Polymorphisms of haemostasis genes as risk factors for preterm delivery

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Summary
Clinical trials evaluating the potential benefit of anticoagulant treatment in pregnant women with inherited thrombophilia are based on the observation that a genetic predisposition to thrombosis is associated with frequent abortions and preterm birth. It was the aim of our study to delineate the impact of genetic polymorphisms with prothrombotic and antithrombotic effects on the occurrence of preterm birth in a large cohort of very-low-birth-weight (VLBW)-infants and their mothers. We examined the factor V Leiden and the prothrombin G20210A mutation, the factor VII 121del/ins and the factor XIII Val34Leu polymorphism in preterm very-low-birth-weight (VLBW, n=593) and term-born-infants (n=278) and their mothers (n=785). The primary outcome was preterm vs. term birth. From all polymorphisms tested, the maternal factor VII-121del/ins polymorphism (26.2 vs. 17.6 %; p=0.009) and the infant’s factor VII-121del/ins polymorphism (29.0 vs. 20.0 %; p=0.009) were more frequent in singleton VLBW and their mothers compared to term infants and their mothers. Furthermore, the frequency of the factor XIII-Val34Leu polymorphism was significantly lower in singleton VLBW than in term infant controls (5.1 vs. 9.6%, p=0.025). In a multivariate regression analysis, previous preterm delivery (OR=3.8, 95% CI: 1.7–8.4), the maternal carrier status of the factor-VII-121del/ins polymorphism (OR=1.7, 95% CI: 1.12–2.5, p=0.007) and the lower frequency of infant’s factor-XIII-Val34Leu polymorphism (OR=0.53; 95% CI: 0.29–0.96; p=0.038) were found to be independently associated with preterm delivery. In VLBW mothers with pathological CTG as cause of preterm delivery, the frequency of factor V Leiden mutation was significantly increased compared to VLBW mothers without pathological CTG (14.1 vs. 6.1%, p=0.01). The investigated haemostasis gene polymorphisms have a much lower impact on subsequent preterm delivery than known risk factors such as previous preterm birth. The reported association of the factor-VII-121del/ins polymorphism on preterm delivery and its clinical relevance needs to be further elucidated.

Introduction
Preterm birth is still the leading cause of perinatal morbidity and mortality with a prevalence of up to 11% (1). Despite considerable efforts to understand the pathogenic pathways associated with preterm birth, the mediating factors that predispose women to preterm delivery are relatively unknown. As substantial progress has been made in the identification of inherited hypercoagulable disorders, recent investigations suggest a potential role of inherited thrombophilia in adverse pregnancy outcomes (2–4). These thrombophilic disorders include the factor V Leiden mutation, which renders factor V resistant to cleavage by activated protein C, and the prothrombin G20210A mutation which was found to be associated with raised plasma concentrations of prothrombin (3). In a recent meta-analysis, factor V Leiden was suggested to be associated with early and late recurrent foetal loss and late non-recurrent foetal loss, while the prothrombin G20210A mutation has a significant impact on early recurrent and late non-recurrent foetal loss (4). In line with this, a significantly increased prevalence of these prothrombotic mutations was described in a cohort of preterm VLBW-infants compared to healthy term infants (5).

Based on these observations, several studies suggest a potential benefit of anticoagulation prophylaxis with heparin or as-
pirin for pregnant women carrying the factor V Leiden or the prothrombin G20210A-mutation (6–9). However, the issue of anticoagulation prophylaxis for women with thrombophilia and pregnancy complications is critical (8, 10), since randomised placebo-controlled trials have not been completed so far (11) and higher rates of miscarriage were reported in unselected groups of pregnant women exposed to non-steroidal anti-inflammatory drugs during pregnancy (12–14). In addition to that, the limited information on the effect of inherited thrombophilia and preterm delivery cannot justify anticoagulation prophylaxis at this stage (15).

Besides hypercoagulable states, lower levels of coagulation factors were reported in pregnant women with history of previous preterm delivery or abortion (2, 16). Furthermore, women with specific coagulation factor deficiencies are prone to pregnancy loss (17). One candidate of genetic risk factors for preterm delivery is the factor VII–121del/ins promoter polymorphism which results in an approximately 20% decrease of factor VII coagulant activity (18–20). The factor XIII-Val34Leu polymorphism is biochemically associated with the formation of a fibrin meshwork having thinner fibers, smaller pores and altered permeation characteristics compared to fibrin clots formed by individuals with factor XIII-Val34 homozygosity (21). In addition to that, the factor-XIII-Leu 34 allele was demonstrated to have a protective effect against thrombosis and infarction in various clinical entities (21–26).

