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REVIEW

Hereditary thrombophilic risk factors for recurrent pregnancy loss

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Abstract This review summarizes current knowledge about the role of hereditary hypercoagulation factors predisposing to thrombophilia-associated recurrent fetal loss. Thrombophilias are a major cause of adverse pregnancy outcome, playing a role in the etiology of up to 40% of cases worldwide. Hereditary thrombophilic predispositions to recurrent pregnancy wastage include genetic lesions in blood coagulation factors II and V as well as natural anticoagulants antithrombin, protein C, and protein S. Furthermore, methylenetetrahydrofolate reductase gene variants conferring higher thrombophilia risk in combination with these mutations and the newly described annexin A5 gene M2 promoter allele are associated with repeated fetal loss. The review gives a concise description of the molecular defects arising from the genetic changes, of the role these factors play in the timing and definition of fetal loss, and risk estimates from available studies and meta-analysis. This knowledge is instrumental for a more precise assessment of individual risks for repeated fetal loss and should guide therapeutic strategies, where relevant. Since the average childbearing age increases in Western societies, the importance of a timely diagnosis of fetal loss predisposition is increasing.

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Heritable thrombophilic lesions

Pregnancy loss is a major problem of women's health. About one fifth of all women worldwide have suffered at least one abortion, and 1/20 has had two or more spontaneous pregnancy losses (Branch et al. 1992). More than 500,000 women per year experience a recurrent abortion in the USA (Bick and DRW Metroplex Recurrent Miscarriage Syndrome Cooperative Group 2000). Mostly, adverse pregnancy outcome in the first trimester is caused by chromosomal abnormalities incompatible with life. Nevertheless, routine gynecological, endocrine, and cytogenetic tests cannot unravel the reason for recurrent fetal losses in 30–40% of cases (Branch et al. 1992).

Hereditary or acquired hypercoagulation disorders promoting thrombosis, collectively termed "thrombophilias," form the molecular basis for the majority of otherwise unexplained fetal loss (Kupferminc et al. 1999). Histological studies have demonstrated an increased prevalence of microthrombi in the placental vessels of women with recurrent miscarriage (Out et al. 1991; Rai et al. 1996), although there are some controversies (Alfirevic and Kurjak 1990). Normal pregnancies are characterized by a hypercoagulability state that predisposes to thrombosis (Sanson et al. 1999; Simioni et al. 1999). Hereditary thrombophilic defects in combination with these physiological changes may increase the risk of fetal loss (Kupferminc 2003).

Table 1 gives an overview of inherited defects found in the majority (70%) of thrombophilic patients. For two of these defects, factor V Leiden (FVL) mutation and the prothrombin G20210A mutation (PTm), together account-

Table 1 Known hereditary thrombophilia risk factors

Prevalence in the white population (%)				
Thrombophilic defect	Incident VTE	Recurrent VTE	Normal population	Relative thrombotic risk
Antithrombin deficiency	1–2	2–5	0.02–0.04	5
Protein C deficiency	2–5	5–10	0.2–0.5	6–10
Protein S deficiency	1–3	5–10	0.1–1	2
Factor V Leiden	20	40–50	3–7	3–7 (heterozygotes) 50–100 (homozygotes)
Prothrombin G20210A	3–8	15–20	1–3	2–8 (heterozygotes)

VTE venous thromboembolism

ing for more than half of all cases with inherited thrombophilia, direct DNA analysis is performed when indicated.

Factor V Leiden

Factor V Leiden mutation is an adenine to guanine substitution at position 1691 of the coagulation factor V gene (Bertina et al. 1994; Greengard et al. 1994; Voorberg et al. 1994). The resulting amino acid replacement, arginine (R) to glutamine (Q), at position 506 occurs exactly at one of the three contact residues where activated protein C (APC) would normally cleave and inactivate procoagulant factor Va. As a result, activated factor V Leiden becomes partially resistant to the anticoagulant action of APC and is inactivated at an approximately tenfold slower rate than normal, thereby resulting in increased thrombin generation and a prothrombotic state.

