Does thrombophilia play an aetiological role in Legg-Calvé-Perthes disease?

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**Heritable thrombophilic disorders have been proposed as one of the causes for Legg-Calvé-Perthes disease. A total of 62 patients diagnosed with this disease between 1988 and 1997 and 50 controls were screened for thrombophilia. The incidence and relationship of thrombophilia to the severity of the disease were evaluated.**

One patient and none of the controls had protein S deficiency. One of the control group and one of the patients had protein C deficiency with the latter child also having a combined deficiency with a mutant factor V gene.

The number of children with a mutant factor V gene, protein C deficiency, who were homozygous for the C 677T polymorphism of methylenetetrahydrofolate reductase or were heterozygous for mutant G20210A prothrombin did not differ statistically in the study and the control groups. No patient had antithrombin deficiency or positive lupus anticoagulant.

We found no correlation between thrombophilia and the extent of the disease. The most common risk factors for arteriovenous thromboembolism showed no statistical significance in our patients compared with the control group or with the general population. These data do not confirm an aetiological role for thrombophilia in Perthes’ disease.

The aetiology of Legg-Calvé-Perthes disease, or Perthes’ disease, a common disorder of the hip in children, is unclear. The most widely accepted theories involve repeated vascular interruption of the blood supply to the femoral head which results in venous occlusion with subsequent venous hypertension and bone death. Recent reports have suggested that heritable thrombophilic disorders may be one of the pathogenetic causes of Perthes’ disease.

Congenital thrombophilia, leading to an increased tendency to early thrombosis, may be caused by various disorders. Deficiencies of natural anticoagulants such as antithrombin III (AT III), protein C and protein S are rare in normal populations.

Homozygosity for a relatively common polymorphism in the methylenetetrahydrofolate reductase (MTHFR) gene is associated with high homocysteine levels and increased risk of thrombosis. Another mutation contributing to early thrombosis was recently discovered in the gene of prothrombin.

In 1994, Glueck et al tested eight children with Perthes’ disease and found that five of them had a thrombophilic disorder. This inspired another study in which 33 out of 44 patients (75%) with the disease were found to have low levels of protein C or S, a high level of lipoprotein a or hypofibrinogen. Glueck et al proceeded to check resistance to APCR in 64 children, finding a low APCR ratio in 23 (35.9%) of them, eight (12.5%) of whom had a mutant factor V gene. Other authors conducted similar studies in patients with Perthes’ disease; Gallistl et al checked the APCR in 40, Dilley et al compared 57 with a control group and Aruda et al analysed 61 patients and 41 controls. None of these investigators found evidence to
support the hypothesis that congenital thrombotic disorders are a risk factor for the development of Perthes’ disease.

Our aim was to evaluate the incidence of thrombophilia in our patients. We extended the tests carried out by previous authors by adding the evaluation of MTHFR mutant prothrombin and lupus anticoagulant. We also attempted to correlate the extent and severity of the disease with the presence of thrombophilia.

Patients and Methods

All patients with Perthes’ disease, diagnosed between 1988 and 1997, were considered suitable for the study. The diagnosis was made by clinical findings and radiological assessment. Details of the age of onset and the extent of the disease were noted. The severity of the disease was graded using the classification of Catterall. 23

A total of 50 consecutive children admitted for elective surgery served as a control group.

Of the 77 patients with Perthes’ disease treated by us, 62 were available for participation in our investigation. They included 52 boys and 10 girls whose mean age was 9.6 ± 3.6 years at the time of the study. The mean age at diagnosis was 6.0 ± 2.4 years. The control group included 27 boys and 23 girls with a mean age of 8.2 ± 4.6 years. The age difference between both groups was marginal (p = 0.05).

Four of the patients (6.45%) and three of the control group (6%) were of Arabic origin; the rest were Jewish.

A total of 23 patients (37%) had been diagnosed within the two years before the study period and 39 (63%) had had the disease for longer than two years.

An in-depth family history of early thromboembolic events was taken. The probands’ parents were questioned about the incidence of early myocardial infarcts, cerebrovascular accidents, DVT and pulmonary embolism in their families. Blood for coagulation assays was drawn after informed consent had been obtained.

Platelet-poor plasma was obtained from anticoagulated blood with 3.8% sodium citrate by centrifugation at 3000 g. The prothrombin, partial thromboplastin, fibrinogen and thrombin times were normal, and lupus anticoagulant was negative in all patients in both groups. We also attempted to correlate the extent and severity of the disease with the presence of thrombophilia.

Results

In the patients studied femoral involvement was on the left in 28, on the right in 26 and bilateral in eight. The Catterall classification showed that five patients had group-1 disease, 18 had group-2 and 39 had group-4. The family history was negative in the control group and no early thrombotic events were documented in our patients. One patient with Perthes’ disease had a grandfather who had had a myocardial infarction at the age of 58 years, and another a grandmother who had had a stroke at the age of 55 years. In both cases the parents of the probands, as well as the rest of their family, were healthy and showed no thrombotic tendencies. A complete hypercoagulability screening test, the same as for the study group, was carried out for both affected grandparents and the results were within normal limits.

