Foetal Growth Restriction in Children with Prothrombotic Risk Factors

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Keywords

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 intervillous 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-University, 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 urgery (recruitment of a control population) or assessment for potential bone marrow donation (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, heparin 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

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5 because of twin pregnancies. For 19 children information as to twin pregnancy was not available. 35 children had a gestational age below 37 weeks; in 3 the gestational age was above 42 weeks (no reference data for birth weight available). For 16 children information regarding gestational age was missing. The final study population covered 451 children and complete laboratory tests had been performed in 375 subjects (83%) enrolled.

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 polystyrene tubes, stored at –70 °C and thawed immediately before the assay procedure. For genetic analysis, which was performed between 1996 and 1999 in all study patients, we obtained venous blood in EDTA-treated sample tubes (Sarstedt, Nücmbrecht, Germany), from which cells were separated by centrifugation at 3000 g for 15 min. The buffy coat layer was then removed and stored at –70 °C pending DNA extraction by a spin column procedure (Qiagen, Hilden, Germany).

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 activities were measured on an ACL 300 analyser (Instrumentation Laboratory, Germany) using chromogenic substrates (Chromogenix, Sweden). Free protein S antigen, total protein S and protein C antigen were measured using commercially available ELISA assay kits (Stago, France).

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 interdependencies between prothrombotic risk factors and birth weight were "birth weight in the lowest quartile" and "birth weight below the lowest decile" in singletons 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 antithrombin deficiency or the mutations FV G1691A, or PT G20210A or an Lp(a) concentration > 30 mg/dl was found either in 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 stratified by single heterozygous and multiple or homozygous 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/m^3) 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 children (83% of the study population) aged two months to 16 years, classified 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; homozygous 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 mutation (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 prothrombotic 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 subgroups (cOR (95%-CI), 1.10 (0.62-1.96)). In contrast, the risk of intrauterine 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 heterozygous defect whereas the second included infants with at least two heterozygous and/or at least one homozygous defect (Table 1). The latter comprised 25 infants of whom 18 had two heterozygous defects, and 7 had homozygous defects. In addition, the impact of any inherited risk factor for thrombophilia on birth weight was calculated. The proportion 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 homozygous defects respectively (below the lowest decile 9.1%, 15.3% and 28.0%).

Table 2 illustrates the dose effect of single heterozygous or homozygous 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 homozygous defects. Another important finding is that the effects were greater at the more extreme end of

Risk factors for thrombophilia	Birth weight above the lowest quartile	Birth weight below the lowest quartile	Birth weight above the lowest decile	Birth weight below the lowest decile
Absent	76.3 %	23.7 %	90.9 %	9.1 %
N = 232	N=177	N=55	N=211	N=21
Single heterozygous defects[1,4]				
N= 118	69.5 %	30.5 %	84.8 %	15.2 %
	N=82	N=36	N=100	N=18
Multiple or homozygous defects[2,5]				
N = 25	52.0 %	48.0 %	72.0 %	28.0 %
	N=13	N=12	N=18	N=7
All defects combined[3,6]				
N = 143	66.4 %	33.6 %	82.5 %	17.5 %
	N=95	N=48	N=118	N=25

[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)

Table 1 Proportion of low birth weight defined as birth weight in the lowest quartile or below the lowest decile in relation to the presence of any foetal risk factor for thrombophilia: no, single heterozygous, multiple or homozygous defects or all defects combined

low birth weight (below the lowest decile instead of the lowest quartile).

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

Risk factor for thrombophilia	Birth weight in the lowest quartile		Birth weight below the lowest decile	
	Crude odds ratio (95 % CI)	Adjusted odds ratio* (95% CI)	Crude odds ratio (95 % CI)	Adjusted odds ratio* (95% CI)
Single heterozygous defects N=118	1.41 (0.86-2.32)	1.29 (0.78-2.14)	1.81 (0.92-3.55)	1.53 (0.76-3.08)
Multiple or homozygous defects N =25	2.97 (1.28-6.89)	3.19 (1.35-7.49)	3.91 (1.46-10.43)	4.01 (1.48-10.84)
All defects combined N =143	1.63 (1.03-2.58)	1.52 (0.95-2.44)	2.13 (1.14-3.97)	1.92 (1.02-3.63)

*adjusted for maternal smoking and low maternal weight (<50 kg)

Table 2 Relative risk (measured as odds ratio and 95% CI of being in the lowest birth weight quartile or below the lowest decile, associated with thrombophilia) stratified by foetal defects of thrombophilia given in comparison with children above the lowest quartile or the lowest decile

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 heparin 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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