Factor V Leiden and Antiphospholipid Antibodies in Either Mothers or Infants Increase the Risk for Perinatal Arterial Ischemic Stroke

Michal J. Simchen, MD; Gal Goldstein, MD; Aaron Lubetsky, MD; Tzipi Strauss, MD; Eyal Schiff, MD; Gili Kenet, MD

Background and Purpose—The objective was to investigate the role of infant and maternal thrombophilia in a cohort of mothers and infants presenting with perinatal arterial ischemic stroke.

Methods—Forty-seven infants with clinically and radiologically confirmed perinatal arterial ischemic stroke underwent thrombophilia workup: factor V Leiden (FVL), PII20210A mutation, Methylene-tetrahydrofolate reductase 677T polymorphism, protein C, protein S, antithrombin, FVIII, and antiphospholipid antibodies. Thrombophilia data were available for 23 mother–infant pairs and compared with control populations to evaluate the risk for PAS.

Results—Thirty of 47 (64%) infants and 15 of 22 mothers (68%) had evidence of thrombophilia. In 18 of 23 (78%) mother–infant pairs, there was at least 1 thrombophilic risk factor, but 15 pairs were mismatched in pathology. Among infants, FVL, protein C deficiency, and presence of antiphospholipid antibodies prevailed (OR, 4.2; 95% CI, 1.5–11.3; OR, 12.2; 95% CI, 2.5–59.9; OR, 4.1; 95% CI, 1.4–12.2, respectively). Interestingly FVL prevailed in almost one-third of mothers (OR, 8.5; 95% CI, 4.1–17.5) and 18% of mothers had antiphospholipid antibodies (OR, 3.8; 95% CI, 1.5–10.0).

Conclusions—Maternal and neonatal thrombophilia, especially presence of FVL or antiphospholipid antibodies, may be important in the pathogenesis of perinatal arterial ischemic stroke. The nature of thrombophilic mother–infant risk potential interactions warrants further investigation. (Stroke. 2009;40:65-70.)

Key Words: antiphospholipid antibodies ▪ FVL ▪ perinatal ischemic stroke ▪ thrombophilia

Perinatal arterial ischemic stroke (PAS) is defined as a focal (or multifocal) cerebral ischemic infarction, confirmed by radiographic or pathological assessment, attributable to an arterial event, and occurring between 28 weeks’ gestation and 28 days of postnatal age.1 Perinatal stroke occurs in ~1 in every 4000 to 5000 live births,2,3 and in settings with high frequency of neuroimaging may reach to 1 in 2300 live births.4,5 Most perinatal strokes involve the middle cerebral artery. Cerebral arterial infarction in newborns either may present acutely during the neonatal period with neurological symptoms such as seizures3,6 or may be clinically asymptomatic until several months of age, when signs of motor impairment or seizures are first noted, leading to a delayed presumed diagnosis of PAS.5,7 PAS has received increased attention as an important cause of cerebral palsy and other neurological disabilities, including epilepsy and cognitive impairment.8-10

The pathophysiology of perinatal stroke is complex and multifactorial. Risk factors may relate to both maternal and placental problems, as well as fetal and neurological disorders. Maternal preeclampsia, chorioamnionitis, fetal cardiac anomalies, polycythemia, birth asphyxia, and systemic infection are associated with PAS.8,11-13

The role of genetic and acquired thrombophilias in the pathogenesis of PAS is quite controversial and not completely understood.14-16 Maternal thrombophilias, both genetic and acquired, have been widely investigated in their association with various pregnancy complications,17-21 but their role in PAS is not clear.

Our aim in the present study was to investigate the role of maternal and infant thrombophilia in a cohort of mother–infant pairs in whom the infant has experienced a perinatal ischemic stroke. This information may be important in assessing predisposition to future potentially hazardous thrombotic events.

