Germinal Matrix Hemorrhage: Intraventricular Hemorrhage in Very-Low-Birth-Weight Infants
The Independent Role of Inherited Thrombophilia

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Background and Purpose—The etiology of germinal matrix hemorrhage–intraventricular hemorrhage (GMH-IVH) is multifactorial and the role of genetic polymorphisms is unclear. The aim of this prospective study was to evaluate prothrombotic genetic mutations as independent risk factors for the development of all grades of GMH-IVH in very-low-birth-weight infants.

Methods—The presence of both factor V Leiden and prothrombin gain-of-function gene mutations were prospectively assessed in 106 very-low-birth-weight infants. Infants with GMH-IVH were compared to those without GMH-IVH according to genetic and clinical characteristics.

Results—Twenty-two out of 106 infants had GMH-IVH develop (20.7%). Infants with GMH-IVH had significantly lower gestational ages and birth weights. In the multivariate Poisson regression model, the prevalence of GMH-IVH appeared to be inversely related to gestational age, with a risk ratio of 0.83 (95% CI, 0.72–0.97; \( P = 0.02 \)) per week. Risk ratio of GMH-IVH for carriers of either prothrombotic mutation was 2.65 (95% CI, 1.23–5.72; \( P = 0.01 \)), similar to the risk ratio associated with need for resuscitation at birth (2.30; 95% CI, 1.02–5.18; \( P = 0.04 \)).

Conclusions—Very-low-birth-weight infants who are carriers for either prothrombotic mutations are at increased risk for development of GMH-IVH. Genetic factors act as independent risk factors of the same magnitude as other known risk factors. (Stroke. 2011;42:1889-1893.)

Key Words: factor V Leiden ■ germinal matrix intraventricular hemorrhage ■ preterm infants ■ prothrombin mutation ■ risk factors

The etiology of germinal matrix hemorrhage–intraventricular hemorrhage (GMH-IVH) affecting preterm babies is multifactorial but is likely to depend also on individual genetic factors. Qualitative and quantitative, as well as congenital and acquired, abnormalities in coagulation and fibrinolysis have received increasing attention as predisposing factors.1 Thrombophilic patterns have been associated with an increased risk of hemorrhagic and ischemic perinatal cerebral lesions,2,3 although the role of thrombophilia in the pathogenesis of GMH-IVH in preterm infants remains unclear.

Factor V Leiden (Arg506Gln) and prothrombin (G20210A) gain-of-function gene mutations are common prothrombotic polymorphisms in white populations4 associated with cerebral venous thrombosis, the most frequently recognized cause of symptomatic IVH in term neonates.5 The association between factor V Leiden and IVH was first suggested in a report of 8 patients with hydrocephalus.6 Because of liver immaturity, neonates are born with a natural deficiency in 3 anticoagulant factors (protein C, protein S, and antithrombin), potentially predisposing to thrombotic events7 if other congenital or acquired risk factors are present, although conventional coagulation tests are often inadequate to explore hypercoagulability.8 This peculiarity of the neonatal coagulation system tends to become more severe in sick neonates.9 A possible direct role of thrombophilia in triggering GMH-IVH is reinforced by the evidence of the venous origin of this form of intracranial bleeding,10,11 because thrombophilia increases the risk of venous thrombosis in the small venules of germinal matrix. Accordingly, factor V Leiden and prothrombin mutations seem to increase the risk of GMH-IVH developing in preterm infants,12,13 although the methodology and the statistical evidence of these observations deserve further studies.1 However, these mutations were not found to be associated with an increased risk of IVH in a Israeli population of 166 premature infants,14 and other authors even suggest a protective role for thrombophilia versus the severest
forms of GMH-IVH.15,16 The aim of this prospective study was to assess the potential role of these prothrombotic genetic mutations as independent risk factors for the development of all grades of GMH-IVH in very-low-birth-weight infants.

