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Hereditary Thrombophilic Risk Factors for Recurrent Pregnancy Loss

Arseni Markoff[1]*, Nadja Bogdanova[2] and Meyer Michel Samama[3]

[1]Institute of Medical Biochemistry, ZMBE and IZKF, Münster
[2]Institute of Human Genetics, UKM, Westfalian-Wilhelms University of Munster, Munster, Germany
[3]Hotel-Dieu University Hospital, Paris, France

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, these gene defects confer higher thrombophilia risk in combination. They, as well as 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-analyses. 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.

Keywords: Fetal loss; Recurrent pregnancy loss; Thrombophilia; Genetic predisposition; Risk factors

Heritable Thrombophilic Lesions

Pregnancy loss is a major problem of women's health. About 1/5 of all women worldwide have suffered at least one abortion, and 1/20 have had two or more spontaneous pregnancy losses [1]. More than 500,000 women per year experience a recurrent abortion in the United States of America [2]. 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 [1].

Hereditary or acquired hypercoagulation disorders promoting thrombosis, collectively termed 'thrombophilias', form the molecular basis for the majority of otherwise unexplained fetal loss [3]. Histological studies have demonstrated an increased prevalence of microthrombi in the placental vessels of women with recurrent miscarriage [4,5], although there are some controversies [6]. Normal pregnancies are characterized by a hypercoagulability state that predisposes to thrombosis [7,8]. Hereditary thrombophilic defects in combination with these physiological changes may increase the risk of fetal loss [9].

Table 1 gives an overview of inherited defects found in the majority (70%) of thrombophilic patients. For two of these defects, the factor V Leiden (FVL) mutation and the prothrombin G20210A mutation (PTm), together accounting for more than half of all cases with inherited thrombophilia, direct DNA analysis is performed when indicated.

It should be noted that the predisposing role of hereditary thrombophilic factors to venous thrombosis has been demonstrated in several clinical studies and associated risks are significant (Table 1). Historically, the predispositions to recurrent fetal loss associated with these lesions have been identified in retrospective analyses of pregnancies (at baseline) of patients included in the European Prospective Cohort on Thrombophilia (EPCOT) study [10]. This analysis demonstrated that women with familial thrombophilia had an increased risk for fetal loss, particularly stillbirth. Later, the data on pregnancy follow-ups were collected from the prospective study and evaluated, which to a great extent confirmed the results obtained previously, namely the increased fetal loss risk, but no conclusions on thromboprophylaxis could be made, because of the small treated patients' number and the varying therapies [11].

The moderate pregnancy loss risk increase in carriers of the hereditary thrombophilic factors known at the time was also concurrent with pregnancy outcomes comparable to the controls. This is why it is necessary for recurrent fetal loss to a) precisely evaluate the role of newly identified hereditary thrombophilia risk factors with higher population incidence, such as M2/ANXA5 and b) check the efficiency of thromboprophylaxis for recurrent pregnancy loss (RPL) patients that are carriers of hereditary thrombophilia lesions in adequately powered and properly controlled clinical trials.

Factor V Leiden

The factor V Leiden mutation is an adenine to guanine substitution at position 1691 of the coagulation factor V gene [12-14]. 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 ten-fold 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

***Corresponding author:** Arseni Markoff, Institute of Medical Biochemistry, ZMBE, Von Esmarch str. 56, 48149 Münster, E-mail: markoff@uni-muenster.de

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extraction are heterozygous FVL carriers. FVL heterozygosity has been shown to be associated with a 3- to 7-fold increase of venous thrombosis risk, while homozygotes have a 50- to 100-fold increased risk [15-17], (Table 1).

An elevated thrombosis risk may be due to a combined defect of factor V Leiden carriage and yet another unequivocally diagnosed risk factor such as homozygosity for MTHFR C677T [17-19].

