A Genome-Wide Association Study Identifies KNG1 as a Genetic Determinant of Plasma Factor XI Level and Activated Partial Thromboplastin Time

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Objective—Elevated plasma levels of coagulation factor XI (FXI) are implicated in the pathogenesis of venous thromboembolism and ischemic stroke, and polymorphisms in the F11 gene are associated both with risk of venous thromboembolism and an elevated plasma FXI level.

Methods and Results—Here, we report the first hypothesis-free genome-wide genetic analysis of plasma FXI levels. Two genome-wide significant loci were detected in the family-based Genetic Analysis of Idiopathic Thrombophilia 1 cohort: one located in the kininogen I gene (KNG1) (rs710446; P=7.98×10−10) and one located in the structural F11 gene (rs4241824; P=1.16×10−8). Both associations were replicated in a second population-based Swedish cohort. A significant effect on KNG1 mRNA expression was also seen for the 2 most robustly FXI-associated single nucleotide polymorphisms located in KNG1. Furthermore, both KNG1 single nucleotide polymorphisms were associated with activated partial thromboplastin time, suggesting that FXI may be the main mechanistic pathway by which KNG1 and F11 influence activated partial thromboplastin time and risk of thrombosis.

Conclusion—These findings contribute to the emerging molecular basis of venous thromboembolism and, more importantly, help in understanding the biological regulation of a phenotype that has proved to have promising therapeutic properties in relation to thrombosis. (Arterioscler Thromb Vasc Biol. 2012;32:2008-2016.)

Key Words: factor XI  ■  kininogen  ■  genome-wide associations  ■  activated partial thromboplastin time  ■  thrombosis

Coagulation factor XI (FXI) is a component of the contact (activation) pathway and circulates in the blood in complex with high-molecular-weight kininogen (HK). FXI has an important role in propagation and stabilization of the evolving thrombus in vivo, which is independent of coagulation factor XII (FXII).1 Interestingly, congenital FXI deficiency only confers a mild bleeding phenotype compared with deficiencies in coagulation factors VIII and IX (hemophilia A and B, respectively). There is also growing evidence that FXI plays a key role in the pathogenesis of thrombosis.2-6 Individuals with elevated plasma levels of FXI (defined as values above the 90th percentile of the distribution seen in control subjects) run an ≥2-fold increased risk of venous thrombosis.4,7 Smaller studies of younger patients also support an association with ischemic stroke, whereas the FXI level seems to be of minor importance in myocardial infarction.2,6,8-11 Conversely, FXI-deficient mice are protected from experimentally induced thrombosis or stroke,12 and human subjects with severe FXI deficiency have a reduced incidence of venous thromboembolism (VTE) and stroke.10,13,14

Recent molecular genetic studies of VTE have identified polymorphisms in the F11 gene located on chromosome 4, which are associated both with risk of disease and elevated plasma FXI level,15-17 findings that have subsequently been replicated in other VTE studies in whites and blacks.18-20 Of note, the fact that common genetic factors underlie both traits had been suggested earlier based on a family study of thrombophilia, indicating a significant genetic correlation between FXI levels and risk of VTE (0.56; P=0.02).21

Although the evidence of an important role for FXI in the pathogenesis of VTE is strong, knowledge about the regulation of the plasma concentration of this protein remains sparse. However, it is notable, in this context, that the additive heritability of plasma FXI has been estimated to be as high as 45%.22 Despite the substantial genetic regulation, a hypothesis-free genome-wide association study (GWAS) has so far not been

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conducted to reveal additional gene loci, influencing the variation in FXI level. Against this background, we performed a GWAS of the plasma FXI concentration in extended Spanish families, half of which were ascertained through thrombophilia, with replication of the key findings in a population-based sample of healthy Swedish subjects. Expression analysis in human liver was also undertaken to confirm the functional significance of the identified candidate gene.

