# *F11* Gene Duplication Causes Elevated FXI Plasma Levels and Is a Risk for Venous Thrombosis

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Coagulation factor XI (FXI) is the zymogen of a plasma protease encoded by *F11* located on 4q35.2 and is mainly synthesized by hepatocytes.[1,2] FXI is activated by coagulation factor XIIa and plays an important role in the intrinsic coagulation pathway of thrombin generation resulting in fibrin clot formation.[3,4] Bleeding problems can be due to FXI deficiency, but without clear correlations between FXI plasma levels and bleeding severity.[2] FXI deficiency is a rare disorder (1 in 1 million in white population) but with a higher prevalence in some ethnic groups, for instance 9% in Ashkenazi Jews.[5] In contrast, elevated plasma FXI levels are correlated with a higher risk for venous thromboembolism (VTE) and ischemic stroke.[6–9] There is no clear correlation between FXI levels and their contribution to myocardial infarction.[8] Different reports describe an association between genetic variability in *F11* locus and venous thrombosis with elevated FXI levels.[10,11] These genetic factors are noncoding frequent single nucleotide polymorphisms (SNPs) located in *F11* introns, e.g., rs2036914 and rs2289252.[10,12,13] Due to these insights related to a potential prothrombotic role of FXI, there is a growing interest in targeting FXI with new anticoagulant therapies.[8]

We here describe a family with inherited thrombophilia (►**Fig. 1A**). The 58-year-old proband (II.6) with a history of recurrent VTE and negative routine thrombophilia laboratory screening was screened in the ThromboGenomics study using a targeted panel for known genes associated with bleeding and thrombosis.[14] A *F11* duplication was found while no other pathogenic variants could be detected in known thrombophilia genes (►**Fig. 1B**). As only *F11* exonic regions were captured, the exact size of the duplication remains unknown but it involves at least the complete *F11* gene (arr[GRCh37] 4q35.2:(187185980–187209984)x3). Cosegregation analysis of this duplication was performed in the family using real-time polymerase chain reaction to calculate the gene dosage ratio for *F11* and its neighboring genes *FGG* and *KLKB1* (upstream) and *FAT1* (downstream) (►**Fig. 1C**, ►**Supplementary Table S1**, available in the online version). In addition, the *F11* gene duplication was confirmed in the proband (II.6) and his brother (II.5) using Multiplex Ligation-dependent Probe Amplification (MLPA). For this, 300 ng of DNA from II.5 and II.6 and four normal control samples were assayed using the MRC-Holland MLPA kit for the *F11* gene (SALSA MLPA Probemix P440-A2 F10 + F11) as per manufacturer's instructions. Fragments were analyzed using an ABI 3730XL sequencer and interpreted using GeneMarker software (SoftGenetics).

FXI plasma activity levels were measured with the ACL TOP analyzer (Werfen, Brussels, Belgium) according to the manufacturer's recommendations. For patients receiving direct oral anticoagulant (DOAC) therapy, sample pretreatment with DOAC-STOP (Nodia, Boom, Belgium) was performed. The normal range for FXI was 70 to 130%. Control experiments showed

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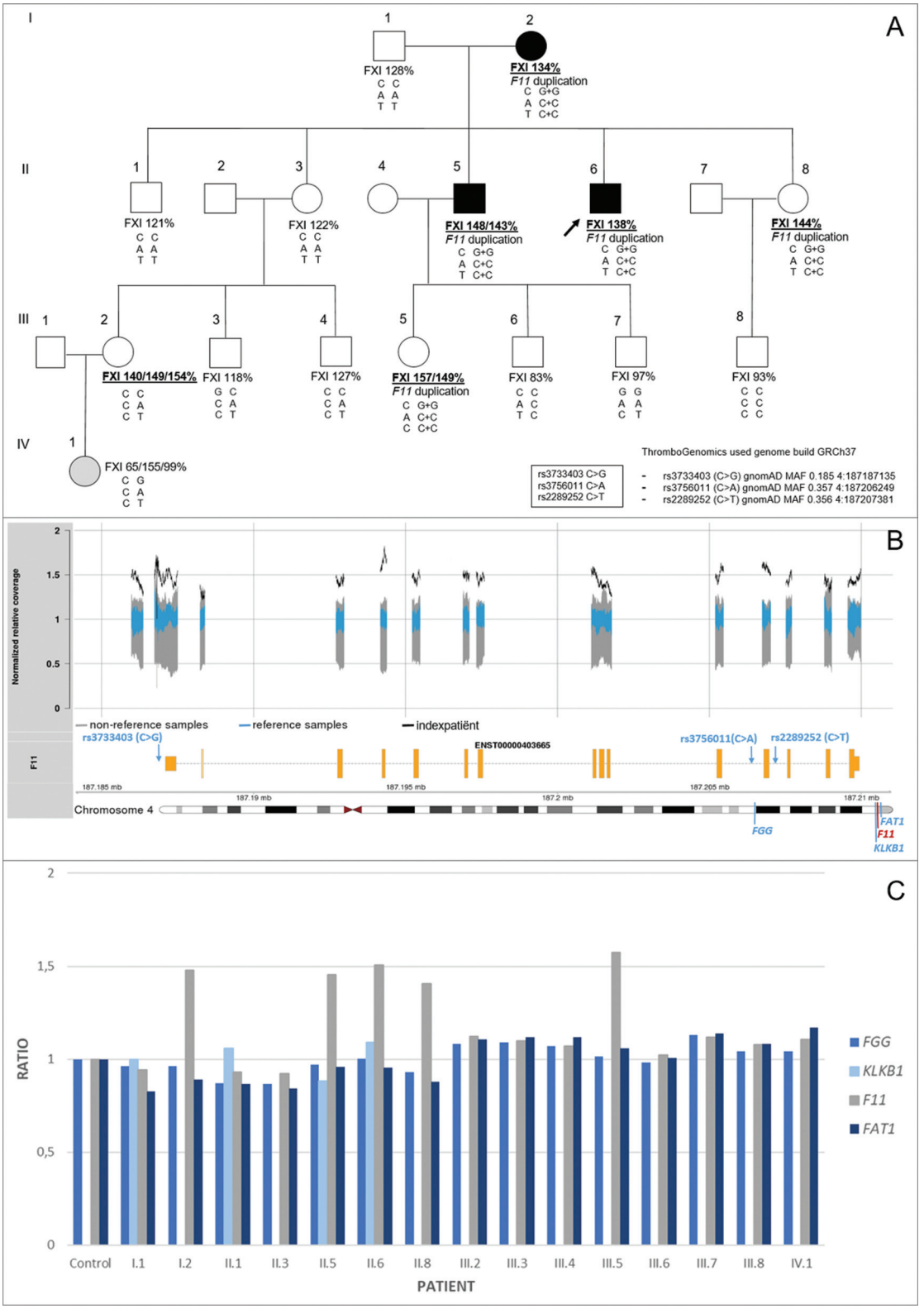


