

# LEIDEN'S V-FACTOR IN LEGG-CALVÉ-PERTHES DISEASE

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## ABSTRACT

Inherited tendency to hypercoagulability has been suggested as a cause of vascular thrombosis resulting in Legg-Calvé-Perthes disease. An investigation of the most common inherited risk factor for hypercoagulability - the mutation in the V-factor gene (Leiden's V-factor) - was carried out among 20 Patients diagnosed with Legg-Calvé-Perthes disease. Patients were compared with 214 healthy controls. The prevalence of the Leiden's V-factor was higher in

patients with Legg-Calvé-Perthes disease than in controls (30% vs. 1,87%). The odds ratio for the development of Legg-Calvé-Perthes disease in the presence of the Leiden's V-factor mutation was 22,5 ( $p < 0,05$ ; confidence interval: 5,68-89,07). These data suggest the Leiden's V-factor as an inherited risk factor for hypercoagulability associated with the development of Legg-Calvé-Perthes disease.

**Keywords:** *Legg-Perthes disease. Thrombophilia. V-Factor.*

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## INTRODUCTION

Legg-Calvé-Perthes (LCP) disease, which is a hip dysfunction affecting children, is the result of a degeneration of the proximal femoral epiphysis due to a failure of the local blood flow (avascular necrosis). While its etiology remains unclear, inherited thrombophilia has been suggested as a potential cause of the disease.<sup>1,2</sup>

Thrombophilia can be defined as an increased trend to thromboembolic phenomena, either inherited or acquired. Once thrombosis is an uncommon event in children, the occurrence of thrombotic phenomena during childhood is frequently related to a genetic cause.<sup>3</sup> Mutations to prothrombin gene (G20210A), methylene-4-hydrofolate reductase (MTHFR) (C677T) and V factor (G1691A) are well-established inherited risk factors to thrombosis according to literature.<sup>4-6</sup>

The most common risk factor to arterial and venous thrombosis is the resistance to activated C protein resulting from a genetic mutation of V-factor.<sup>5,7-9</sup> The mutation to V-factor gene (Leiden's V-factor) leads to a hypercoagulability state due to the resistance to the anticoagulative action of the activated C protein.<sup>5</sup> Usually, the activated C protein degrades the activated V factor (Va), cleaving amino acids Arg306 and Arg506. Factor V molecules with the Leiden's mutation - in which Arg506 is replaced by glutamine (resultant from A being replaced by G at the 1691 position of the gene) - are resistant to degradation caused by C protein.<sup>5,10</sup>

The correlation between thrombophilia and Legg-Calvé-Perthes disease has been shown by several studies<sup>1,2,11-14</sup>, with some controversies.<sup>15-21</sup> This study was aimed to examine the association between Leiden's V factor as an inherited risk factor to hypercoagulability and the development of Legg-Calvé-Perthes disease.

## MATERIALS AND METHODS

### Patients

Fifty non-related patients diagnosed with LCP - based on clinical and X-ray criteria - recruited at the Clinical Orthopaedics Outpatient Facility of the Federal University of Ceará (UFC) were contacted, usually by mail (most of the patients live in the countryside of the state, far away from the capital city, where usually there is no telephone available). Of the fifty contacted patients, blood samples were collected from 20 patients who were able to get to the campus. The investigation protocol was conducted after the approval by UFC's Committee of Ethics and with informed consent terms signed by parents or caregivers of the children included in the study. Twenty patients diagnosed with LCP were assessed. The group included fifteen boys and five girls. The mean age at diagnosis was 6.55 years (standard deviation: 2.03; median: 6.5). The disease was unilateral in 90% of the cases, and surgery was performed in 10 (50%). The father of one child had an objective diagnosis of thrombosis.

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## Controls

In order to assess the prevalence of Leiden's V factor mutation in our population, a genetic analysis of 214 healthy individuals representing the local population with no previous history of thromboembolic events was provided (110 men, 104 women). The mean age was  $30.44 \pm 9.39$  years (median: 28 years). In our region, where the characteristic ethnical group is the 'mestiço', predominantly descending from indigenous and European people, and rarely descending from Africans.

## METHODS

High molecular weight DNA was withdrawn from peripheral blood leukocytes using the "salting out" method.<sup>22</sup> In order to assure quality on DNA extraction, electrophoresis with agarose stained with ethidium bromide was performed.

