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A Quantitative-Trait Locus in the Human Factor XII Gene Influences Both Plasma Factor XII Levels and Susceptibility to Thrombotic Disease

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One approach to the identification of genetic loci that influence complex diseases is through the study of quantitative risk factors correlated with disease susceptibility. Factor XII (FXII) plasma levels, a related phenotype correlated with thrombosis, is such a risk factor. We conducted the first genomewide linkage screen to localize genes that influence variation in FXII levels. Two loci were detected: one on chromosome 5 and another on chromosome 10 (LOD scores 4.73 and 3.53, respectively). On chromosome 5, the peak LOD score occurred in the 5q33-5ter region, near the *FXII* gene. Addition of a 46C/T mutation in the *FXII* gene increased the multipoint LOD score to 10.21 ($P = 3.6 \times 10^{-12}$). A bivariate linkage analysis of FXII activity and thrombosis further improved the linkage signal (LOD = 11.73) and provided strong evidence that this quantitative-trait locus (QTL) has a pleiotropic effect on the risk of thrombosis ($P = .004$). Linkage analysis conditional on 46C/T indicated that this mutation alone cannot explain the chromosome 5 signal, implying that other functional sites must exist. These results represent the first direct genetic evidence that a QTL in or near the *FXII* gene influences both FXII activity and susceptibility to thrombosis and suggest the presence of one or more still unknown functional variants in *FXII*.

Introduction

Thrombosis is a common cause of morbidity and mortality in industrialized nations. Both venous and arterial thrombosis can be life-threatening events, and both are of great public health importance. Although there is little direct information on prevalence, retrospective and prospective data (Coon et al. 1973; Nordstrom et al. 1992; Anderson et al. 1991) suggest a minimum lifetime prevalence of 5%–10% for deep-vein thrombosis alone. After the inclusion of arterial thromboses, other venous thromboses, and undiagnosed thrombotic conditions, the true lifetime prevalence of thrombosis must be substantially larger than 10%.

The canonical causes of thrombosis include both environmental and genetic factors. The high prevalence of thrombosis and its known environmental influences (e.g., oral contraceptive use) suggest that multiple genes of varying effects will be involved in determining susceptibility to thrombosis. There are several well-characterized genetic defects that lead to increased thrombotic risk (Lane et al. 1996). However, very little information is available on the relative importance of genetic factors in thrombosis risk in the general population. Moreover, it is unlikely that these known mutations, with their comparatively low frequencies, constitute the primary genetic influences on the risk of common late-onset thrombosis. Recently, as part of the GAIT (Genetic Analysis of Idiopathic Thrombophilia) project, we quantified the genetic component of susceptibility to thrombosis and related phenotypes (Souto et al. 2000*a*, 2000*b*). Among the clotting factors studied, factor XII (FXII) levels exhibited one of the highest heritabilities (67%) and a significant positive genetic correlation (0.351) with thrombotic disease (Souto et al. 2000*a*), indicating that some of the genes that influence variation in this physiological risk factor also influence liability to thrombosis. Therefore, we performed a genome scan to identify genes influencing FXII levels. To our knowledge, our study represents the first genomewide scan undertaken to identify regions containing genes that influence variation in susceptibility to thrombotic disease and its intermediate phenotypes. The identification of such regions and genes may help to elucidate the mecha-

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nism underlying the risk of common thrombosis and therefore may suggest preventive strategies to reduce thrombosis-related morbidity and mortality.

Subjects and Methods

Subjects and Phenotypes

The recruitment, sampling, and phenotyping methods used in the GAIT project have been extensively described elsewhere (Soria et al. 2000; Souto et al. 2000*b*;). In brief, the sample included 21 families (398 individuals) selected primarily for pedigree size, to maximize the power to detect genetic effects. To be included, a family was required to have ⩾10 living individuals in three or more generations. Twelve families were selected through a proband with idiopathic thrombophilia, which was defined as a personal history of multiple thrombotic events (at least one of which spontaneous), a history of a single spontaneous episode of thrombosis plus a first-degree relative who was also affected, or onset of thrombosis at <45 years of age. Of the 12 probands, 10 had onset at age <45 years, 8 experienced multiple thromboses, and only 2 were ascertained because of a single episode of thrombosis with a relative also affected. Diagnoses of the thrombophilic probands were verified by objective methods. A proband's thrombophilia was considered idiopathic if all known (during the recruitment period of 1995–1997) biological causes (e.g., antithrombin deficiency, protein S and C deficiencies, activated protein C resistance, plasminogen deficiency, heparin cofactor II deficiency, Leiden factor V, dysfibrogenemia, lupus anticoagulant, and antiphospholipid antibodies) of thrombophilia were excluded. These thrombophilic factors were also absent in all affected relatives. The remaining 9 of the 21 families were selected without regard to phenotype.