Subjects and Methods
All inborn preterm infants weighing <1500 g (very-low-birth-weight infants) at birth admitted to the neonatal tertiary care center (intensive care unit) IRCCS Fondazione Ca’ Granda–Ospedale Maggiore Policlinico, Milan, Italy, over the course of 1 year (since January 2007) were included in the study. Written consent from both parents was obtained. The presence of factor V Leiden (Arg506Gln) and G20210A prothrombin gene mutations was prospectively assessed in all newborns; 0.5 mL of venous blood was sampled from infants and collected in EDTA. The 2 mutations were searched with standardized methods.17,18

We also explored the relationship between the occurrence of GMH-IVH and the following prenatal and perinatal variables: intraventricular hemorrhage; IUGR, intrauterine growth restriction; birth weight <10th percentile, prenatal steroids administration, single or multiple pregnancy, mode of delivery (vaginal delivery versus cesarean delivery), gender, gestational age (GA; weeks), birth weight (grams), and Apgar score ≤5 at 1 and 5 minutes.

The following acute and chronic neonatal outcomes were examined: respiratory distress syndrome requiring any kind of respiratory support, chronic lung disease (defined as oxygen dependency up to 36 weeks corrected age), hemodynamically significant patent ductus arteriosus (defined by echocardiographic criteria)19 with pharmacological treatment of patent ductus arteriosus, necrotizing enterocolitis (requiring surgical treatment), and retinopathy of prematurity (stage). Requirement for inotropic drugs during the first 5 days of life was also recorded. For statistical analysis, hypotension was defined as need of any kind of inotropic drugs for at least 1 day.

The infants were subdivided into 2 groups in relation to the occurrence of GMH-IVH, defined according to the criteria of Papile.20 The prevalence of GMH-IVH was estimated by brain ultrasound; the infants underwent brain ultrasound scans according to the routine clinical assistance for the diagnosis of GMH-IVH in preterm infants (days 1, 3, and 7 of life and follow-up scans at 14, 21, and 28 days, and then monthly until discharge). Brain ultrasound scans were reviewed by 2 independent examiners.

Statistics
Descriptive statistics are presented as mean ± SD for continuous variables and number and percentages for categorical variables. Categorical outcomes were compared with use of χ² tests for trends. Continuous outcomes were compared with use of independent Student t tests. A multivariate Poisson regression model with robust standard error23 was used to estimate the risk ratios (RR) and 95% CI of GMH-IVH according to selected clinical and genetic newborn characteristics, including the presence of a gain-of-function gene mutation, GA (weeks), Apgar score ≤5 at 1 minute, and hypotension (as defined).

We evaluated the association between gain-of-function gene mutations and severity of GMH-IVH with Fisher exact test and by fitting a multiple polytomous ordinal logistic model22 in which the outcome, given the low number of cases, was categorized as 0 (no GMH-IVH), 1 (GMH or IVH grade 2), or 2 (IVH grade 3 or 4). All the analyses were performed with Stata 11 (StataCorp).

Results
One hundred six premature infants were enrolled in the study. Infants were subdivided into 2 groups (group with GMH-IVH and group without GMH-IVH) according to the ultrasound detection of GMH-IVH. Characteristics of the study population and postnatal variables are shown in Tables 1 and 2.

The overall prevalence of GMH-IVH was 20.7% (22 out of 106 infants): 12 infants had GMH develop, 4 had IVH grade 2, 5 had IVH grade 3, and 1 had IVH grade 4. GMH-IVH was diagnosed in 18 out of the 22 patients within the third day of life; the remaining instances of GMH-IVH were diagnosed at the time of the third scan. Birth weight and GA were significantly lower in the GMH-IVH group compared to the group without GMH-IVH (mean birth weight, 884.77 ± 257.95 versus 1135.42 ± 261.31 g; P = 0.001; mean GA, 27.81 ± 2.38 versus 30.25 ± 2.71 weeks). Thirteen babies died before discharge, 5 of 22 were in the GMH-IVH group and 8 of 84 were in the group without GMH-IVH (P = 0.09).