**Patients and Methods**

**Subjects**

The discovery GWAS was performed in the Genetic Analysis of Idiopathic Thrombophilia 1 (GAIT-1) cohort, which is a family-based cohort consisting of 398 individuals belonging to 21 Spanish families, 12 of whom were selected through a proband with idiopathic thrombophilia and 9 who were randomly selected. Of these, 339 had FXI and aPTT measurements. A detailed description of the GAIT-1 sample has been reported elsewhere. All subjects gave informed consent to the use of their data, and the GAIT-1 study protocols were approved by the Institutional Review Board of the Hospital de la Santa Creu i Sant Pau.

Replication was performed in population-based, unrelated Spanish individuals, 340 men and 322 women with a similar age distribution (33–80 years), who had been recruited at random from the greater Stockholm area for inclusion as control subjects in the Precocious Coronary Artery Disease (PROCARDIS) study (cohort acronym OLIVIA). Of these, 658 had FXI measurements. Informed consent was provided by all participants, and the study was granted approval by the Ethics Committee of the Karolinska Institutet.

Expression analyses in human liver were conducted in 211 liver biopsy samples from the Advanced Study of Aortic Pathology (ASAP) study. ASAP enrolls patients undergoing aortic valve surgery at the Karolinska University Hospital, Stockholm, Sweden. Biopsies were obtained during open-heart surgery. Informed consent was provided by all the participants, and approval was given by the ethics committee of the Karolinska Institutet.

**Phenotype and Genotype Determinations**

Venous blood samples were obtained after an overnight fast, and DNA was extracted from whole blood samples using a standard protocol in both cohorts. Plasma FXI concentration was determined in GAIT-1 using platelet-poor citrate plasma, which had been prepared by centrifugation at 2000g for 20 minutes at room temperature. For activated partial thromboplastin time (aPTT) and FXI, assays were performed on fresh plasma samples. Briefly, aPTT was measured in an automated coagulometer (ACL 3000; Instrumentation Laboratory SpA, Milan, Italy) with the use of bovine cephalin and silica, and FXI was assayed with deficient plasma from Diagnostica Stago (Asnières, France). Intra-assay and interassay coefficients of variation were between 2% and 6% for all phenotypes in GAIT-1.22 For OLIVIA, FXI antigen level was determined in EDTA plasma samples by ELISA, using a matched pair antibody set for assay of human FXI antigen from Affinity Biologicals Inc (Ontario, Canada), essentially according to the instructions provided by the manufacturer. The VisuCal Antigen from Affinity Biologicals Inc was used as calibrator, and results were expressed as U/mL. Coagulation reference plasma from Technoclone GmbH (Vienna, Austria) was used as control plasma. The interassay coefficient of variation was 10.6%.

Genome-wide, high-coverage, single nucleotide polymorphism (SNP) genotyping in GAIT-1 was performed using the Illumina Infinium 317K Beadchip (Illumina, San Diego, CA), as previously described.23 SNPs with a call rate <0.95, a minor allele frequency <0.02, or deviating from Hardy-Weinberg equilibrium at \( P < 5 \times 10^{-8} \) were excluded. After quality control (QC) filtering, 283,437 SNPs remained for analysis. OLIVIA samples were genotyped using the Illumina 1M Beadchip. QC filters (call rate \( \leq 0.95 \), minor allele frequency \( < 0.01 \), and deviation from Hardy-Weinberg equilibrium \( P < 10^{-8} \)) were applied before imputation. After QC filtering, 498,717 SNPs remained available for imputation. Dosage imputation was then conducted using MaCH 1.0.16 (Center for Statistical Genetics, University of Michigan) and the 1000 Genomes Caucasian (CEU) reference panel providing a total of 2,543,887 SNPs for analysis. Genotyping in ASAP was performed on Illumina Human 610W-Quad Bead arrays (Illumina), and filters identical to the ones applied in OLIVIA were used for QC.

As GAIT-1 sample is a family-based study and the origin of individuals is homogeneous, we did not perform a stratification analysis. However, in OLIVIA, multidimensional scaling coordinates, reflecting genetic distances between individuals (population substructure), were calculated based on the genotype data using PLINK v1.05 (PLINK Center for Human Genetic Research, Massachusetts General Hospital, and the Broad Institute of Harvard and MIT), and outliers in the nearest neighborhood analysis for multidimensional scaling 1 versus multidimensional scaling 2 were excluded.