Materials and Methods

Study Group
Pediatric patients with cerebral infarction diagnosed between April 1996 and October 2005 were entered into the Israeli Pediatric Stroke
Control Group
As the rates of thrombophilia vary significantly between different populations, we used as comparison groups the rates of thrombophilia from a locally derived pediatric population including 145 healthy neonates and children referred for complete thrombophilia workup before elective surgery.24 We also used a previously published cohort of 631 healthy nulliparous pregnant women tested for thrombophilia at our center.17 The definition of perinatal stroke was applied by experienced neuroradiologists, referring to porencephalic lesions, periventricular cysts, volume loss, and brain atrophy as signs of nonacute strokes. Children with symptoms beyond the perinatal period without radiographic confirmation of nonacute presumed perinatal stroke were excluded. Among 136 registry patients, whose mean age at the occurrence of stroke was 5.9±5.2 years, 58 children had perinatal stroke diagnosed. Follow-up and outcome data were obtained from medical records and assessment during visits at ambulatory clinics. Children with seizures, hemiparesis, or other neurological abnormalities requiring speech or motor therapy or prolonged neurodevelopmental follow-up were defined as neurologically impaired, whereas children with no such abnormalities were considered neurologically intact. Children in the study group were consecutively referred for laboratory evaluation of thrombophilic risk factors. All thrombophilia testing was performed in the same tertiary referral center laboratory, using standard assays. Maternal thrombophilia evaluation was also recommended in all cases, but not all mothers complied with this request.

Coagulation Tests for Analysis of Thrombophilia
Blood samples were obtained on the first referral visit. Blood samples were collected into 3.8% (9:1 ratio) trisodium citrate centrifuged within 30 minutes of blood sampling at 2000 rpm (20 min), and plasma aliquots were stored at −80°C until analysis. Prothrombin time (PT), activated partial thromboplastin time (APTT), and fibrinogen levels were measured using standard methods. Factor V Leiden (FVL) was measured using the LA screen and the dilute Russell viper venom test time expressing the ratio of assay times using a poor phospholipids-containing reagent (LA Confirm; Gradipore). Prolonged ratios (>1.7 for Circulating Anticoagulant (CAC)-Russell viper venom test and CAC-PTT, respectively) were repeated for confirmation at least 12 weeks after the initial tests. Anticardiolipin antibodies were measured using Autoenzyme Anticardiolipin (ACL) Kit (Cambridge Life Sciences). ACL were considered positive if medium-to-high titers (>20 GPL or MPL units [IgG phospholipids units or IgM phospholipids units]) were present on ≥2 occasions at least 6 weeks apart. B-2 glycoprotein-1 anticardiolipin antibodies were measured using a standard kit (Asco Diagnostica). Borderline (15 to 20 U/mL) or higher values were repeated at least twice to confirm the diagnosis.

Factor 8:C (one stage clotting assay by Dade Behring) testing was performed for mothers only and not for the children presenting with PAS at least 3 months after delivery and repeated at least twice to confirm activity levels >160%.

Genomic DNA was extracted from EDTA-anticoagulated blood samples using standard methods. Factor V Leiden (FVL) was detected by polymerase chain reaction amplification of a 267-bp fragment and MnlI digestion, as previously described.23 The C677T polymorphism in the methylene-tetrahydrofolate reductase gene was identified using the Hinfl cleavage of a 198-bp polymerase chain reaction-amplified product.26 For identification of the G20210A substitution in the factor II gene (FIIG20210A), a modification of the method used by Poort et al27 was performed, as previously described.28

Statistical Analysis
Frequencies and distribution of various thrombophilias were compared within the study group as well as to frequencies of genetic thrombophilia in the population. The χ² tests and Fisher exact test were performed as appropriate. A 2-tailed P<0.05 was considered statistically significant.

Results
One-hundred thirty-six children fulfilling our inclusion criteria are registered to date in the Pediatric Stroke Registry. All had evidence of ischemic stroke documented by computed tomography or MRI before 18 years of age. Of these, 58 children had perinatal ischemic stroke diagnosed and all were requested to undergo a complete thrombophilia workup.

