Prevalence of Three Prothrombotic Polymorphisms: Factor V G1691A, Factor II G20210A and Methylene tetrahydrofolate Reductase (MTHFR) C 677T in Argentina

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Each year, venous thromboembolism (VTE) affects 50–150/100,000 individuals in the global population [1]. Acquired (surgery, pregnancy, contraceptives, cancer, antiphospholipid antibodies, immobilization, etc.), as well as hereditary, factors interact to promote the development of deep vein thrombosis and pulmonary embolism. Hereditary factors that induce VTE development can be classified as classical (e.g., ATIII, Protein C and Protein S deficiencies or dysfunctions) and polymorphisms. In recent years, Factor V Leiden, MTHFR CT 677, and Prothrombin 20,210 mutations [2] were found to be responsible for producing Activated Protein C Resistance (APCR), hyperhomocysteinemia and increasing levels of Factor II, respectively. Some acquired conditions may have similar effects. For instance, the presence of antiphospholipid antibodies, pregnancy or oral contraceptives (OC) may induce APCR [3]; folic acid deficiency or renal insufficiency can increase homocysteine levels; and OC, and presumably other yet unknown mutations, might increase plasmatic levels of prothrombin. Thus, genetic analyses are the better tool to detect hereditary thrombophilic cases in general population studies.

Factor V Leiden, which consists of a point mutation in factor V gene (nucleotide 1691 G to A substitution), had so far emerged as the most frequent genetic condition associated with VTE, with a frequency of 18% in clinical series of VTE (11.5–37.0%) [4–8]. Heterozygous individuals have a 5–10 times higher risk of VTE during their lives compared to wild type population; this risk increases 10 times in homozygous subjects compared to heterozygous.

A G→A variant in Factor II (Prothrombin 20,210) has been associated with high plasma levels of this coagulation factor, and with a higher risk of venous and arterial thrombosis. This characteristic has been reported in 18% of VTE patients with a positive familial clinical history of the disease and in 6.2% of consecutive patients with a first deep vein thrombosis. The risk of VTE attributed to the mutation is 2.8 times higher when compared to the wild type [9–11].

Abbreviations: MTHFR, methylenetetrahydrofolate reductase; VTE, venous thromboembolism; AT III, Antithrombine III; APC R, Activated Protein C Resistance; OC, oral contraceptives.

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Since mutation of methylenetetrahydrofolate reductase at nucleotide 677 (C to T) results in a thermolabile variant with reduced enzymatic activity, homocysteine levels may increase, particularly in conjunction with low plasma folate levels (<15.4 nmol/L). Although generally considered a mild risk (2.5 times) for VTE, there are still some controversial data about its contribution as an isolated risk factor or as an element that enhances other thrombogenic conditions [12–15].

A previous study carried out in Argentina showed 1.6% prevalence of Factor V Leiden [16], but this survey included data mainly from Buenos Aires, the capital city. Thus, it does not actually represent the whole country, which might show many ethnic differences. We are not aware of any previous studies carried out in our country on the prevalence of Prothrombin 20,210 and MTHFR.

1. Materials and Methods

1.1. Patients

Samples from 418 healthy unrelated blood donors (21–65 years old) from all the different regions of our country, and devoid of previous hemorrhagic or thrombotic disorders, were analyzed. The number of samples from each place was calculated proportionally to the population of the region, with slight overrepresentation of the Patagonian and northern provinces in order to study a relative large proportion of Amerindians.

1.2. DNA Extraction and Mutations Detection

DNA extraction was performed as originally described [17] from an entire blood sample kept at −20°C. To identify Factor V Leiden, a 267-bp DNA fragment of factor V gene that included nucleotide 1691 was amplified by PCR technique and digested with Mnl I as previously described [18]. To identify G→A mutation of the prothrombin gene, a 345-bp fragment was obtained and then digested using Hind III endonuclease as reported [9]. Screening for the MTHFR C→T677 substitution was performed by amplification of a 198-bp DNA fragment and followed by Hinf I digestion, as originally described [19].

1.3. Statistical Analysis

Differences in prevalence between provinces were tested for heterogeneity by the chi-square test.

