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Risk for Early Pregnancy Loss by Factor XIII Val34Leu: The Impact of Fibrinogen Concentration

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Background: We have already described a significantly elevated overall risk for recurrent pregnancy loss (RPL) in women carrying the coagulation factor XIII (FXIII) Val34Leu and/or the plasminogen activator inhibitor-1 (PAI-1) 4G/5G polymorphism assuming that these polymorphisms contribute synergistically to RPL because of impaired hypofibrinolysis. Recent studies on FXIII indicate that the impact of the FXIII 34Leu genotype on fibrin structure and fibrinolysis is affected by fibrinogen concentration. Therefore, we reinvestigated the association between fibrinogen concentrations and FXIII Val34Leu with early RPL. *Materials and Methods:* In this case-control study, we enrolled 49 women with a history of two consecutive or three to six nonconsecutive pregnancy losses between the 8th and 12th week of gestation and 48 healthy controls. The risk for RPL in carriers of FXIII 34Leu at fibrinogen levels above or below the median and first tertile of controls was evaluated. *Results:* In carriers of the 34Leu allele, fibrinogen levels below the median (i.e., $\leq$300 mg/dl) and the first tertile (i.e., $\leq$284 mg/dl) of controls were associated with an increased risk for RPL [(2.9 (1.1–7.7), 3.9(1.0–15.0)]. *Conclusions:* The FXIII Val34Leu polymorphism may be associated with the development of early RPL in association with fibrinogen concentrations. At fibrinogen levels in the low normal range, FXIII 34Leu may modify fibrin structure toward an increased resistance to fibrinolysis. J. Clin. Lab. Anal. 27:444–449, 2013.

Key words: recurrent pregnancy loss; factor XIII; fibrinogen; factor XIII Val34Leu polymorphism; coagulation

INTRODUCTION

A successful outcome of pregnancy depends on a regular placental formation. In the very beginning of this process, a functional balance of fibrinolysis and coagulation is necessary. A well-regulated fibrinolysis may secure adequate trophoblast invasion, which is crucial for a regular implantation, and well-balanced fibrin deposition into the walls of the decidual veins may allow proper formation of the placental basal plate.

Coagulation factor XIII (FXIII) may have a significant impact on placental formation since FXIII covalently cross-links fibrin and affects fibrinolysis. For FXIII, a common polymorphism is known that is associated with earlier cross-linking, formation of a finer fibrin meshwork, and reduced susceptibility to fibrinolysis (1).

Hence, this FXIII Val34Leu polymorphism may impair fibrinolysis and has been reported to carry an elevated overall risk for recurrent pregnancy loss (RPL) in women homozygous for the FXIII 34Leu as well as in compound carriers of the FXIII 34Leu and the hypofibrinolytic 4G variant of the common 4G/5G plasminogen activator inhibitor-1 (PAI-1) polymorphism (2). However, Lopez Ramirez et al. (3) and Barbosa et al.

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(4) have published controversial results stating that the FXIII Val34Leu polymorphism is not a risk factor for early pregnancy complications.

Our original findings demonstrating an association of impaired fibrinolysis with RPL (2) are in line with later studies by Lim et al. (5) who showed the considerable influence of the FXIII 34Leu allele on the structure of fibrin clots. It was further demonstrated that this modification is strongly affected by fibrinogen levels (5). Carriers of homozygous FXIII 34Leu show clots of looser structure, thicker fibers, and increased permeability for fibrinolytic molecules at fibrinogen concentrations in the upper normal range, whereas levels of fibrinogen in the low normal range are associated with thinner, more tightly packed fibers and lower susceptibility to fibrinolysis in FXIII 34Leu homozygotes.

In line with this observation, Boekholdt et al. described that the homozygous 34Leu genotype represented a significant risk for coronary artery disease (CAD) at fibrinogen concentration below the first tertile of controls (6). In contrast, fibrinogen concentrations above the upper tertile of controls showed a protective tendency against CAD in carriers of the homozygous 34Leu genotype (6). Moreover, fibrinogen concentrations in the upper quartile of controls were statistically significant associated with protection against myocardial infarction (MI) (6, 7).

