RESEARCH LETTER

Updated Analysis of Gene Variants Associated With Deep Vein Thrombosis

To the Editor: A single-nucleotide polymorphism (SNP) association study assessing variants associated with deep vein thrombosis found that, of 19,682 gene-centric SNPs, 18 were consistently associated with deep vein thrombosis in the Leiden Thrombophilia Study (LETS) and in a subset of the Multiple Environmental and Genetic Assessment of Risk Factors for Venous Thrombosis study (MEGA-1). Nine of these SNPs were genotyped in the remaining part of the MEGA study (MEGA-2). Assays for the other 9 SNPs in MEGA-2 were not available at that time; the associations of these 9 SNPs with venous thrombosis in 1314 cases and 2877 controls of MEGA-2 were subsequently assessed.

Methods. Details of the study population, DNA collection, SNP selection, and genotyping were described previously.1 Chromosome 1 associations were stratified by factor V Leiden (rs6025, chr 1:167,785,673) to avoid reporting false-positive associations due to linkage disequilibrium. This analysis was previously performed for rs2227589 (SERPINC1 [NCBI Entrez Gene 462]) and rs670659 (RG57 [NCBI Entrez Gene 6000]), which appeared to be independently associated.1 The SNPs rs6016 (chr 1:167,778,744), rs4524 (chr 1:167,778,379), rs3820059 (chr 1:167,657,778), and rs6131 (chr 1:167,847,509) were among the 9 SNPs pending genotyping in MEGA-2. The rs6016 SNP (F5 [NCBI Entrez Gene 2153]) was in linkage disequilibrium with rs4524 (F5, D’ = 0.99 and r2 = 0.97 in LETS and MEGA-1), which has been reported to be associated with venous thrombosis.2 The SNP rs3820059 (C1orf114 [NCBI Entrez Gene 57821]) was in linkage disequilibrium with factor V Leiden (D’ = 0.91 and r2 = 0.09) and not associated with venous thrombosis after adjustment for factor V Leiden in LETS and MEGA-1. The SNP rs6131 (SELP [NCBI Entrez Gene 6403]) was also not associated with venous thrombosis after adjustment for factor V Leiden, although linkage disequilibrium was not strong (D’ = 0.34 and r2 = 0.03).

Six additional SNPs in MEGA-2 were genotyped: rs4524 (F5), rs1801690 (APOH [NCBI Entrez Gene 350]), rs3087546 (EPS8L2 [NCBI Entrez Gene 64787]), rs369328 (CASPA8P2 [NCBI Entrez Gene 9994]), rs881 (TACR1 [NCBI Entrez Gene 6869]), and rs2266911 (ODZ1 [NCBI Entrez Gene 10178]). The false discovery rates for the first 9 SNPs in MEGA-2 were previously reported.1 For the present analysis, the false discovery rate was manually recalculated for all 15 SNPs. Men and women were combined in all analyses including rs6048 (F9 [NCBI Entrez Gene 2158]) because the association with deep vein thrombosis in MEGA-2 was similar in men and women.3 Odds ratios, 95% confidence intervals, and P values were calculated by logistic regression using SPSS for Windows release 16.0.2 (SPSS Inc, Chicago, Illinois).

Results. The associations between SNPs and deep vein thrombosis in MEGA-2 are presented in the Table. Of 6 newly genotyped SNPs, only rs4524 in F5 replicated in MEGA-2. The other SNPs had odds ratios close to 1 and corresponding P values > .20. After adjustment for factor V Leiden, rs4524 remained associated with venous thrombosis (odds ratio, 1.21; 95% confidence interval, 1.10-1.34). Linkage disequilibrium between factor V Leiden and rs4524 was low (r2 = 0.02 in MEGA-2).

The previous study1 identified 7 SNPs with a false discovery rate of less than 20%; 3 had P < .05 and a false discovery rate of less than 10% (rs13146272 in CYP4V2 [NCBI Entrez Gene 283440], rs2227589 in SERPINC1, and rs1613662 in GP6 [NCBI Entrez Gene 51206]). After recalculating the false discovery rate for 15 SNPs, 7 SNPs (including rs4524) had a false discovery rate of less than 20% (Table).

Comment. The rs4524 SNP in F5 was consistently associated with deep vein thrombosis in 3 large case-control studies. Five SNPs in APOH, EPS8L2, CASPA8P2, TACR1, and ODZ1 were associated in 2 case-control studies but failed to replicate in the third study. The associations of 3 SNPs in F5, C1orf114, and SELP were due to linkage disequilibrium with other SNPs.

