Lipoprotein (a) and other prothrombotic risk factors in Caucasian women with unexplained recurrent miscarriage

Results of a multicentre case-control study

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Summary
From 1998 to 2003, 133 Caucasian women aged 17–40 years (median 29 years) suffering from unexplained recurrent miscarriage (uRM) were consecutively enrolled. In patients and 133 age-matched healthy controls prothrombotic risk factors (factor V (FV) G1691A, factor II (FII) G20210A, MTHFR T677T, 4G/5G plasminogen activator inhibitor (PAI)-1, lipoprotein (Lp) (a), protein C (PC), protein S (PS), antithrombin (AT), antiphospholipid/anticardiolipin (APA/ACA) antibodies) as well as associated environmental conditions (smoking and obesity) were investigated. 70 (52.6%) of the patients had at least one prothrombotic risk factor compared with 26 control women (19.5%; p<0.0001). Body mass index (BMI; p=0.78) and smoking habits (p=0.44) did not differ significantly between the groups investigated. Upon univariate analysis the heterozygous FV mutation, Lp(a) > 30 mg/dl, increased APA/ACA and BMI > 25 kg/m² in combination with a prothrombotic risk factor were found to be significantly associated with uRM. In multivariate analysis, increased Lp(a) (odds ratio (OR): 4.7/95% confidence interval (CI): 2.0–10.7), the FV mutation (OR:3.8/CI:1.4–10.7), and increased APA/ACA (OR: 4.5/CI: 1.1–17.7) had independent associations with uRM.

Keywords
Lipoprotein (a), recurrent miscarriage

Introduction
Thrombophilia is a hypercoagulable state caused by inherited and acquired risk factors. Among the genetic prothrombotic risk factors deficiency states of protein C (PC), protein S (PS) and antithrombin (AT) as well as the factor V (FV) G1691A, the factor II (FII) G20210A and the methylenetetrahydrofolate reductase (MTHFR) C677T genotype leading to increased homocysteine concentrations in some individuals are well established (1, 2). In addition, increasing evidence in recent years has led to elevated concentrations of lipoprotein (Lp(a)) being identified as a risk factor for premature myocardial infarction, stroke (3, 5) and venous thromboembolism (6, 7). Serum levels of Lp(a) are controlled by the genetically determined variation in the number of kringle IV repeats in different isoforms of apolipoprotein(a) (8). Many pathogenetic mechanisms have been proposed for lipoprotein (a) and its contribution to cardiovascular diseases so far, and the prothrombotic effect of elevated Lp(a) levels are in part explained and discussed via an antifibrinolytic activity (9).

Among other clinical conditions, inherited thrombophilia appears to increase the susceptibility to adverse pregnancy outcomes such as intrauterine growth retardation, preeclampsia, placental abruption, intrauterine fetal death, or abortion (11, 12). Thrombosis in decidual vessels leading to inadequate placental circulation and consequent gestational pathology is a possible pathogenetic mechanism (12). Various studies have demon-
Strated the association of distinct thrombophilic defects with fetal loss (10, 11, 13–16). In a recent meta-analysis, FV, activated protein C resistance, FII and PS deficiency were shown to be significantly associated with fetal loss (17). The underlying causes of recurrent miscarriages, defined as three or more consecutive miscarriages, may be diverse. Known etiologic factors include chromosomal aberrations, uterine abnormalities and endocrine dysfunction, but about 30–40% of all recurrent fetal losses remain unexplained (18), underlining the importance of further research in this area. Identifying thrombophilia as an important etiologic factor in these patients would offer the opportunity for potentially effective prevention with anticoagulant therapy which has already been started in clinical studies (19). The aim of this study was to analyze the relevance of elevated Lp(a) among other inherited or acquired thrombophilic defects as well as the combination of environmental factors such as smoking or overweight associated with established and new prothrombotic risk factors.

Patients and methods

Ethics

The present multicentre follow-up study was performed in accordance with the ethical standards laid down in the updated version of the 1964 Declaration of Helsinki and was approved by the medical ethics committee of the University of Münster, Germany.

