Foetal Growth Restriction in Children with Prothrombotic Risk Factors

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Keywords

Foetal growth restriction, factor V G1691A, prothrombin G20210A, lipoprotein (a), protein C, protein S, antithrombin

Summary

Placental infarction is frequently observed in low birth weight children. To evaluate whether low birth weight in healthy term neonates is associated with foetal inherited prothrombotic risk factors this retrospective study was conducted. Outcome measures were “birth weight in the lowest quartile” and “birth weight in the lowest decile” in singletons with a gestational age of ≥37 weeks.

The analyses were based on 375 Caucasian children screened at the Münster childhood thrombophilia centre with complete data for all prothrombotic risk factors (factor V G1691A, prothrombin G20210A, elevated lipoprotein (a), protein C, protein S, antithrombin-deficiency). The proportion of children in the lowest birth weight quartile increased from 23.7% to 30.5% to 48.0% for children with no, only single heterozygous and multiple or homozygous defects respectively. The respective adjusted odds ratios (95% confidence intervals) of thrombophilia for birth weight in the lowest quartile (lowest decile) were 1.53 (0.76-3.08) in carriers of one prothrombotic risk factor and 4.01 (1.48-10.84) in subjects carrying multiple or homozygous defects. We identified foetal thrombophilia as an additional cause of low birth weight.

Introduction

Obstetric complications such as placental abruption, foetal loss, and stillbirth are associated with intervillosus or spiral-artery thrombosis, and placental infarction is frequently observed in low birth weight children (1). Previous work on the potential role of prothrombotic risk factors has focused mainly on the maternal side of the placenta and showed that placental infarction and thrombosis are associated with maternal thrombophilia, i.e. antiphospholipid antibodies, hyperhomocysteinemia, protein C deficiency, the heterozygous factor V (FV) G1691A mutation, the heterozygous prothrombin (PT) G20210A variant and increased lipoprotein (Lp) (a) concentrations (2-8).

However, prothrombotic risk factors in the foetus need to be considered as well, because these inherited prothrombotic risk factors may become relevant early in the life of the affected child. Homozygous antithrombin deficiency is a well known cause of stillbirth, and homozygous protein C or S deficiency can lead to purpura fulminans during the first days of life (9). Recently several prothrombotic risk factors, i.e. the factor V G1691A mutation, the prothrombin G20210A variant, increased Lp (a), deficiencies of protein C, have been linked to neonatal thromboembolism (10, 11), and the FV G1691A mutation or the PT G20210A variant in foetuses seem to be related to premature birth (12). There are also some data suggesting that the FV G1691A mutation in the foetus might account for placental infarction and miscarriage (13). We therefore retrospectively tested the hypothesis that inherited prothrombotic risk factors in the foetus might have caused a shift towards lower birth weight in the affected newborns in a Caucasian cohort of children.

Materials and Methods

Ethics. The present multicenter study was performed in accordance with the ethical standards laid down in the 1994 Declaration of Helsinki and was approved by the Medical Ethics Committee at the Westfälische Wilhelms-Universität, Münster, Germany.

Sampling frame. At the Münster childhood thrombophilia centre, screening for prothrombotic risk factors had been performed in 720 Caucasian children from all catchment areas of Germany between October 1996 and December 1999. The data bank of all subjects investigated contained extensive information regarding birth weight, obstetric and perinatal complications. Screening for prothrombotic risk factors had been performed either in relation to elective surgery (recruitment of a control population) or because of previous thrombosis in the child or other family members. As described the subjects investigated have been recruited from all catchment areas of Germany.

Questionnaire. Information on gestational age, weight assessment within 24 h after birth, maternal height and weight before pregnancy, the number of births preceding the index child and the cigarette consumption during pregnancy was obtained by means of a one-page questionnaire either mailed to the families or completed during a subsequent routine visit to the hospital.

Exclusion criteria. Preterm infants, twins, healthy neonates with incomplete obstetric records, first weight assessment > 24 h after birth, cases with known maternal pregnancy complications (history of recurrent foetal loss or stillbirth, deep venous thrombosis, myocardial infarction or stroke, maternal diabetes, preeclampsia, severe viral or bacterial infections, hepatic administration), and congenital infections were not enrolled in the present study. Since body weight obtained > 24 h after birth is influenced by fluid intake, fluid loss and intensive care treatment modalities sick neonates with symptomatic thromboembolism in the perinatal period were not included in the analyses presented here.