Total RNA from human ASAP liver specimens was isolated using RNeAlter (Ambion, Austin, TX), Trizol (BRL-Life Technologies, Grand Island, NY), and Rneasy Mini kit (Qiagen, Stockholm, Sweden), including treatment with RNase-free DNase set (Qiagen) according to the manufacturer’s instructions. RNA quality was determined with an Agilent 2100 bioanalyzer (Agilent Technologies Inc, Palo Alto, CA), and quantity was measured by a NanoDrop (Thermo Scientific, Waltham, MA). Global gene expression was generated using Affymetrix ST 1.0 Exon arrays (Affymetrix, Santa Clara, CA) and the core subset of Affymetrix meta probe sets (Affymetrix). QC procedures and preparations preceding meta probe set level investigations (ie, the analysis in whole-gene variation with genotype) have been reported in detail.

**Statistical Analysis**

Association analyses in GAIT-1 between SNPs and the natural logarithm-transformed FXI level were performed using variance components methods in the SOLAR version 4.0 software package (Southwest Foundation for Biomedical Research, San Antonio, TX), with sex and age as covariates. From the genome-wide significant loci, we selected the 5 most significantly associated SNPs for further exploration in the OLIVIA replication cohort, comprising 658 Swedish controls. Linear regression analysis according to an additive model of inheritance was applied in OLIVIA to test for associations between the selected SNPs and natural logarithm-transformed FXI antigen level, using STATA version 11 (StataCorp, College Station, TX), and adjusting for sex and age. Fine mapping of candidate regions using 1000 Genomes–imputed SNP data was conducted in OLIVIA by analyzing associations between all imputed SNPs located within 150kb of the 2 lead SNPs showing the strongest association in GAIT-1 (rs410446 in the KNG1 locus and rs4241824 in the FXI locus) and logarithm-transformed FXI level, applying an additive model of inheritance with adjustment for sex and age. The 3 SNPs that reached Bonferroni-corrected significant \( P \) values in the replication analysis (rs710446, rs5030062, and rs4241824) were subsequently tested for association with expression levels of F11 and KNG1 in a total of 211 human liver samples obtained from the ASAP study. Genotypes from the 3 candidate SNPs were tested for association with gene expression levels using additive linear models in R, with each genotype being coded as 0, 1, or 2.

**Results**

**Sample Characteristics**

Table 1 shows sample characteristics of participants in the GAIT-1 and OLIVIA studies. Briefly, both were European samples with similar proportion of men and women and similar body mass index distributions, with mean values going from 24.7 to 25.9 kg/m². OLIVIA differed from the GAIT-1 sample, in that all participants were healthy (compared with 10.5% of individuals with VTE in GAIT-1) and it was a population-based sample compared with the family-based
Two SNPs passed the genome-wide significance level of $P<5\times10^{-8}$ for their association with the plasma FXI activity level (Table 2); the most robustly associated SNP (rs710446; $P=7.98\times10^{-10}$) was located in the kininogen 1 (KNG1) gene. This gene encodes HK, which acts as a cofactor for the activation of kallikrein and FXII, initiating the contact pathway of coagulation. The other SNP attaining genome-wide significance (rs4241824; $P=1.16\times10^{-8}$) was located in an intron within $F11$, the structural gene coding for FXI. Further adjustments for disease status resulted in minor alterations to the strength of the associations (rs710446; $P=3.49\times10^{-10}$, and rs4241824; $P=3.78\times10^{-8}$). No additional genome-significant signals appeared when we conducted the analysis conditioned on the 2 most significant SNPs (rs710446 and rs4241824).