Inclusion criteria

With written or oral consent, consecutively admitted Caucasian women (obstetric departments: therapy and diagnostic work up) with unexplained recurrent miscarriage (uRM), defined as three or more abortions < 23 gestational weeks with the same partner, were enrolled (20).

Diagnostic work up

Gynecologic diagnostic procedures included vaginal ultrasound, hysteroscopy, laparoscopy, screening for genital urinary tract infections, a comprehensive hormonal status including progesterone and estradiol serum levels, as well as paternal / maternal karyotyping. In addition, all symptomatic women with uRM were screened for thyroid function.

Table 1: Characteristics of patients and controls, showing number of cases, percentages, median (range) values and p-values.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Patients (n=133)</th>
<th>Controls (n=133)</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age, years (range) at first abortion</td>
<td>29 (17–40)</td>
<td>28.5 (18–40)</td>
<td>0.7</td>
</tr>
<tr>
<td>Obstetrical history</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total number of pregnancies</td>
<td>605</td>
<td>257</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Total number of abortions</td>
<td>494</td>
<td>210</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Total number of live births</td>
<td>111</td>
<td>255</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Live births/pregnancies (%)</td>
<td>111/605 (18.3)</td>
<td>255/237 (99.2)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Environmental conditions</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.7</td>
<td>22.9</td>
<td>0.8</td>
</tr>
<tr>
<td>BMI &gt; 25 kg/m² (%)</td>
<td>39 (29.3)</td>
<td>38 (28.6)</td>
<td>0.9</td>
</tr>
<tr>
<td>Smoking (%)</td>
<td>32 (24.1)</td>
<td>32 (24.1)</td>
<td>0.5</td>
</tr>
</tbody>
</table>

* chi-square test ** induced abortions

Exclusion criteria

Women with chromosomal abnormalities, congenital uterine malformations, abnormal thyroid function, abnormal hormonal status, autoimmune diseases, infectious diseases, cases of non-Caucasian origin, patients with incomplete clinical or laboratory work-up (established prothrombotic risk factors), and subjects lost for follow-up or without consent were not enrolled in the present study.

Environmental conditions

Overweight defined as BMI > 25 kg/m² and smoking > 10 cigarettes per day during pregnancy were classified as underlying environmental conditions.

Final study population

From January 1998 to December 2003, 133 consecutively admitted Caucasian patients with uRM from three different geographic areas of Germany were enrolled into the present multicentre study. With written consent, 133 age-matched healthy women of the same ethnic origin not related to the index cases, with a similar distribution of BMI and smoking habits, who had delivered at least one child without complications and who had no history of spontaneous abortion served as a control group. The controls were enrolled within the same time period from similar geographic areas as the patients (Table 1).

Laboratory analysis

Blood samples were collected three to six months after fetal loss in patients or normal delivery in controls. The factor V (FV) G1691A and the factor II (FII) G20210A mutations, the MTHFR C677T and 4G/5G plasminogen activator inhibitor (PAI)-1 genotypes, levels of protein C, protein S, antithrombin, and anti-phospholipid/antiphospholipid antibodies (APA/ACA) were investigated as established prothrombotic risk factors, using standard laboratory techniques (6, 21–25). A type I deficiency (antithrombin, protein C) state was diagnosed when functional plasma activity and antigen concentration of a protein were repeatedly found to be below the 50% age-related percentile. A type II deficiency (antithrombin, protein C) was diagnosed by the repeated finding of low functional activity levels along with normal antigen concentrations of the respective proteins (abnormal in three independent blood samples at least six weeks apart). The diagnosis of protein S deficiency was based on reduced free protein S antigen levels combined with decreased or normal total protein S antigen concentrations. For APA/ACA, titers above cut-off values > 20 IU/ml (IgG) and >11 IU/ml (IgM) were considered abnormal in three independent blood samples at least six weeks apart.

