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Brief report

KNG1 Ile581Thr and susceptibility to venous thrombosis

Pierre-Emmanuel Morange,[1,2] Tiphaine Oudot-Mellakh,[3,4] William Cohen,[1,2] Marine Germain,[3,4] Noémie Saut,[1,2] Guillemette Antoni,[3,4] Marie-Christine Alessi,[1,2] Marion Bertrand,[4,5] Anne-Marie Dupuy,[6] Luc Letenneur,[7,8] Mark Lathrop,[9] Lorna M. Lopez,[10,11] Jean-Charles Lambert,[12-14] Joseph Emmerich,[15] Philippe Amouyel,[12-14,16] and David-Alexandre Trégouët[3,4]

[1]Inserm UMR_S 626, Marseille, France; [2]Université de la Méditerranée, Marseille, France; [3]Inserm UMR_S 937, Paris, France; [4]Université Pierre et Marie Curie (UPMC, Paris 6), Paris, France; [5]Inserm UMR_S 708, Paris, France; [6]Inserm U888, Hôpital La Colombière, Montpellier, France; [7]Inserm U897, F-33076, Bordeaux, France; [8]Université Victor Segalen, Bordeaux, France; [9]Commissariat à l'Energie Atomique, Institut de Génomique, Centre National de Génotypage, Evry, France; [10]Department of Psychology, The University of Edinburgh, Edinburgh, United Kingdom; [11]Centre for Cognitive Ageing and Cognitive Epidemiology, The University of Edinburgh, Edinburgh, United Kingdom; [12]Inserm U744, Lille, France; [13]Institut Pasteur de Lille, Lille, France; [14]Université de Lille Nord de France, Lille, France; [15]Inserm U765, Médecine Vasculaire-HTA, Hôpital Européen Georges-Pompidou, Université Paris-Descartes, Paris, France; and [16]CHRU de Lille, Lille, France

Three single nucleotide polymorphisms (SNPs) were recently found to be associated with activated partial thromboplastin time (aPTT). Because shortened aPTT levels have been observed in patients experiencing venous thrombosis (VT), we investigated the effects of these 3 aPTT-associated SNPs, rs2731672, rs9898, and rs710446, on the risk of VT in a sample of 1110 healthy patients and 1542 patients with VT. Among the 3 tested SNPs, only rs710446 was associated with VT risk; the rs710446-C allele was associated with an increased risk of VT (odds ratio 1.196, 95% confidence interval 1.071-1.336, *P* = .0012). This association also was observed in an independent sample of 590 controls and 596 patients (odds ratio 1.171, 95% confidence interval 0.889-1.541, *P* = .059). We also confirmed that the rs710446-C allele was associated with decreased aPTT levels, making this nonsynonymous Ile581Thr variant a new genetic risk factor for VT. (*Blood*. 2011; 117(13):3692-3694)

Introduction

Activated partial thromboplastin time (aPTT) is a global coagulation test that has been used during the last 50 years as a standard screening test in clinical laboratories throughout the world.[1] aPTT levels are considered to reflect global coagulation activity. This test cumulatively explores factors belonging to the classic intrinsic (FXI, FIX, and FVIII) or common (FII and fibrinogen) coagulation cascade. Besides its sensitivity toward variation levels of these coagulation factors, aPTT also is associated with age, female sex, estrogen use, and obesity.[2] Shortened aPTT levels have been proved reliable as a predictor of venous thrombosis (VT).[2,3] Very recently, the authors of a genome-wide association study (GWAS) reported 3 single nucleotide polymorphisms (SNPs) associated with aPTT levels.[4] These 3 SNPs were located in 3 coagulation cascade genes, *F12* (rs27431672), *HRG* (rs9898), and *KNG1* (rs710446). We reasoned that alleles associated with shortened aPTT levels might be associated with an increased risk of VT. To test this hypothesis, the genotype distributions of rs27431672, rs9898, and rs710446 observed in 2 independent samples of VT patients from the MARseille THrombosis Association study (MARTHA) 08 (n = 972) and MARTHA10 (n = 570) were compared with those observed in 1110 healthy subjects from the Three-City Study.[5]

Methods

Patients

MARTHA patients are unrelated Europeans consecutively recruited at the Thrombophilia center of La Timone Hospital (Marseille, France) between January 1994 and October 2005 (supplemental Table 1, available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article). All participants provided written informed consent, and the protocol was approved by the ethics committee of each participating institution.