Study population. Questionnaires regarding known risk factors were available for 658 children (91.0%). 37 of these children were excluded because of neonatal stroke and 66 because of venous thromboembolism in the neonatal period, 26 because of problems during pregnancy or in the perinatal period, and...
ties were measured on an ACL 300 analyser (Instrumentation Laboratory, 6.12. odds ratios were calculated. All calculations were performed in SAS version 9.2.

weight were included in the final logistic regression model if a significant
square statistics and Fisher's exact test). Other known risk factors for low birth
logistic regression. The significance level (p-value: p) was set at 0.05 (chi-
exact tests were calculated, followed by analysis of the risk of being in the
this population respectively and no versus any cigarette smoking in pregnancy.

Laboratory investigations. Blood sample collection. In the infants and
children blood samples were collected by peripheral venipuncture into plastic
tubes containing 1/10 by volume of 3.8% trisodium citrate (Sarstedt, Nümbrecht, Germany) and placed immediately on melting ice. Platelet poor plasma was prepared by centrifugation at 3000 g for 20 min at 4 °C, aliquoted in poly-

Assays for genotyping. The FV G1691A mutation and the prothrombin
G20210A variant were determined by polymerase chain reaction and analysis of
restriction fragments as previously reported (14,15).

Assays for plasmatic factors. Amidolytic protein C and antithrombin activi-

Classification of deficiency states. A heterozygous type I deficiency state
(antithrombin, protein C) was diagnosed when functional plasma activity and
immunological antigen concentration of a protein were below 50% of normal of
the age-related limit (16). 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 respectively (17). Lp(a) was determined with
the COALIZA Lp(a) assay kit (Chromogenix, Sweden). Lp(a) levels
> 30 mg/dl were defined as elevated (10, 11).

Statistical analyses. The main outcome measures to assess potential inter-
dependencies between prothrombotic risk factors and birth weight were "birth
weight in the lowest quartile" and “birth weight below the lowest decile" in
singleton with a gestational age of more than 37 weeks. National reference
data (18) were used to define the lowest decile and the lowest quartile. The
lowest decile corresponds to the widely used definition of "small for gestational
age" (19). The lowest quartile was used to confirm that the effects are not only
confined to the extremes of the distribution and to increase power.

A combined parameter for any prothrombotic risk factor was generated:
This indicator was one if protein C, protein S or anti thrombin deficiency or the
mutations FV G1691A, or PT G20210A or an Lp(a) concentration > 30 mg/dl
found in either isolation or as combinations of these factors. The indicator
was zero when none of these risk factors could be detected. In order to assess
potential dose effects of these risk factors, the cumulative indicator was strati-
fied by single heterozygous and multiple or homozgyous defects. All analyses
regarding the effect of the cumulative indicator were confined to children in
whom all of the laboratory tests had been performed.

The known risk factors for low birth weight (20) considered in the present
study were classified as: first born versus any number of previous births,
maternal height, weight or body mass index below versus above the lowest
decile of the entire study population [159 cm, 50 kg and BMI 18.83 (kg/m²) in
this population respectively] and no versus any cigarette smoking in pregnancy.

Cross tabulations with the appropriate chi-square statistics and Fisher’s
exact tests were calculated, followed by analysis of the risk of being in the
lowest birth weight quartile and of birth weight below the lowest decile using
logistic regression. The significance level (p-value: p) was set at 0.05 (chi-
square statistics and Fisher's exact test). Other known risk factors for low birth
weight were included in the final logistic regression model if a significant
association with low birth weight was observed in our data. Crude and adjusted
odds ratios were calculated. All calculations were performed in SAS version
6.12.

Results

Prevalence rate of prothrombotic risk factors. 451 infants and chil-
dren (83% of the study population) aged two months to 16 years, class-
ified according the criteria mentioned in the Method part have been
investigated (10, 11, 16, 17). The prevalence rate of prothrombotic risk factors
in 451 children investigated was 17.4% for the factor V G1691A
mutation (heterozygous n = 73; homozgyous n = 5), 2.3% for protein C
deficiency, 1.1% for protein S deficiency, and 0.5% for antithrombin
deficiency. In addition, 3.1% carried the prothrombin G20210A muta-
tion (heterozygous n = 12; homozygous n = 2), and 18.4% of children
showed increased Lp(a) concentrations respectively. In addition, the
heterozygous factor V G1691A mutation was combined with further
prothrombotic risk factors in 16 cases (increased Lp(a) levels n = 13;
protein S deficiency n = 1, protein C deficiency n = 1, PT G20210A
mutation n = 1), and increased Lp(a) was additionally found in one
child with protein S deficiency and in a further subject carrying the PT
G20210A mutation.

