C for 1 min and synthesis at 72°C for 1 min then a final synthesis at 72°C for 5 min. Conditions for exons 5 and 16 were similar except annealing was at 55°C. The specificity of the amplification was confirmed by agarose gel electrophoresis prior to further analysis.

SSCP analysis

Single-stranded conformation polymorphism (SSCP) analysis was used identify possible polymorphisms in the exons listed above.

A non-denaturing 8% polyacrylamide gel (49:1) was prepared using a mixture of: 3.75 ml 10× Tris/borate/EDTA buffer, 30 ml 20% (49:1) acrylamide, 40.6 ml double distilled water (ddH2O), 600 ul 10% ammonium persulphate (Sigma, UK) and 75 ul tetramethylethylenediamine (Sigma). Once assembled, the gel was left for at least 1 h at ambient temperature, then for a further 1 h in a cold room at 4°C to polymerise. The PCR products were diluted with ddH2O and formamide loading buffer according to the strength of the DNA bands. Prior to loading, the samples were denatured at 95°C for 3 min, then immediately snap-cooled on ice. They were then loaded onto the gel and run overnight at 350 V.

Silver staining

The DNA on the SSCP gel was visualized by silver staining as follows: each gel was washed in 0.5% glacial acetic acid and 10% industrial methylated spirit for 3 min and then incubated in 0.1% silver nitrate on a shaking platform for 20 min. The gel was then rinsed with distilled water for 30 s followed by incubation in 0.3% formaldehyde, 1.5% sodium hydroxide solution until the DNA was visible. The reaction was stopped by addition of 0.75% sodium carbonate solution for 10 min the developed gel was then dried down between two sheets of cellophane.

DNA cycle sequencing

Sequencing of PCR-amplified DNA was performed on a 310A Sequencer with the Big Dye RR Terminator Cycle Sequencing Kit (ABI, UK) according to the manufacturer’s recommended procedure. The sequencing patterns, which indicated the different polymorphisms, are represented in Figs. 2–6.

Restriction fragment length polymorphisms

Following sequencing, two polymorphisms were identified on exon 13a, and one each on exons 13b, 13c, 5 and 16. These were subsequently analysed by restriction enzyme digestion. EcoRI and Taq I (Invitrogen Life Technologies) were used to detect variations in exon 13a; Hpy188I was used for exon 13b, DdeI for exon 13c, Na III (New England Biolabs, USA) for exon 5 and Hpy81 for exon 16.

Statistical analysis

The chi-squared test was employed to compare the detection of any frequencies of the single-nucleotide polymorphisms (SNPs) in the three groups. The non-parametric Kruskal–Wallis test was used to compare the presence of SNPs in different exons with APCR ratios. A P-value of <0.05 was deemed statistically significant.

Results

The characteristics of the study and control groups are depicted in Table 1 and the distribution of the APCR ratios is represented in Fig. 1.

The intense analysis of the relevant exons in the factor V gene revealed nine SNPs. These are specified in Figs. 2–6. The polymorphism nomenclature is based on GenBank Accession No AF119360 and is tabulated (Table 2).

Following the sequencing analysis, four SNPs, 2379 A>G, 2298 C>T, 2325 T>C and 2391 A>G, were identified in exon 13a. The last three polymorphisms were found to be in complete linkage disequilibrium with each other. The 2379 A>G SNP was elicited in the heterozygous form in 7 of the 51 study samples (14%); 3 of the 24 parous control samples (12.5%) and in 1 of the 15 idiopathic control samples (7%). The other SNPs (2298 C>T; 2325 T>C and 2391 A>G) in exon 13a, were found in both the heterozygous and homozygous forms in the study group. A frequency of 42.5% (20/51) in the heterozygous state and 6% (3/51) in the 15 idiopathic control samples (7%). The other SNPs were identified in exon 13b. The SNP was absent in the parous control and idiopathic control groups. No SNPs were identified on exon 13d.