In a meta-analysis published by Rey et al. [20], 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. Later systematic review of 40 studies confirmed similar associations for FVL (Table 2) and unexplained pregnancy loss [21]. Magnitudes of the observed associations are rather modest and come from case-control studies that tend to overestimate odds ratios, nevertheless they document the role of FVL as a contributing factor to recurrent pregnancy loss. Another trend to overestimate odds ratios comes from inclusion of unrecognized antiphospholipid syndrome (APS) patients in such studies, who have a rather significant risk of fetal losses. A recent systematic review and meta-analysis of systematic prospective trials confirms a small absolute increased risk of late pregnancy loss among FVL carriers (OR 1.52, 95% CI 0.80-1.25) but excludes association with preeclampsia and fetal growth restriction [22].

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 [23], and may be an acquired condition resulting from pregnancy [24], and oral contraceptive use [25]. 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 [26,27]. One mutation (R306T, factor V Cambridge) was indeed causative of APC resistance. The other lesion (R306G) was found in a Hong 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 accumulation of messenger RNA (prolonged turnover) and greater protein synthesis of prothrombin [28].

Both homozygous and heterozygous carriage 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 2- to 8-fold increased risk for venous thrombosis [29]. Very few cases of homozygosity for this mutation have been described [30]. 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 [31]. From the meta-analysis [20], there is a significant association found between PTm carriage and recurrent abortion before 13 weeks of pregnancy (OR 2.70, 95% CI 1.37-5.34) as well as with non-recurrent fetal loss after 20 weeks (Table 2), confirmed in the later systematic review of studies (OR 2.43, 95% CI 1.12-4.79) [21]. As with FVL, observed RPL associations with PTm are modest and their magnitudes are estimated from case-control

	Prevalence in the white population (%)			
Thrombophilic defect	Incident VTE[a]	Recurrent VTE	Normal population	Relative Thrombotic Risk
Antithrombin deficiency [7,8,30]	1-2	2-5	0.02-0.04	5-10
Protein C deficiency [7,8,30,31]	2-5	5-10	0.2-0.5	6-10
Protein S deficiency [7,8,30]	1-3	5-10	0.1-1	2-10
Factor V Leiden [10,11,12]	20	40-50	3-7	3-7 (heterozygotes) 50-100 (homozygotes)
Prothrombin G20210A [25,26,27]	3-8	15-20	1-3	2-8 (heterozygotes)

[a] VTE is abbreviated for venous thromboembolism

Table 1: Known hereditary thrombophilia risk factors.

Thrombophilic defect	Recurrent pregnancy loss before 13 weeks (95% CI)	Non-recurrent pregnancy loss (95% CI)	Non-recurrent pregnancy loss after 19 weeks (95% CI)
Antithrombin deficiency	0.88 (0.17-4.48)	1.54 (0.97-2.45)	7.63 (0.30-196.36)*
Protein C deficiency	1.57 (0.23-10.54)	1.41 (0.96-2.97)	3.05 (0.24-38.51)*
Protein S deficiency	14.72 (0.99-218)	7.39 (1.28-42.83)	20.09 (3.70-109-15)*
Factor V Leiden	2.01/1.91* (1.13–3.58)/(1.01-3.61)*	1.73 (1.18-2.54)	3.26/2.06* (1.82-5.83)/(1.10-3.86)*
Prothrombin G20210A	2.05/2.70* (1.18-3.54)/(1.37-5.35)*	2.32 (1.12-4.79)	2.30/2.66* (1.09-4.87)/(1.28-5.53)*

Table 2: Relative risk for relative fetal loss associated with hereditary thrombophilic defects according to ref. [21] and [22]*.

studies, because of methodological limitations. Nevertheless inherited thrombophilia with the PTm mutation should be considered in the multifaceted pathology of recurrent pregnancy loss.

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 [32,33]. 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 [34]. Although they have been the target of intense clinical research, taken together they account for less than 10% of patients with thrombophilia [35].