### Replication Analysis and Fine Mapping

From the 2 genome-wide significant loci we selected the 5 most significant SNPs for further exploration in the OLIVIA replication cohort, comprising 658 Swedish controls. These 5 SNPs were all associated with log-transformed FXI levels at $P<10^{-7}$ in the discovery cohort. Linkage disequilibrium measures between rs710446 and rs5030062 were $R^2=0.93$ and $D’=1$, indicating that the 2 SNPs are highly linked according to the 1000 Genomes Pilot 1 release. The pairwise linkage disequilibrium measures between the other combinations of these 5 SNPs were all <0.8. Nonetheless, we decided to apply a standard Bonferroni correction for replication of 5 SNPs ($P<0.01$). Three of these SNPs passed the Bonferroni-corrected level for replication, 2 of which were located in the $KNG1$ gene (rs710446; $P=1.81\times10^{-4}$, and rs5030062; $P=1.57\times10^{-4}$), and 1 was located in the $F11$ gene (rs4241824; $P=5.06\times10^{-3}$) (Table 2). Only 1 SNP reached nominal significance (rs4253399; $P=3.96\times10^{-2}$), whereas 1 SNP (rs4686799) did not replicate at all. The ranges of

### Discovery Analysis

Figure 1 illustrates the results of the discovery association analyses performed for 283 437 SNPs in relation to FXI level, organized by chromosomal position in a Manhattan plot.

**Table 1. Characteristics of Study Participants in the Discovery and Replication Cohorts**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>GAIT-1</th>
<th>OLIVIA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Counts</td>
<td>339</td>
<td>658</td>
</tr>
<tr>
<td>Age (years)</td>
<td>37.7 (19.83)</td>
<td>60.3 (7.01)</td>
</tr>
<tr>
<td>Men, %</td>
<td>48.7</td>
<td>51.16</td>
</tr>
<tr>
<td>European ancestry, %</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>24.7 (4.9)</td>
<td>25.9 (4.0)</td>
</tr>
<tr>
<td>Arterial disease, %</td>
<td>6.5</td>
<td>0</td>
</tr>
<tr>
<td>Venous disease, %</td>
<td>10.5</td>
<td>0</td>
</tr>
<tr>
<td>T2D, %</td>
<td>3.8</td>
<td>4.47</td>
</tr>
<tr>
<td>FXI, %</td>
<td>100.7 (21.1)</td>
<td>105.9 (44.9)</td>
</tr>
<tr>
<td>FXI quartiles</td>
<td>86.5; 100; 113.5</td>
<td>76.0; 98.0; 125.2</td>
</tr>
<tr>
<td>aPTT</td>
<td>0.95 (0.10)</td>
<td>ND</td>
</tr>
<tr>
<td>aPTT quartiles</td>
<td>0.87; 0.95; 1.02</td>
<td>ND</td>
</tr>
</tbody>
</table>

Values are mean (SD) or number of subjects or percentage of subjects in group. aPTT levels are expressed as a ratio of the aPTT time (in s) between the individual value and the aPTT reference value of the laboratory.

GAIT-1 indicates Genetic Analysis of Idiopathic Thrombophilia 1; BMI, body mass index; T2D, type-2 diabetes mellitus; FXI, coagulation factor XI; aPTT, activated partial thromboplastin time; ND, not determined.

Figure 1. Manhattan plot representing the genome-wide association results between the 283 437 single nucleotide polymorphisms (SNPs) in the discovery cohort and natural log-transformed FXI levels adjusted for age and sex. Every dot represents an SNP organized by chromosomal order and position. The y axis is proportional to statistical significance (expressed as $-\log_{10}$ of the $P$ values).
plasma FXI levels associated with the different genotypes are shown in Table 3.

We then looked at all imputed SNPs in OLIVIA that were located within ±50 kb of the lead SNP in the KNG1 locus detected in GAIT-1 (rs710446) (Figure 2A). Among the 90 SNPs located within this region, 9 had \( P \) values ≤0.01. The strongest associations in this locus in OLIVIA were found for SNPs located within this region, 9 had \( P \) values of 0.048 and 0.031, respectively that were consistent with the effects observed on plasma levels of FXI in GAIT-1 and OLIVIA. Furthermore, we found a highly significant correlation \( (R^2=0.62, P=0.001) \) between KNG1 mRNA expression and FXI mRNA expression in liver. The corresponding association between the strongest plasma FXI-associated SNP in the FXI locus (rs4241824) and expression level of FXI mRNA in the liver was not significant.