Genotyping

As part of an ongoing GWAS on VT risk, MARTHA08 patients were typed in 2008 with the Illumina Human610-Quad Beadchip, whereas the Illumina Human660W-Quad Beadchip was used in early 2010 for typing MARTHA10 patients. Control patients from the Three-City Study also were typed with the Illumina Human610-Quad Beadchip. The 3 tested SNPs were available on the GWAS arrays, and none of them showed significant deviation from Hardy-Weinberg equilibrium at $P < 10^{-2}$. The genotype success rates were greater than 99.8% for all SNPs, except for rs9898 in MARTHA10 patients, where it only reached 98.1%. Replication of the results observed in MARTHA was investigated in the Facteurs de Risque et de Récidives de la Maladie Thromboembolique Veineuse (FARIVE) study,[6] where genotyping was performed with the use of TaqMan technology.

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Table 1. Genotype distribution of aPTT hit SNPs in the Three-City Study control patients and in MARTHA VT patients

	Three-City Study	MARTHA08	MARTHA10
rs2731672 (*F12*)			
CC	659 (59)	599 (62)	365 (64)
CT	394 (36)	322 (33)	183 (32)
TT	57 (5)	51 (5)	22 (4)
MAF (T)	0.229	0.218	0.199
P		.124*	
rs9898 (*HRG*)			
CC	454 (41)	386 (40)	222 (40)
CT	534 (48)	453 (46)	264 (47)
TT	120 (11)	133 (14)	73 (13)
MAF (T)	0.349	0.370	0.367
P		.141	
rs710446 (*KNG1*)			
TT	383 (35)	280 (29)	173 (30)
TC	545 (49)	497 (51)	280 (49)
CC	182 (16)	194 (20)	117 (20)
MAF (C)	0.409	0.455	0.451
P		.00117	

Values are n (%).

aPTT indicates activated partial thromboplastin time; MAF, minor allele frequency; MARTHA, MARseille THrombosis Association study; SNP, single nucleotide polymorphisms; VT, venous thrombosis.

**P* value of the Cochran-Armitage trend test.

Statistical analysis

Association between SNPs and disease status was assessed by use of the Cochran-Armitage trend test and logistic regression, whereas linear regression analysis was used to test the association with aPTT levels. Haplotype association analyses were performed by the use of THESIAS[6] and Gridhaplo software.[7]

Results and discussion

As indicated in Table 1, no association with VT risk was observed for the *F12* rs2731672 or the *HRG* rs9898. Conversely, the *KNG1* rs710446-C allele was found more frequently in MARTHA08 and MARTHA10 patients (0.455 and 0.451, respectively) compared with healthy patients of the Three-City Study (0.409). When the 2 sets of VT patients were combined and compared with the Three-City Study, the rs710446-C allele was associated with an increased odds ratio (OR) for VT of 1.196 (95% confidence interval [CI] 1.071-1.336, $P = .0012$) Adjustment for sex, ABO blood group (tagged by the *ABO* rs8176746, rs8176704, and rs505922),[8] and FV Leiden (tagged by rs2420371) with the use of logistic regression analysis did not modify this association because the corresponding allelic OR became 1.204 (95% CI 1.071-1.354, $P = .0019$).

To further replicate the association of *KNG1* rs710446 with VT risk, we investigated its effect in a sample of 590 healthy patients and 596 VT patients who were part of FARIVE.[8] Because of technical problem of genotyping this SNP with a Taqman assay, we instead genotyped the rs698078 (genotype success rate of 99%), which serves as a perfect proxy ($r^2 = 1.00$ according to the SNAP database[9]). As indicated in Table 2, the genotype distribution of the rs698078 paralleled that observed for rs710446 in Table 1. The rs698078-G allele that corresponds to the rs710446-C allele was found more frequently in VT patients compared with control patients (0.456 vs 0.422, $P = .113$). After adjusting for age, sex, ABO blood group, and FV Leiden mutation, we found that the OR for VT associated with the rs698078-G allele was 1.171 (95% CI 0.889-1.541, $P = .059$). The association was borderline, and this finding is likely because of the modest power (estimated to 37% by use of the CatS software[10]) the FARIVE study has to detect such an association.

