No clear link between VKORC1 genetic polymorphism and the risk of venous thrombosis or peripheral arterial disease.

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Dear Sir,

Vitamin K, discovered in the 1930s, functions as a cofactor for the posttranslational carboxylation of glutamate residues (1). The vitamin K-epoxide reductase complex (VKOR) allows vitamin K epoxide to be converted back into reduced vitamin K. Its activity is the rate-limiting step of vitamin K-dependent protein gamma-carboxylation. Gamma-carboxylation is essential for the biological activity of clotting factors (factors II, VII, IX and X, and proteins C, S, and Z). Genetic and acquired disorders of some of these factors are linked to venous thromboembolism (VTE). Matrix GlA protein and bone GlA protein (osteocalcin), two vitamin K-dependent proteins involved in calcium homeostasis, have been implicated in the pathogenesis of atherosclerosis, myocardial infarction and stroke (2, 3). Moreover, VKOR has been implicated in angiogenesis, a process with an important role in cardiovascular disease (4). Studies of two families showing vitamin K-dependent clotting factor deficiency type 2 (VKCFD2) permit to identify the VKOR subunit 1 (VKORC1) gene. Another four patients resistant to vitamin K antagonists (VKA) point to VKORC1 as a candidate locus at which certain mutations or polymorphisms confer resistance to pharmacotherapy (5). VKORC1 as a target of coumarin-based drugs was confirmed in several single patients with coumarin resistance, showing heterozygous mutations in VKORC1 exhibiting impaired coumarin inhibition (5, 6). Common VKORC1 polymorphisms have since been found to affect the VKA dose response and warfarin dose requirements (7–10). The frequency of these polymorphisms differs among ethnic populations (8).

Various nomenclature for VKORC1 haplotypes were proposed: VKORC1*2 haplotype, strictly corresponds to the haplotype combination called “A” by Rieder’s study (7), while VKORC1*1, *3 and *4 haplotype, correspond to the haplotype combination called “B”. Two polymorphisms, the C1173T and G-1639A, are in complete linkage disequilibrium and can be used to distinguish between the haplotype combinations A and B.

The simple genotyping of VKORC1 G-1639A or C1173T with the CYP2C9*3 polymorphism could predict a high risk of overdose before initiation of anticoagulation with acenocoumarol (9–12). Indeed, muted allele A or T (G-1639A or C1173T) explains about one third of the variability of the pharmacologic response (37% of factor VII decrease and 30% of international normalised ratio [INR] change) (9). Here we studied the functional promoter polymorphism of VKORC1 G-1639A (rs9923231) to identify the major VKORC1 haplotype 2 (group A of Rieder’s classification) (7) and “non VKORC1*2” haplotype (group B). The G allele of the G-1639A SNP corresponds to the group B VKORC1 haplotype and the A allele to the group A VKORC1 haplotype.

Two recent studies showed a link between VKORC1 haplotypes and the risk of cardiovascular disease. The first, a Chinese case-control study, suggested that VKORC1 B/B haplotype was a major risk factor for coronary heart disease (CHD), stroke, and aortic dissection, probably through the role of osteocalcin in vascular calcification (13). The second, a French case-control study, showed a protective effect of the VKORC1 A/A haplotype on VTE (14). However, several other studies have failed to show any association between the VKORC1 genotype and the risk of cardiovascular disease (15, 16). To test the hypothesis that VKORC1-dependent effects on the coagulation cascade and vascular calcification would contribute to susceptibility to vascular diseases, we investigated the previously unexplored association of VKORC1 A and B haplotypes with peripheral arterial disease (PAD). Conflicting findings prompted us to seek also a link between VKORC1 A and B haplotypes and VTE.

We studied two matched case-control studies, namely the Pallas study (Paris Lower Limb Atherothrombosis Study) for PAD and the FARIVE study (Facteurs de Risques et de récidives de la maladie thromboembolique Veineuse) for first episodes of VTE. All the participants gave their written informed consent, and the Paris-Cochin Ethics Committee approved the protocols. The PAD cases were consecutive Caucasian men under 70 years of age, who are described in detail elsewhere (17, 18). The FARIVE study included men and women with a first proximal venous thrombosis and/or pulmonary embolism. For each case, age- and sex-matched controls were recruited among patients hospitalized during the same period with no history of arterial or venous thrombosis or cancer.

VKORC1 A/B status was determined with the Taqman allelic discrimination assay (Applied Biosystems) targeting the discriminant SNP G-1639A. The primers and probes (available on
The G allele of the G-1639A SNP corresponds to the group B VKORC1 haplotype of Rieder’s classification and the A allele to the group A VKORC1 haplotype.