The recent systematic review and meta-analysis of systematic prospective trials on pregnancy complications among FVL and PTm carriers excludes the association of PTm carriage with preeclampsia and fetal growth restriction [22].

MTHFR

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 methylentetrahydrofolate 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 [36]. Although initial data suggested an association between homozygosity for MTHFR C677T and venous thrombosis, prospective studies could not confirm these results [37,38].

A second common polymorphism in the MTHFR gene, A1298C, has been described by van der Put [39]. 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 [36]. 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 [36].

Over the last fifteen years, a number of studies on the association between inherited thrombophilia and pregnancy loss have been published [40-49]. 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 [20]. 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. The data of this meta-analysis are confirmed and expanded by another systematic review of 40 studies [21] (Table 2). The initial observation that homozygosity for MTHFR C677T could be related to pregnancy loss [50,51], supported by more recent studies [52,53], could not be confirmed, neither in another sample [42], nor by meta-analysis [20,21]. The combined carriage of MTHFR polymorphisms C667T or A1298C with FVL or PTm mutation does not seem to increase the risk of fetal losses [21]. On the other hand, results of the Hordaland Homocysteine Study, performed on individuals from the Hordaland country in Western Norway and including over 5800 women analyzed, confirm a doubled risk of placental abruption for C677T homozygotes [54], and possible interaction of C667T with FVL carriage that seems to significantly increase the risk of stillbirth, 3.3-fold [55]. Discrepancies in the studies on the role of C677T as a risk factor in pregnancy loss can be partly explained with poorly characterized patient cohorts, specific population and lifestyle determinants, and interactions with unconsidered environmental and genetic factors.

Hyperhomocysteinaemia is a risk factor for placenta-mediated diseases such as pre-eclampsia and placenta abruption as well as for fetal neural-tube defects [39,51]. 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 conform to 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 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 [56]. 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 [57]. Reduced annexin A5 expression in the placental trophoblasts has also been demonstrated immunohistochemically in patients with preeclampsia [58]. Based upon these observations and the reported anticoagulation activity of the protein [59], 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 [60]. This hypothesis has received additional support from *in vitro* studies employing atomic force microscopy and functional assays [61]. Very recent data demonstrate that annexin A5 molecules forming 2D arrays on cellular membranes promote membrane repair and herewith enhance membrane stability [62]. In addition, in a mouse model of atherosclerosis, AnxA5 reduced local vascular and systemic inflammation and vascular remodeling and improved vascular function [63].

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 [64]. Genomic analysis of a German 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=533), respectively. Carriers of M2 were found to exhibit a more than two-fold higher RPL risk than non-carriers (OR 2.42, 95% CI 1.27 - 4.58) in comparison to unselected controls (PopGen), and an almost four-fold 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 [65]. The same study demonstrated that the abundance of placental ANXA5 mRNA in 26 women with obstetric complications (preeclampsia, PE and fetal growth restriction, FGR) was threefold lower than in a control group of seven women without pregnancy complications. Another study confirmed same reduced ANXA5 mRNA levels in placentas of FGR complicated pregnancies [66]. A more recent work communicated that decreased ANXA5 expression in M2/ANXA5 placentas, including such from women with PE and/or FGR, results of the carriage of the M2 allele, regardless of parental origin [67].

An analysis of the role of M2 in Italian women with repeated fetal loss or pregnancy-related hypertension corroborated the initial findings of the original RPL work [68]. The study reported a similar prevalence of M2 carriers (15%) in women from Southern Italy as in the German population. In addition, the authors also demonstrated a significant association between M2 carriage 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 to 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).

A recent work by the same group demonstrated the role of M2/ANXA5 in pregnancy-related venous thromboembolism, contributing about a three-fold associated risk for this condition [69].

Independent confirmation of the M2/ANXA5 association with recurrent fetal loss was recently obtained in the Japanese population, where carriage of the haplotype results in similar risk as observed for populations of Central Europe, but the population incidence is lower (5,5 vs. 15%) [70].