The findings of Lim et al. (5) and Boekholdt et al. (6) encouraged us to reevaluate our results on FXIII Val34Leu regarding its effect on the development of RPL in association with fibrinogen concentrations assuming that like for CAD the hypofibrinolytic effect of the 34Leu allele would be pronounced in women showing fibrinogen levels in the lower normal range.

MATERIAL AND METHODS

Study Population

In this case–control study, we enrolled 49 unrelated Caucasian women with a history of two consecutive or three to six nonconsecutive early pregnancy losses and 48 unrelated healthy controls from 2001 to 2002 (2, 8, 9). The patients were referred from local hospitals or gynecologists to the Department of Obstetrics and Gynecology, Division of Gynecologic Endocrinology and Reproductive Medicine, Medical University of Vienna, for evaluation of common causes for RPL. Apart from possible anatomical, hormonal, chromosomal, or infectious causes, all participating women were evaluated for thrombophilic or suspected risk factors for RFP (antiphospholipid syndrome, antithrombin, protein C, protein S, factor V Leiden, prothrombin G20210A gene mutation, methylenetetrahydrofolate reductase C677T, and A1298C polymorphisms). The study groups, gynecological examinations, exclusion criteria, and methods are described in detail elsewhere (2, 8).

The control group of 48 healthy women matched for ethnic background, age, and smoking status was concomitantly recruited. Each participating control had delivered at least one full-term infant and had never experienced pregnancy loss. None of the controls used oral contraceptives, hormonal intrauterine devices, or any other kind of medication.

The study was performed in accordance with the Declaration of Helsinki and written informed consent was given by all participating women.

Laboratory Methods

Blood samples were collected, processed, and stored at $-80^{\circ}C$ according to standard procedures, at least three months after the last pregnancy or at least three months after cessation of lactation to rule out any pregnancy-related alterations of coagulation or fibrinolysis. Routine coagulation screening tests as well as plasmatic and thrombophilic or suspected risk factors for RFP (antiphospholipid syndrome, antithrombin, protein C, protein S, factor V Leiden, prothrombin G20210A gene mutation, methylenetetrahydrofolate reductase C677T, and A1298C polymorphisms) were determined as described (2, 8).

Genetic Analysis

Genomic DNA was isolated from peripheral blood leukocytes using the protocol of the ViennaLab Gene Mutation Assays (ViennaLab Diagnostics GmbH, Vienna, Austria) according to the manufacturers' recommendations. We used PCR and reverse hybridization to genotype samples for FXIII Val34Leu as described previously (2).

The entire hybridization and detection procedure was carried out in a fully automated device (profiBlot II T 30; TECAN Austria, Groeding, 5080 Salzburg). The different genotypes were confirmed in selected samples by automatic fluorescent sequencing, using the ABI 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA; Prism Big Dye Terminator Cycle Sequencing Ready Reaction Kit, Version 2.0).

Statistical Analysis

For the statistical reanalyses of the FXIII Val34Leu effect on the development of RPL in conjunction with fibrinogen levels, SAS® software (version 9.1.3, SAS Institute, Cary, NC) was used.

Odds ratios (ORs), 95% confidence intervals (CIs), and *P*-values were calculated as described (2).

TABLE 1. Demographic Data of Participating Women

	Cases	Controls
Number of participants	49	48
Age at enrolment (mean ± SD)	35.6 ± 6.0	36.6 ± 5.1
Smokers	14	13
Number of overall pregnancies	196	71*
Partus per participant (mean ± SD)	0.37 ± 0.7	1.48 ± 0.6*
Number of miscarriages	178	0*
8th–12th week of gestation	173	0
13th–20th week of gestation	5	0
Number of miscarriages per participant (mean ± SD)	3.6 ± 1.2	0
Women with two consecutive miscarriages	5	0
Women with –three to six miscarriages	44	0
Ratio of successful pregnancies	0.07	1.00*

The study included 49 Caucasian women with a history of two consecutive or —three to six nonconsecutive early pregnancy losses and 48 healthy parous women who had no previous history of pregnancy loss. Cases and controls were unrelated and were matched for age, smoking behavior, and ethnic background.
* $P < 0.0001$.
SD, standard deviation.

