Hereditary thrombophilia and recurrent pregnancy loss: a retrospective cohort study of pregnancy outcome and obstetric complications

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BACKGROUND: The association among hereditary thrombophilia, recurrent pregnancy loss (RPL) and obstetric complications is yet uncertain. The objective of the study was to assess the prognostic value of the factor V Leiden (FVL) and prothrombin (PT) mutations for the subsequent chance of live birth for women with RPL.

METHODS: Pregnancy outcome was recorded in a retrospective cohort of 363 women with a minimum of three consecutive pregnancy losses (early miscarriage, late miscarriage or stillbirth/neonatal death) who were not treated with anticoagulation therapy.

RESULTS: Of the 363 women, 29 were FVL-mutation carriers and 6 were PT-mutation carriers. The unadjusted live birth rate was 45.7% in FVL/PT carriers versus 63.4% in FVL/PT non-carriers, \( P = 0.04 \). The adjusted odds ratio for live birth in FVL/PT carriers was 0.48 (95% CI = 0.23–1.01), \( P = 0.05 \). Among the obstetric complications, only excessive bleeding was found to be associated with FVL/PT mutations.

CONCLUSIONS: In the unadjusted analysis, FVL and PT mutations have a negative prognostic impact on the live birth rate in women with RPL; however, when adjusting for significant covariates, the results no longer reach statistical significance. Strong conclusions on the association between obstetric complications and hereditary thrombophilia cannot be drawn from this study. Whether anticoagulation therapy would improve the prognosis in women with RPL and FVL/PT mutations remains to be documented in large randomized controlled trials.

Key words: recurrent pregnancy loss / hereditary thrombophilia / obstetric complications / retrospective cohort study / pregnancy outcome

Introduction

Approximately 1% of women attempting pregnancy experience at least three consecutive losses of intrauterine pregnancy: recurrent pregnancy loss (RPL). The role of hereditary thrombophilia, such as the factor V G1691A Leiden (FVL) mutation and the prothrombin (PT) G20210A mutation, has been addressed in case–control studies (Ridker et al., 1998; Martini et al., 2000; Younis et al., 2000; Rai et al., 2001) and meta-analyses of case–control studies (Rey et al., 2003; Robertson et al., 2006) where the mutations have been associated with RPL as well as obstetric complications, such as intrauterine growth restriction, pre-eclampsia and placental abruption.

The association between the FVL mutation and RPL seems stronger for non-recurrent second-trimester pregnancy loss compared with recurrent early pregnancy loss (Rey et al., 2003; Robertson et al., 2006). Only three cohort studies have addressed the impact of the FVL/PT mutations on pregnancy outcome in RPL patients, and these studies provide contradicting results. The live birth rate in untreated pregnancies among women with a history of early RPL was, in one study, significantly lower in 25 carriers of the FVL mutation compared with 198 non-carriers, and the same trend was found for women with a history of at least one late miscarriage (Rai et al., 2002). Carp et al. (2002), on the other hand, did not find a lower live birth rate in RPL patients with hereditary thrombophilia.
However, only a small proportion of these had the FVL or the PT mutations. Furthermore, in a group of women with unexplained recurrent miscarriage, Jivraj et al. (2009) found an insignificant difference in the live birth rate among FVL-positive women compared with FVL-negative RPL women: 48% (12 of 25) versus 57% (175 of 307). To add further to the uncertainty of the results, no difference was found in the rate of miscarriage between carriers and non-carriers of the FVL mutation among a group of women with previous thromboembolism (van Dunne et al., 2005).

Thus, in spite of evidence for an association between RPL and hereditary thrombophilia in meta-analyses, the prognostic value of the FVL and the PT mutations regarding the live birth rate remains uncertain. Further investigation of this is relevant since the screening for thrombophilia factors in RPL patients has already become standard practice in many fertility centres (Cleary-Goldman et al., 2007; Norrie et al., 2009). The results of randomized controlled trials do not support anticoagulation therapy for women with unexplained RPL (Clark et al., 2010; Kaandorp et al., 2010); however, the studies were not powered to be conclusive regarding the specific effect of anticoagulation therapy in women with hereditary thrombophilia and unexplained RPL.