In no patient was the basic coagulation screening profile test abnormal. The prothrombin, partial thromboplastin, fibrinogen and thrombin times were normal, and lupus anticoagulant was negative in all patients in both groups. AT III activity was within normal limits (Table I).

Table I. Results of thrombophilia analysis in 62 patients with Perthes’ disease compared with 50 control subjects

<table>
<thead>
<tr>
<th>Test</th>
<th>Patients with Perthes’ disease (n = 62)</th>
<th>Controls (n = 50)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Circulating anticoagulant</td>
<td>All negative</td>
<td>All negative</td>
<td>1.00</td>
</tr>
<tr>
<td>AT III</td>
<td>All normal</td>
<td>All normal</td>
<td>1.00</td>
</tr>
<tr>
<td>Protein C</td>
<td>1 (1.6)</td>
<td>1 (2)</td>
<td>0.69</td>
</tr>
<tr>
<td>Protein S</td>
<td>1 (1.6)</td>
<td>None</td>
<td>0.69</td>
</tr>
<tr>
<td>APCR</td>
<td>5 (8.1)</td>
<td>5 (10)</td>
<td>0.48</td>
</tr>
<tr>
<td>Factor V Leiden</td>
<td>3 (4.8) (1 homozygous)</td>
<td>5 (10)</td>
<td>0.25</td>
</tr>
<tr>
<td>Homozygous for MTHFR</td>
<td>8 (12.9)</td>
<td>6 (12)</td>
<td>0.88</td>
</tr>
<tr>
<td>Mutation of prothrombin</td>
<td>1 (1.6)</td>
<td>2 (4)</td>
<td>0.41</td>
</tr>
</tbody>
</table>

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One patient with Perthes’ disease had a low level of protein S free antigen (52%) compared with the normal levels of 65% to 130%. This patient’s mother had low free protein S levels of 54%. Protein S levels were normal in the control group (Table I).

Another patient with Perthes’ disease aged 17 years was deficient in protein C with an activity of 57%. In the control group one patient, aged 11 years, was deficient in protein C having 44% activity. The levels of protein C rise with age; the normal range for 11- to 16-year-old children is 55% to 111% while the normal adult levels are above 70% (60 to 123) (Table I).

The APC ratios were within normal limits in 45 of the 50 in the control group; the other five (of Arabic origin) had ratios ranging from 2.1 to 2.5. All were heterozygous for factor V Leiden. Five of 62 patients (8.1%) with Perthes’ disease had APC ratios ranging from 1.5 to 2.5. Of these, three (4.8%) had factor V Leiden, two being heterozygous and one, a boy of Arabic origin, was homozygous for the mutation correlating with the lowest APC ratio. Two patients showed low APC ratios (2.4 and 2.45) without factor V Leiden mutation (Table I).

Eight patients (12.9%) were homozygous for the polymorphism of thermolabile MTHFR compared with six (12%) of the control group. One patient (1.6%) was heterozygous for factor-II polymorphism in the patient group and two (4%) were heterozygous in the control group. There was no statistical significance for all the variables tested (Table I).

In the control group no combined coagulopathies were found. One patient with Perthes’ disease (Catterall group III) showed deficiency of protein C combined with heterozygosity for factor V Leiden.

There was no correlation between the extent of the disease and the number of patients who had positive findings (p = 0.431). One of five with group-2 disease was homozygous for factor V Leiden (20%). Of the 18 with group-3 disease, one was deficient in protein C, two were heterozygous for factor V Leiden and three were homozygous for MTHFR; a total of 33%. Of the 39 patients with group-4 disease, one was deficient in protein S, five were homozygous for MTHFR and one was heterozygous for G20210A, a total of 17.9% (Table II).

**Discussion**

The aetiology of Perthes’ disease is still unknown. Intra-vascular thrombosis has been suggested as a potential causative factor. Glueck et al.\(^5,6\) detected inherited thrombophilia in 75% of patients with the disease and claimed that thrombophilia may result in thrombotic venous occlusion of the femoral head leading to Perthes’ disease. In their later article, they suggested that activated protein C was a pathogenetic cause of the disease. They further stated that early initiation of anticoagulation therapy may retard or reverse the disease process. These findings, however, have not been supported by more recent studies.\(^20-22\)

The incidence of thromboembolic complications in children is rare, estimated at 0.07 per 10 000.\(^25\) While plasma concentrations of natural anticoagulants are low at birth, AT-III levels increase to adult values within three months. Free protein S levels are almost normal in infancy, compensating for low total protein S levels, whereas protein C activity remains significantly decreased throughout the first decade of life.\(^25,26\) The incidence of genetic mutations causing thrombophilia such as factor V Leiden, MTHFR and mutant prothrombin does not change throughout life.