TABLE 2. Odds Ratios for Early Pregnancy Loss in the Factor XIII Val34Leu Polymorphism

	OR (95% CI)	*P*-value
Val/Leu vs. Val/Val	1.7 (0.7–3.9)	0.217
Leu/Leu vs. Val/Leu	3.1 (0.3–30.0)	0.320
Leu/Leu vs. Val/Val	5.2 (0.5–49.3)	0.119
Val/Val vs. Val/Leu + Leu/Leu	1.9 (0.8–4.3)	0.121

OR, odds ratio; CI, confidence interval.
* *P*-values < 0.05 were considered statistically significant.

RESULTS

The study population included 49 Caucasian women with a history of two consecutive or —three to six nonconsecutive early pregnancy losses and 48 healthy controls. Demographics of the participating women are shown in Table 1. A total of 178 first and second trimester pregnancy losses were studied, of which 173 occurred between the 8th and 12th week of gestation and five between the 13th and 20th week. None of the women experienced second trimester pregnancy loss only. By study design, the total number of pregnancies, the number of miscarriages, the number of miscarriages per participant, as well as the ratio of successful pregnancies (i.e., the number of successful pregnancies per group referred to the number of overall pregnancies per group) differed significantly between cases and controls.

A total of 21 cases and 16 controls (43% vs. 33%) were heterozygous for the FXIII Val34Leu polymorphism and four cases were homozygous for FXIII 34Leu versus 1 control (8% vs. 2%) (2). We calculated the ORs for the possible combinations of wild type, heterozygosity, and homozygosity for these polymorphisms. There was an increase of ORs with an increasing number of FXIII 34Leu alleles (Table 2). In carriers of at least one 34Leu allele, the OR for developing RPL was increased to 1.9 (95% CI 0.8–4.3). However, results were not statistically significant (Table 2).

Plasma levels of fibrinogen did not differ between cases and controls (mean ± SD: 298 mg/dl ± 58.3 vs. 300 mg/dl ± 48.4). At fibrinogen concentrations above the median and the first tertile of controls (i.e., >300 mg/dl and >284 mg/dl), no elevation of risk for RPL was observed in carriers of the FXIII 34Leu allele with ORs of 0.9 (95% CI 0.3–2.3) and 1.1 (95% CI 0.5–2.6), respectively (Table 3).

In contrast, women carrying the 34Leu allele showed a significantly higher risk for RPL with an OR of 2.9 (95% CI 1.1–7.7) and 3.9 (95% CI 1.0–15.0) when fibrinogen levels were below the median or the first tertile of controls (Table 3).

DISCUSSION

Inherited and acquired thrombophilia can be found in more than 50% of women suffering from RPL of unknown cause (10). Thrombophilic risk factors are also frequent in women with other vascular placental pathologies such as preeclampsia, intrauterine growth retardation, placental abruption, and late fetal loss (11–19). The common denominator for these pregnancy complications seems to be placental insufficiency (20), of which increased fibrin deposition and insufficient invasion of trophoblast are believed to be major pathological determinants (21–23). Adequate fibrinolysis with fine-tuned endometrial vascular remodeling seems mandatory for a sufficient trophoblast invasion into maternal spiral arteries and hence successful implantation and development of a low-resistance uteroplacental circulation (24–26).

Several genetic variants modulate fibrinolysis in early pregnancy. Examples are the PAI 4G variant that may interfere with regular implantation because of overexpression of PAI-1 and hence reduced fibrinolytic activity (26) or the FXIII Val34Leu polymorphism where the 34Leu allele together with fibrinogen levels exhibit a considerable influence on the structure of fibrin clots (5). Carriers of homozygous FXIII 34Leu show clots of looser structure, thicker fibers, and increased permeability for fibrinolytic molecules at fibrinogen concentrations in the upper normal range, whereas levels of fibrinogen in the low normal range are associated with thinner, more tightly

TABLE 3. The Impact of Fibrinogen Concentration on the Risk for Early Pregnancy Loss in Women Carrying the FXIII 34Leu Allele

	Women carrying at least one FXIII 34Leu allele			
Fibrinogen (mg/dl)	Cases *n* (%)	Controls *n* (%)	OR (95% CI)	*P*-values
≤300 [a]	16 (32)	7 (15)	2.9 (1.1–7.7)*	0.036*
≤284[b]	10 (20)	3 (6)	3.9 (1.0–15.0)*	0.040*
>300[a]	9 (18)	10 (21)	0.9 (0.3–2.3)	0.764
>284[b]	15 (31)	14 (29)	1.1 (0.5–2.6)	0.887

[a]Median of fibrinogen levels of controls.
[b]First tertile of fibrinogen levels of controls.
*A *P*-value < 0.05 was regarded statistically significant.
OR, odds ratio; CI: confidence interval; FXIII: coagulation factor XIII.

packed fibers and lower susceptibility to fibrinolysis in FXIII 34Leu homozygotes (5).