Factor V Leiden is the most common inherited cause of thrombophilia, being present in heterozygous form in about 12–20% of patients with venous thrombosis and in 40–50% of those with recurrent venous thrombosis. The mutation is very common in the white population: About 3% to 7% of individuals from Northern European extraction are heterozygous FVL carriers. FVL heterozygosity has been shown to be associated with a three to sevenfold increase of venous thrombosis risk, while homozygotes have a 50- to 100-fold increased risk (Griffin et al. 1993; Koster et al. 1993; Ridker et al. 1997; Table 1).

An elevated thrombosis risk may be due to a combined defect of factor V Leiden carriership and yet another unequivocally diagnosed risk factor such as homozygosity for MTHFR C677T (Cattaneo et al. 1997; Eldibany and Caprini 2007; Ridker et al. 1997).

In a meta-analysis published by Rey et al. (2003), factor V Leiden (Table 2) was found to be associated with early and late recurrent fetal loss (OR 2.01, 95%CI 1.13–3.58) and with late non-recurrent fetal loss (OR 7.83, 95%CI 2.83–21.67). Upon the exclusion of women with other pathologies that could explain fetal loss, the association between factor V Leiden and recurrent abortions increased. While protein S deficiency was related to non-recurrent pregnancy loss occurring after 22 weeks, activated protein C resistance not due to factor V Leiden was associated with recurrent early pregnancy loss. In contrast, no significant association was found between protein C or antithrombin deficiency and recurrent or non-recurrent fetal loss, respectively.

APC resistance not related to factor V Leiden

APC resistance not associated with factor V Leiden has been identified as an additional, independent risk factor for deep vein thrombosis (de Visser et al. 1999) and may be an acquired condition resulting from pregnancy (Cumming et al. 1995) and oral contraceptive use (Olivieri et al. 1995). Some laboratory phenotypes such as lupus anticoagulant and high factor VIII levels may be also due to a reduced sensitivity to APC. Reduced APC sensitivity may also be due to other genetic causes including, for example, two mutations affecting the R205 APC cleavage site of factor V (Chan et al. 1998; Williamson et al. 1998). One mutation (R306T, factor V Cambridge) was indeed causative of APC resistance. The other lesion (R306G) was found in a Hong

Table 2 Relative risk for relative fetal loss associated with hereditary thrombophilic defects according to a meta-analysis by Rey et al. (2003)

Thrombophilic defect	Recurrent pregnancy loss before 13weeks	Non-recurrent pregnancy loss	Non-recurrent pregnancy loss after 19weeks
Antithrombin deficiency	0.88	1.54	Not analyzed
Protein C deficiency	1.57	1.41	Not analyzed
Protein S deficiency	14.72	7.39	Not analyzed
Factor V Leiden	2.01	1.73	3.26
Prothrombin G20210A	2.05	2.32	2.30

Kong Chinese and was reported not to be associated with APC resistance.

Factor II PTm

The 20210G>A mutation (PTm) in the 3′ untranslated region of the factor II gene encoding prothrombin causes a gain of function due to an enhanced recognition of the 3′ end cleavage signal and increased 3′ end processing. This results in the accumulation of messenger RNA (prolonged turnover) and greater protein synthesis of prothrombin (Gehring et al. 2001).

Both homozygous and heterozygous carriership of factor V Leiden or PTm mutations increases the risk of venous thromboembolism (Table 1). The overall prevalence of the prothrombin mutation (PTm) in Europe is approximately 2%. The highest prevalence has been observed in Southern Europe (approximately 3%) and the lowest in the northern parts of the continent (approximately 1.7%). Heterozygous carriers of the 20210A allele have a two to eightfold increased risk for venous thrombosis (Poort et al. 1996). Very few cases of homozygosity for this mutation have been described (Rosendaal et al. 1998). Although the severity of the phenotype and the concurrent thrombosis risk would be expected to be higher in the homozygous state, a broad clinical spectrum with striking heterogeneity has emerged in a very small case number (Bosler et al. 2006). From the meta-analysis (Rey et al. 2003), there is a significant association found between PTm carriership and recurrent abortion before 13 weeks of pregnancy (OR 2.56, 95%CI 1.04–6.29) as well as with non-recurrent fetal loss after 20 weeks (Table 2).

