The haplotype M2 within the ANXA5 gene is independently associated with the occurrence of deep venous thrombosis

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Dear Sir,

Despite increased awareness of the risk factors (1, 2) of venous thromboembolism (VTE), it remains the third most common cardiovascular affliction after ischaemic heart disease and stroke (3, 4).

Annexin V (ANXA5), the most abundant member in the family of annexins, is found in many tissues and in the bloodstream, and plays a potent antithrombotic role (5). A common haplotype (M2) within the ANXA5 gene has been demonstrated to diminish the ANXA5 promoter activity and to be a risk factor for obstetric complications (6, 7).

In a cohort of patients with documented deep venous thrombosis (DVT) occurring in a leg, we investigated whether the presence of the M2 haplotype is associated with the occurrence of VTE.

Between January 2004 and December 2008, we investigated 231 not anticoagulated patients (median age: 69 years; range: 17–82), 98 men and 133 women, with a documented episode of DVT in a leg, who were referred at least three months after the thrombotic episode for a work-up to the Thrombosis Centre at the IRCCS „Casa Sollievo della Sofferenza”, San Giovanni Rotondo (FG), Italy. The median age (range) at the time of the first thrombotic episode was 39 years (18–78).

A complete clinical summary with emphasis on personal and family history for thromboembolic disease was obtained; pulmonary embolism (PE) was diagnosed by helical computed tomography (CT), angiography, or high probability ventilation-perfusion lung scan, as required during the routine diagnostic work-up. DVT were defined as idiopathic when none of the circumsstantial vascular risk factors (surgery, immobilisation, pregnancy, trauma) was recorded. All others were classified as provoked. Patients with malignancy or varicose veins were not included. Overall, 36 (15.6%) individuals (19 men, 17 women) also experienced an objectively confirmed episode of PE. A recurrence of DVT was observed in 43 (18.6%, 95% confidence interval (CI): 14.1–24.2) individuals (21 men and 22 women). A reported recurrent VTE was accepted as such if it had been confirmed by objective diagnostic testing, i.e. ultrasonography showing a newly non-compressible venous segment or perfusion lung scan revealing defects in previously perfused areas or in the original area if the initial episode was previously demonstrated to be resolved in case of PE.

Three hundred-eighty-two apparently healthy subjects (187 men and 195 women; median age 34 years, range 18–66) randomly selected from a Southern Italian general population without a history of VTE were referred at least three months after the documented episode of DVT in a leg, who were investigated whether the presence of the M2 haplotype is associated with the occurrence of VTE.

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Three hundred-eighty-two apparently healthy subjects (187 men and 195 women; median age 34 years, range 18–66) randomly selected from a Southern Italian general population without a history of VTE served as controls. Both cases and controls were Caucasian and from the same region. The two groups were comparable for sex and social status.

After approval of the local Ethics Committee, the study was carried out according to the Principles of the Declaration of Helsinki; informed consent was obtained from all the subjects.

Platelet-poor plasma was obtained by blood samples collected in 3.8% trisodium citrate and centrifuged at 2,000 g for 15 minutes. Plasma was frozen and stored in small aliquots at −70°C until tested. Antiphospholipid antibodies, antithrombin, protein C, and total and free protein S antigen were determined in all patients (8).

DNA was extracted from peripheral blood leukocytes according to standard protocols and factor (F) V Leiden, FIIA20210 mutations and the M2 haplotype were investigated (6, 8, 9).

All the analyses were performed using SPSS version 11.0 (SPSS Inc. Chicago, IL, USA). Between groups, differences in means were evaluated by the Mann-Whitney U-test, after grouping homozygous and heterozygous carriers of the FV Leiden mutation and homozygous and heterozygous carriers of the FIIA20210 allele, whereas differences in frequencies were calculated using chi-squared statistics. The observed numbers of ANXA5 haplotypes were compared with those expected for a population in Hardy-Weinberg equilibrium using a chi-square test. Adjusted odds ratios (OR) and 95% CI were calculated by logistic regression models that controlled for potential confounding variables (age at VTE, sex, FV Leiden and FIIA20210 mutations, and the presence of the M2 haplotype).

Clinical features and prevalences of thrombophilic risk factors in patients and in controls are shown in ►Table 1. A significant different median age was observed between cases (39.0 years, range 21–82) and controls (34.0 years, range 17–66; Mann-Whitney U test p <0.001). Among patients enrolled, 133 (57.6%) were women, 51 (22.1%) had an history of an idiopathic DVT.