### Molecular diagnosis

Mutation Arg506Gln on V factor gene (Leiden's V factor) was identified by amplifying the fragment corresponding to exon 10 of the V factor gene using polymerase chain reaction (PCR) method with primers and conditions as described by Bertina et al.<sup>5</sup> Subsequently, 10 $\mu$ L of the PCR product was digested with 2.5U of *Mnl* I, an enzyme cleaving the normal allele in two sites, producing three fragments of 116bp (base pairs), 67bp and 37bp. Modified alleles lose one cleavage site, producing only two fragments of 153bp and 67bp after the referred enzyme's action. Heterozygous individuals originate both normal and mutant fragments. In each analysis, samples of modified patients, normal patients and distilled water were used as control. After enzymatic digestion, fragments were examined in 6% polyacrylamide gel, silver stain. In order to assure data legitimacy, positive results for mutation were reassessed.

### Statistical Analysis

The statistical analysis of the differences between groups was provided by the Fisher's exact test. The odds ratio was calculated to estimate the risk of developing Legg-Calvé-Perthes disease. Local healthy population served as control group.

## RESULTS

Leiden's V factor (mutation of the V factor gene) was more frequently found in patients with Legg-Calvé-Perthes disease (six out of twenty patients, 30%) as compared to control subjects (four out of 214, 1.87%). The odds ratio for developing the disease in the presence of Leiden's V factor compared to the absence of the mutation was 22.5 (confidence interval: 5.683-89.075;  $p < 0.05$ ; Fisher's Exact Test). Among modified patients, five presented the heterozygous pattern and one homozygous for the mutation.

## DISCUSSION

The most common risk factor for arterial and venous thrombosis is the resistance to activated C protein due to a mutation of V factor<sup>5,7-9</sup>, Leiden's V factor, which leads to a risk of developing venous thrombosis 2-7 times higher among heterozygous individuals and 20-80 times higher among homozygous subjects.<sup>23-28</sup>

While several studies suggest that Leiden's V factor is the potential causative factor for Legg-Calvé-Perthes disease<sup>1,2,12-14</sup> some studies do not show any correlation between this disease and thrombophilia.<sup>16-20</sup> The results reported by Hayek et al.<sup>21</sup> do not imply thrombophilia in the pathogenesis of Perthes disease (4.83% in patients, 10% in controls). Similarly, the study by Franco et al.<sup>15</sup> does not corroborate the association between Leiden's V factor and LCP (odds ratio: 1.36; 95% confidence interval: 0.32-5.84). The prevalence of Leiden's V factor among the patients assessed in this study (30%) is higher than the one previously described. Arruda et al.<sup>12</sup> reports mutation presence in 4.9% of the patients with LCP in the state of São Paulo, Brazil (0.7% in controls;  $p = 0.03$ ). Eldridge et al.<sup>1</sup> describe a prevalence of 9% among patients (5% in controls; odds ratio 1.8), while Glueck et al.<sup>13</sup> report a prevalence of 12.5% in patients and 1% in control group. The mutation prevalence found in our control population (1.87% vs. 0.7%) is similar to that described on the Brazilian study by Arruda et al.<sup>29</sup>

Of particular interest is the study by Balasa et al.<sup>11</sup>, comparing 72 patients with 197 healthy controls. V factor mutation was more commonly seen in patients (11%) than in controls (5%,  $p = 0.017$ ). The odds ratio for the development of the disease in the presence of mutation was 3.39, showing an association between Leiden's V factor and Perthes' disease.

In this study, 20 children with LCP were assessed. Mutation was found in six of twenty patients and in four of 214 controls (OR 22.5;  $p < 0.05$ ; Fisher's Exact Test). Although the small number of patients does not allow us to establish a prevalence of the Leiden's V factor among patients with the disease, these results unveil an association between mutation and LCP. As an illustration, if the fifty originally intended patients had been assessed and no other mutation had been identified in addition to the six mutations shown, an OR of 6.81 would have still indicated some correlation.

Another limitation that can be argued is the fact that no children were included in the control population. Once the prevalence of inherited thrombophilia such as Leiden's V factor does not change with time, we could estimate the odds ratio considering as a control group 214 healthy adult individuals with no previous history of thrombotic events, representing local population.

Finally, a study by Szepesi et al.<sup>14</sup> suggests that the V factor mutation negatively influences the course of the disease, especially in homozygous individuals. Of 47 patients diagnosed with Legg-Calvé-Perthes disease, four were homozygous and showed a more severe form of LCP. In the present study, among modified patients, only one showed the homozygous patterns. Therefore, conclusions concerning the association between homozygosity and disease severity cannot be inferred.

## CONCLUSION

Despite of the small number of patients, this study indicates that, in our population, Leiden's V factor may be associated to the development of Legg-Calvé-Perthes disease. Although these results cannot be regarded as the real risk, they point out to the importance of this factor in the pathogenesis of Perthes' disease in our region. Further studies are warranted to quantify this association.

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