Individuals carrying both an FVL and a prothrombin G20210A mutation have a 20-fold increased risk for venous thrombosis, which is higher than for heterozygous carriers of FVL or prothrombin G20210A alone. DNA analysis of both mutations is therefore highly recommended in patients with a personal or family history of thrombosis (McGlennen and Key 2002; Press et al. 2002). The hereditary deficiencies of anticoagulant proteins antithrombin, protein C, and protein S are heterogeneous in nature and can be caused by many different genetic lesions (Seligsohn and Lubetsky 2001). Although they have been the target of intense clinical research, taken together, they account for <10% of patients with thrombophilia (Florell and Rodgers 1996).

Methylenetetrahydrofolate reductase

It has been suggested that elevated total plasma homocysteine levels (hyperhomocysteinemia) could represent another factor predisposing to thrombosis. Homocysteine is a non-protein-building sulfhydryl amino acid resulting from the intracellular demethylation of methionine. In hepatocytes, homocysteine is remethylated to methionine by the acquisition of a methyl group from methyltetrahydrofolate, derived in a reaction catalyzed by methylenetetrahydrofolate reductase (MTHFR). A quite common variant in the MTHFR gene, namely a C to T substitution at cDNA position 677 leading to a change from alanine to valine, may cause increased levels of plasma homocysteine. This variant shows reduced activity at 37°C and increased thermolability at 46°C. Approximately 12% of the white population is homozygous for the mutation that would cause typical manifestation of moderate hyperhomocysteinemia when folate levels are at the lower end of the normal range (Hanson et al. 2001). Although initial data suggested an association between homozygosity for MTHFR C677T and venous thrombosis, prospective studies could not confirm these results (Cattaneo 1999; Tsai et al. 2001).

A second common polymorphism in the MTHFR gene, A1298C, has been described by van der Put et al. (1998). The prevalence of homozygotes for this variant in the white population is approximately 10%, and 23% of people are compound heterozygotes for C677T and A1298C (Hanson et al. 2001). It has been shown that compound heterozygosity for C677T and A1298C, but not homozygosity for A1298C, is associated with increased fasting and post-methionine load homocysteine plasma levels (Hanson et al. 2001).

Over the last 10 years, a number of studies on the association between inherited thrombophilia and pregnancy loss have been published (Brenner et al. 1999; Finan et al. 2002; Foka et al. 2000; Kovalevsky et al. 2004; Pihusch et al. 2001; Reznikoff-Etiévan et al. 2001; Ridker et al. 1998; Sarig et al. 2002; Wramsby et al. 2000; Younis et al. 2000). In view of the somewhat conflicting results of these studies, and because screening tests for thrombophilia become increasingly available, a meta-analysis has been performed on 31 association reports published in the literature (Rey et al. 2003). In addition to estimating the actual strength of association between inherited thrombophilia and fetal loss, this meta-analysis also served to clarify whether the associations vary by the timing or definition of fetal loss (Table 2). The initial observation that homozygosity for MTHFR C677T could be related to pregnancy loss (Nelen et al. 2000; van der Molen et al. 2000), supported by more recent studies (Goodman et al. 2006; Subrt et al. 2008), could not be confirmed, neither in another sample (Foka et al. 2000) nor by meta-analysis (Rey et al. 2003). However, when additional thrombophilic factors were considered, MTHFR C677T seemed to significantly increase the risk of pregnancy wastage (Coulam et al. 2006; Kupferminc et al. 1999; Tranquilli et al. 2004). The A1298C MTHFR variant

alone showed no significant association with recurrent pregnancy loss, but a good correlation was confirmed in the presence of other prothrombotic mutations (Goodman et al. 2006; Subrt et al. 2008).