Among the 3 newly discovered SNPs found genome-widely associated with aPTT in the work by Houlihan et al,[4] in our study we found *KNG1* rs710446 to be associated with VT but not *F12* rs2731672 or *HRG* rs9898. We cannot exclude that the latter 2 could be associated with VT risk, but the MARTHA project is likely underpowered to detect their effects, if any. According to the strength of associations observed in Table 1, the power to detect at $P = .05$ the effects of rs2731672 and rs9898 was less than 35% for both. Other SNPs mapping the *HRG* and *F12* genes were available as part of our ongoing GWAS on VT, but none of them showed evidence for association with the disease (supplemental Table 2). Conversely, other *KNG1* SNPs demonstrated evidence of association with VT, but their association was because of their linkage disequilibrium with rs710446 (supplemental Table 3).

Altogether, these results provide strong evidence that the *KNG1* rs710446, a nonsynonymous variant (Ile581Thr) predicted to be damaging,[4] is associated with the risk of VT. Several arguments are in favor of the implication of *KNG1* locus, which encodes high molecular weight fibrinogen (HK) in VT physiopathology. HK plays an important role in blood coagulation by positioning prekallikrein and FXI near factor XII.[11] In addition, *KNG1* knockout mice demonstrated prolonged aPTT and delayed arterial thrombosis.[12] Moreover, antibody against mouse FXI, by directly interfering with the FXI-HK interaction, prevented arterial occlusion induced by FeCl3 to a similar degree to total FXI deficiency.[13] HK is also an important member of the plasma kallikrein-kinin system,[14] the activation of which may contribute to the manifestations of disorders such as hereditary angioedema,[15] sepsis,[16] ulcerative colitis,[17] and Alzheimer disease.[18]

It would be tempting to assert that the effect of rs710446 on VT risk is mediated by its effect on aPTT levels recently discovered.[4] We also observed a strong association between rs710446 and plasma aPTT levels in MARTHA08 ($R^2 = 5.96\%$, $P = 8.74 \times 10^{-12}$) and MARTHA10 ($R^2 = 9.22\%$, $P = 9.01 \times 10^{-11}$), with the rs710446-C allele associated with decreased aPTT levels (supplemental Table 4). However, because aPTT measurements were not available in the Three-City Study, it was not possible to really test this hypothesis.

Other *KNG1* SNPs also were found to influence aPTT levels in MARTHA (supplemental Table 2) but, again, the strongest association was observed for rs710446. We were also able to confirm the results of Houlihan et al[4] by noting that, at the *F12* and *HRG* loci, the strongest associations were observed for rs2731672 and rs9898, respectively (supplemental Table 2). To a lesser extent, *F11* also was suggested to modulate aPTT levels,[4] and we also observed this phenomenon in our samples (supplemental Table 2). Surprisingly, some, but not all, aPTT-associated *F11* SNPs showed some promising evidence of association with VT, and the converse also

Table 2. Genotype distribution of the *KNG1* rs698078 in FARIVE

rs698078	Control patients, n = 590	Cases, n = 596
AA	205 (35)	174 (29)
AG	271 (46)	301 (51)
GG	114 (19)	121 (20)
MAF (G)	0.422	0.456

Values are n (%). *P* value of the Cochran-Armitage trend test was .113.

FARIVE indicates Facteurs de Risque et de Récidives de la Maladie Thromboembolique Veineuse; and MAF, minor allele frequency.

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was observed (supplemental Table 2). The association of *F11* SNPs with VT risk is not new because 2 *F11* SNPs, rs2036914 and rs2289252, were previously found to be associated with VT in the Leiden Thrombophilia study.[19] None of these 2 SNPs was available in our GWAS dataset, nor were they in strong linkage disequilibrium with any of the *F11* SNPs associated with VT in the present work (supplemental Table 5). In-depth haplotype analysis would be further required to disentangle the exact contribution of *F11* SNPs to VT risk and aPTT variability, but this research is out of the scope of the present report. In conclusion, in this report we identify the *KNG1* Ile581Thr variant as a new candidate risk factor for VT and encourage further study of the genetic determinants of pertinent phenotypic intermediate traits for identifying new risk factor of VT.

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Authorship

Contribution: P.-E.M., M.L., J.E., P.A., and D.A.T. designed the research analyzed data and wrote the paper; and T.O.-M., W.C., M.G., N.S., G.A., M.-C.A., M.B., A.-M.D., L.L., L.M.L., and J.-C.L. performed research and analyzed data.

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L.M.L. has previously published under her maiden name, Lorna M. Houlihan.

Correspondence: Professor Pierre-Emmanuel Morange, Laboratory of Haematology, CHU Timone, 246, rue Saint-Pierre, 13385 Marseille Cedex 05, France; e-mail: pierre.morange@ap-hm.fr.

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