All children (except for 1 child) had diagnoses during the first year of life, with a mean age at diagnosis of 3.7±4.4 months. Of the 23 PAS patients, 13 presented with seizures during the neonatal period, 9 had nonacute presumed perinatal stroke diagnosed because of late diagnosis of hemiparesis or hemiplegia, and only 1 infant, who was examined for increasing head circumference, had PAS diagnosed at age 2 years. This child has a brother with diagnosed PAS.

Thrombophilia workup results were available in 47 infants and in 22 mothers. In 30 of 47 (64%) infants at least 1 hereditary or acquired thrombophilic risk factor could be demonstrated. Twenty-five infants (53%) had genetic thrombophilia, whereas 11 (23%) had antiphospholipid antibodies (APLA), either alone or in combination with genetic thrombophilia (Table 1).

Infant and Maternal Thrombophilia
To look at the contribution of maternal thrombophilia to the risk for perinatal stroke, we examined mother–infant pairs for whom a complete set of thrombophilia workup studies was performed. Because only 22 mothers complied with our request for testing, we studied 23 mother–infant pairs for whom we had complete data sets (1 mother had 2 children with PAS).

To look for potential selection bias in our analysis of this subgroup of mother–infant pairs, we compared thrombophilia risk factors between the group of infants for whom thrombophilia results were available for both infants and their mothers (n=23) to those for whom no maternal information was available (n=24). As can be seen in Table 1, the distribution of thrombophilia risk factors was very similar, indicating lack of any undesired selection.
Of the mother–infant pairs, 14 (61%) infants had at least 1 genetic or acquired thrombophilic risk factor, of whom 12 infants had genetic thrombophilia markers. Four infants had genetic risk factors. Six infants had APLA, of whom 3 were also found to have genetic thrombophilia.

Fifteen of 22 (68%) mothers were positive for thrombophilia markers. Fourteen mothers had genetic thrombophilic risk factors, whereas 4 mothers had APLA. Three mothers had combined genetic thrombophilia risk factors, and 3 had combined genetic and APLA. Six mothers had previous thromboembolic episodes: 3 had previous strokes, 1 had a transient ischemic attack during pregnancy, and 2 others had previous episodes of deep vein thrombosis. One mother had 2 children with PAS and a previous pregnancy that ended in stillbirth.

In 18 of 23 mother–infant pairs (78%), at least 1 thrombophilic risk factor could be identified for either mother or infant (Table 2). Of the 18 thrombophilic pairs, matching thrombophilia markers were found in 3 pairs, whereas a partial match (in which either mother or infant had an additional thrombophilia marker) were found in 7 additional pairs. Eight mother–infant pairs had a complete mismatch in thrombophilia markers. More than half of the cases of nonsimilar thrombophilia patterns (8 of 15 cases) were characterized by the presence of APLA in either mother or infant. Five mother–infant pairs had no evidence of maternal or infant thrombophilia.

Table 3 presents the distribution and relative frequencies of thrombophilic risk factors in mothers and infants compared with our control populations. In children with PAS, the presence of FVL mutation, protein C deficiency, or APLA exerted a significant risk for stroke as compared to our controls. Interestingly, mothers carrying the FVL mutation or APLA also increased the risk for PAS in their children. The relative risk of mothers with FVL mutation was 8.5-times (95% CI, 4.1–17.5) higher and the risk of APLA was 3.8-times (95% CI, 1.5–10.0) higher as compared to that of control mothers (Table 3).

To test whether presence of thrombophilia had an impact on the neurological outcome, we compared children with residual neurological deficit to children who were neurologically intact after their stroke. Fifteen children had residual neurological deficit; 10 of the 15 were carriers of thrombophilia (67%) compared to 4 of 9 (44%) children without residual neurological damage. This trend was not statistically significant ($P=0.285$).

