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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 VTE, CHD or stroke (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

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Received December 17, 2007
Accepted after minor revision March 14, 2008

Prepublished online April 9, 2008
doi:10.1160/TH07-12-0742

Thromb Haemost 2008; 99: 970–972

Table 1: Association of the *VKORC1* genotype with venous thromboembolism (VTE) and peripheral arterial disease (PAD). 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.

	Venous thromboembolism			Peripheral arterial disease (overall)			Severity of peripheral arterial disease	
							2 vs. 1 N=36 vs. 92	3 vs. 1 N=37 vs. 92
RS 9923231 G→A	**Controls N=257 (%)**	**Cases N=257 (%)**	**OR (95% CI)**	**Controls N=303 (%)**	**Cases N=165 (%)**	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**
G allele	309 (60)	311 (61)	1 (Ref.)	355 (59)	194 (59)	1 (Ref.)	1 (Ref.)	1 (Ref.)
A allele	205 (40)	203 (39)	0.98 (0.77, 1.26)	251 (41)	136 (41)	0.97 (0.73, 1.29)	1.12 (0.65, 1.95)	1.07 (0.62, 1.85)
G/G genotype	90 (35.0)	100 (38.9)	1 (Ref.)	105 (34.7)	62 (37.6)	1 (Ref.)	1 (Ref.)	1 (Ref.)
G/A genotype	129 (50.2)	111 (43.2)	0.78 (0.54, 1.14)	145 (47.9)	70 (42.4)	0.81 (0.53, 1.24)	1.46 (0.61, 3.47)	1.34 (0.57, 3.16)
A/A genotype	38 (14.8)	46 (17.9)	1.09 (0.66, 1.82)	53 (17.5)	33 (20)	1.02 (0.58, 1.81)	1.14 (0.38, 3.36)	1.05 (0.36, 3.06)
G/A or A/A genotype	167 (65.0)	157 (61.1)	0.85 (0.60, 1.21)	198 (65.4)	103 (62.4)	0.86 (0.57, 1.28)	1.35 (0.60, 3.02)	1.24 (0.56, 2.75)

request) were chosen with Primer Express Software (Applied Biosystems, Courtaboeuf, France). STATA 8 software was used for statistical analyses. Odds ratios (ORs) and 95% confidence intervals (CIs) were estimated by means of conditional logistic regression. The genotype was modelled by assuming either a dominant model (1 or 2 copies of the minor allele) or a model with heterozygotes and homozygotes modelled separately. All analyses adjusted for the matching variables of age and gender. We adjusted only for matching factors, since acquired risk factors are not expected to confound genetic associations except through selection bias.

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 were carried by 61.1% of VTE cases and 65% of controls (OR=0.85; 95% CI: 0.6–1.21). 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.

Acknowledgements

We thank Veronique Remones, Fouad Dali-Ali, Patricia Verrier, Marjorie Starck and Isabelle Martinez for their technical assistance. This work was supported by research grants from Programme Hospitalier de Recherche Clinique FARIVE (PHRC AOR 02057), Fondation pour la Recherche Médicale, Fondation de France and the Leducq foundation (Leducq Transatlantic Network of Excellence on Atherothrombosis Research-LINAT).

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