### Association With Expression Level in Human Liver

Because FXI is primarily synthesized in the liver, we investigated associations between the 2 most robustly associated SNPs in the KNG1 locus (rs710446 and rs5030062) and expression levels of KNG1 in a total of 211 human liver samples. As shown in Figure 3, both SNPs showed significant associations with KNG1 mRNA expression in the liver (\( P \) values of 0.048 and 0.031, respectively) that were consistent with the effects observed on plasma levels of FXI in GAIT-1 and OLIVIA. Furthermore, we found a highly significant correlation \( (R^2=0.62, P=0.001) \) between KNG1 mRNA expression and FXI mRNA expression in liver. The corresponding association between the strongest plasma FXI-associated SNP in the FXI locus (rs4241824) and expression level of FXI mRNA in the liver was not significant.

### Associations With aPTT and Other Related Phenotypes

To explore the effects of the most robustly FXI-associated SNPs on thrombosis-related proteins involved in the contact pathway, we also tested the associations between the 5 top SNPs and FXII, histidine-rich glycoprotein (HRG), and prekallikrein in the GAIT-1 sample. We also tested the effects on aPTT, as an indicator of effects on coagulation factors belonging to the intrinsic pathway. Interestingly, all 3 top SNPs located in the KNG1 locus were also found to be strongly associated with aPTT (\( P=1.77\times10^{-4}, P=3.35\times10^{-7}, \) and \( P=4.67\times10^{-7} \)). This association was substantially attenuated when adjustment was made for FXI levels (in addition to sex and age), but it did not disappear.

### Table 2. Associations Between Plasma FXI Concentration and the Most Significantly FXI-Associated SNPs in the Discovery and Replication Cohorts

<table>
<thead>
<tr>
<th>CHR</th>
<th>SNP</th>
<th>Gene</th>
<th>Position</th>
<th>A1</th>
<th>A2</th>
<th>FreqA1</th>
<th>FreqA1</th>
<th>( P ) Value</th>
<th>( \beta ) GAIT-1</th>
<th>SE GAIT-1</th>
<th>( P ) Value</th>
<th>( \beta ) OLIVIA</th>
<th>SE OLIVIA</th>
<th>Imputation Quality OLIVIA</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>rs710446</td>
<td>KNG1</td>
<td>187942621</td>
<td>C</td>
<td>T</td>
<td>0.45</td>
<td>0.42</td>
<td>7.98E−10</td>
<td>0.094</td>
<td>0.016</td>
<td>1.81E−04*</td>
<td>0.08</td>
<td>0.021</td>
<td>0.9979</td>
</tr>
<tr>
<td>3</td>
<td>rs5030062</td>
<td>KNG1</td>
<td>187363874</td>
<td>C</td>
<td>A</td>
<td>0.43</td>
<td>0.38</td>
<td>1.19E−07</td>
<td>0.083</td>
<td>0.017</td>
<td>1.57E−04*</td>
<td>0.082</td>
<td>0.022</td>
<td>0.9987</td>
</tr>
<tr>
<td>3</td>
<td>rs4686799</td>
<td>KNG1</td>
<td>187933930</td>
<td>C</td>
<td>T</td>
<td>0.76</td>
<td>0.77</td>
<td>2.00E−07</td>
<td>0.099</td>
<td>0.019</td>
<td>7.80E−01</td>
<td>0.007</td>
<td>0.024</td>
<td>0.9999</td>
</tr>
<tr>
<td>4</td>
<td>rs4241824</td>
<td>F11</td>
<td>187428781</td>
<td>G</td>
<td>A</td>
<td>0.48</td>
<td>0.5</td>
<td>1.16E−08</td>
<td>−0.088</td>
<td>0.016</td>
<td>5.06E−03*</td>
<td>−0.062</td>
<td>0.022</td>
<td>0.9758</td>
</tr>
<tr>
<td>4</td>
<td>rs4253399</td>
<td>F11</td>
<td>187425088</td>
<td>T</td>
<td>G</td>
<td>0.58</td>
<td>0.62</td>
<td>6.22E−08</td>
<td>−0.088</td>
<td>0.017</td>
<td>3.96E−02</td>
<td>−0.047</td>
<td>0.023</td>
<td>0.9628</td>
</tr>
</tbody>
</table>

**CHRS indicates chromosome; SNP, single nucleotide polymorphism; GAIT-1, Genetic Analysis of Idiopathic Thrombophilia 1; SE, standard error.**

*Significant after Bonferroni correction for multiple testing.