Patients with congenital thrombophilia rarely present with their first thromboembolic complication until early adult life.\(^25\) If thrombosis does occur in children it is usually in those who have combined defects, such as factor V Leiden and one of the protein deficiencies.

The results of our study offered no support for a causative relationship between thrombophilia and Perthes’ disease. The family histories of patients with Perthes’ disease showed no abnormal tendency to thrombosis. The test results were negative in two cases suspected of thrombophilia.

In the study group of 62 patients, three (4.83%) had mutated factor V Leiden (APCR). One patient was homozygous and two were heterozygous, compared with 10% in our control group. The heterozygous prevalence of factor V trait is 5% to 8% in the general Caucasian population and is slightly higher in the Arab population, whereas it is 20% to 60% in various cohorts of patients with venous thrombosis.\(^27,28\) These data further emphasise that factor V Leiden has no pathognomonic role in Perthes’ disease. The fact that two patients demonstrated APCR without the mutation could imply acquired APCR or another type of mutation. Our group is too small to establish this finding.

A homozygous MTHFR gene was detected in eight (12.9%) out of 62 of our patients with Perthes’ disease compared with six (12%) of our 50 controls. Mutant factor II was detected in one patient (1.6%) compared with two (4%) of the control group. Of 336 healthy Jewish control subjects previously studied in our laboratory by Salomon et al.,\(^29\) 3.9% were heterozygous for factor V Leiden. Our results therefore do not differ statistically from these controls (p = 0.56). Heterozygosity of MTHFR was found in 14.3% of the same controls, and 5.4% of them were heterozygous for factor II polymorphism.

Factor V Leiden, mutant factor II and homozygous MTHFR have recently been described as the most common risk factors for thrombosis.\(^17,18,27\) Thus, according to our results, an inherited tendency to hypercoagulability seems to be an unlikely cause of Perthes’ disease.

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**Table II.** Thrombophilia in each Catterall group of Perthes’ disease

<table>
<thead>
<tr>
<th>Number of patients (%)</th>
<th>Catterall group</th>
<th>Factor V Leiden</th>
<th>MTHFR</th>
<th>Factor II</th>
<th>Protein S</th>
<th>Protein C</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 (20)</td>
<td>II</td>
<td>1 hom</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18 (33)</td>
<td>III</td>
<td>2</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>339 (17)</td>
<td>IV</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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One patient with the disease and one of the control group were found to be deficient in protein C. Hereditary protein C deficiency is inherited as an autosomal dominant trait. Normal levels of protein C activity are defined as 70% to 130% of a normal adult plasma pool. 26 Adult heterozygotes for protein C deficiency have protein C levels of activity of 30% to 70% of normal. The level of activity of protein C in children is low and rises with age. At one to five years of age the mean activity level is 66% (40 to 92), at six to ten years of age it is 69% (45 to 93) and at 11 to 16 years of age 83% (55 to 111).

Antithrombin deficiency, although rare, is found in 1% to 5% of patients with thrombosis. 30 No such deficiency was demonstrated in our patients or in the control group. Similarly, no patient had positive lupus anticoagulant, a relatively defined risk factor, especially for early arterial thromboembolism. 18

In our patients with Perthes' disease we found one patient with deficiency of protein S and one with a combined deficiency of protein C and factor V Leiden mutation. No protein S deficiency and no combined defects were found in the control group. Antithrombin, protein C and protein S deficiency are all rare conditions. 8 Since one patient with Perthes' disease had protein S deficiency and one had combined defects, it may be that inherited thrombophilic conditions are important in the pathogenesis of the disease in a small minority of children. This evidence cannot support the suggestion that thrombophilia is more widely responsible.

Thrombosis is rare in children between the age of one month and adolescence. When it does occur, it is usually in those with combined defects such as factor V Leiden mutation and one of the protein deficiencies. 32 This gives more relevance to our finding of combined defects in the study patients with Perthes' disease.

We could not find any correlation between the presence of thrombophilia and the severity of Perthes' disease. If thrombophilia did have a causative effect, we would expect a relatively higher incidence of thrombophilic patients in the more severely involved group (Table II). The patient with low protein C levels combined with heterozygosity for factor V Leiden was diagnosed with bilateral group-3 disease.

Our results differ from those obtained by Glueck et al. 5,6,19 This could be related to the selection or different ethnic background of the patients. In a similar population, however, the same risk factors which we analysed were related to the development of other vascular diseases. 29

Hereditary hypercoagulable disorders present a potential risk for early thromboembolism. The risk could be provoked by other conditions, resulting in thrombosis and requiring anticoagulant treatment. When considering the treatment of a thrombophilic patient, proof of thrombosis or a combination of other risk factors is mandatory. Our study has produced no evidence to support a role of thrombophilia in the pathogenesis and evolution of Perthes' disease.

No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

References
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