Discussion
The role of genetic and acquired thrombophilias in the pathogenesis of PAS is quite controversial and not completely understood. FVL mutation, the prothrombin mutation ($\textrm{FII}G_{20210A}$), hyperhomocystinemia, and elevated Lp(a) have all been described with increased frequency in infants with PAS when compared with healthy control sub-

| Table 1. Thrombophilic Risk Factors Among 47 Infants With Perinatal Stroke and Comparison Between Infants With Perinatal Stroke With and Without Available Maternal Information |
|-----------------------------------------------|-----------------|-----------------|-----------------|
| All Infants With Perinatal Stroke, N=47 (%) | Infants With Maternal Information, N=23 (%) | Infants With No Maternal Information, N=24 (%) | *P |
| At least 1 thrombophilic risk factor | 30 (63.8) | 14 (60.9) | 16 (66.7) | 0.67 |
| Genetic thrombophilia (at least 1) | 25 (53.2) | 12 (52.2) | 13 (54.2) | 0.9 |
| Decreased protein C activity | 9 (19.2) | 5 (21.7) | 4 (16.7) | 0.66 |
| Decreased free protein S | 6 (12.8) | 2 (8.7) | 4 (16.7) | 0.43 |
| FVL mutation | 10 (21.3) | 6 (26.1) | 4 (16.7) | 0.43 |
| $\textrm{FII}G_{20210A}$ mutation | 3 (6.4) | 2 (8.7) | 1 (4.2) | 0.55 |
| MTHFR C677T | 9 (19.2) | 5 (21.7) | 4 (16.7) | 0.66 |
| Acquired (APLA) | 11 (23.4) | 6 (26.1) | 5 (20.8) | 0.68 |
| Combined genetic and acquired thrombophilia | 7 (14.9) | 4 (13) | 3 (12.5) | 0.62 |

*Comparison of thrombophilia distribution between infants with and without available maternal information.

| Table 2. Relative Risks of Thrombophilia in Infants With Perinatal Stroke and Their Mothers Compared With Controls |
|-----------------------------------------------|-----------------|-----------------|-----------------|
| Risk Factor | Infants, n=23 | Prevalence in Controls | Relative Risk (95% CI) | Mothers, n=22 | Prevalence in Controls | Relative Risk (95% CI) |
| 677T MTHFR homozygote | 21.7% (5) | 15.1% | 1.4 (0.6; 3.5) | 13.6% (3) | 12.7% | 1.1 (0.4; 3.1) |
| FVL-Het | 26.1% (6) | 6.25% | 4.2 (1.5; 11.3) | 31.8% (7) | 3.8% | 8.5 (4.1; 17.5) |
| $\textrm{FII}G_{20210A}$-Het | 8.7% (2) | 3.6% | 2.4 (0.5; 12.5) | 9% (2) | 4.2% | 2.1 (0.5; 7.5) |
| Protein C deficiency | 21.7% (5) | 1.8% | 12.2 (2.5; 59.9) | 0% (0) | NA | NA |
| Free protein S deficiency | 8.7% (2) | 0% | NA | 13.6% (3) | NA | NA |
| Antithrombin deficiency | 0% (0) | 0% | NA | 0% (0) | NA | NA |
| APLA | 21.7% (5) | 5.4% | 4.1 (1.4; 12.2) | 18.2% (4) | 4.7% | 3.9 (1.5;10.0) |

Relative risks are presented for infants vs controls (derived from Kenet et al) and for mothers vs controls (derived from Salomon et al), separately. Het indicates heterozygous; NA, not available.
In contrast, a recent cohort study found no difference in the incidence of genetic polymorphisms between newborns with stroke and a random newborn control sample. Although the role of infant thrombophilia in the pathogenesis of PAS is still undergoing investigation, the role of maternal thrombophilia in the pathogenesis of PAS has not been studied extensively to date.

In our cohort of well-defined PAS cases, we found a high rate of thrombophilia in both mothers and infants. Among infants with PAS, we found at least 1 thrombophilic marker in 64% (30/47), and among mothers 68% (15/22) were found to be thrombophilia carriers. These high rates of thrombophilia are in concordance with a recently published study of 60 mother–infant pairs in whom the rates of thrombophilia were 55% and 50% in mothers and infants with PAS, respectively.