<table>
<thead>
<tr>
<th>RS 9923231 G→A</th>
<th>Controls (N=257) (%)</th>
<th>Cases (N=257) (%)</th>
<th>OR (95% CI)</th>
<th>Controls (N=303) (%)</th>
<th>Cases (N=165) (%)</th>
<th>OR (95% CI)</th>
<th>OR (95% CI)</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G allele</td>
<td>309 (60)</td>
<td>311 (61)</td>
<td>1 (Ref.)</td>
<td>355 (59)</td>
<td>194 (59)</td>
<td>1 (Ref.)</td>
<td>1 (Ref.)</td>
<td>1 (Ref.)</td>
</tr>
<tr>
<td>A allele</td>
<td>205 (40)</td>
<td>203 (39)</td>
<td>0.98 (0.77, 1.26)</td>
<td>251 (41)</td>
<td>136 (41)</td>
<td>0.97 (0.73, 1.29)</td>
<td>1.12 (0.65, 1.95)</td>
<td>1.07 (0.62, 1.85)</td>
</tr>
<tr>
<td>G/G genotype</td>
<td>90 (35.0)</td>
<td>100 (38.9)</td>
<td>1 (Ref.)</td>
<td>105 (34.7)</td>
<td>62 (37.6)</td>
<td>1 (Ref.)</td>
<td>1 (Ref.)</td>
<td>1 (Ref.)</td>
</tr>
<tr>
<td>G/A genotype</td>
<td>129 (50.2)</td>
<td>111 (43.2)</td>
<td>0.78 (0.54, 1.14)</td>
<td>145 (47.9)</td>
<td>70 (42.4)</td>
<td>0.81 (0.53, 1.24)</td>
<td>1.46 (0.61, 3.47)</td>
<td>1.34 (0.57, 3.16)</td>
</tr>
<tr>
<td>A/A genotype</td>
<td>38 (14.8)</td>
<td>46 (17.9)</td>
<td>1.09 (0.66, 1.82)</td>
<td>53 (17.5)</td>
<td>33 (20)</td>
<td>1.02 (0.58, 1.81)</td>
<td>1.14 (0.38, 3.36)</td>
<td>1.05 (0.36, 3.06)</td>
</tr>
<tr>
<td>G/A or A/A genotype</td>
<td>167 (65.0)</td>
<td>157 (61.1)</td>
<td>0.85 (0.60, 1.21)</td>
<td>198 (65.4)</td>
<td>103 (62.4)</td>
<td>0.86 (0.57, 1.28)</td>
<td>1.35 (0.60, 3.02)</td>
<td>1.24 (0.56, 2.75)</td>
</tr>
</tbody>
</table>

The VKORC1 genotype frequencies in the VTE and PAD patients and controls are reported in Table 1. The VKORC1 genetic variants were in Hardy-Weinberg equilibrium. The observed frequencies of minor allele A in cases and controls of the two studies ranged from 0.39 to 0.41. VKORC1 A was carried by 61.1% of VTE cases and 65% of controls (OR=0.85; 95% CI: 0.60–1.21). The VKORC1 genotype was not associated with VTE: the ORs were 0.78 (95% CI: 0.54–1.14) and 1.09 (95% CI: 0.66–1.82), respectively, for VKORC1 A haplotype heterozygosity and homozygosity. This confirmed that the VKORC1 genotype does not influence the risk of VTE (16). VKORC1 A was carried by 62.4% of PAD cases and 65.4% of control subjects (OR=0.86, CI 0.57–1.28). The VKORC1 genotype was not associated with PAD: the ORs were 0.81 (95% CI: 0.53–1.24) and 1.02 (95% CI: 0.58–1.28), respectively, for VKORC1 A haplotype heterozygosity and homozygosity. We also studied the possible influence of the VKORC1 genotype on the severity of PAD, categorized as follows: group 1: intermittent claudication (IC) with a walking distance above 100 m (N=92); group 2: IC with a walking distance below 100 m (N=36); and group 3: critical limb ischemia (N=37). Among cases, the genotype frequencies were not significantly different in group 2 compared with group 1 (OR 1.12, 95% CI 0.69–1.83) or in group 3 compared with group 1 (OR 0.99, 95% CI 0.61–1.62). VKORC1 could play a role in VTE or PAD by affecting gamma-carboxylation of vitamin K-dependent proteins, namely clotting factors in VTE and osteocalcin in PAD. In a Chinese study, the VKORC1 B/B haplotype was associated with 1.7– and 1.8-fold increased risks of stroke and CHD, while VKORC1 A/A was associated with protection from VTE in a French population (14). However, these results were not confirmed in other Caucasian populations (15, 16). The VKORC1 A genotype frequencies in the two case-control studies analyzed here are similar to those previously reported in European Caucasians (14, 15), but slightly higher than in a recent US study (16). However, differences in the geographic distribution of VKORC1 polymorphisms are unlikely to explain the lack of correlation with PAD and VTE observed here. Our study size was modest, and results were consistent with a wide range of possible associations, as evidenced by the wide CIs.

Our results suggest the absence of a clear association between VKORC1 polymorphisms that determine warfarin sensitivity and the risk of VTE and PAD. Further investigations are needed to clarify the role of VKORC1 variants in the pathogenesis of arterial and venous thrombosis, in particular with implications of Gas6 or extra-hepatic Gla protein levels. Interactions with other candidate genes or local environmental factors might explain why VKORC1 gene variants are associated with arterial or venous thrombosis in certain populations.

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References