The aim of our study was to investigate the prognostic value of the FVL and the PT mutations with regard to the live birth rate in a sufficiently powered cohort of RPL patients not treated with anticoagulation therapy. As secondary outcomes, we also studied the cumulative incidence of live births and obstetric and neonatal complications after referral.

**Materials and Methods**

**Definitions**

RPL was, in our study, defined as a minimum of three consecutive pregnancy losses (early or late miscarriage or stillbirth/neonatal death). The previous pregnancy losses were all verified either by positive urine hCG/serum hCG and ultrasound or uterine curettage and histology. Patients in whom none of the pregnancy losses had been verified as intrauterine by ultrasound or histology of evacuated tissue were not included since it cannot be excluded that the pregnancies verified by positive urine hCG/serum hCG had in fact been spontaneously resorbed ectopic pregnancies. Pregnancy loss was classified as either early (<gestational week 13 + 6) or late (gestational week 14–21 + 6), stillbirth was defined as loss after 22 weeks of gestation and neonatal death was defined as live birth where the baby died within the first 28 days of life. Live birth was classified as birth of a child surviving beyond 28 days, which is the neonatal period. To avoid bias, if the patient was pregnant beyond Week 12 at the time of first consultation, the outcome of this pregnancy was not included as the first pregnancy after referral.

**Patients**

Figure 1 illustrates the selection of the study population. The entire cohort comprised 1088 consecutive women referred to the Danish Recurrent Miscarriage Clinic in the period from 1986 to 1 May 2008. Inclusion criteria: all women referred with RPL and who were Danish-speaking and had not immigrated or died after referral. Exclusion criteria were: (i) abnormal uterine anatomy evaluated by hysterosalpingography,
hysteroscopy or uterine hydrosonography, (ii) abnormal blood karyotype in either partner, (iii) positive titre for the lupus anticoagulant, (iv) irregular menstrual cycles defined as cycle lengths outside the interval between 23 and 35 days, (v) non-Caucasian origin (primarily because of the lower prevalence of the hereditary thrombophilias in these populations) or (vi) treatment with anticoagulation therapy (apart from low-dose aspirin) during the first pregnancy after referral. Demographic data of the study group are presented in Table I.

**Table I** Demographic data, pregnancy outcome at time of referral and after referral in a group of women with unexplained RPL, n = 363.

<table>
<thead>
<tr>
<th>FVL positive (n = 35)</th>
<th>FVL negative (n = 328)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>At time of referral</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median maternal age at first consultationa</td>
<td>31 (29–33) 22–42</td>
<td>32 (19–43 22–43)</td>
</tr>
<tr>
<td>Median number of pregnancy lossesb</td>
<td>4 3–4 3–13</td>
<td>4 3–4 3–10</td>
</tr>
<tr>
<td>Median number of early pregnancy losses</td>
<td>4 3–4 2–12</td>
<td>4 3–4 0–10</td>
</tr>
<tr>
<td>Median number of late pregnancy losses</td>
<td>0 0–0 0–1</td>
<td>0 0–0 0–2</td>
</tr>
<tr>
<td>Median number of stillbirths/neonatal deaths</td>
<td>0 0–0 0–2</td>
<td>0 0–0 0–3</td>
</tr>
<tr>
<td>≥5 previous lossesb</td>
<td>12 (34.3)</td>
<td>81 (24.7)</td>
</tr>
<tr>
<td>Previous live birth (≥ 1)</td>
<td>11 (31.4)</td>
<td>145 (44.2)</td>
</tr>
<tr>
<td>BMIc,e</td>
<td>21.2 19.9–23.8 15.7–36.3</td>
<td>21.9 20.3–24.5 16.2–42.2</td>
</tr>
<tr>
<td>Smoking</td>
<td>11 (31.4)</td>
<td>80 (24.4)</td>
</tr>
<tr>
<td><strong>After referral</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Live birth rate, unadjusted</td>
<td>16/35 (45.7)</td>
<td>208/328 (63.4)</td>
</tr>
<tr>
<td>Live birth rate, unadjusted, after spontaneous conceptionf</td>
<td>5/13 (38.5)</td>
<td>76/128 (59.4)</td>
</tr>
<tr>
<td>Live birth rate, unadjusted, after ARTg</td>
<td>4/9 (44.4)</td>
<td>29/43 (67.4)</td>
</tr>
<tr>
<td>Stillbirth/neonatal Death</td>
<td>1 (2.9)</td>
<td>4 (1.2)</td>
</tr>
<tr>
<td>Late miscarriage</td>
<td>1 (2.9)</td>
<td>8 (2.4)</td>
</tr>
<tr>
<td>Early miscarriage</td>
<td>17 (48.6)</td>
<td>107 (32.6)</td>
</tr>
<tr>
<td>Cumulative live birth incidenceh</td>
<td>22 (62.9)</td>
<td>274 (83.5)</td>
</tr>
</tbody>
</table>