It was the aim of this study to delineate the associations of genetic polymorphisms with prothrombotic (factor V Leiden, prothrombin G20210A) and antithrombotic (factor-VII-121del/ins, factor XIII-Val34Leu) effects with the occurrence of preterm birth in a large cohort of very-low-birth-weight (VLBW)-infants and their mothers compared to healthy term infants and their mothers, who served as controls.

Materials and methods

Study population

We prospectively studied the influence of gene polymorphisms involved in the blood coagulation system on preterm delivery in a large cohort of VLBW infants (inclusion criteria: birth weight <1500g, gestational age <37 weeks, exclusion criteria: outborn VLBW, lethal disabilities). The study population consisted of 871 infants (593 VLBW-and 278 term-born-infants) and their mothers (507 mothers of VLBW infants and 278 mothers of term infants), and was prospectively enrolled at neonatal intensive care units (VLBW, consecutive births) or the nurseries (random selection of controls) of participating centres. This multicenter trial started in 2000 and finished in October 2003. Written informed consent was given by all mothers participating in our study. The primary outcome of this study was preterm vs. term birth. The cause for preterm delivery (pre-eclampsia, intrauterine infection, placental abruption, pathological cardiocography (CTG), premature labor) was defined by the attending physician. Clinical data of all infants and their mothers were documented and coded before mutation analysis.

Genotyping

DNA-samples were extracted from dried blood spots (retrospective study) and from buccal swabs (prospective study). DNA was extracted with a commercially available kit (Qiagen, Hilden, Germany). All polymorphisms were detected by PCR and restriction enzyme digestion. Primer and DNA-sequences were selected from previously published reports. Primer pairs for detection of the FVL-mutation were 5'-TCA GGC AGGA AAC AAC GCAC TA-3' (DdeI-digest; 29) and 5'-GGCC TG GTC TGG AAGC T-3' (HindIII-digest, 27), for the F2A20210G-mutation 5’-GCA C AGC GCT TTC TTG AGGC TC TCT TC-3' and 5'-GAGC GG ACGG TT TTG TTG GAGC GAG A-3' (HindIII-digest; 28), for the F13Val/Leu-mutation 5’-CAT GCC TTT TCT GTT GTC TTC-3’ and 5’-TAC CTT GCA GGT TGA CGC CCC GGG GCA CTA-3’ (Ddel-digest; 29) and 5’-GGG CTG TGG TGG AGG CTC TCT TC-3’ and 5’-GAG CGG ACG GTT TTG TGG CCA GCG-3’ (HindIII-digest) for the factor VII ins/del-mutation (19).

The expected carrier frequencies for Caucasian populations for the homo- or heterozygous factor V Leiden-, prothrombin G20210A-, factor-VII-121 ins/del- and homozygous factor XIII-Val34Leu polymorphism were 4.9, 3.0, 19.9 and 3% (30). Based on these expected frequencies, an alpha error < 0.05 (twosided) and a power of 0.8, our sample size (term born singletons, n=278 vs. preterm VLBW-singletons, n=593) would be sufficient to detect differences of carrier-frequencies between cases and controls of 5% (prothrombin G20210A, factor XIII-Val34Leu ) to 9% (factor VII ins/del).

Statistical analysis

Data analysis was performed using the SPSS 9.0 data analysis package (Munich, Germany). Hypotheses were evaluated with Fisher’s exact test (two sided) and multivariate logistic regression models. A p value < 0.05 was considered as statistically significant.

Ethics

All parts of the study were approved by the local committee on research in human subjects of the University of Lübeck.

### Table 1: Clinical characteristics of mothers and infants.

<table>
<thead>
<tr>
<th></th>
<th>Mothers term born infants (n=278)</th>
<th>Mothers VLBW-infants (n=597)</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years; mean/SD)</td>
<td>30.0 / 5.4</td>
<td>29.7 / 5.5</td>
<td>0.56</td>
</tr>
<tr>
<td>Previous preterm delivery (%)</td>
<td>2.9</td>
<td>8.5</td>
<td>0.002</td>
</tr>
<tr>
<td>Maternal descendence (%)</td>
<td>92.5</td>
<td>78.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>- Germany</td>
<td>4.3</td>
<td>11.4</td>
<td></td>
</tr>
<tr>
<td>- other European countries</td>
<td>1.4</td>
<td>6.5</td>
<td></td>
</tr>
<tr>
<td>- Turkey, Middle East</td>
<td>0.4</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>- Asia</td>
<td>0.0</td>
<td>1.4</td>
<td></td>
</tr>
<tr>
<td>- Africa</td>
<td>1.4</td>
<td>1.3</td>
<td></td>
</tr>
<tr>
<td>Multiple pregnancy (%)</td>
<td>-</td>
<td>21.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Gestational age at delivery (weeks; mean/SD)</td>
<td>39.5 / 1.2</td>
<td>28.3 / 2.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Birth weight of infants (grams; mean/SD)</td>
<td>3554 / 471</td>
<td>1062 / 291</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Infant gender (male, %)</td>
<td>49.3</td>
<td>50.9</td>
<td>0.71</td>
</tr>
</tbody>
</table>