Hyperhomocysteinaemia is a risk factor for placenta-mediated diseases such as preeclampsia and placenta abruption as well as for fetal neural tube defects (van der Molen et al. 2000; van der Put et al. 1998). However, it does not appear that homozygosity for MTHFR C667T, the genetic abnormality most commonly associated with hyperhomocysteinemia, is linked to an increased risk of venous thromboembolism (VTE) in pregnant women. As clinical events in homozygotes are likely to reflect the interaction of the genotype with a relative deficiency of vitamins such as B12 and folic acid, the absence of an association of this genotype with gestational VTE may reflect pregnancy-related physiologic reduction in homocysteine levels and/or the effects of folic acid supplements that are now taken widely by women in pregnancy for the prevention of neural tube defects. According to the guidelines of the Italian Society for Haemostasis and Thrombosis (SISET), evidence of association between pregnancy complications and MTHFR polymorphisms is not sufficient (Lussana et al. 2009). The use of folic acid is suggested for the whole pregnancy in women with mild hyperhomocysteinemia.

M2 haplotype of ANXA5

Annexin A5 (placental anticoagulant protein) occurs in normal placental villi and appears to be reduced when antiphospholipid antibodies are present (Rand et al. 1994). Reduced annexin A5 expression in the placental trophoblasts has also been demonstrated immunohistochemically in patients with preeclampsia (Shu et al. 2000). Based upon these observations and the reported anticoagulation activity of the protein (Romisch et al. 1991), it has been suggested that annexin A5 molecules form an antithrombotic shield on the apical surface of placental syncytiotrophoblasts that may in pregnancy be disrupted by antiphospholipid antibodies (Rand and Wu 1999). This hypothesis has recently received additional support from in vitro studies employing atomic force microscopy and functional assays (Rand et al. 2003).

A few years ago, we observed that a sequence variation in the promoter of the annexin A5 (ANXA5) gene represents a risk factor for recurrent pregnancy loss (Bogdanova et al. 2007). Genomic analysis of a German recurrent pregnancy loss (RPL) patient sample, all known to carry neither factor V Leiden nor a prothrombin mutation, revealed an overrepresentation of four consecutive nucleotide substitutions in the ANXA5 promoter, transmitted as a joint haplotype (M2). Reporter gene assays showed that M2 reduces the in vitro activity of the ANXA5 promoter to 37–42% of the normal level. The possible relationship between M2 and RPL was assessed by comparing RPL patients (n = 70) with two independent control groups, namely women from the registry of the Institute of Human Genetics in Münster (n = 500) and from the PopGen biobank in Kiel (n = 500), respectively. Carriers of M2 were found to exhibit a more than twofold higher RPL risk than non-carriers (OR 2.42, 95%CI 1.27–4.58) in comparison to unselected controls (PopGen) and an almost fourfold higher risk relative to the Münster "super-controls," i.e., women with successful pregnancies and no previous history of pregnancy loss (OR 3.88, 95%CI 1.98–7.54).

Recently, the expression of ANXA5 in placentas from M2 haplotype carriers has been shown to be reduced by a factor of two at the mRNA level compared to women lacking M2 (Chinni et al. 2009). The same study demonstrated that the abundance of placental ANXA5 mRNA in 26 women with obstetric complications (preeclampsia and fetal growth restriction) was threefold lower than in a control group of seven women without pregnancy complications.

An analysis of the role of M2 in Italian women with repeated fetal loss or pregnancy-related hypertension recently corroborated our initial findings (Tiscia et al. 2009). The study reported a similar prevalence of M2 carriership (15%) in women from Southern Italy as in the German population. In addition, the authors also demonstrated a significant association between M2 carriership and both RPL (defined as three or more fetal losses at ≤23 weeks; OR 3.1, 95%CI 1.1–9.5) and pregnancy-related hypertensive disorders (OR 2.1, 95%CI 1.2–3.5). The results of the Italian study also suggested that the role of M2 could be more pronounced in early fetal loss (≤15 weeks) than in later events (15–23 weeks). This is in contrast to the trend noted for the FVL and PTm thrombophilic mutations for which the risk of fetal loss increases after the 19th week of pregnancy (Table 2).