Twelve of 106 babies were heterozygous for either gain-of-function mutation (9 had prothrombin mutation and 3 had factor V Leiden); 6 of 12 babies (27.3%) were in the GMH-IVH group (4 with prothrombin mutation and 2 with factor V Leiden) whereas 6 (7.1%) carriers (5 with the prothrombin mutation and 1 with factor V Leiden) had no intracranial bleeding.

During the univariate analysis, lower birth weight (categorized as <750 g, 750–999 g, ≥1000; P = 0.000), younger GA (categorized as ≤30 weeks or ≥30 weeks; P = 0.005), low 5-minute Apgar score ≤5; P = 0.014), and hypotension (P = 0.001) were more common in infants with GMH-IVH than in infants without GMH-IVH. Infants in the GMH-IVH group had a higher prevalence of gain-of-function mutations (P = 0.008).

To rule out confounding variables, we performed Poisson regression (Table 3) to adjust analysis for GA, Apgar score ≤5 at 5 minutes, hypotension (as defined), and gain-of

<table>
<thead>
<tr>
<th>Table 1. Characteristics of the Study Population</th>
<th>Group Without GMH-IVH (N=84)</th>
<th>Group With GMH-IVH (N=22)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>42 (50.0)</td>
<td>11 (50.0)</td>
<td>1.00</td>
</tr>
<tr>
<td>Female</td>
<td>42 (50.0)</td>
<td>11 (50.0)</td>
<td></td>
</tr>
<tr>
<td>Gestational age (wk)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;30</td>
<td>33 (39.3)</td>
<td>16 (72.7)</td>
<td>0.005</td>
</tr>
<tr>
<td>≥30</td>
<td>51 (60.7)</td>
<td>6 (27.3)</td>
<td></td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;750</td>
<td>8 (9.5)</td>
<td>7 (31.8)</td>
<td>0.000</td>
</tr>
<tr>
<td>≥750–999</td>
<td>12 (14.3)</td>
<td>10 (45.5)</td>
<td></td>
</tr>
<tr>
<td>&gt;1000</td>
<td>64 (76.2)</td>
<td>5 (22.7)</td>
<td></td>
</tr>
<tr>
<td>IUGR</td>
<td>36 (42.9)</td>
<td>9 (40.9)</td>
<td>0.87</td>
</tr>
<tr>
<td>Multiple pregnancy</td>
<td>30 (35.7)</td>
<td>8 (36.4)</td>
<td>0.955</td>
</tr>
<tr>
<td>Prenatal steroids</td>
<td></td>
<td></td>
<td>0.17</td>
</tr>
<tr>
<td>Yes</td>
<td>18 (23.1)</td>
<td>6 (40.0)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>60 (76.9)</td>
<td>9 (60.0)</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>6</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Vaginal delivery</td>
<td>8 (9.5)</td>
<td>2 (9.1)</td>
<td>0.951</td>
</tr>
<tr>
<td>Apgar score ≤5 at 1 min</td>
<td>38 (45.2)</td>
<td>10 (45.4)</td>
<td>0.986</td>
</tr>
<tr>
<td>Apgar score ≤5 at 5 min</td>
<td>3 (3.6)</td>
<td>4 (18.2)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

GMH-IVH indicates germinal matrix hemorrhage–intraventricular hemorrhage; IUGR, intrauterine growth restriction.

*χ² test.
function gene mutations. Prevalence of IVH was related to GA, with RR of 0.83 per week of GA (95% CI, 0.72–0.97; \(P = 0.02\)). RR of GMH-IVH in carriers of either mutation was \(0.29\) in the group without GMH-IVH; the mean value was 1.35±0.26, which we considered not different from the control values. No differences in hemoglobin levels and platelet counts were observed on the first sample at admission (hemoglobin 15.6±1.0 g/dL in GMH-IVH group versus 14.6±3.25 in the group without GMH-IVH; \(P = 0.34\); platelet count 190 800±77 007/mm³ and 151 005±56 195/mm³, respectively; \(P = 0.29\)).