IQR, inter-quartile range; ART, assisted reproductive technology.
aThis variable was binomially distributed.
bIncludes early and late pregnancy losses and stillbirths/neonatal deaths.
cData on the method of conception were available on 215 of 363 women and of these, the method of conception was unknown in 22 cases.
dSee text for definition.
eData for BMI were available for 315 women.

Data collection

The project information was mailed to 666 women whom we asked to return a form stating whether or not they wished to participate in the project. Non-responders received a reminder after 4 and 7 weeks, respectively, resulting in a final response rate of 89% (n = 594) of which 23% did not wish to participate (n = 136). Interested women (n = 458) were contacted by telephone and were given oral information about the project and subsequently mailed a questionnaire of which 434 were returned. Topics of the questionnaire were: outcome of first pregnancy (if any) after the first consultation, outcome of pregnancies subsequent to this pregnancy, obstetric complications (placental abruption, pre-eclampsia, excessive bleeding defined as bleeding resulting in subsequent blood transfusion) if birth and smoking habits at the time of first consultation. All returned questionnaires were carefully checked, and in the case of inconsistent or missing data, the patients were contacted by telephone for clarification. Of the 434 women, 71 had not been pregnant after referral and so the remaining 363 women represent the final study group.

Outcome analysis

Primary outcome was defined as unadjusted and adjusted incidence rate of live birth after referral defined as: (the number of live births in the first pregnancy after referral)/(all first pregnancies after referral). As the secondary outcomes, we studied (i) the cumulative incidence of live birth after referral defined as: (the proportion of women who in any one pregnancy after referral had a live birth)/(number of women included in the study), i.e. achievement of a live birth in the total follow-up-period after referral, and (ii) the incidence rate of obstetric or neonatal complications in the first pregnancy after referral defined as: (the number of specified obstetric or neonatal complications in connection with birth in the first pregnancy after referral)/(all pregnancies ending with birth in the first pregnancies after referral).
Statistical analysis
A sample size calculation regarding the primary study question was performed based on the finding of the study by Rai et al. (2002) with an acceptable Type I error of 0.05, Type II error of 0.10, experienced frequency of live birth in carriers versus non-carriers of 69.3 and 37.5% and an expected prevalence of carriers of the FVL/PT mutations of 8%. This resulted in a required sample size of \(~365\) (29 FVL-/PT-positive women and 336 FVL-/PT-negative women).