Exon 13b exhibited two different SNPs 2627 A>G and 2684 A>G in complete linkage disequilibrium with each other. This pair of SNPs was frequent in the heterozygous form in 33 (17/51); 21 (5/24) and 0% in the study group, parous controls and idiopathic control group, respectively. The homozygous form was identified only in the study group with a frequency of 24% (12/51).

2863 A>G was the SNP identified in exon 13c in the heterozygous form in 24% (12/51) of the samples in the study group. The SNP was absent in the parous control and idiopathic control groups. No SNPs were identified on exon 13d.

In exon 5, the heterozygous form of the SNP 910+7 C>T was evident in 18 (9/51); 17 (4/24) and 7% (1/15) of the study group, parous controls and idiopathic controls, respectively. Finally, exon 16 exhibited SNP 5470 A>G in the heterozygous form in 55% (28/51) of the study group. The SNP was completely absent in both control groups.

Of all the patients in the study group, only two did not have a demonstrable SNP in the exons analysed. Two patients in the study group had a combination of seven SNPs [one patient displayed the heterozygous 2298 A>G; 2325 C>T; 2391 T>C (exon 13a)]; heterozygous 2627 A>G;
2684 A→G (exon 13b); heterozygous 910+7 C→T (exon 5) and heterozygous 5470 A→G (exon 16), whereas the other displayed the same SNPs in exon 13a, 13b and 16, but had the heterozygous 2863 A→G (exon 13c) SNP instead of the one in exon 5. Of the 51 patients, 31 patients (61%) displayed a combination of more than one SNP, mostly a combination of the SNPs on exon 13a and 13b in conjunction with the SNP on exon 16.

From the results presented in Table 2, it would appear that RM and acquired APCR may be associated with homozygosity for 2627 A→G and 2684 A→G on exon 13b, or heterozygosity for 2863 A→G on exon 13c, or heterozygosity for 5470 A→G on exon 16.

Furthermore, we analysed the relationship between the presence of individual SNPs, combinations of SNPs or an absence of SNPs and APCR ratios. The presence of the SNP

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**Figure 2:** Exon 13a: polymorphisms indicated on study sample above; control sample below

**Figure 3:** Exon 13b: polymorphisms indicated on study sample above; control sample below
in exon 5 appears independent of the APCR ratio. The SNP 5470 A→G (exon 16) and the 2863 A→G (exon 13c) are both associated with lower APCR ratios, and this effect also appears in combination with the SNP in exon 5. This analysis is illustrated in Fig. 7.

**Discussion**

The detection of SNPs that appear to be relatively common in the general population have been previously described in some clotting factors including the factor V and prothrombin genes. However, many mutations that have been identified appear to be loss of function polymorphisms failing to elucidate a clear relationship between the polymorphisms and clinical disease.

Thus far, the FVL mutation has been the only genetic defect for which a causal relationship to APCR has been clearly delineated. Other low frequency factor V mutations have been described, such as the factor V Cambridge (Arg306Thr) (Williamson et al., 1998) and factor V Hong Kong (Arg306Gly) (Chan et al., 1998). These mutations may result in APCR but the clinical association with thrombosis is less clear.
An R2 haplotype has also been described in association with APCR (Lunghi et al., 1998). The R2 haplotype has been associated with mild APCR (both in the presence and the absence of FVL). However, not all studies have been convincing regarding the role of the haplotype in clinical disease (Luddington et al., 2000). The polymorphic sites within the HR2 haplotype do not explain why the haplotype should alter APCR. The two amino acid substitutions coded by the haplotype, His1299Arg and Met1736Val also appear to be neutral (Soria et al., 2003).

Some data suggest that the R2 allele may represent a marker in linkage with an unknown defect rather than a functional polymorphism (Pecheniuk et al., 2001).

We have intensely analysed the B domain of the factor V gene in women with RM and identified several different SNPs. To the best of our knowledge, this is the first study describing these polymorphisms in women with a thrombophilic tendency involving the protein C coagulation pathway and RM.