As a potentially novel prothrombotic risk factor, lipoprotein (a) (Lp(a)) was investigated in 124 of the 133 patients and in the entire control group from fresh–frozen serum samples (80°C maximum 4 weeks) with an apo(a) isofrom-independent ELISA technique (Apo-Tek Lp(a), Sigma Diagnostics, St. Louis, USA): Serum levels of Lp(a) >30 mg/dl (>90th percentile: healthy Caucasian controls) were considered elevated (6). Phenotyping of apo(a) isofroms was performed in a selected subgroup of 54 patients and 54 controls (origin no different from the entire study group) recruited in Münster (6). A cut-off of 28 kringle IV re-
peats (< 10th percentile: healthy Caucasian controls) was used to
differentiate small and large apo(a) isoforms. Criteria for the her-
editary nature of a hemostatic defect were the identification of a
causative gene mutation.

Statistics
Statistical analyses were performed using the StatView 5 soft-
ware package (SAS Institute Inc., Cary, NC, USA). To compare
the rate of prothrombotic risk factors between patients and con-
trols, to evaluate an independent contribution of thrombophilia
and underlying environmental conditions to the onset of uRM,
and to adjust for potential confounders, the odds ratios (OR) and
95% confidence intervals (CI) were estimated from the con-
ditional logistic regression model (PHREG procedure, SAS, V8:
(26)). Because of their apparent non-Gaussian distribution, con-
tinuous data are presented as medians and ranges and are evalu-
ated by non-parametric statistics using the Wilcoxon-Mann-
Whitney U test. In addition, the frequency distribution of under-
lying conditions was compared using the chi-square test or
Fisher's exact test. P-values < 0.05 were considered significant.

Results
Patient population
Over a five-year period, 133 Caucasian patients with confirmed
uRM were enrolled in the participating obstetric departments
(Table 1). The median/range age at first abortion was 29.0
(17–40) years. Until miscarriage occurred the mean (standard
deviation) pregnancy duration was 9 (± 3.5) gestational weeks.
The number of total pregnancies, abortions and live births com-
pared to healthy control women are shown in table 1. In addition,
accompanying environmental conditions, e.g. median BMI
prevalences of BMI > 25 (kg/m²), and smoking individuals in pa-
ients and controls are presented.

Prothrombotic risk factors
In 70 of the 133 patients (52.6%), at least one established pro-
thrombotic risk factor was found compared with 26 among the
133 controls (19.5%; p < 0.0001). The distribution of single and
combined prothrombotic risk factors in patients and controls is
shown in table 2. Interestingly, 17 of the 133 patients (12.8%)
had a combination of at least two prothrombotic risk factors
compared with two (1.5%) of the healthy control individuals
(p=0.0005).

Upon univariate analysis and compared to controls, patients
showed significantly higher prevalences of elevated Lp(a) (OR:
4.5/CI: 2.3–9.0; p<0.000), FV G1691A (OR: 4.0/CI: 1.7–9.6;
p=0.002), elevated APA/ACA (OR: 5.4/CI: 1.5–19.2; p=0.009),
and BMI > 25 kg/m² in combination with at least one prothrom-
bolic risk factor (OR: 2.8/CI: 1.1–7.0; p=0.02). In parallel with
the increased risk associated with the elevated Lp(a) serum con-
centration observed in the total study group, we found in a sub-
group of patients and controls with Lp(a) serum concentrations
similar to the total study group (subgroup 14.2 mg/dl, total co-
hort 14.1 mg/dl; p=0.99) that the presence of small apo(a) iso-
forms is also increased in the group of patients with uRM (38.9% versus 12.7%, p= 0.002; OR: 4.3/CI: 1.6–11.2; p=0.003). How-
ever, no significant differences were found for frequencies of FII