The $\chi^2$ test and the Fisher’s exact test were used to compare unadjusted live birth rates between FVL-/PT-positive and FVL-/PT-negative women. The adjusted outcome measure of live birth versus pregnancy loss (odds for live birth) was studied by a logistic regression analysis performed to identify the possible prognostic variables that best predicted the outcome of live birth. The variables to be entered into the model were identified in a univariate analysis performed to identify variables significantly ($P < 0.05$) associated with live birth outcome. The following variables were tested in the univariate analysis: previous live birth prior to referral, smoking (cigarettes) at the time of referral, $\geq 5$ pregnancy losses prior to referral, age $\geq 35$ years at the time of referral, the presence or the absence of the FVL and the PT mutation and treatment with intravenous immunoglobulin (Ivlg) in the first pregnancy after referral. Of these variables, three significantly influenced live birth outcome and were directly entered into the model: the number of previous pregnancy losses less than or equal to Week 21 + 6, the presence or the absence of smoking (cigarettes) at the time of referral and the presence or the absence of the FVL and the PT mutations. There was only a trend towards maternal age influencing the live birth outcome ($<35$ years: 64.1% live birth versus $\geq 35$ years: 56.3% live birth, $P = 0.2$). However, since it is widely accepted that there is an association between maternal age and risk of pregnancy loss (Nybo Andersen et al., 2000), we decided to include the variable in the model. Maternal age and the number of previous pregnancy losses were entered as continuous variables, whereas the other variables were converted into binary categories. The FVL and the PT mutations were grouped together as one variable, and heterozygotes and homozygotes for the mutations were categorized together.

To compare the cumulative live birth rate and obstetric complications between FVL-/PT-positive and FVL-/PT-negative women, we used the $\chi^2$ test and the Fisher’s exact test. For comparing birthweight in subsequent births, the independent samples $t$-test was used.

$P$-values of $<0.05$ and odds ratios (ORs) with confidence intervals (CI) not including the value of 1.0 were accepted as proof of statistical significance. Data analyses were performed using the SPSS (Statistical Package for Social Sciences) version 15.0.

Verification of live births and stillbirths
Using the Danish Civil Registration number of each of the women, live births and stillbirths, obstetric complications [premature birth with rupture of membranes: (PPROM), excessive bleeding, placental abruption, asphyxia and pre-eclampsia], gestational age at birth (weeks), birthweight (g), parity and sex of subsequent children were identified in the Danish National Birth Registry and the Danish National Hospital Discharge Registry. In cases of inconsistency between the register-reported data and the self-reported data, we decided to accept the register data as correct. In the cases where the obstetric complications turned out to be significantly associated with the FVL/PT mutations, we additionally calculated the difference between the FVL-/PT-positive and FVL-/PT-negative women using the self-reported data. The register data were also used to establish the cumulative live birth rate among non-responders and women who did not wish to participate in the project. For identification of pregnancies and obstetric complications, the following International Classification of Diseases (ICD)-10 codes were explored: DO14, DO15, DO312D, DO364, DO42.0, DO42.2, DO42.4, DO45, DO67–68, DO80–84, DP00–DP02, DP20, DP21, DP95.9, DZ37 and DZ38, diagnostic codes for Caesarean section: DO82.0, DO82.1, DO82.8, DO82.9, DO84.2, DO84.3 and DP03.4 and surgical codes for Caesarean section: KMCA (after 1996) and 66020/66040 (before 1996). For identification of pregnancies before 1 January 1994, a list of complementary ICD-8 codes was made.

Laboratory testing for hereditary thrombophilia
A total of 363 women were tested for the FVL and the PT mutations. In 139 cases, the test had been performed as part of the standard laboratory screening performed at the time of referral, whereas the testing in 224 women was based on DNA from an existing biobank of blood samples from the couples with RPL referred to our clinic since 1986. Where no DNA was left, new blood samples were collected ($n = 21$).

Genomic DNA was extracted from EDTA-anticoagulant blood samples using the Maxwell®16 System Blood DNA Purification Kit (Promega, Madison, WI, USA). The FVL and the PT genotypes were determined using real-time PCR assays performed in a LightCycler® 480 instrument (Roche, Mannheim, Germany). Both the FVL assay and the PT assay were based on methods previously described in the literature (van den Bergh et al., 2000) to identify the two alleles F5 c1691 G*A (R506Q, Leiden; rs6052) and F2 c20210 G*A (PT; rs1799963). Women carrying the specific alleles (in hetero- or homozygotic forms) are called in this article FVL-positive or PT-positive women, respectively.

Ethics
The project was approved by the Regional Ethics Committee of the Copenhagen Region (ref. no.: H-C-2008-061) and was reported to the Danish Data Protection Agency according to Danish legislation. All patients gave informed oral and written consent to participation.