As shown in Table 1, 18 (7.8%, 95% CI: 4.9–12.1) patients showed a persistent LAC positivity, and three (1 also with LAC positivity) an aCL titre > 14 GPL (1.3%, 95% CI: 0.03–3.9). Two cases (0.9%, 95% CI: 0.00–3.3) showed a protein S deficiency, whereas none had protein C or antithrombin deficiency. In two controls and one case, genetic data were not available for technical reasons.

The observed distribution of ANXA5 genotypes showed no significant difference when compared to that predicted from the
Hardy-Weinberg equilibrium (Chi-square test, p: >0.1).

As a whole, 62 (2 homozygotes) patients (27.0%) and 63 (all heterozygotes) controls (16.6%) carried the M2 haplotype (OR: 1.9, 95% CI: 1.24–2.76).

The independent nature of the contribution of the M2 haplotype to the occurrence of DVT was assessed in a multiple logistic regression model in which, in addition to the M2 haplotype, a series of relevant covariates (age when the thrombotic event occurred, sex, FV Leiden and FII A20210 mutations) were included. The analysis showed a significant increase (OR: 2.2; 95% CI: 1.3–3.7) of the occurrence of DVT in carriers of the M2 haplotype as compared to non-carriers. Carriership of the FV Leiden or FII A20210 allele was independently related to the occurrence of DVT (OR: 4.1, 95% CI: 1.9–8.8 and OR: 3.7, 95% CI: 1.8–7.6, respectively). Among patients with idiopathic DVT (n=51), 24 (47.1%, 95% CI: 34.1–60.5) carried the M2 haplotype. Of them, four and two patients showed the co-segregation of the M2 haplotype and of the FV Leiden or the FIIA20210 gene variant, respectively. Carriership of the M2 haplotype was significantly associated with a history of idiopathic DVT (OR: 4.5, 95% CI: 2.4–8.3).

In the present report, haplotype and genotype M2 frequencies in controls were comparable to those reported in the German PopGen controls (6).

Present data show that the M2 haplotype is significantly and independently associated with the occurrence of DVT.

Table 1: Clinical characteristics and thrombophilic risk factors in controls and patients investigated.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Controls N=382</th>
<th>Patients N=231</th>
</tr>
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<tbody>
<tr>
<td>Age at enrolment median (range)</td>
<td>34 (18–66)</td>
<td>43 (18–82)</td>
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<tr>
<td>Age at time of DVT median (range)</td>
<td>---------------</td>
<td>---------------</td>
</tr>
<tr>
<td>Men n (%)</td>
<td>187 (49)</td>
<td>98 (42.4)</td>
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<tr>
<td>Circumstantial DVT n (%)</td>
<td>---------------</td>
<td>---------------</td>
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<tr>
<td>Idiopathic n (%)</td>
<td>---------------</td>
<td>---------------</td>
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<tr>
<td>Pulmonary embolism n (%)</td>
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<tr>
<td>Recurrence n (%)</td>
<td>---------------</td>
<td>---------------</td>
</tr>
<tr>
<td>FV Leiden n (%)*</td>
<td>14 (3.7)</td>
<td>32 (13.9)</td>
</tr>
<tr>
<td>FII A20210 allele n (%)*</td>
<td>16 (4.2)</td>
<td>33 (14.4)</td>
</tr>
<tr>
<td>Protein S deficiency n (%)</td>
<td>n.a.</td>
<td>2 (0.9)</td>
</tr>
<tr>
<td>LAC positivity n (%)</td>
<td>n.a.</td>
<td>18 (7.8)</td>
</tr>
<tr>
<td>IgG aCL &gt;14 GPL n (%)</td>
<td>n.a.</td>
<td>3 (1.3)</td>
</tr>
<tr>
<td>ANXA5 M2 haplotype n (%)°</td>
<td>63 (16.6)</td>
<td>62 (27.0)</td>
</tr>
</tbody>
</table>

DVT, deep enous thrombosis; LAC, lupus anticoagulant; aCL, anticardiolipin antibodies. No antithrombin or protein C deficiency was recorded. ° In two controls and one patient no genetic data were available. °° 2 homozygotes. ▲ Among them, one carried the M2 haplotype. Neither of patients with protein S deficiency carried the M2 haplotype. ▲ Eight (7 women) cases (3.5%) and two (0.5%) controls carried the FV Leiden and the M2 haplotype, nine (7 women, 1 homozygote) cases (4.0%) and no controls carried the FIIA20210 and the M2 haplotype.

References