**Table 2: Distribution of single and combined established pro-
thrombotic risk factors in patients and controls.**

<table>
<thead>
<tr>
<th>Prothrombotic Risk</th>
<th>Patients Numbers (%)</th>
<th>Controls Numbers (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elevated lipoprotein (a) total</td>
<td>41/124 (33.1%)</td>
<td>14/133 (10.5%)</td>
</tr>
<tr>
<td>Single</td>
<td>30/41</td>
<td>12/14</td>
</tr>
<tr>
<td>Combined:</td>
<td>11/41</td>
<td>2/14</td>
</tr>
<tr>
<td>&amp; factor II G20210A</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>&amp; factor II G20210A and lipoprotein (a) &gt; 30 mg/dl</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>&amp; lipoprotein (a) &gt; 30 mg/dl</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>&amp; antithromboplastic/anticardiolipin antibodies</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Factor V G1691A total</td>
<td>24/133 (18.0%)</td>
<td>7/133 (5.3%)</td>
</tr>
<tr>
<td>Single</td>
<td>12/24</td>
<td>6/7</td>
</tr>
<tr>
<td>Combined:</td>
<td>12/24</td>
<td>1/7</td>
</tr>
<tr>
<td>&amp; factor II G20210A</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>&amp; Factor II G20210A and lipoprotein (a) &gt; 30 mg/dl</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>&amp; antithromboplastic/anticardiolipin antibodies</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Factor II G20210A total</td>
<td>6/133 (4.5%)</td>
<td>3/133 (2.3%)</td>
</tr>
<tr>
<td>Single</td>
<td>3/6</td>
<td>1/3</td>
</tr>
<tr>
<td>Combined:</td>
<td>3/6</td>
<td>1/3</td>
</tr>
<tr>
<td>&amp; factor V G1691A</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>&amp; Factor V G1691A and lipoprotein (a)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Protein C type I deficiency total</td>
<td>1/133 (0.8%)</td>
<td>1/133 (0.8%)</td>
</tr>
<tr>
<td>Single</td>
<td>1/133 (0.8%)</td>
<td>1/133 (0.8%)</td>
</tr>
<tr>
<td>Combined:</td>
<td>1/133 (0.8%)</td>
<td>1/133 (0.8%)</td>
</tr>
<tr>
<td>Antithromboplastic/anticardiolipin antibodies total</td>
<td>14/127 (11.0%)</td>
<td>3/123 (2.3%)</td>
</tr>
<tr>
<td>Single</td>
<td>7/14</td>
<td>2/12</td>
</tr>
<tr>
<td>Combined:</td>
<td>7/14</td>
<td>1/12</td>
</tr>
<tr>
<td>&amp; factor V G1691A</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>&amp; Lipoprotein (a)</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>&amp; protein C type I deficiency</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>&amp; antithromboplastic/anticardiolipin antibodies</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 3: Conditional logistic regression model: To evaluate the in-
dependent contribution to the risk of recurrent miscarriage and to ad-
just for potential confounders, thrombophilic risk factors and the com-
bination of a prothrombotic risk factor with an underlying environmental
condition were analyzed.

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Odds Ratio</th>
<th>95% Confidence Interval</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No prothrombotic risk factor or underlying condition</td>
<td>1 (reference)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lipoprotein(a) &gt;30 mg/dl</td>
<td>4.7</td>
<td>2.0–10.7</td>
<td>0.000</td>
</tr>
<tr>
<td>Factor V G1691A</td>
<td>3.8</td>
<td>1.4–10.7</td>
<td>0.01</td>
</tr>
<tr>
<td>Antithromboplastic/anticardiolipin antibodies</td>
<td>4.5</td>
<td>1.1–17.7</td>
<td>0.03</td>
</tr>
<tr>
<td>Prothrombotic risk factor &amp; BMI &gt; 25 kg/m²</td>
<td>0.8</td>
<td>0.2–2.3</td>
<td>0.6</td>
</tr>
</tbody>
</table>


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just missed the level of statistical significance (OR: 2.3/CI 0.9–5.9; p=0.07). The association of prothrombotic risk factors with uRM remained stable in the multivariate analysis, including all thrombophilic risk factors significantly associated with uRM in the univariate analysis. In contrast, the combination of BMI > 25 kg/m² with a prothrombotic risk factor did not qualify as an independent risk factor for RM in the logistic regression model used (Table 3).