Comparative results were obtained when the multiple Poisson regression was used to analyze the 2 prothrombotic mutations separately. We observed 2 infants with GMH-IVH (66.7%) among the 3 infants with the factor V Leiden mutation, and 4 (44.4%) infants among the 9 infants with G20210A prothrombin mutation compared to 16 infants with GMH-IVH (17.0%) and without these mutations (\(P = 0.02\)). The analysis confirmed the elevated RR for factor V and prothrombin gene mutations: 3.48 (95% CI, 1.89–6.41; \(P < 0.001\)) and 2.50 (95% CI, 1.07–5.80; \(P = 0.03\), respectively. The presence of gain-of-function gene mutations was associated (\(P = 0.03\)) with the severity of GMH-IVH (Table 4). This association was confirmed (\(P = 0.02\)) after adjusting for GA, hypotension, and Apgar score.

The prothrombin time international normalized ratio was performed at 7 days of life according to the clinical routine protocol in all infants. The results were abnormal, as expected in very-low-birth-weight infants, but no significant differences were observed between the 2 groups (prothrombin time/international normalized ratio 1.42±0.40 and 1.30±0.29 in the group with GMH-IVH and the group without GMH-IVH, respectively; \(P = 0.40\) and 1.30±0.26, which we considered not different from the control values. No differences in hemoglobin levels and platelet counts were observed on the first sample at admission (hemoglobin 15.6±1.0 g/dL in GMH-IVH group versus 14.6±3.25 in the group without GMH-IVH; \(P = 0.34\); platelet count 190 800±77 007/mm³ and 151 005±56 195/mm³, respectively; \(P = 0.29\)).

### Table 3. Risk of Germinal Matrix Hemorrhage–Intraventricular Hemorrhage According to Selected Clinical and Genetic Characteristics by Multivariate Analysis

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>RR*</th>
<th>95% CI</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GA (wk)</td>
<td>0.83</td>
<td>0.72–0.97</td>
<td>0.02</td>
</tr>
<tr>
<td>Apgar score ≤5 at 5 min</td>
<td>2.30</td>
<td>1.02–5.18</td>
<td>0.04</td>
</tr>
<tr>
<td>Hypotension</td>
<td>2.05</td>
<td>0.74–5.69</td>
<td>0.17</td>
</tr>
<tr>
<td>Gain-of-function gene mutations</td>
<td>2.65</td>
<td>1.23–5.72</td>
<td>0.01</td>
</tr>
</tbody>
</table>

*RR calculated with Poisson regression with robust standard error; each variable is adjusted for the others in the Table.

CI indicates confidence interval; GA, gestational age; RR, risk ratio.

### Table 4. Germinal Matrix Hemorrhage–Intraventricular Hemorrhage Grades and Presence ofGain-of-Function Gene Mutations

<table>
<thead>
<tr>
<th>G20210A Prothrombin or Arg506Gln Factor V Leiden Mutations (N=12)</th>
<th>No Prothrombotic Mutations (N=94)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N (%)</td>
<td>N (%)</td>
</tr>
<tr>
<td>Without GMH-IVH</td>
<td>6/12 (50.0)</td>
</tr>
<tr>
<td>GMH</td>
<td>3/12 (25.0)</td>
</tr>
<tr>
<td>IVH grade 2</td>
<td>1/12 (8.3)</td>
</tr>
<tr>
<td>IVH grade 3</td>
<td>2/12 (16.7)</td>
</tr>
<tr>
<td>IVH grade 4</td>
<td>0/12 (0)</td>
</tr>
</tbody>
</table>

\(P\) (Fisher exact test) 0.03

GMH indicates germinal matrix hemorrhage; IVH, intraventricular hemorrhage; GMH-IVH, germinal matrix hemorrhage–intraventricular hemorrhage.
MRI was performed only in the group of infants with GMH-IVH at term-corrected age. No direct sign (ie, clots) of sinovenous thrombosis or abnormalities potentially resulting from it were detected. No clinical complication related to thrombotic events was observed.