In the future, it would appear reasonable to study the role of M2 and other ANXA5 haplotypes in other populations and ethnic backgrounds. An additional avenue of further studies could be to clarify the interaction between M2 and other known hereditary RPL risk factors. This notwithstanding, the ANXA5 promoter M2 haplotype undoubtedly represents an established predisposition to fetal loss and should thus be included in the analytical panel of inherited thrombophilic factors. This would not only improve the available prognostic algorithms for RPL, allowing a more precise assessment of individual disease risk, but should also provide a guide to adequate therapies where relevant.

aPL antibodies and M2 haplotype of ANXA5

The presence of circulating maternal antiphosholipid antibodies is yet another established major risk factor for

recurrent pregnancy loss. A higher incidence of RPL has been documented for both low-risk and high-risk pregnancies with antiphospholipid antibodies (aPL; (Empson et al. 2002; Subrt et al. 2008). Antiphospholipid antibodies are thought to lead to fetal loss by causing thrombosis of the placental vessels, although the observed variability in placental pathology somehow argues against such a direct involvement (Nilsson et al. 1975; Salafia and Cowchock 1997). Lowered expression of ANXA5 in placentas of M2 haplotype carriers (Chinni et al. 2009) could be potentially responsible for reduced coverage of phospholipid trophoblast surfaces and hence lead to an increase in the number of exposed available antigenic determinants for the generation of aPL. Preliminary results (Cherkelova et al., unpublished observations) suggest about a twofold higher incidence of M2/ANXA5 in SLE and aPL patients with obstetric complications. The possible predisposition of M2/ANXA5 carriers to develop aPL warrants further studies in larger patient groups.

Conclusive remarks

There is an increasing tendency for childbearing to occur later in women's lives, particularly in Western Europe, Australia, New Zealand, Canada, and the USA (RCOG 2009). However, the biologically optimal period for childbearing is between 20 and 35 years of age. After this period, it turns increasingly difficult to fall pregnant and the chances of miscarriage increase with progressing age. This is why it is becoming even more important to diagnose common risk factors and hereditary predispositions to fetal loss in a timely fashion. Although data on the combined effect of maternal age and genetic risk factors are still lacking, it is generally expected that the latter would have even stronger bearing on mothers older than 35. In any case, mothers at later childbearing age should have their fetal loss risk minimized not the least because of the impact of fetal losses on subsequent pregnancies.

Based upon our current knowledge, some forms of hereditary thrombophilia clearly appear to be associated with RPL. SISET guidelines recommend testing for FVL and PTm in pregnancy (Lussana et al. 2009). They do not recommend testing for polymorphisms of FXII, MTHFR, and PAI-1 genes and polymorphisms of FV and FII genes, different from FVL and PTm. It is advisable to perform the screening before pregnancy, and if performed during pregnancy, results should be interpreted very carefully and where applicable completed with family history. Genetic testing for the FVL mutation and PTm G20210A variant is indicated for women with RPL or non-recurrent late miscarriage. Since homocysteine is an established risk factor for obstetric complications, it seems more indicated to dose homocysteine plasma levels than testing for MTHFR polymorphisms. Testing MTHFR A1298C variant could be optional, but its relevance should be judged with caution and only in conjunction with C677T. Evaluation of APC resistance not due to FVL or protein S deficiency, using plasma-based functional assays, is indicated in women with early recurrent abortions, whereas women with late miscarriage should be tested for protein S deficiency alone.

It is advisable to refer women tested positive for one or more thrombophilic mutations to specialized centers for monitoring future pregnancies and for considering low-molecular-weight heparin (LMWH) prophylaxis during pregnancy. The American College of Chest Physicians of USA guidelines recommend for RPL women with thrombophilia antepartum administration of prophylactic or intermediate-dose ultrafractionated heparin or prophylactic LMWH combined with aspirin (Bates et al. 2008).

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