Coexistence of C677T and A1298C mutations in the 5,10 methylene-tetrahydrofolate reductase enzyme in pediatric thrombotic patients

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Abstract
Background. One of the thrombophilic conditions that has been widely studied is the C677T mutation in the gene encoding the enzyme 5,10 methylene-tetrahydrofolate reductase (MTHFR). The presence of the A1298C mutation in the same gene is also considered as one factor that predisposes thrombosis.

Methods. Nine pediatric patients diagnosed with thrombophilia were studied: seven males and two females with an age range from 1 month to 13 years. We performed real-time polymerase chain reaction (RT-PCR), study of the C677T and A1298C mutations in the MTHFR enzyme, G1691A mutation (Leiden) and Factor V, and prothrombin mutation G20210A. Using conventional methods the following analyses were made: activated C-reactive protein (CRPa) and protein C and S coagulation, as well as antithrombin (AT).

Results. All patients had co-existing mutations of C677T and A1298C in the MTHFR. Only one patient was homozygous for C677T and heterozygous for A1298C. The other eight patients presented heterozygous mutations. The nine patients did not demonstrate the presence of mutations G1691A factor V (Leiden) and G20210A prothrombin or alterations in the CRPa, AT and proteins C and S.

Conclusions. Coexistence of the C677T and A1298C mutations should be considered for investigation in all patients presenting with thrombophilia.

Key words: thrombosis, methylene-tetrahydrofolate reductase, Factor V Leiden

Introduction
The incidence of thrombotic events is rare in children but appears to be increasing. The Canadian Commission on Pediatrics estimates that there are 0.67 cases/100,000 children per year.1 The cause of the thrombotic events has been attributed to a combination of several risk factors (infection, central venous line or chemotherapy). International guidelines recommend screening for risk factors such as those acquired and hereditary as an important part in managing thrombosis.2 Multiple investigations in our country have contributed to the identification of predisposing factors for thrombosis. In most cases studied, it has been identified that there is presence of multiple thrombophilic conditions when a vaso-occlusive episode occurs. These findings have also been reported by several researchers in other countries.2-10
One of the thrombophilic conditions that has been extensively studied is the enzymatic activity of 5,10 methylenetetrahydrofolate reductase (MTHFR), which is involved in the metabolism of homocysteine in which the presence of two mutations, C677T and A1298C, have been identified that cause a decrease in its enzymatic activity.11-16

In the Caucasian population, the incidence of the C677T mutation in MTHFR is ~40% for heterozygotes and 10% for homozygotes.14 There are reports that thrombophilic Mexican patients who are carriers of hetero- or homozygous states for the C677T mutation in MTHFR do not express an increase in plasma homocysteine. This does not rule out the involvement of the C677T mutation in MTHFR in the pathogenesis of thrombosis.16

The presence of the A1298C mutation in the MTHFR enzyme is also considered as a predisposing factor for thrombosis, and there are no reports in the Mexican literature on the investigation of this mutation in patients with thrombophilia.14 Heterozygous coexistence of C677T/A1298C mutations in MTHFR has been described in some studies;14-15 however, there are no reports of the coexistence of these mutations in pediatric patients with thrombosis. Therefore, the objective of this work is to present nine pediatric patients diagnosed with thrombosis and, after performing various tests, to identify the most common markers of thrombophilia. Coexistence of mutations C677T and A1298C in the MTHFR gene was evidenced as the only associated genetic factor.