Thus, the haplotype M2/ANXA5 appears likely RPL risk factor for European and Asian populations.

M2/ANXA5 is unique as RPL risk factor because of three main reasons:

- high carriage rate among populations of Central Europe, estimated as 15%;
- M2/ANXA5 heterozygtes are at similar RPL risk as FVL and PTm carriers;
- the only thrombophilia related risk factor for comparatively early fetal losses, between gestational weeks (GW) 10 and 15

In the future, it would appear reasonable to study the role of M2 and other ANXA5 haplotypes in various 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.

Antiphospholipid Antibodies (aPL) 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 aPL [53,71]. Since aPL are a strong risk factor associated with fetal loss, great care should be taken by the selection of RPL patient groups for studies on hereditary thrombophila, as not to include any APS patients. 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 [72,73]. Lowered expression of ANXA5 in placentas of M2 haplotype carriers [65,67] could be potentially responsible for reduced coverage of phospholipid enriched trophoblast surfaces and hence lead to an increase in the number of exposed available antigenic determinants for generation of aPL. Preliminary results [Cherkelova et al., 2010, 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 Abs warrants further studies in larger patient groups.

Conclusive Remarks

Rising maternal age and growing fetal loss risk

There is an increasing tendency for childbearing to occur later in women's lives, particularly in Western Europe, Australia, New Zealand, Canada and the United States of America [74]. However, the biologically optimal period for childbearing is between 20 – 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 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.

Hereditary thrombophilia diagnostics in pregnant women. Evidence of embryonal factors

It should be noted that hereditary thrombophilic defects have been hitherto known for the majority (70%) of hereditary thrombophilia patients. All of these, listed in Table 1, are maternally transmitted lesions. The rest 30% of heritable thrombophilia are largely unknown and might be due to mutations in proteins auxiliary or co-factory to coagulation cascades. The M2/ANXA5 haplotype is yet another lesion affecting expression levels of the protein with potent anticoagulant function in placenta. Although it cannot be generally responsible for the rest of heritable thrombophilias, it is still a factor to consider when diagnosing the condition. It should also be noted that this defect is conveyed embryonally. Reduced placental expression is observed independent of parental carriage [67], and preliminary analysis demonstrates equal risk for paternal carriers in RPL couples [unpublished results].

Based upon our current knowledge, some forms of hereditary thrombophilia, including M2/ANXA5, clearly appear to be associated with RPL. SISET guidelines recommend testing for FVL and PTm in pregnancy [55]. 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.

The clinical guidelines for testing for heritable thrombophilia of the British Society for Haematology suggest testing of pregnant women with a family history of venous thrombosis if an event in a first-degree relative was unprovoked, or provoked by pregnancy, combined oral contraceptive exposure or a minor risk factor [75]. The result will be more informative if the first-degree relative has a known thrombophilia.

Anticoagulant therapy in RPL patients with heritable thrombophilia

A weak recommendation for heritable thrombophilia screening in pregnant women is made at the last American Society of Hematology meeting [76], because available studies do not exclude a beneficial effect of thromboprophylaxis in such patients. Data from clinical trials with anticoagulants (aspirin and low molecular weight heparin, LMWH) in idiopathic recurrent fetal loss are clearly insufficient to draw a justified conclusion about treating women with heritable thrombophilia. A thoroughly conducted trial on thrombophilic patients with a fetal loss, treated with aspirin, vs, aspirin plus LMWH demonstrated beneficial effect of the combined treatment, but lacked a placebo control group [77]. Another recently reported clinical study, the ALIFE trial, was properly controlled with a placebo group but did not report any significant benefit in both treated groups (aspirin and aspirin + LMWH) [78]. Last trial apparently included notable fraction of women with embryonic losses (< wk.10). Both trials were obviously underpowered to address the possibility of improved outcomes among carriers of heritable thrombophilia.