### Table 3. Ranges of Plasma FXI Levels According to Genotype for the 3 Replicated SNPs

<table>
<thead>
<tr>
<th>N GAIT-1</th>
<th>Mean GAIT-1</th>
<th>SD GAIT-1</th>
<th>N OLIVIA</th>
<th>Mean OLIVIA</th>
<th>SD OLIVIA</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs710446</td>
<td>CC</td>
<td>80</td>
<td>111.209</td>
<td>22.22307</td>
<td>112</td>
</tr>
<tr>
<td></td>
<td>CT</td>
<td>192</td>
<td>103.461</td>
<td>20.83135</td>
<td>307</td>
</tr>
<tr>
<td></td>
<td>TT</td>
<td>115</td>
<td>90.6566</td>
<td>17.62139</td>
<td>228</td>
</tr>
<tr>
<td>rs5030062</td>
<td>AA</td>
<td>126</td>
<td>91.8909</td>
<td>17.77347</td>
<td>248</td>
</tr>
<tr>
<td></td>
<td>CA</td>
<td>185</td>
<td>104.323</td>
<td>21.05324</td>
<td>303</td>
</tr>
<tr>
<td></td>
<td>CC</td>
<td>70</td>
<td>110.807</td>
<td>22.97315</td>
<td>96</td>
</tr>
<tr>
<td>rs4241824</td>
<td>AA</td>
<td>102</td>
<td>109.522</td>
<td>20.92292</td>
<td>170</td>
</tr>
<tr>
<td></td>
<td>GA</td>
<td>178</td>
<td>101.343</td>
<td>19.3923</td>
<td>325</td>
</tr>
<tr>
<td></td>
<td>GG</td>
<td>87</td>
<td>90.9067</td>
<td>20.63006</td>
<td>152</td>
</tr>
</tbody>
</table>

FXI indicates coagulation factor XI; SNP, single nucleotide polymorphism; GAIT-1, Genetic Analysis of Idiopathic Thrombophilia 1.
Figure 2. A, Regional plot showing the fine mapping within the KNG1 locus performed in OLIVIA. Squares represent single nucleotide polymorphisms (SNPs) plotted by chromosome position. The y axis reflects –log P of the associations between SNPs and coagulation factor XI (FXI) concentration. Color intensity represents the level of linkage disequilibrium between all SNPs and the reference SNP (rs710446). B, Regional plot showing the fine mapping within the F11 locus performed in OLIVIA. Squares represent SNPs plotted by chromosome position. The y axis reflects –log P of the associations between SNPs and FXI concentration. Color intensity represent the level of linkage disequilibrium between all SNPs and the reference SNP (rs4241824) (figures created with SNAP software).
Harbored in a handful of population-based, healthy Swedish individuals, we provided evidence that the most robustly FXI-associated SNPs identified in our discovery analysis were also found to be strongly associated with prekallikrein (rs710446; \( P = 7.00 \times 10^{-4} \)), and rs4686799; \( P = 3.37 \times 10^{-5} \)). Moreover, the proportion of variance in aPTT accounted for by these 3 SNPs (after adjustment for age and sex) dropped from nearly 20% to almost 0 (Table 4). However, the 3 top SNPs located in the KNG1 locus were also found to be weakly associated with prekallikrein (rs710446; \( P = 7.15 \times 10^{-4} \)), rs5030062; \( P = 2.19 \times 10^{-9} \), and rs4686799; \( P = 0.016 \)). In addition, SNP rs4686799 was also significantly associated with FXII (\( P = 0.032 \)) and HRG (\( P = 6.09 \times 10^{-7} \)). The corollary of these findings is that SNPs in KNG1 affect aPTT mainly by influencing not only the regulation of FXI, but also by regulating other closely related proteins contained in the contact pathway. This effect would be mediated by an increase in KNG1 levels, as indicated in our RNA expression analyses.