The most significant finding is the demonstration of a significantly increased frequency of the SNP 5470 A→G on exon 16 in the study group compared with our two control groups. The complete absence of 5470 A→G in both the control groups, including the idiopathic RM group, suggests that this SNP may exert a modifier effect in both APCR as well as in RM. However, 5470 A→G has not been described in the context of thrombosis. The question arises as to whether this is a random finding or whether it is a distinct entity attributable to placental influence, manifesting as RM. We venture an explanation for this below.

The SNP in exon 13c 2863 A→G appears to be positively associated with a lower APCR ratio, both when it is present alone or in combination with 5470 A→G on exon 16. The SNP in exon 5, 910+7 C→T does not associate with a lower APCR ratio, however, when in combination with 5470 A→G (exon 16), there is a definite lowering of the APCR ratio, highlighting the significance of the SNP in exon 16.

Ostensibly, among the other SNPs that we identified, those in exon 13a and exon 13b that display complete linkage disequilibrium, are frequent polymorphisms that are evident not only in the study group but also in both the control groups. Although we identified significantly more of these SNPs of exon 13a and 13b in the study group than in the control groups, they most likely represent common non-functional polymorphic changes. Of the six SNPs that we describe in exon 13, three SNPs, at nucleotide positions 2684, 2863 and 2391, have been previously described as representing mis-sense mutations in a group of male and female patients with venous thrombosis (Kostka et al., 2000).

Our study is limited by small numbers, but we adhered to stringent inclusion criteria in order to obtain a refined and clinically biochemically purified group. As the presence of non-FVL APCR or acquired APCR may be influenced by many confounding variables, obtaining a large study number was precluded by the avoidance of “contaminating” clinical and laboratory factors. We concede that a larger number of subjects in each control group would have increased our power calculations. It would also be interesting to consider

<table>
<thead>
<tr>
<th>Table 1: Characteristics of patient groups</th>
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<tbody>
<tr>
<td>Study group</td>
</tr>
<tr>
<td>(n = 51)</td>
</tr>
<tr>
<td>Age in years median range</td>
</tr>
<tr>
<td>Number of past pregnancy losses median (range)</td>
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</tbody>
</table>

Figure 6: Exon 16: polymorphism indicated on study sample above; control sample below
another control group of women with acquired APCR without RM. This was intended as a hypothesis-generating study, but now that we have a suggestion of a possible modifier effect for the 5470 A→G SNP within exon 16 and the 2863 A→G SNP in exon 13c, it would be ideal to further explore these exons in the factor V gene in a larger number of participants.

We realize that the distribution of the APCR ratios may seem higher than is normally reported. A similar range (2.25–3.54) was detected in a control group of patients in a study of thrombosis using the same test (Kostka et al., 2000). It is worthwhile noting that in the same group of patients, they obtained different APCR ratios using different assays. In another study, the assertion has been made that analysis of APC response by different assays is an influential factor in disclosing genomic variations (Siegert et al., 2001). Important genetic variations may be missed by some coagulation assays of the APCR (Siegert et al., 2001).

The complex nature of thrombophilia and indeed genetic polymorphisms makes it arduous for one to prove a definite causal relationship between acquired APCR, polymorphisms and RM. Indeed, the pathogenesis of thrombophilia-associated pregnancy loss is poorly understood. Despite a significant association between thrombophilia and recurrent pregnancy loss (Rey et al., 2003), placental thrombosis has not been a consistent finding with these losses (Mousa and Alfirevic, 2000; Sikkema et al., 2002). Furthermore, antithrombin III deficiency, i.e. a very strong risk factor for venous and arterial thrombosis has not been associated with RM (Rey et al., 2003).