Because of uncertainties associated with the magnitudes of risk concerning heritable thrombophilia and uncertainties on possible benefits of thromboprophylaxis in pregnant women, current American College of Chest Physicians guidelines do not make firm recommendations on the use of antithrombotic therapy in this patient population [79]. These guidelines deleted previous weak recommendations [80], for hereditary thrombophilia screening in women with recurrent fetal loss and for antithrombotic therapy in RPL women with hereditary thrombophilia. The results of ongoing randomized clinical trials are necessary, to prove potential therapeutic efficiency of antithrombotic therapy in women with heritable thrombophillia and recurrent pregnancy loss (Effectiveness of Dalteparin Therapy as Intervention in Recurrent Pregnancy Loss [http://www.ClinicalTrials.gov; identifier: NCT00400387]; Prevention of Unexplained Recurrent Abortion by Enoxaparine [http://www.ClinicalTrials.gov; identifier: NCT00740545; TIPPS: Thrombophilia in Pregnancy Prophylaxis [http://www.ClinicalTrials.gov; identifier: NCT00967382]).

Noteworthy, individualized VTE risk assessment is recommended in pregnant patients with thrombophilia but no prior venous thrombosis, as opposed to routine pharmacologic prophylaxis. Antepartum clinical surveillance or LMWH/UFH therapy plus postpartum anticoagulants are suggested for thrombophilic women at risk for VTE. The French national recommendations for clinical practice (RPC) under the topic 'prevention of pre-surgical and obstetric VTE', grade the VTE risk for carriage of FVL of PTm mutations as 'moderate' and recommend LMWH/UFH therapy upon antepartum clinical surveillance [81].

Thrombophilic complications in assisted reproductive technology (ART) patients

Taking in account developments of the last 25 years, including the trend of progressing maternal age with concomitantly growing miscarriage risk in modern societies, it is clear that the use of ART is on the increase. Along with its use, the reports of thromboembolic complications (TEC) resulting in significant maternal morbidity, even mortality, are not rare [82]. TEC associated with ART generally occur as a feature of the ovarian hyperstimulation syndrome (OHSS) by an altogether unclear pathogenic mechanism. On average arterial thrombotic complications (ATC) are present earlier in *in vitro* fertilization (IVF) pregnancies and are almost always concurrent with OHSS symptoms development. In contrast, venous thrombotic complications (VTC) occur later, days to weeks after the resolution of OHSS. Although the true incidence of ATC and VTC resulting from ART is difficult to establish from the literature, it is clear from available reports that inherited thrombophilia has been detected in altogether 1/3 of the women tested, with 41% prevalence in the VTC vs. 19% in the ATC group [82]. Much research remains to be done to minimize the potentially devastating effects of TEC in IVF pregnancies, but testing for inherited thrombophilia might be an indication in ART.

M2/ANXA5 diagnostics and questions of therapy in RPL patients

Genotyping analyses of RPL cohorts from Central Europe (German, Italian and French) demonstrate incidence of the M2 haplotype in 24 – 34% of the patients, depending on the strength of the selection criterion (≥2, vs. ≥3 losses). In contrast, the incidence of the marker in the general Central European population is 15%, so the calculated relative risk for RPL carriers is between 2.5 and 3. Since this risk is very similar to the relative risks contributed by the 'classic' thrombophilia factors, PTm and FVL and the incidence of M2 in the general population is 3 - 5 fold higher, genetic testing should be recommended for women with comparatively early (10 – 15 GW) unexplained recurrent pregnancy losses, or pregnant women with unexplained VTE.

Taken the possibility that M2 carriage might be a predisposing factor for aPL Abs development, a treatment with anticoagulants that has proven successful in obstetric APS syndrome might be applicable at least to a subset of M2 carriers suffering RPL.

The possible therapeutic relevance of LMWH therapy in M2 carriers should be evaluated from maximum patient resources enrolled in completed and ongoing clinical trials to reach the necessary statistical power and because of the difficulties in obtaining sponsorship and organizing new trials with the necessary cohorts size.

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