Risk of intrauterine growth restriction with respect to single pro-
thrombotic risk factors. The highest risk of suffering from intrauterine
growth restriction in children with a complete laboratory evaluation
was found in carriers of defects within the protein C pathway (factor V
G1691A mutation, protein C deficiency, protein S deficiency) A: below
the lowest birth weight quartile vs. above the lowest quartile: 31.1% vs.
18.0%, Crude Odds ratio (95% confidence intervals) (cOR (95%-CI)) 2.05 (1.22-3.45); B: below the lowest birth weight decile vs. above the
lowest birth weight decile: 34.8% vs. 19.8%, cOR (95%-CI), 2.17
(1.11-4.21), followed by children carrying the prothrombin G20210A
mutation (A: 5.8% vs. 2.2%, cOR (95%-CI), 2.74 (0.86-8.71); B: 4.3%
vs. 3.0%, cOR (95%-CI), 1.45 (0.31-6.84)).

In addition, the risk of intrauterine growth restriction in children
with increased Lp(a) concentrations was 19.4% in the lowest quartile
(30.4% below lowest decile) compared with 18.0% in children above the
lowest quartile (16.7% above the lowest decile). However, the risk
of being a small for date baby in children with elevated Lp(a) levels did
not reach statistical significance when comparing both quartile sub-
groups (cOR (95%-CI), 1.10 (0.62-1.96)). In contrast, the risk of in-
trauterine growth restriction in carriers of elevated Lp(a) concentrations
was clearly increased in paediatric subjects with a birth weight below
the lowest decile in comparison to subjects carrying a birth weight
above the lowest decile (cOR (95%-CI), 2.18 (1.09-4.35)).

Multivariate logistic regression analysis with respect to single and
combined prothrombotic risk factors. Assessment of the impact of the
effect of foetal prothrombotic risk factors for thrombophilia on birth
weight was conducted for the 375 children. Analyses were stratified by
genotype i.e. one subgroup comprised children with only one hetero-
zygous defect whereas the second included infants with at least two
heterozygous and/or at least one homozgyous defect (Table 1). The
latter comprised 25 infants of whom 18 had two heterozygous defects,
and 7 had homozgyous defects. In addition, the impact of any inherited
risk factor for thrombophilia on birth weight was calculated. The propor-
tion of children in the lowest birth weight quartile increased from
23.7% to 30.5% to 48.0% for children with no, a single heterozygous
and multiple heterozygous or homozgyous defects respectively (below
the lowest decile 9.1%, 15.3% and 28.0%).

Table 2 illustrates the dose effect of single heterozygous or homo-
zgyous or multiple heterozygous defects or for any inherited risk factor
for thrombophilia: Single heterozygous defects increased the risk less
than half as much as multiple or homozgyous defects. Another impor-
tant finding is that the effects were greater at the more extreme end of

von Kries et al.: Foetal Growth Restriction
Influence of maternal risk factors. Maternal smoking and low maternal weight before pregnancy were significantly (p < 0.05: Chi-square statistics) associated with birth weight in the lowest quartile, whereas no significant association with being the firstborn and with maternal age above 35 years was found in our data (data not shown). Adjustment for these risk factors, however, did not account for consistent changes of the odds ratio.

Discussion

The data presented in this retrospective study clearly demonstrate that inherited risk factors for thrombophilia in the foetus increase the risk for low birth weight. This effect is more pronounced in children with either homozygous defects or multiple combined prothrombotic defects than in children with single heterozygous defects only. The latter finding is in line with results recently published on adult and childhood patients with venous thromboembolism, suggesting earlier and more severe clinical signs of vascular accidents in patients suffering from combined prothrombotic risk factors (21-24).

The main finding in this study is that the presence of prothrombotic risk factors in the foetus, mainly the factor V G1691A mutation, is a risk factor for intrauterine growth restriction. Few papers have addressed the impact of genetic risk factors of thrombophilia in the foetus with respect to miscarriage and placental infarction (13), and to premature birth (12). To our knowledge this is the first systematic study on the impact of prothrombotic risk factors on low birth weight. Since both sides of the placenta – the maternal and the foetal side – are important for the substrate supplied to the foetus, it appears plausible that inherited risk factors for thrombophilia in the foetus itself has an impact on

<table>
<thead>
<tr>
<th>Risk factors for thrombophilia</th>
<th>Birth weight above the lowest quartile</th>
<th>Birth weight below the lowest quartile</th>
<th>Birth weight above the lowest decile</th>
<th>Birth weight below the lowest decile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absent</td>
<td>76.3 %</td>
<td>23.7 %</td>
<td>90.9 %</td>
<td>9.1 %</td>
</tr>
<tr>
<td>N=232</td>
<td>N=177</td>
<td>N=55</td>
<td>N=211</td>
<td>N=21</td>
</tr>
<tr>
<td>Single heterozygous defects</td>
<td>69.5 %</td>
<td>30.5 %</td>
<td>84.8 %</td>
<td>15.2 %</td>
</tr>
<tr>
<td>N=118</td>
<td>N=82</td>
<td>N=36</td>
<td>N=100</td>
<td>N=18</td>
</tr>
<tr>
<td>Multiple or homozygous defects</td>
<td>52.0 %</td>
<td>48.0 %</td>
<td>72.0 %</td>
<td>28.0 %</td>
</tr>
<tr>
<td>N=25</td>
<td>N=13</td>
<td>N=12</td>
<td>N=18</td>
<td>N=7</td>
</tr>
<tr>
<td>All defects combined</td>
<td>66.4 %</td>
<td>33.6 %</td>
<td>82.5 %</td>
<td>17.5 %</td>
</tr>
<tr>
<td>N=143</td>
<td>N=95</td>
<td>N=48</td>
<td>N=118</td>
<td>N=25</td>
</tr>
</tbody>
</table>