Results
The FVL mutation was diagnosed in 29 patients (8.0%) of whom 1 was homozygous and 28 were heterozygous and the PT mutation was diagnosed in 6 patients (1.7%), all of whom were heterozygous. Of the 139 patients tested as part of the standard laboratory screening, 19 tested positive for the FVL mutation (13.7%) and 2 were positive for the PT mutation (1.4%). As seen in Table I, FVL-/PT-positive and FVL-/PT-negative women were not significantly different in terms of the prognostic factors: maternal age, median number of previous miscarriages and the proportion of women with a live birth prior to referral.

Clinical outcome
In the first pregnancy after referral, 224 of the 363 patients (61.7%) experienced live birth (this included four pregnancies with twins and five ongoing pregnancies presumed to result in live birth), 5 had a stillbirth/neonatal death, 133 miscarried again and 1 pregnancy was terminated in Week 14 because of an abnormal fetal karyotype. Karyotyping of products of conception was performed in 26 of the 134 miscarriages (18 with normal and 6 with abnormal karyotypes, whereas 2 cultures failed). These were women referred after 2000, whereas we do not have exact figures before this date.
The unadjusted live birth rate was 45.7% (n = 16 of 35) versus 63.4% (n = 208 of 328) in FVL-/PT-positive versus FVL-/PT-negative women, P = 0.04. When the analysis was performed separately for the two mutations, the unadjusted live birth rate was 48.3% (n = 14 of 29) versus 62.9% (n = 210 of 334) in FVL-positive versus FVL-negative women, P = 0.12, and 33.3% (n = 2 of 6) versus 62.2% (n = 222 of 357) in PT-positive versus PT-negative women, P = 0.21. Logistic regression analysis showed that after adjusting for significant covariates, FVL-/PT-positive women were less likely to experience a live birth compared with FVL-/PT-negative women, OR = 0.48 (95% CI = 0.23–1.01, P = 0.05; Table II). The model was systematically tested for all possible interaction terms and none was found to be significant. The cumulative live birth rate in the follow-up-period after referral was 62.9% among the FVL-/PT-positive women versus 83.5% among the FVL-/PT-negative women, P < 0.01.

In the first pregnancy after referral for FVL-/PT-positive versus FVL-/PT-negative women, the frequencies of stillbirth/neonatal death were 2.9% (n = 1) versus 1.2% (n = 4), P = 0.40, of late miscarriage 2.9% (n = 1) versus 2.4% (n = 8), P = 0.60 and of early miscarriage 48.6% (n = 17) versus 32.6% (n = 107), P = 0.06. Of the five women with stillbirth/neonatal death as primary outcome, one FVL-positive woman experienced a neonatal death; the child was delivered by Caesarean section in Week 27 because of extreme intrauterine growth restriction (birthweight 590 g) but died at 28 days old. The four FVL-negative women experienced an intrauterine death at gestational age 31 weeks/birthweight = 1620 g, an intrauterine death during delivery caused by placental abruption at gestational age 41 weeks/birthweight = 3950 g, neonatal death 1 week after delivery by emergency Caesarean section because of placental abruption at gestational age 29 weeks/birthweight = 1372 g and a neonatal death soon after spontaneous delivery at 24 weeks with a birthweight = 435 g. None of the stillbirths/neonatal deaths had any malformations detected.

Among the 224 women who gave birth after referral (not including the five women for whom we do not have obstetric register data because their pregnancies were still ongoing at the time of the register withdrawal), no significant differences were seen between the FVL-/PT-positive and the FVL-/PT-negative women in the prevalence of the obstetric complications, birthweight <2500 g, Caesarean section, preterm birth with rupture of membranes/PPROM, placental abruption, asphyxia, pre-eclampsia or preterm birth/birth before the beginning of Week 37. However, the diagnosis of excessive bleeding was registered more frequently among the FVL-/PT-positive women compared with the FVL-/PT-negative women, 11.8% (n = 2) versus 0.5% (n = 1), P = 0.02. When self-reported data were used, excessive bleeding was reported in 3 of 206 (1.5%) of the FVL-/PT-negative women compared with 2 of 17 (11.8%) of the FVL-/PT-positive women, P = 0.05. One woman did not answer the questions on obstetric complications in the questionnaire. Mean birthweight was 3140 ± 872 g in FVL-/PT-positive women compared with 3370 ± 696 g in FVL-/PT-negative women, P = 0.20.