The role of the endothelial receptor protein C involved in the protein C anticoagulant pathway and the placental maintenance of pregnancy has been firmly established in mice (Gu et al., 2002). Mutations have also been described in other components of the protein C pathway (thrombomodulin and endothelial protein C receptor genes) in mice but a functional relationship for these mutations could not be extrapolated to humans with RM (Kaare et al., 2007). Human placenta is known to express the same factors that control the protein C anticoagulant pathway as that in mice; thrombomodulin (a membrane glycoprotein that activates protein is localized to the apical membranes of syncytiotrophoblast), a variant of tissue factor protein that was identified in the syncytiotrophoblast cells, and annexin V (an anticoagulant that binds to negative membrane phospholipids) is abundant on normal placentas (Lanir et al., 2003).

More recent work from mouse models has suggested a role for maternal carriage of the FVL mutation in causing fetal losses in the absence of placental thrombosis. It is suggested that the FVL mutation caused fetal losses in mice by a disruption to the materno-fetal interaction controlling the protein C anticoagulant pathway on the surface of the trophoblast which led to poor placental development (Sood et al., 2007). Hence this new animal model provides a possible explanation for the difference in the SNPs detected in our study and those that have previously been to be found in the context of venous and arterial thrombosis. We therefore suggest that the 5470 A→G (exon 16) and the 2863 A→G (exon 13c) described in this study may possibly inflict RM by disrupting the protein C anticoagulant pathway leading to placental failure as was demonstrated in mice (Sood et al., 2007).

Furthermore, there is emerging evidence from knock-out mice models that the fetal genotype exerts an important procoagulative effect on placental trophoblasts (Sood et al., 2007). We support the hypothesis that a synergistic effect of maternal and fetal prothrombotic mutations has implications for pregnancy loss (Sood et al., 2007), and may be an exaggerated response in RM.

Ultimately, we have identified SNPs in the functional B domain of the factor V gene in women with non-FVL APCR and RM. We conclude that the SNPs in exon 13a and 13b and in exon 5 are common and most likely non-functional SNPs that have also been described in patients with acquired

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### Table 2: Number (percentage) of women with each SNP in each group

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Study group (n = 51)</th>
<th>Parous control group (n = 24)</th>
<th>Idiopathic RM control group (n = 15)</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exon 13a 2379 A→G</td>
<td>7 (14)</td>
<td>3 (12.5)</td>
<td>1 (7)</td>
<td>P = 0.7631</td>
</tr>
<tr>
<td>Exon 13b 2298 C→T</td>
<td>20 (42.5)</td>
<td>7 (29)</td>
<td>5 (33)</td>
<td>P = 0.6845</td>
</tr>
<tr>
<td>Exon 13c 2298 C→T</td>
<td>3 (6)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>P = 0.305</td>
</tr>
<tr>
<td>Exon 13b 2627 C→T</td>
<td>17 (33)</td>
<td>5 (21)</td>
<td>0 (0)</td>
<td>P &lt; 0.0273</td>
</tr>
<tr>
<td>Exon 13b 2627 C→T</td>
<td>12 (24)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>P = 0.005</td>
</tr>
<tr>
<td>Exon 13c 2863 T→C</td>
<td>12 (24)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>P = 0.005</td>
</tr>
<tr>
<td>Exon 5 910+7 C→T</td>
<td>9 (18)</td>
<td>4 (17)</td>
<td>1 (7)</td>
<td>P = 0.5656</td>
</tr>
<tr>
<td>Exon 16 5470 A→G</td>
<td>28 (55)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>P &lt; 0.0001</td>
</tr>
</tbody>
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**Figure 7:** APCR ratio for some SNPs detected

Medians, inter-quartile and complete ranges are shown. 5, heterozygous for SNP in exon 5 (n = 10); 16, heterozygous for SNP in exon 16 (n = 19); 5 and 16, heterozygous for SNPs in both 5 and 16 (n = 3); 13c, heterozygous for SNP in exon 13c (n = 4); 13c and 16, heterozygous for SNPs in both 13c and 16 (n = 8); 0, no SNPs detected (n = 29)
ACPR and venous thrombosis. However, the SNPs 5470 A→G (on exon 16) and 2863 A→G (on exon 13c) may be important modulators of a genetic susceptibility at the placental interface manifesting in RM.

References


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