**Discussion**

Our results confirm the primary hypothesis and show that very-low-birth-weight infants heterozygous for a prothrombotic mutation (Arg506Gln factor V Leiden or G20210A prothrombin) are at increased risk for development of GMH-IVH. Genetic thrombophilia acts as an independent risk factor of the same magnitude of other known environmental risk factors.23 The univariate analysis confirmed the previously reported association of GMH-IVH with younger GA, lower birth weight, and postnatal resuscitation.24–26 We were not able to confirm a protective role of prenatal steroids,27,28 perhaps because of the low rate of administration in this studied population.

The multivariate regression demonstrated a higher RR of GMH-IVH in infants carrying prothrombotic mutations, as well as those needing resuscitation at birth, whereas for those having hypotension develop in the first days of life the RR failed to reach statistical significance. The role of thrombophilia as an independent risk factor for the development of GMH-IVH was further investigated by performing the statistical subanalyses of the 2 mutations separately. Despite the small total number of infants with genetic thrombophilia, the multiple Poisson regression confirmed the elevated RR for carriers of each single prothrombotic mutation.

Our study highlights the potential effect of prothrombotic mutations in triggering GMH-IVH, although the understanding of the pathological steps in the microvasculature of germinal matrix is beyond the possibilities of this study. There is consensus on the venous origin of GMH-IVH,10,11 because extravasated red blood cells are observed in a perivenular distribution in the early pathology stages of GMH-IVH. How thrombophilia may account for the increased risk of GMH-IVH remains to be understood. There is evidence that among the potential triggers of GMH-IVH, venous stasis or thrombosis in the microvasculature of germinal matrix plays a significant role; therefore, thrombophilia is likely to increase the risk of occurrence of these events.11 The subsequent increase in the local venous pressure induces transudation of red blood cells and secondary rupture of venules wall, determining blood tunneling along the perivenous space and further distortion and tethering of these smaller connecting venous tributaries.10,11

Our findings are consistent with those of the retrospective study by Petaja et al,12 who reported a similar prevalence (23% versus 27% in our study) of factor V Leiden and prothrombin mutations in preterm infants with GMH-IVH: the OR for being a carrier of factor V Leiden in patients with IVH was 6.2 when compared to premature infants without IVH. The association between factor V Leiden and IVH was also suggested in a report of 8 patients with hydrocephalus.6 On the contrary, Göpel et al15 found no significant differences in the overall prevalence of IVH in infants with and without prothrombotic mutations. However, the multivariate analysis showed that carriers of factor V Leiden or prothrombin mutation have a significantly reduced risk for development of extension of IVH to grade II or higher,15 which are the most severe forms. We could not confirm this protective effect of prothrombotic mutations toward the most severe forms of IVH, although each study has too few patients to address this specific question. In addition, we believe it is more appropriate to investigate the triggering capacity of thrombophilia as an independent factor in promoting any grade of GMH-IVH, because the causative correlation between different levels of severity is less known.

We observed only 1 patient with grade 4 IVH, so no speculation can be performed on the potential protective effect of factor V Leiden or prothrombin mutation and developing the most severe forms with parenchymal involvement, as suggested by Göpel et al.15 However, it has been recently observed that the risk of grade 4 versus grade 3 IVH is not related to other commonly cited clinical factors, but it increases with declining GA.20

A limitation of this study comes from the lack of information on the thrombophilic patterns in the parents, because it is known that the risk of development of perinatal ischemic lesions in the brains of infants can be increased by the presence of maternal thrombophilia.3,30,31 Nevertheless, we have shown that among the postnatal risk factors, the innate thrombophilia of neonates seems to play a role equivalent to those of postnatal environmental risk factors. The importance of genetic factors may increase over the next few years because we are trying to minimize perinatal environmental risk factors.

**Disclosures**

None.

**References**


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