**Fig. 1** (**A**) Pedigree of the VTE patients (*black symbols* and index indicated with *arrow*), case IV.1 with a neonatal arteria cerebri media infarct (*gray symbols*), and their unaffected relatives (*white symbols*). Five family members carry the *F11* duplication as indicated. FXI plasma values are indicated (normal values between 70 and 130%). The analysis results for Sanger sequencing of *F11* SNP rs3733403 (C > G), rs3756011 (C > A), and rs2289252 (C > T) are indicated. (**B**) Coverage profile for the *F11* gene obtained for reference samples and the index patient II.6 (*black line*) showing significantly higher coverage over the complete *F11* locus. The locations of studied SNP rs3733403, rs3756011, and rs2289252 are indicated on the map for *F11*. The locations of studied genes *FGG* (exon 1), *KLKB1* (intron 6–7), *F11* (intron 10–11), and *FAT1* (exon 10) are indicated on the chromosome 4 map. (**C**) Gene dosage ratios for *F11*, *FGG*, *KLKB1*, and *FAT1* (on *Y*-axis) of different patients (on *X*-axis) determined with real-time PCR. A ratio of 1.5 is indicative for a gene duplication. PCR, polymerase chain reaction; SNP, single nucleotide polymorphism; VTE, venous thromboembolism.

 

that pretreatment of the samples with DOAC-STOP did not bias the FXI assay (data not shown). All five family members (I.2, II.5, II.6, II.8, III.5) carrying the *F11* duplication have elevated FXI plasma levels (►**Fig. 1A**). The proband (II.6) and his brother (II.5) both had a history of unexplained VTE while his sister (II.8) had no history of thrombosis (►**Supplementary Table S2**, available in the online version). Their mother (I.2) had pulmonary embolism at the age of 70. Patient III.5, a 30-year-old woman, had no history of thrombosis. Remarkably, elevated FXI levels were also measured for III.2 and IV.1, who have no *F11* duplication (►**Fig. 1C**). The 33-year-old woman III.2 is healthy while her daughter (IV.1) presented with a neonatal arteria cerebri media infarct with subarachnoid and plexus choroideus hemorrhage. Her FXI level was normal for age at birth (65%), while it was temporary elevated (155%) at the age of 3 years but normalized on a control sample (99%). She carried no pathogenic variants in any of the known thrombosis and hemostasis genes using a panel test.[15] Sanger sequencing of *F11* SNP rs3733403, rs3756011, and rs2289252 in members of this pedigree showed that II.3 and III.2 inherited the other *F11* allele from affected (grand)mother I.2 than the allele present in index case II.6 and the other family members with duplicated *F11* (►**Fig. 1A**, ►**Supplementary Table S3**, and ►**Supplementary Figs. S1–S3**, available in the online version). Family members III.7 and III.8 with normal FXI levels and no *F11* deletion carried other *F11* SNP than their affected father and mother, respectively, pointing out the presence of frequent chromosomal rearrangements on the duplicated *F11* allele. The genetic variant rs2289252 was previously associated with elevated FXI levels and contributed to the risk of VTE, but these values were never higher than the upper value of the normal range.[10,16,17]

This report is the first described case of a family with *F11* gene duplication leading to elevated FXI activity levels. As described above, these increased FXI activity levels can be an additional risk factor for thrombosis. In this family the presence of *F11* gene duplication is important for thrombophilia risk management.

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Conflict of Interest
None declared.

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