In comparison, SNPs located in the F11 locus showed weaker associations with aPTT (rs4253399; \( P = 3.97 \times 10^{-3} \)) and rs4241824; \( P = 9.49 \times 10^{-4} \)) that completely disappeared when controlling for FXI concentration (Table 4). As for the KNG1 SNPs, the proportion of variance in aPTT accounted for by FXI SNPs (after adjustment for age and sex) became almost 0 (Table 4) after adjusting for FXI levels. SNPs located in the F11 locus were not associated with any of the other tested proteins belonging to the contact pathway.

### Discussion

Elevated plasma levels of FXI have been associated with venous thrombosis\(^4\) and ischemic stroke,\(^5,33\) which constitute important, disabling, and potentially fatal diseases with an incidence of 0.1% to 0.3% per year in whites.\(^26,27\) Although recent studies have identified some common polymorphisms within the F11 gene showing an association with FXI levels both in white and black populations,\(^15,17\) a hypothesis-free genome-wide study, which could potentially identify additional genetic loci influencing the plasma FXI level, had yet to be performed. In this report, we present the first GWAS aimed at identifying novel genetic factors that contribute to FXI regulation conducted in European-ancestry Spanish families.

The most robustly associated locus in our discovery analysis is the KNG1 gene. This association was replicated in a second sample of population-based, healthy Swedish individuals, and genotype-gene expression level association analyses provided evidence that the most robustly FXI-associated SNPs harbored in KNG1 are related in an allele-specific manner to KNG1 mRNA expression. Taken together, these findings suggest that KNG1 is a good candidate gene for the regulation of plasma FXI concentration. Of note, KNG1 encodes HK, which is known to play an important role in the contact pathway of coagulation. Almost all FXI and 70% to 90% of prekallikrein circulate in blood as a complex with HK, HK enhances FXI activation by FXII,\(^28-30\) and severe HK deficiency is usually associated with low FXI levels.\(^31\) Thus, it is plausible to conclude that SNPs in KNG1 influence FXI levels by altering levels of HK, which complexes with and stabilizes FXI in plasma. In this regard, the lead SNP in KNG1 (rs710446) causes a nonsynonymous substitution (Ile581Thr) in HK, which is predicted to be benign according to SIFT and PolyPhen software (Craig Venter Institute, Polyphen Harvard University) but which is suggested to be part of a potential regulatory region (functional-SNP).\(^32\) Supporting this prediction, our analysis of RNA expression in the liver showed that SNP rs710446 (associated with higher FXI level) was also associated with higher KNG1 expression.

Interestingly, we also found a significant association between the lead KNG1 rs710446 SNP and aPTT, an association which is in agreement with a recent GWAS analysis that identified this SNP as one of the SNPs that is most strongly associated with aPTT.\(^32\) When we performed the same analysis, except that we adjusted for FXI levels, the associations between SNPs located in the KNG1 locus and aPTT, as well as the proportion of variance explained by these SNPs, were substantially attenuated. This is in agreement with the interpretation of our results that SNPs in KNG1 affect aPTT mainly by altering FXI regulation. However, the remaining association may indicate that KNG1 also influences other members of the contact pathway that together alter aPTT. In this regard, we found highly significant associations of KNG1 SNPs with HRG and prekallikrein levels in the GAIT-1 cohort. HRG, which was found to be an important determinant of aPTT,\(^33,34\) has been shown to bind FXIIa with high affinity, a mechanism by which it may modulate the contact pathway.\(^31\) It should also be emphasized in this context that a highly significant association has been previously found between aPTT and SNP rs9898 in the HRG gene,\(^35\) an association that could be confirmed in GAIT-1 (\( P = 6.6 \times 10^{-6} \)). This SNP was, however, not associated with FXI level in our sample, indicating that KNG1 SNPs affect FXI and HRG independently and that these 2 proteins, probably among others, determine the variation in aPTT in the population.

### Figure 3

Box-plots (median, 50% and 95% CIs) showing the differences in expression levels of KNG1 and F11 according to genotypes.

A. Expression level of KNG1 transcript in the liver according to genotype distribution of KNG1 SNP rs710446 (\( P = 0.031 \)).

B. Expression level of KNG1 transcript in the liver according to genotype distribution of KNG1 SNP rs5030062 (\( P = 0.048 \)).