1 \(p=0.197\); 2 \(p=0.015\); 3 \(p=0.043\) (Fisher’s exact test, birth weights above versus in first quartile)

4 \(p=0.105\); 5 \(p=0.014\); 6 \(p=0.022\) (Fisher’s exact test, birth weights above versus below the lowest decile)

The data presented in this retrospective study clearly demonstrate that inherited risk factors for thrombophilia in the foetus increase the risk for low birth weight. This effect is more pronounced in children with either homozygous defects or multiple combined prothrombotic defects than in children with single heterozygous defects only. The latter finding is in line with results recently published on adult and childhood patients with venous thromboembolism, suggesting earlier and more severe clinical signs of vascular accidents in patients suffering from combined prothrombotic risk factors (21-24).

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foetal growth, accounting for low birth weight. However, as one limitation of the study presented here, we have only investigated the foetal site of the placenta, i.e. the child itself. Thus, no conclusions can be drawn from the present data with respect to the possible interaction between foetal and maternal thrombophilia and intrauterine growth restriction.

The analyses were confined to children who had not experienced neonatal stroke or thrombosis since symptomatic intrauterine thromboembolic events in the foetus may produce organ shrinking, and organ damage with possible limb amputation and thereby leading to reduced birth weight (25-27). In order to rule out interference by maternal acquired risk factors foetuses who were exposed to maternal pregnancy complications, i.e. history of recurrent foetal loss or stillbirth, deep venous thrombosis, myocardial infarction or stroke, maternal diabetes, preeclampsia, severe viral or bacterial infections, and maternal hepatic administration have not been enrolled in the study presented here. In addition, in the statistical multivariate analyses we included only those cases with complete information on all prothrombotic risk factors. Thus, our risk estimate for inherited prothrombotic risk factors in the foetus is therefore conservative.

On the one hand, a further limitation of the study presented here is that the information on potential confounding risk factors for low birth weight was obtained retrospectively. However, on the other hand, information/recall bias is unlikely, as the parents had to extract most of the information from their personal obstetric records (“Mutterpass”) or the well-baby booklet and were unaware of the study hypothesis. In addition, selection bias is unlikely because 91% of the consecutively recruited eligible Caucasian population were covered by the questionnaire.

These findings reported here have major implications for research into the role of risk factors of thrombophilia on foetal growth, prematurity or foetal loss. Previous work on these issues has focussed on the maternal side, e.g. by linking placental infarction and thrombosis to maternal thrombophilia due to antiphospholipid antibodies (2), hyperhomocysteinemia (4, 5), protein C deficiency (6), the FV G1691A gene mutation (3), the PT G20210A variant (3, 8) and increased Lp(a) concentrations (7). Based on our data it appears evident that the presence of prothrombotic gene mutations in the foetus is a risk factor for low birth weight. Besides the established prothrombotic risk factors mentioned, elevated Lp(a) serum concentrations above 30 mg/dl, clearly correlated with the presence of small apo(a) isoforms, were identified as independent risk factors for the occurrence of venous thromboembolic events in children and adults (10). In vitro, Lp(a) inhibits the activation of plasminogen by streptokinase and tissue plasminogen activator (tPA) and competes with plasminogen for binding to fibrin as well as for binding to annexin II, the plasminogen/tPA receptor on endothelial cells and platelets (28-35). Because of these properties and the great structural homology between Lp(a) and plasminogen it has been hypothesised that Lp(a) inhibits fibrinolysis (28). Thus, these anti-fibrinolytic properties of Lp(a) have been made responsible in part for the association of elevated Lp(a) and risk for atherosclerotic vessel diseases as well as for venous thromboembolic disease. Further studies on the impact of thrombophilia on low birth weight therefore have to take account of the foetal side and potential interdependencies with maternal thrombophilia as well.

In conclusion we have identified inherited thrombotic risk factors in Caucasian foetuses as an additional cause for low birth weight. These findings might be of relevance to therapeutic strategies aimed at preventing low birth weight and morbidity associated with foetal thrombophilia.

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