*p-test for maternal age, Mann-Whitney U test for gestational age and birth weight, chi-square test for previous preterm delivery, maternal descendence, multiple pregnancy and infant gender
Results

The clinical data of mothers and infants are given in table 1. Distribution of polymorphisms of haemostatic genes in preterm VLBW vs. term infants and their mothers were in Hardy-Weinberg equilibrium. From all polymorphisms tested, the maternal factor-VII-121del/ins polymorphism (26.2 vs. 17.6%; p=0.009) and the infant’s factor-VII-121del/ins polymorphism (29.0 vs. 20.0%; p=0.009) were more frequent in singleton VLBW and their mothers compared to term infants and their mothers. In addition to that, the frequency of the factor XIII-Val34Leu polymorphism was significantly lower in singleton VLBW than in term infant controls (5.1 vs. 9.6%, p=0.0025, table 2). Although the frequency of the factor-V-Leiden mutation tended to be higher in VLBW mothers (8.3% vs. 5.2%), this observation proved not to be significant (p=0.17).

To rule out possible confounding, we did a multivariate logistic regression analysis including previous preterm delivery <37 gestational weeks and all gene polymorphisms studied as independent variables and preterm delivery as dependent variable. Multiple VLBW infants were excluded from this analysis as multiple pregnancy is a known risk factor for preterm delivery. After stepwise exclusion of non-significant independent variables, only previous preterm delivery <37 gestational weeks (OR=3.8, 95% CI: 1.7–8.4, p=0.001), the maternal carrier status of the factor-VII-121del/ins polymorphism (OR=1.7, 95% CI: 1.12–2.5, p=0.007), and the lower frequency of infant’s factor XIII-Val34Leu polymorphism (OR=0.53, 95% CI: 0.29–0.96, p=0.038) were significant predictors of preterm delivery.

Causes of preterm delivery in the whole group were intrauterine infection (21.3%), pre-eclampsia (17.9%), placental abruption (6.3%), pathological CTG (24.3%) and premature labor (33.7%). There has been no difference between mothers with or without polymorphisms of haemostatic genes in causes of preterm delivery except for the higher frequency of factor V Leiden mutation in VLBW mothers with pathological CTG compared to VLBW mothers without pathological CTG (14.1% vs. 6.8%, p=0.01; table 3).

Discussion

Our present study showed the impact of functionally relevant gene variants involved in haemostasis on preterm delivery in a large cohort of VLBW infants, term infant controls and their mothers.

The maternal factor-V-Leiden and prothrombin G20210A genotype are well recognized prothrombotic risk factors associated with late foetal loss (4). In contrast, both mutations were found to have no significant impact on preterm delivery in our cohort. These data suggest that more information is needed before potentially harmful antithrombotic prophylaxis/treatment is offered based on genetic predisposition (10, 15, 31).

In addition, we could not confirm our previous data on the significant impact of the infant’s factor V Leiden and prothrombin G20210A genotype on preterm delivery. Although the prothrombin G20210A genotype proved to be an independent predictor of preterm birth in the retrospective cohort (5), this mutation was found to have no effect in our prospective cohort.

Haemostasis during normal pregnancy is considered to be a fine balance between ensuring proper implantation and placental development, adequate supply of oxygen and transport of waste products through the placental-uterine interface. In later pregnancy, haemostatic balance changes in the direction of hypercoagulability, thus decreasing bleeding complications during de-

Table 2: Frequency of polymorphisms of haemostatic genes in infants and mothers. Frequencies for homozygous (factor XII-Val34Leu) and heterozygous or homozygous (factor-V-Leiden, prothrombin-G20210A, factor-VII-ins/del) mutations (all data as %) are described.

<table>
<thead>
<tr>
<th>Polymorphisms of Haemostasis Genes</th>
<th>Term singletons</th>
<th>VLBW singletons</th>
<th>VLBW multiples</th>
<th>p*</th>
<th>Mothers term</th>
<th>Mothers VLBW</th>
<th>Mothers VLBW mult.</th>
<th>p**</th>
</tr>
</thead>
<tbody>
<tr>
<td>factor-V-Leiden</td>
<td>6.0</td>
<td>6.0**</td>
<td>5.6**</td>
<td>0.68</td>
<td>5.2</td>
<td>8.3</td>
<td>8.2</td>
<td>0.17</td>
</tr>
<tr>
<td>prothrombin G20210A</td>
<td>2.5</td>
<td>3.0**</td>
<td>2.0**</td>
<td>0.69</td>
<td>2.5</td>
<td>3.6</td>
<td>3.6</td>
<td>0.51</td>
</tr>
<tr>
<td>factor-VII-121del/ins</td>
<td>20.0</td>
<td>29.0</td>
<td>21.0</td>
<td>0.009</td>
<td>17.6</td>
<td>26.2</td>
<td>19.1</td>
<td>0.009</td>
</tr>
<tr>
<td>factor-XIII-Val34Leu</td>
<td>9.6</td>
<td>5.1</td>
<td>3.2</td>
<td>0.025</td>
<td>8.3</td>
<td>5.8</td>
<td>3.6</td>
<td>0.21</td>
</tr>
</tbody>
</table>