Among our infants FVL, protein C deficiency, and presence of APLA prevailed as significant risk factors for PAS. Twenty-six percent of our PAS infants were carriers of FVL as compared to only 6.25% of controls. This prevalence of FVL is much higher than the 5% rate reported in the recent mother–child study by Curry et al, but quite similar to a previously published Israeli case-controlled study in which FVL and APLA prevailed among pediatric patients with stroke and the rate of FVL carrierness was 17.2%. FVL was also recently reported as increasing the risk of arterial ischemic stroke occurrence in children of Mediterranean origin, in whom the rate of FVL carrierness among

Table 3. Patterns of Thrombophilic Risk Factors for Mother–Infant Pairs

<table>
<thead>
<tr>
<th>Case</th>
<th>Infant</th>
<th>Mother</th>
<th>Mother–Infant Mismatch</th>
<th>Mat. Hx. Thrombosis</th>
</tr>
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<tr>
<td>1</td>
<td>Normal</td>
<td>FVL*</td>
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<td>No</td>
</tr>
<tr>
<td>2</td>
<td>Normal</td>
<td>PS,* factor VIII*</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>3</td>
<td>Normal</td>
<td>PS,* APLA*</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>4*</td>
<td>Normal</td>
<td>Factor VIII*</td>
<td>Yes</td>
<td>IUFD</td>
</tr>
<tr>
<td>5</td>
<td>MTHFR*</td>
<td>Normal</td>
<td>Yes</td>
<td>CVA @ 18 years</td>
</tr>
<tr>
<td>6</td>
<td>PC*</td>
<td>Normal</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>7</td>
<td>FVL, PT, PC,* PS,* APLA*</td>
<td>FVL, PT</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>8</td>
<td>PT, MTHFR, PC*</td>
<td>PT, MTHFR</td>
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<td>No</td>
</tr>
<tr>
<td>9</td>
<td>MTHFR, PC,* APLA*</td>
<td>MTHFR</td>
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<td>No</td>
</tr>
<tr>
<td>10</td>
<td>APLA</td>
<td>PS,* APLA*</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>11</td>
<td>FVL</td>
<td>FVL, APLA*</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>12</td>
<td>FVL, APLA*</td>
<td>FVL</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>13</td>
<td>MTHFR*</td>
<td>APLA*</td>
<td>Yes</td>
<td>TIA in pregnancy</td>
</tr>
<tr>
<td>14†</td>
<td>APLA*</td>
<td>Factor VIII*</td>
<td>Yes</td>
<td>IUFD</td>
</tr>
<tr>
<td>15</td>
<td>FVL, PC,* PS*</td>
<td>FVL</td>
<td>CVA ×2</td>
<td></td>
</tr>
</tbody>
</table>

*Mismatched pathologies.
†These 2 children are brothers.

APLA indicates antiphospholipid antibodies (anticardiolipin Ab IgG and IgM, lupus anticoagulant dilute Russell viper venom test, B2glycoprotein 1 IgG and IgM); CVA, cerebrovascular accident; DVT, deep vein thrombosis; IUFD, intrauterine fetal demise; MTHFR, 677T methyltetrahydropholate reductase, only homozygotes were considered positive; PT, prothrombin mutation G20210A heterozygote; PC, Protein C deficiency; PS, Protein S deficiency; Mat. Hx., maternal history.
patients was 16.7% to 23.3%.35,36 Despite the fact that FVL was definitely a significant risk factor for pediatric and perinatal stroke in our study as well as in other Mediterranean population studies, its role in the pathogenesis of arterial thrombosis in the young remains to be elucidated.37

The contribution of APLA to the multifactorial nature of PAS is not yet well-defined. In our study, 8 mother–infant pairs had evidence of APLA, in either mother or child. More than one-fifth of our PAS infants tested positive for APLA, compared to 5.4% in controls. Again, this result is in agreement with the Israeli study of pediatric stroke patients in which 15.5% of patients were APLA-positive. These data were not available in the study by Curry et al.33

Protein C deficiency was more prevalent in PAS infants compared with controls (21.7% vs 1.8%, respectively), significantly increasing the risk for PAS. This was not observed in our previous pediatric stroke study in which protein C deficiency was found in 6.9% of older children with stroke. We had no data on protein C prevalence in the mothers control group; therefore, risk could not be estimated.