Discussion

The aim of the present multicentre study was to evaluate the role of elevated lipoprotein (a) concentration and its possible interaction with other established prothrombotic risk factors or predefined environmental conditions in Caucasian women with unexplained recurrent miscarriage. Univariate analysis revealed that elevated serum levels of Lp(a), the FV mutation, elevated APA/ACA titers as well as the combination of overweight or obesity (BMI > 25 kg/m²) with any prothrombotic risk factor were significantly more frequent in patients as compared to healthy controls, whereas frequencies of the FII G20210A variant, the MTHFR T677T and 4G/4G PAI-1 genotypes, protein C-, protein S-, or antithrombin deficiency did not differ significantly between the two groups. In this context, we wish to emphasize that the prevalence rates of prothrombotic risk factors in the control women presented here did not differ from those reported for other healthy control populations (6, 7, 24, 25). Furthermore and interestingly, the combination of daily smoking during pregnancy in combination with the presence of at least one prothrombotic risk factor just missed the level of statistical significance as a risk factor for uRM. In the multivariate statistical model, elevated Lp(a) serum concentration, heterozygosity for the factor V G1691A mutation as well as increased APA/ACA, but not the combination of overweight or obesity with at least one thrombophilic risk factor, retained their significant and independent association with recurrent fetal loss.

Heterozygosity for factor V G1691A and increased APA/ACA titers are well accepted risk factors not only of venous thrombosis but also of pregnancy complications including uRM, eclampsia and growth retardation (12, 27, 28). Lp(a) has previously been identified as a risk factor of arterial thrombotic diseases including myocardial infarction and stroke at a young age (3, 29–31). Evidence is accumulating that elevated Lp(a) is also a risk factor of venous thrombosis, at least in childhood and adolescence and in patients with prothrombotic underlying diseases such as nephrotic syndrome and rheumatic diseases (6, 32, 33). Thus, Lp(a) appears to be harmful to various vessel systems including arteries, veins and the placenta. Interestingly, although elevated Lp(a) has also been associated with eclampsia and fetal growth retardation (12, 27, 28), in the latter clinical conditions Lp(a) is less well accepted as a potential risk factor. The mechanism by which Lp(a) favours thrombosis is not fully understood so far. As an inactive homologue of plasminogen, the protein constituent of Lp(a) may inhibit fibrinolysis (34, 35). Very recently, Hancock et al. have revisited the pathomechanism of Lp(a) with respect to t-PA mediated plasminogen inactivation: Not consistent with a model in which apo(a) decreases the amount of plasminogen bound to fibrin, the authors postulate a novel equilibrium template model, in which the binding interactions between apo(a)/Lp(a) and t-PA, fibrin or fibrin degradation products, and plasminogen is taken into account. In this model a quaternary complex is formed in the presence of apo(a)/Lp(a) that exhibits a reduced turnover number thus resulting in inhibition of plasminogen activation (36).

Lp(a) levels are under strong genetic control exerted by the apo(a) gene (37, 38). The main determinant is a variable number of repeat polymorphisms in the apo(a) gene which encodes for a variable number of kringle IV repeats in apo(a), i.e., the specific protein component of Lp(a). In fact, Lp(a) levels are inversely correlated with the size of apo(a) isoforms. In agreement with this we found that, in addition to elevated Lp(a) serum concentration, the presence of a small apo(a) isoform < 10⁶ percentile of Caucasian healthy controls is also associated with increased risk of uRM. The mechanism by which elevated Lp(a) could interfere with fibrinolysis may support the hypothesis that a hypofibrinolytic state is associated with complicated pregnancies (39). In addition, a down regulation of fibrinolysis along with established and new prothrombotic risk factors clearly underline the need for antithrombotic treatment regimens in women with uRM and further pregnancy complications, such as the use of low molecular weight heparin (40).

Although one of the largest controlled series of Caucasian women with uRM, the size of the cohort in the present study was still too small, hence the statistical power of this study is too low to exclude the possibility that the FII G20210A variant, the MTHFR T677T genotype, protein C-, protein S-, or antithrombin deficiency or the combination of at least one prothrombotic risk factor with smoking or overweight may be independent risk factors of uRM. In fact, they have been identified in other studies including a meta-analysis as independent factors of recurrent miscarriage (17, 41).

In summary, the data presented here underline the multifactorial etiology of uRM in Caucasian women. In the future it will be important to investigate larger cohorts of women with recurrent miscarriage to obtain more information on interactions between all prothrombotic risk factors reported (17, 41), and thereby derive algorithms which are helpful in the individual prognosis and thus in treatment decisions, for example in applying anticoagulant therapies.

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References