Two-sided P-values < 0.05 were regarded as significant (Fisher’s exact test). *Term singletons vs. preterm VLBW singletons, **Mothers of term singletons vs. mothers of preterm VLBW singletons.

Table 3: Frequency of polymorphisms of haemostatic genes in mothers of VLBW and the cause of preterm delivery. Frequencies for heterozygous and homozygous polymorphisms for factor-V-Leiden and prothrombin A G20210A, factor-VII-121 del/ins and homozygous factor-XIII-Val34Leu were indicated as %. Two-sided P-values < 0.05 were regarded as significant (cause of preterm delivery yes vs. no; Fisher’s exact test.).
livery (32). In histological studies of abortion tissue, the frequent finding of structural and thrombotic changes in placental capillaries suggested placental thrombosis or infarction to be risk factors for preterm delivery (33, 34). In contrast, haemostatic balance during normal pregnancy may be affected by the deficiency of coagulation factors (17), such as decreased levels of factor VII (2, 16).

In this study, we demonstrated a relationship of the factor-VII-121del/ins polymorphism with premature delivery for the first time. The factor-VII-121del/ins polymorphism is associated with decreased factor VII levels (18–20). In line with this, Nelson et al. (2, 16) demonstrated that women with factor VII levels below 94% had a threefold risk of spontaneous abortion, suggesting a role of factor VII levels in sustaining pregnancy. As factor VII deficiency is regarded as phenotypically pleomorphic ranging from asymptomatic patients to those with severe bleedings (35), the biochemical effect of the factor-VII-121del/ins polymorphism at the fetal-maternal interface may be variable. Because the identification of genetic predictors for preterm delivery has implications for risk stratification and prevention of preterm birth, further studies are needed to (a) determine the extent to which the polymorphism influences factor VII levels and placental bleeding complications in pregnant women, and (b) confirm the association between the factor-VII-121del/ins polymorphism and preterm delivery.

We further demonstrated an inverse association of the factor-XIII-Val34Leu polymorphism with preterm delivery. In uncomplicated pregnancy, placental extracellular matrix and fibrin formation are well modulated by pro- and anticoagulant cascades. However, in spontaneous abortion tissue, morphological abnormalities such as increased intervillous space fibrin indicate haemostatic dysfunction (36). This fact draws attention to factor XIII which catalyzes the formation of bonds between fibrin monomers causing increased mechanical strength of the thrombus. Factor XIII deficiency has been associated with severe bleeding diathesis in pregnant women and a higher risk of miscarriage (37). The factor-XIII-Val34Leu polymorphism is known to alter the fibrin meshwork to thinner fibers, smaller pores and variable permeation characteristics (21). So far, no experimental proof has been made to determine whether the thinner fibrin meshwork leads to changes in the stability of the thrombus. Notably, the factor-XIII-Val34Leu polymorphism has been previously described as a protective factor against venous thrombosis, myocardial infarction, stroke, and white matter disease in VLBW (21–26). The protective role of the factor-XIII-34Leu allele against preterm delivery needs to be further elucidated.

Finally, the causes of preterm delivery were not different in carriers of polymorphisms of haemostatic genes compared to non-carriers, except for the increased frequency of factor-V-Leiden mutation in VLBW mothers with pathological CTG as the cause of preterm delivery. Pathological CTG may be the result of a multifactorial process including fetal, maternal and placental factors, such as affected oxygen supply. As the prothrombotic factor V Leiden is likely to cause impairment of blood flow in the placenta, a cause-effect-correlation may be suggested. However, total numbers are too small to warrant conclusions at this stage of investigation. Therefore, evaluation in a larger cohort of mothers with preterm delivery on the basis of pathological CTG is necessary to exclude that this association occurred by chance.

In conclusion, our data suggest that polymorphisms of the blood coagulation system have a much lower impact on subsequent preterm delivery than known risk factors such as previous preterm birth. The significant influence of the maternal factor-VII-121del/ins polymorphism on preterm delivery needs to be further elucidated.

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References

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