In our study 15 of 23 (65%) infants had some form of neurological impairment during follow-up. This high rate of neurological impairment is common. In other studies cerebral palsy developed in 68% of children with PAS,4 epilepsy was common among PAS patients presenting with seizures,10 and some neurological sequelae were documented in 38% to 58% of mother–child pairs study.33 We found no significant association between neurological outcome and thrombophilia, although infants with impaired neurological outcomes tended to test positive for thrombophilia more often when compared to the unimpared infants (67% vs 44%, respectively).

Thrombophilia was documented in more than two-thirds of the mothers who underwent thrombophilia screening. We found that maternal carriership of FVL or presence of APLA significantly increased the risk of PAS in their offspring. In our study 31.8% of mothers were heterozygous carriers of the FVL mutation compared with only 3.8% in a large cohort of nulliparous pregnant women17 and with 8.2% of the mothers in the study by Curry et al.33 We also found that infants of mothers carrying APLA had an increased risk for stroke. APLA was found in 18.2% of mothers compared with 4.7% of control mothers. A potential association of maternal APLA with fetal thrombophilias leading to an increased risk of PAS has already been raised by Nelson et al;1 however, studies of the outcome of infants born to mothers with APLA syndrome and of studies of children with primary APLA syndrome revealed very rare cases of perinatal stroke.39 The presence of APLA in neonates with PAS may therefore represent an acquired comorbid trigger, increasing the risk for stroke, especially when other thrombophilic risk factors coexist.40 To our knowledge our study is the first to report such significant associations.

The importance of demonstrating the presence of thrombophilia in cases of PAS extends further beyond the index pregnancy, because children with thrombophilia as well as their mothers, apart from carrying an increased risk for PAS, may be predisposed to higher risks of thrombosis or further pregnancy complications throughout their lives.

There are several possible therapeutic implications of thrombophilia testing are several, including the need for anticoagulation required after initial diagnosis, the length of anticoagulation, and potential prophylaxis required in subsequent life events such as surgery or prolonged immobilization. Moreover, for mothers who tested positive for thrombophilia, special additional therapeutic considerations include management during subsequent pregnancies or hormonal treatments (ie, oral contraceptives).

Our study has several limitations. We did not have complete thrombophilia information on all of our patients. Among 47 PAS patients in our registry for whom we have thrombophilia results, we had information on only 22 mothers. Nevertheless, we found no difference in the distribution of thrombophilia risk factors between PAS patients for whom maternal thrombophilia was available and those for whom it was not; therefore, we believe our results are valid to the group as a whole. Furthermore, selection of severe cases can be ruled out because the neurological outcome of the 23 infants tested is not different from that of previously published reports.

Another limitation of our study is the relatively small number of cases. This could potentially limit our ability to accurately estimate the magnitude of risk factors for PAS. Effort is currently being made to enlarge the group to strengthen our conclusions.

We have not evaluated the potential role of paternal thrombophilic on PAS risk. We found several cases of mismatch in mother and infant thrombophilias in our cohort. The presence of other genetic thrombophilic factors in the infant implies a possible contribution of paternal thrombophilia to perinatal ischemic thrombosis. A similar finding was reported in by Curry et al.33 Therefore, it would be prudent to investigate both parents for possible prothrombotic tendencies as part of the workup in cases of neonatal thrombosis.

In summary, we found that both maternal and infant thrombophilia may contribute to the higher prevalence of PAS. Both FVL and APLA have been identified as potential risk factors for PAS in the Israeli population of infants and mothers studied, as compared with controls.

We believe that thrombophilia testing should be performed on any child with PAS and that both parents should be tested as well. Positive tests may eventually lead to therapy-related considerations to reduce the risk of PAS in future pregnancies of FVL carriers. Large prospective studies of perinatal stroke are currently lacking and, according to our results, we believe this issue deserves further attention.

Disclosures

None.

References


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