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Table. Associations of SNPs with Deep Vein Thrombosis in MEGA-2\(^a\)

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP</th>
<th>Risk Allele (b)</th>
<th>Alleles, No. (%)</th>
<th>Previous SNP Analysis(^d)</th>
<th>Updated SNP Analysis(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Case</td>
<td>Control</td>
</tr>
<tr>
<td>F9(^c)</td>
<td>rs4524</td>
<td>T</td>
<td>1961 (79)</td>
<td>4037 (73)</td>
<td>1.33 (1.19-1.48)</td>
</tr>
<tr>
<td>CYP4V2(^d)</td>
<td>rs13146272</td>
<td>A</td>
<td>1600 (69)</td>
<td>3366 (64)</td>
<td>1.24 (1.11-1.37)</td>
</tr>
<tr>
<td>SERPING1(^d)</td>
<td>rs2227589</td>
<td>T</td>
<td>308 (12)</td>
<td>539 (19)</td>
<td>1.29 (1.11-1.49)</td>
</tr>
<tr>
<td>GP6(^d)</td>
<td>rs1613662</td>
<td>A</td>
<td>2185 (84)</td>
<td>4683 (82)</td>
<td>1.15 (1.01-1.30)</td>
</tr>
<tr>
<td>RGS7(^d)</td>
<td>rs670659</td>
<td>C</td>
<td>1711 (66)</td>
<td>3632 (64)</td>
<td>1.10 (1.00-1.22)</td>
</tr>
<tr>
<td>NRR1(^d)</td>
<td>rs1523127</td>
<td>C</td>
<td>1038 (40)</td>
<td>2158 (38)</td>
<td>1.09 (0.99-1.20)</td>
</tr>
<tr>
<td>F9(^d)</td>
<td>rs6048</td>
<td>A</td>
<td>1872 (72)</td>
<td>3985 (70)</td>
<td>1.16 (0.98-1.37)</td>
</tr>
<tr>
<td>NART6(^d)</td>
<td>rs2001490</td>
<td>C</td>
<td>1013 (39)</td>
<td>2122 (37)</td>
<td>1.08 (0.98-1.19)</td>
</tr>
<tr>
<td>APOH(^f)</td>
<td>rs1801690</td>
<td>C</td>
<td>2389 (95)</td>
<td>5217 (96)</td>
<td>1.15 (0.92-1.44)</td>
</tr>
<tr>
<td>EPSS8L2(^c)</td>
<td>rs3087546</td>
<td>T</td>
<td>1336 (54)</td>
<td>3027 (65)</td>
<td>1.04 (0.95-1.14)</td>
</tr>
<tr>
<td>CASP8AP2(^c)</td>
<td>rs369328</td>
<td>A</td>
<td>1133 (47)</td>
<td>2566 (48)</td>
<td>1.04 (0.94-1.14)</td>
</tr>
<tr>
<td>ZNF544(^d)</td>
<td>rs6510130</td>
<td>G</td>
<td>60 (2)</td>
<td>145 (3)</td>
<td>1.09 (0.81-1.48)</td>
</tr>
<tr>
<td>TACR1(^c)</td>
<td>rs881</td>
<td>C</td>
<td>2099 (84)</td>
<td>4621 (83)</td>
<td>1.04 (0.91-1.18)</td>
</tr>
<tr>
<td>CD2(^c)</td>
<td>rs2266911</td>
<td>C</td>
<td>2032 (81)</td>
<td>4455 (81)</td>
<td>1.06 (0.86-1.29)</td>
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<tr>
<td>MET(^d)</td>
<td>rs2377712</td>
<td>G</td>
<td>92 (4)</td>
<td>190 (3)</td>
<td>1.03 (0.80-1.33)</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; FDR, false discovery rate; MEGA, Multiple Environmental and Genetic Assessment of Risk Factors for Venous Thrombosis; OR, odds ratio; SNP, single-nucleotide polymorphism.

\(^a\)All gene symbols and rs numbers are from the National Center for Biotechnology Information (NCBI) build 36.

\(^b\)Risk-increasing allele identified in Leiden Thrombophilia Study and MEGA-1.

\(^c\)Previously genotyped SNPs.

\(^d\)In the previous study, men and women were analyzed separately for rs6048 (F9). Combined analysis resulted in a different P value, affecting the rank and consequent FDRs for rs6048 and rs2001490.

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Analysis and interpretation of data: Bezemer, Bare, Arellano, Reitsma.

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