Controversial results have been published regarding the effect of the FXIII Val34Leu polymorphism on the development of CAD, peripheral artery disease, ischemic stroke, and venous thromboembolism (6, 7, 27–29).

In studies of the 34Leu where the impact of fibrinogen levels was taken into consideration, a protective effect of the 34Leu genotype was noted at fibrinogen levels in the upper normal range (6, 27–29). Moreover, Boekholdt et al. have demonstrated an elevated risk for developing disease at fibrinogen levels in the lower normal range (6). These findings are in line with the in vitro results of Lim et al. (5) who demonstrated that in comparison to carriers of the Val34 genotype, homozygous 34Leu carriers form fibrin clots with lower permeability and hence decreased susceptibility to fibrinolysis at low fibrinogen concentrations.

In the large study on factor V Leiden and its association with preterm birth conducted by Hiltunen et al. (30), the effects of several genetic variants have been investigated but no association of late pregnancy complications with the 34Leu allele was found. The authors describe a significantly elevated risk for late preterm birth in carriers of factor V Leiden and in women with a history of venous thrombosis. Based on the fact that factor V Leiden is resistant to inactivation by activated protein C, hypercoagulation followed by placental thrombosis and uteroplacental ischemia may be the reason for late preterm birth found in this study. With regard to early pregnancy complications, several studies underline the importance of impaired fibrinolysis followed by insufficient remodeling of maternal tissue, impaired trophoblast invasion, and abnormal placentation (23, 26, 31–33). However, it does not surprise that in the study of Hiltunen et al., the 34Leu allele was not a risk factor for preterm birth.

Several studies have evaluated the risk for RPL in carriers of the 34Leu allele (3, 4, 34–38). While Coulam et al. (37), Goodman et al. (38), Yenicesu et al. (34), and our own study (2) identified the 34Leu allele as a risk factor for RPL, Lopez Ramirez et al. (3), Barbosa et al. (4), Jeddi-Tehrani et al. (35), and Soltanghoraee et al. (36) did not confirm these results. The significant ethnic heterogeneity of FXIII Val34Leu showed by Attie-Castro et al. (39) might help to explain the different conclusions of the Iranian studies (35, 36). However, according to the data of Boekhold et al. (6) and Lim et al. (5), a simple explanation for the discrepant study results could be that to date no study has taken into account the possible impact of fibrinogen levels on the development of RPL in carriers of the FXIII 34Leu allele.

In an attempt to determine the effect of FXIII Val34Leu variants, Lopez Ramirez et al. (3) analyzed the rates of α- and γ-chain cross-linking formation in the FXIII Val34Leu variants, but they diluted all plasma samples to the same final fibrinogen concentration of 0.47 g/l and used thrombin levels below physiological coagulation conditions. Consequently, a possible effect of the different plasma fibrinogen levels on the formation of the fibrin meshwork in the FXIII Val34Leu variants was completely masked.

Hence, our study is the first that evaluates the effect of FXIII Val34Leu variants with respect to fibrinogen levels in women suffering from RPL. However, it has to be taken into account that the development of coagulation-based pregnancy complications is usually a multifactorial event involving gene–gene and gene–environment interactions (37). For this reason, some carriers of predisposing genotypes will not develop disease while others will, even without carrying distinct risk factors.

Finally, it has to be noted that the size of our study population is small and only suitable to describe trends or generate a hypothesis. Larger studies are needed to further investigate our findings. However, our results point to the importance of FXIII 34Leu as a risk factor for the development of RPL in women with fibrinogen concentrations in the lower normal range. Furthermore, our findings emphasize that, in general, the effect of FXIII 34Leu should only be evaluated concomitantly with fibrinogen concentrations.

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