C. Expression level of F11 transcript in the liver according to genotype distribution of F11 SNP rs4241824 (\( P = 0.145 \)).
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<th>A2</th>
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<th>P</th>
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<th>P</th>
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Accordingly, we hypothesize that KNG1 may play a central role in the regulation of aPTT (and thereby risk of thrombosis) by modulating the FXI concentration, as well as the level of other related proteins of the contact pathway, such as HRG and prekallikrein. Further mechanistic studies are warranted to elucidate these mechanisms.

A prolonged aPTT is an indicator of deficiencies in coagulation factors belonging to the intrinsic pathway of coagulation, and a short aPTT is a stable predictor of risk of thrombosis. In this regard, it is important to point out that SNP rs710446 has also recently been found to be associated with increased risk of venous thrombosis in 2 independent samples from Marseille (odds ratio=1.20 [1.07–1.34]). Our findings, taken together with other observations in KNG1 knockout mice, suggest that genetic variation in KNG1 may be important in determining both the function of the intrinsic pathway of coagulation and the risk of thrombosis. Furthermore, the mechanistic pathway by which KNG1 affects aPTT and risk of thrombosis may be to a large extent through regulation of FXI concentration, which has in fact been proposed as a risk factor for deep vein thrombosis too.

The second genome-wide significant locus in our discovery analysis was found to be contained in the structural gene for FXI, with the most robustly associated SNP being rs4241824. These results were also confirmed in our replication cohort and are in agreement with previous studies showing that genetic variants within or in the vicinity of the F11 gene show suggestive evidence of association with FXI concentration. In fact, among the 2 independent SNPs that were found to significantly influence FXI level by Li et al, one is in almost perfect linkage disequilibrium with SNP rs4241824 (R²=0.97). The second was not genotyped in our discovery analysis but showed significant association with FXI level in our replication cohort (rs2289252; P=0.025). Interestingly, the second most robustly associated SNP in the F11 locus in our study (rs4253399) showed suggestive evidence of association with aPTT both in our study and in a previous study, an effect that disappeared completely after further adjustment for FXI concentration. Finally, SNPs located in the F11 locus have also been associated with VTE both in white and black populations, providing further evidence that the effect of these SNPs on the aPTT and VTE phenotypes is mediated through modulation of FXI concentration.

The importance of FXI in VTE has been supported by work in animal models (mice, rabbits, and rats), in which targeting of FXI with inhibitory antibodies and peptidomimetic inhibitors potently reduced thrombus formation. Moreover, inhibition of FXIa in baboons is efficacious in preventing formation of platelet-rich thrombi in arterial grafts, demonstrating the role of FXI-dependent thrombin generation, platelet activation, and fibrin formation. Given the evidence supporting FXI as a key player in the pathogenesis of thrombosis in humans, and the fact that human subjects with severe FXI deficiency have a reduced incidence of VTE and stroke but nonetheless a mild bleeding phenotype compared with other bleeding disorders, FXI has recently gained increasing attention as an anticoagulant drug target that could potentially improve the ratio of efficacy to bleeding compared with currently available anticoagulant agents.
One limitation of this study is the restricted sample size of GAIT-1, which results in a limited power to detect associations with low effect. Thus, it is plausible that other signals with lower effect sizes have remained undetected; hence, further studies with larger sample sizes are warranted to identify any additional genetic factors determining FXI concentration.

In summary, we report the first hypothesis-free genome-wide genetic analysis of the FXI activity level, which reveals that KNG1 and F11 are the main genetic regulators of plasma FXI level. More importantly, we suggest that FXI may be the main mechanistic pathway by which KNG1 and F11 determine aPTT and risk of venous thrombosis, although other proteins contained in the contact pathway, such as HRG and prekallikrein, may also add to the KNG1 effect on aPTT. These findings may contribute to elucidation of the molecular basis determining risk of VTE, and more importantly, may help in understanding the biological regulation of a phenotype that has proved to have promising therapeutic properties.

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Disclosures
None.

References


A Genome-Wide Association Study Identifies KNG1 as a Genetic Determinant of Plasma Factor XI Level and Activated Partial Thromboplastin Time

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