2. Results

From a total of 418 samples taken throughout the country, heterozygosity for Factor V Leiden was detected in 12 (2.9%), and heterozygosity for Prothrombin 20,210 in 11 (2.6%). No homozygous individuals were found for these two mutations. In the case of MTHFR, on the other hand, 65 subjects were mutant homozygous (15.8%) and 180 were heterozygous (42.8%) (Table 1). No distribution differences were observed in the provinces sampled, perhaps due to the low prevalence found for Factor V Leiden and Prothrombin 20,210 (p=0.54 for both).

Volunteers were asked about the nationality of their ancestors, especially their grandparents, in order to establish the main source of migration to the country. However, no relationship between this data and Factor V Leiden, Prothrombin 20,210 mutation or MTHFR T 677 was found (data not shown).

This study also gave us the opportunity to detect asymptomatic combined mutations in the general population. Thus, three cases of Factor V Leiden with MTHFR T 677, and two cases of Prothrombin 20,210 with MTHFR T 677 were observed. These results represented 1.2% of the general population. No double heterozygosity for Factor V Leiden and Prothrombin 20,210 was discovered.

3. Discussion

Considerable variations of the prevalence of Factor V Leiden have been found in different surveys carried out in many countries throughout the world. In Argentina, there is virtually no black population, with a Caucasian migration that came mainly from Spain and Italy and secondarily from Northern and Eastern Europe. A small number of Asian immigrants arrived during this century. As a result of this distribution, we expected to find similar values of Factor V prevalence to those reported for Southern Europe [20–21] (Italy: 2.5%
Table 1. Distribution of three gene alterations in the Argentine population

<table>
<thead>
<tr>
<th>Province/City</th>
<th>Factor V G1691A</th>
<th>Prothrombin G20210A</th>
<th>MTHFR C677T</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>G/G</td>
<td>G/A</td>
<td>A/A</td>
</tr>
<tr>
<td>Buenos Aires (n=47)</td>
<td>45</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Capital Federal (n=72)</td>
<td>70</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Córdoba (n=40)</td>
<td>39</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Corrientes (n=21)</td>
<td>20</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Chaco (n=20)</td>
<td>19</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Chubut (n=34)</td>
<td>33</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Formosa (n=19)</td>
<td>18</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Mendoza (n=47)</td>
<td>45</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Misiones (n=20)</td>
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<td>1</td>
<td>1</td>
</tr>
<tr>
<td>San Juan (n=20)</td>
<td>20</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Santa Fé (n=38)</td>
<td>38</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Tierra del Fuego (n=20)</td>
<td>19</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Tucumán (n=20)</td>
<td>20</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Totals (n=418)</td>
<td>406</td>
<td>12</td>
<td>12</td>
</tr>
</tbody>
</table>

of 4676 and Spain 3.7% of 132), but with some possible variations between provinces. However, this has not been observed in our survey.

In Spain, Prothrombin 20,210 is reported to be the most prevalent genetic risk factor for VTE, with 6.5% of carriers in the general population. Factor V Leiden prevalence in Argentina (2.9%) is close to the value expected, considering that our main sources of immigration were Caucasians from Southern Europe. Prothrombin 20,210 prevalence (2.6%) is quite comparable to Factor V Leiden. Homozygous MTHFR T 677 was found in a relatively high percentage of the Argentine population (15.8%). Even higher values have been reported in Colombia, another South American country (25.3%) [22]. Regional variations of the genetic background should be considered in order to obtain useful information about the relative weight of hereditary and environmental factors for VTE in each country. This could be useful for making public health decisions such as folic acid intake recommendations.

The detection of a joint concurrence of two prothrombotic risk factors in 1.2% of the asymptomatic population agrees with reports on control groups of cohorts of patients with VTE (0.9%) [23], emphasizing the importance of acquired as well as other yet unknown genetic factors for the development of VTE.

4. Summary

Screening in selected but still wide populations such as women with recurrent fetal loss, placental insufficiency, pre-eclampsia, or needing oral contraception, or programmed high-risk surgery in both sexes, is still controversial. Knowledge of the prevalence of these polymorphisms in the general population is useful in order to take decisions that could considerably increase the burden to health resources, even in developed countries.

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