Table II Adjusted ORs of variables predictive for live birth outcome in a group of patients with unexplained RPL, n = 363.

<table>
<thead>
<tr>
<th>Independent variable*</th>
<th>OR</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of previous pregnancy losses less or equal to Week 21 + 6</td>
<td>0.75</td>
<td>0.63–0.89</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Maternal age</td>
<td>0.94</td>
<td>0.89–0.99</td>
<td>0.02</td>
</tr>
<tr>
<td>Smoking</td>
<td>0.51</td>
<td>0.31–0.84</td>
<td>0.01</td>
</tr>
<tr>
<td>FVL/PT positivity</td>
<td>0.48</td>
<td>0.23–1.01</td>
<td>0.05</td>
</tr>
</tbody>
</table>

*Variables: smoking and FVL/PT positivity are both dichotomous variables. Maternal age and number of previous pregnancy losses less than Week 21 + 6 are nominal variables where an increase in the variable by 1 means a decrease in the odds for live birth by the value of the OR, and an increase in the variable by 2 means a decrease in the odds for live birth by double the OR and so forth. FVL, factor V Leiden; PT, prothrombin. P-values of <0.05 and CIs not including the value 1.00 were accepted as significant.

Treatment with lVg was given as part of randomized controlled trials (Christiansen et al., 2002) or after conclusion of these trials as a routine treatment offered in our clinic to patients with at least four previous miscarriages. lVg treatment during pregnancy was given to 25.7% of the FVL-/PT-positive and 32.6% (n = 107) of the FVL-/PT-negative women, P = 0.41. Among the women referred after 2000, 24% (n = 52) conceived after assisted reproductive treatment (IVF/ICSI, transfer of cryopreserved embryos or intrauterine insemination with partner’s or donor sperm), 66% (n = 141/215) conceived spontaneously and for 10% (n = 22/215) data on the method of conception were not available. With regard to patients referred before 2000, we do not have exact figures on method of conception, but it is estimated that less than 10% of these patients conceived after assisted reproductive treatment.

**Discussion**

On the basis of a large Danish cohort of women with RPL, we found that the unadjusted live birth rate in the first pregnancy after referral was lower in FVL-/PT-positive compared with FVL-/PT-negative women. When adjusting for significant covariates, the difference in the subsequent live birth rate between FVL-/PT-positive and FVL-/PT-negative women was no longer significant. Since the confidence intervals in the adjusted analysis cross unity, a firm conclusion regarding the prognostic value of the FVL/PT mutations for the subsequent chance of live birth cannot be made.

The strength of our study is the analysis of ORs for live birth outcome adjusted for important prognostic variables, as such an analysis has not previously been carried out in RPL patients with and without hereditary thrombophilia. One weakness of our study is that the inclusion criteria differ from those of the previous cohort studies (Carp et al., 2002; Rai et al., 2002; Jivraj et al., 2009) in two aspects: these studies excluded women with i) positive titres for anticardiolipin antibodies and ii) women with a history of thrombosis. We excluded women with the lupus anticoagulant, but we did not directly exclude women who were solely anticardiolipin antibody positive. A reason for this is that the cut-off values for positivity for anticardiolipin antibodies have changed several times during the period in which the study group was referred (Miyakis et al., 2006), making it difficult to introduce a standardized cut-off level. However, we believe that the majority of patients with high titres of anticardiolipin antibodies were indirectly excluded, since in our study we systematically excluded all women having received anticoagulation therapy with heparin or coumadin derivates (n = 37) who were, in most instances, patients...
with lupus anticoagulant, high titres of anticardiolipin antibodies or a previous thromboembolic episode, all of which are established indications for heparin treatment during pregnancy in our as well as other Danish gynaecological/obstetrical clinics.