Materials and Methods
We described nine pediatric patients with diagnosis of thrombophilia. There were seven males and two females with an age range from 1 month to 13 years. Patients were selected by the medical staff of the Hematology Service of Hospital Infantil de Mexico Federico Gómez in Mexico City.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age</th>
<th>Base disease</th>
<th>Location of the thrombosis</th>
<th>Diagnostic method</th>
<th>MTHFR C677T</th>
<th>MTHFR A1298C</th>
<th>FV G1691A</th>
<th>PT G20210A</th>
<th>CRPa</th>
<th>PC</th>
<th>PS</th>
<th>AT</th>
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<tbody>
<tr>
<td>M</td>
<td>11 years</td>
<td>None</td>
<td>Portal vein thrombosis</td>
<td>Clinical</td>
<td>CT</td>
<td>AC</td>
<td>GG</td>
<td>GG</td>
<td></td>
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<td>M</td>
<td>1 month</td>
<td>Dimorphic syndrome</td>
<td>Atrial thrombosis, PTE</td>
<td>ECG</td>
<td>CT</td>
<td>AC</td>
<td>GG</td>
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<tr>
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<td>US</td>
<td>CT</td>
<td>AC</td>
<td>GG</td>
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<tr>
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<td>None</td>
<td>Portal vein thrombosis</td>
<td>US</td>
<td>CT</td>
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<tr>
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<td>CVA</td>
<td>MRI</td>
<td>CT</td>
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<td>Right atrial thrombosis</td>
<td>MRI</td>
<td>TT</td>
<td>AC</td>
<td>GG</td>
<td>GG</td>
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<tr>
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<td>CT</td>
<td>AC</td>
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<tr>
<td>M</td>
<td>12 years</td>
<td>Chronic pneumopathy &amp; pulmonary hyperplasia</td>
<td>CVA</td>
<td>MRI</td>
<td>CT</td>
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<tr>
<td>F</td>
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F, female; M, male; CRPa, activated C-reactive protein; FV G1691A, factor V Leiden mutation; PT G20210A, prothrombin mutation G20210A; MTHFR C677T, mutation C677T in methylenetetrahydrofolate reductase, MTHFR A1298C, mutation A1298C in methylenetetrahydrofolate reductase, PC, protein C deficiency; PS, protein S deficiency; AT-, antithrombin deficiency; PTE, pulmonary thromboembolism, CVA, cerebrovascular accident, AVM, arteriovenous malformation; EC, electrocardiogram; MRI, magnetic resonance imaging; US, ultrasound.
Prior permission was obtained from all parents or guardians of the patients. A 3- to 5-ml sample of peripheral blood anticoagulated with EDTA was taken for DNA analysis. The extraction was performed with reagents and equipment from Roche Diagnostics (MagNA Pure Compact) according to the manufacturer’s recommendations. Investigation of the C677T and A1298C mutations in the gene coding for the MTHFR enzyme, G1691A in the gene coding for factor V (Leiden) and G20210A in the prothrombin gene were performed by real-time polymerase chain reaction (RT-PCR) using the LightCycler 2.0 (Roche Diagnostics). Determination was performed of the resistance to C-reactive protein (CRPα), coagulation activity of proteins C and S, and antithrombin (AT), for which a sample of peripheral blood anticoagulated with sodium citrate (3.8%) was obtained. Commercial reagents used were from Dade Behring (Sysmex CA-1500, Marburg GmbH) following the manufacturer’s instructions.

Results

All patients had coexistence of mutations C677T and A1298C in the MTHFR enzyme. Only one patient was homozygous for C677T and heterozygous for A1298C; the remaining eight patients were heterozygous for both mutations. The presence of mutations of factor V G1691A (Leiden) and prothrombin G20210A were not identified in the nine patients and, similarly, they showed no alterations in CRPα, AT and coagulation of proteins C and S (Table 1).

In conclusion, in this study we focused on the description of the coexistence of C677T and A1298C mutations in the gene coding for MTHFR. We believe that these mutations should be investigated in all patients presenting thrombophilia and for which no predisposing factors, whether inherited or acquired, are identified. Diverse thrombophilic conditions have been identified in the described thrombophilic Mexican patients. Much research has been carried out and it has been postulated that thrombotic events have a genetic predisposition and that, depending on the number of mutations or alterations, will determine the severity of vaso-occlusive events.4-10

In Mexico, Ruiz-Argüelles et al. have made various contributions that help to understand hemostatic alterations in patients with thrombophilia.4-10 In one study with a series of 100 patients, they demonstrated that 94% of the patients studied had at least a marker of thrombophilia and 81% of the patients had two or more alterations.10 Based on various conclusions, it is recommended that the study of the greatest number of mutations or polymorphisms associated with these events be performed on all thrombophilic patients.

The risk associated with each isolated genetic defect may be relatively low, but the simultaneous presence of several mutations may dramatically increase the susceptibility to disease.18 Furthermore, both lifestyle and environmental factors may interact with one or more genetic mutations and increase the susceptibility and severity of the event. The analysis of risk factors such as acquired genetic factors combined with environmental factors has contributed significantly to the understanding of this pathology.

References