Chapter 10

Role of Gene Polymorphisms

Involved In Development of

Thrombophilias In Recurrent

Early Pregnancy Loss
Role of gene polymorphisms involved in development of thrombophilias in recurrent early pregnancy loss

10.1 Introduction

Thrombophilia are hemostatic disorders, classified as inherited and acquired; hereditary disorders include deficiencies of antithrombin III, proteins C and S deficiencies, genetic mutations such as factor V Leiden (FVL), prothrombin gene G20210A and the thermolabile variant of the methylene tetrahydrofolate reductase gene (C677T). Pregnancy is characterized by hemostatic and thrombophilic events which are necessary to maintain sufficient blood volume so that the maternal and fetal requirements are met. These events are exemplified by increased expression of procoagulant factors such as VIII, XII, VII, V, von Willebrand factor and fibrinogen (Martinelli 2002). In addition, protein S and protein C are reduced lowering the fibrinolytic activities (Brenner 2004). All of these modifications, together with an enlarged plasma volume, prepare the mother to face the hemostatic state during delivery. The risk of venous thrombosis in pregnancy is increased seven- to eight-fold, and even more after delivery as a result of all these modifications. The risk will become even more if the individual harbors any genetic factors that enhance the levels of these coagulation factors. Thrombophilias were investigated in relation to recurrent miscarriages in early or late pregnancy (Onderoglu 2006), intrauterine death/intrauterine growth retardation (Yamada 2005), placental abruption (Herman 2006), hypertensive disorders of pregnancy (Cansun Demir 2006) and maternal or neonatal thrombosis.
Chapter 10

Protein C is involved in dissolution of clots and the durability of clot is decided by activation of this protein. Activated protein C resistance is associated with recurrent venous thrombosis (Jastrzebska 2003), cerebral thrombosis (Ghalib 2004) and bad obstetric history (Lindqvist 2006). The most common cause of inherited activated protein C resistance is factor V Leiden (FVL). FVL is due to a single G1691A, R506Q mutation in exon 10 of the factor V gene. This mutation makes factor V refractory to cleavage by activated protein C. In the Western countries, the prevalence of FVL in the general population is 0%-7% and that in patients with recurrent venous thrombosis is 3%-35%.

The prothrombin G20210A mutation is a point mutation at nucleotide position 20210 in the prothrombin gene. This mutation is associated with elevated plasma prothrombin levels (>130%), and increased risk of venous and arterial thrombosis (Franco 1999). After FVL, it is the second most common identified independent risk factor for venous thrombosis.

Neural tube defects arise early in embryogenesis following a failure of the neural tube to close. Anencephaly occurs when the brain and skull bones are not rightly formed. When this happens, part or all of the brain and skull bones might be missing. Babies with this defect die before birth (miscarriage or stillbirth) or shortly after birth. The underlying mechanisms involve a combination of genetic, environmental and nutritional factors. It is well documented that folate supplementation during pregnancy relieves the risk of neural tube defects and low erythrocyte folate levels are associated with pregnancy loss.
Chapter 10

The major circulating form of folate is 5-methyl tetrahydrofolate which is synthesized from 5,10-methyle tetrahydrofolate by the action of MTHFR. Methyl tetrahydrofolate is crucial for the survival of the cell as it is the major source of methyl (single carbon units) groups. Abnormalities in MTHFR activity leads to elevated plasma levels of homocysteine which is routinely monitored to assess the fetal growth and pregnancy complications. Hyper homocysteinemia lead to vascular and metabolic changes which are associated with placental infarction (Goddijn-Wessel 1996), recurrent miscarriages (Raziel 2001) and pre-eclampsia (Ingec 2005). Homocysteine levels are also effected by demographic and life style factors such as age, gender, ethnicity, smoking, obesity, intake of folic acid. Maternal smoking or exposure to cigarette smoke increases homocysteine levels and the risk of having adverse pregnancy outcomes (Sobczak 2004). A common missense mutation in MTHFR is a C to T substitution at nucleotide 677 which converts an alanine to a valine residue and this result in decreased MTHFR activity leading to elevated homocysteine and decreased folate levels in plasma.

Many contradictory reports exist regarding the role of polymorphisms in the hemostatic genes in relation to the risk of REPL. In the absence of such report from the Indian population, we screened the subjects for FVL, G20210A of prothrombin and C677T of MTHFR.
10.2 Results

A total of 145 women with REPL and 92 women with successful pregnancy history were included in the present study. All women were healthy and none had a history of thrombotic events. Restriction fragment length polymorphism analysis as well as sequence analysis was carried out to find the genotype of an individual. The frequencies and the comparative statistics are presented in Table 10.1.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Polymorphism</th>
<th>Cases</th>
<th>Controls</th>
<th>Odds ratio (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FVL</td>
<td>G1691A</td>
<td>Nil</td>
<td>Nil</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prothrombin</td>
<td>G20210A</td>
<td>Nil</td>
<td>Nil</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MTHFR</td>
<td>C677T: CC</td>
<td>98</td>
<td>60</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CT</td>
<td>37</td>
<td>24</td>
<td>1.07 (0.53-2.15)</td>
<td>0.87</td>
</tr>
<tr>
<td></td>
<td>TT</td>
<td>10</td>
<td>8</td>
<td>1.33 (0.42-4.24)</td>
<td>0.79</td>
</tr>
</tbody>
</table>

From the values, it is clear that none of the polymorphisms had any significant association with REPL in the present population. In addition, the factor V and prothrombin variants were not seen in any of the case as well as control women. Sequence analysis revealed the presence of a coding region G→A transition in exon 10 in a woman with REPL (Fig 10.1).
Fig 10.1 A G→A transition in exon 10 of \textit{FVL} observed in woman with REPL.

Arrows indicate the base change due to polymorphism. However, since the frequency is below 1%, this can be a rare substitution rather than a genuine polymorphism. This leads to a substitution of arginine by lysine at 513 position (1683 in mRNA) in the protein.

10.3 Discussion

Since 1996, when Sanson et al. correlated fetal loss with protein C, protein S and antithrombin deficiencies, many studies have investigated the relationship between thrombophilias and spontaneous abortions. Despite the substantial amount of data gathered, there is still a certain amount of controversy in the results. In a report from Netherlands and Ireland, a significant association was observed between carriers of homozygous FVL with early pregnancy loss (<20 weeks) (Murphy 2000). However, Caucasian as well as Japanese populations failed to show similar association (Hashimoto 1999). Similar controversies also exist in prothrombin and MTHFR polymorphisms. This
necessitates the analysis of these variants in a population specific manner to evaluate the risk and to formulate appropriate therapeutic strategies. Indian population is unique in terms of the genetic status because of the extensive inbreeding linked to the religious and social issues. Hence, we tried to find the status of these polymorphisms in south Indian population. It is interesting to observe that FVL and prothrombin variants were not seen in any of the subjects. A report from north India with coronary artery disease also observed a similar absence of these mutations. This indicates that these mutations are either completely missing or present at such a low level that they cannot be scored as polymorphism (<1%). A statistically similar distribution of MTHFR variant between cases and controls suggests that this variation cannot explain the REPL seen in south Indian subjects. In conclusion, variations in thrombophilic genes are not useful as predictors of pregnancy loss.