Other weaknesses of our study concern: (i) treatment during the first pregnancy after referral, (ii) the low proportion of karyotyping of products of conception and (iii) the retrospective as opposed to prospective study design. Regarding treatment during first pregnancy after referral, a considerable proportion of the women in the study received treatment with lIg, but the decision to offer lIg was completely independent of the presence or the absence of the FVL-/PT-mutations, and in our study, there was no significant difference in the proportion of women treated with lIg when comparing FVL-/PT-positive and FVL-/PT-negative women. lIg-treatment can cause thromboembolic complications (Katz and Shoenfeld, 2005) as an adverse effect, although none of the women in our study experienced thromboembolic episodes due to lIg treatment. Still, this risk factor should be taken into account, when considering treatment with lIg to RPL patients with other risk factors for thromboembolic episodes. Since lIg is known to cause thromboembolic complications, an influence on the association between hereditary thrombophilia and increased risk of new pregnancy loss cannot be excluded; however, the univariate analysis did not find that lIg treatment was significantly influencing live birth rate. Concerning other treatments that could influence pregnancy outcome, some patients may have taken low-dose aspirin. We have always in our clinic advised women with RPL with or without hereditary thrombophilia not to use low-dose aspirin, since this is not evidence-based (Clark et al., 2010; Kaandorp et al., 2010); however, we cannot exclude that a few of the women had taken this due to advice from other doctors. Furthermore, we have treated very few (<2%) with progesterone in our study because the effect of this still remains to be documented by means of randomized controlled trials (Haas and Ramsey, 2008). Karyotyping of miscarriages was only performed in a minority of cases because in our country, the recommended procedure in the case of early miscarriage (<8th week) is not to do curettage, but rather to offer medical abortion or treat expectantly resulting in poor possibilities for successful karyotyping. Because of the low proportion of karyotyped products of conception, we decided to include all first pregnancies after referral as primary outcome including those with an abnormal karyotype. Without doubt, some of the miscarriages have been aneuploid; however, the incidence of these is expected to be similar in carriers and non-carriers of the thrombophilia mutations. The ideal study design would be a straightforward prospective study; however, this is not possible if an adequate study population is to be reached. The retrospective cohort design is thus the second-best option, but recall bias is likely to have influenced the data collected in our questionnaire. We tried to avoid recall bias by use of register validation whenever possible, and a substantial part of the study group was not aware of their FVL-/PT-carrier status at the time of filling out the questionnaire. The five stillbirths/neonatal deaths registered as prospective outcome were all caused by placental dysfunction (placental insufficiency or abortion) or extreme preterm birth. These are complications that can theoretically be caused by the FVL/PT mutations and we consider them as relevant outcomes.

We were not able to demonstrate a significant impact of hereditary thrombophilia regarding the obstetric complications apart from excessive bleeding, which may be due to placental abruption. However, the number of women with excessive bleeding was extremely small, and the sample size calculation is based on the assessment of the primary outcome, live birth. Thus, the observed difference could be due to chance. Also excessive blood loss is difficult to estimate and a marker such as number of blood transfusions given would have been ideal. Still, the fact that excessive bleeding was the only obstetric complication which turned out to be significant with a larger proportion among the women with hereditary thrombophilia, emphasizes the need to test the effect of anticoagulation therapy in these patients in randomized controlled trials since the possibility of anticoagulation doing more harm (increase bleeding in patients with placental abruption) than good in these patients cannot be excluded.

In conclusion, in women with RPL, unadjusted data show that hereditary thrombophilia has a negative impact on prospective pregnancy outcome; however, after adjusting for significant covariates, the results are no longer significant. The results of randomized controlled trials do not support anticoagulation therapy for women with unexplained RPL. Specifically, whether anticoagulation therapy for women with RPL and hereditary thrombophilia will improve the prognosis remains to be documented.

Authors’ roles

M.L., H.S.N., A.N.A. and O.B.C. contributed to the study design. M.L., R.S. and O.B.C. collected the blood samples. R.S. performed the DNA extraction. T.V.H. performed the laboratory analyses. M.L. and H.S.N. performed the statistical analyses. M.L. and O.B.C. wrote the article. All authors critically revised the article and approved the final version.

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