FXII was assayed using deficient plasma from Diagnostica Stago (Asnières) by automated coagulometry as described elsewhere (Souto et al. 2000*b*). To reduce measurement error, assays were performed in duplicate, and the average value calculated for each person. The inter-assay coefficient of variation was estimated in 5.5%.

All procedures were reviewed by the institutional review board of the Hospital de la Santa Creu i Sant Pau (Barcelona). Adult subjects gave informed consent for themselves and their minor children.

Genotypes

DNA was extracted using a standard protocol (Miller et al. 1988). The present genome scan used a total of 363 highly informative microsatellite markers, spaced at ~9.5 cM. The microsatellites consisted primarily of the ABI-Prism genotyping set MD-10. Linkage mapping was undertaken, using multiplex PCR with the PE LMS II fluorescent marker set; in a few instances, nearby Généthon markers were substituted for LMS II markers, to improve robustness. The PCR products were analyzed on PE 310, PE 377, and PE 3700 automated sequencers and were genotyped using the PE Genotyper software. Information on microsatellite markers can be found in the publicly accessible Genome Database. The average heterozygosity of these markers was 0.79.

Markers in or near several hemostasis-related candidate genes were used to augment this genome scan. The 46T/C polymorphism in the FXII gene was amplified as reported elsewhere (Kanaji et al. 1998), with minor modification. In brief, we used *Sfa*NI, which provided better resolution of the digested bands. The genotypic data were entered into a database and were analysed for discrepancies (i.e., violations of Mendelian inheritance), using the INFER (PEDSYS) program (Dyke 1995). Discrepancies were checked in the laboratory for mistyping, and markers for discrepant individuals were either corrected or excluded from the analysis.

Linkage Analysis

Standard oligogenic multipoint variance-component linkage methods, as implemented in the SOLAR software program, were used for the genome scan (Almasy and Blangero 1998). Previous studies have suggested that such methods may be vulnerable to deviations from multivariate normality and particularly to high levels of kurtosis in the trait distribution (Allison et al 1999). Levels of FXII in the GAIT sample exhibited a kurtosis of 0.05. Recent statistical genetic theory demonstrates that this level of kurtosis will not affect the distribution of LOD scores and that the standard nominal *P* values for LOD scores are appropriate for the FXII linkage screen (Blangero et al. 2000). Allele frequencies were estimated from the GAIT sample, and marker maps for multipoint analyses were obtained from ABI-Prism and from the Marshfield Center for Medical Genetics. Because 12 of the families were ascertained through thrombophilic probands, all analyses included an ascertainment correction achieved by conditioning the likelihood of these pedigrees on the likelihoods of their respective probands (Boehnke and Lange 1984). genomewide *P* values were calculated using the method of Feingold et al. 1993. Bivariate linkage analyses using the mixed discrete/continuous trait multivariate model were conducted with a modified version of SOLAR (Williams et al. 1999).

Measured Genotype and Conditional Linkage Analyses

Quantitative-trait association analysis was performed using the measured genotype approach (Hopper and Mathews 1982) by testing for genotype-specific differ-

Table 1

Results from the Initial and Conditional Genomewide Linkage Screens of FXII

Chromosome	Position (cM)	LOD Score for		
		First Pass	Second Pass	Third Pass
5	193	4.73	...	...
10	38	3.53	3.43	...
2	9	2.26	1.83	.59
11	10	1.30	.44	.19
14	63	1.16	.75	.90
15	79	1.03	.41	.35

NOTE.—All loci with LOD scores >1.0 in the initial pass are shown. The second linkage pass was conditional on a locus on chromosome 5q, and the third pass was conditional on loci on 5q and 10p.

ences in the means of traits while allowing for the nonindependence among family members. These analyses were performed using SOLAR (Almasy and Blangero 1998). To assess linkage conditional on the observed association (Amos 1994), an extension of the variance-component–based linkage test was performed by simultaneously incorporating the genotype-specific means of the measured genotype test and estimating a linkage component.

Results

Multipoint variance-component methods were used to assess linkage between highly informative autosomal DNA markers, spaced at ~9.5 cM, and plasma levels of FXII. The results of the initial FXII linkage screen are shown in figure 1 and table 1. The linkage analysis revealed strong evidence that quantitative-trait loci (QTLs) on chromosomes 5q influence FXII levels (LOD = 4.73; nominal $P = 1.5 \times 10^{-6}$; genomewide $P = .00046$), as do QTLs at chromosome 10p (LOD = 3.43, nominal $P = 3.5 \times 10^{-5}$; genomewide $P = .0114$). Additionally, suggestive evidence of linkage (LOD scores of 1.0–2.3) were observed on chromosomes 2p, 11p, 14q, and 15q. Given the strong evidence for multiple QTLs that influence FXII levels, we performed a series of multilocus linkage screens, in which QTLs were sequentially incorporated into the linkage model and the genome

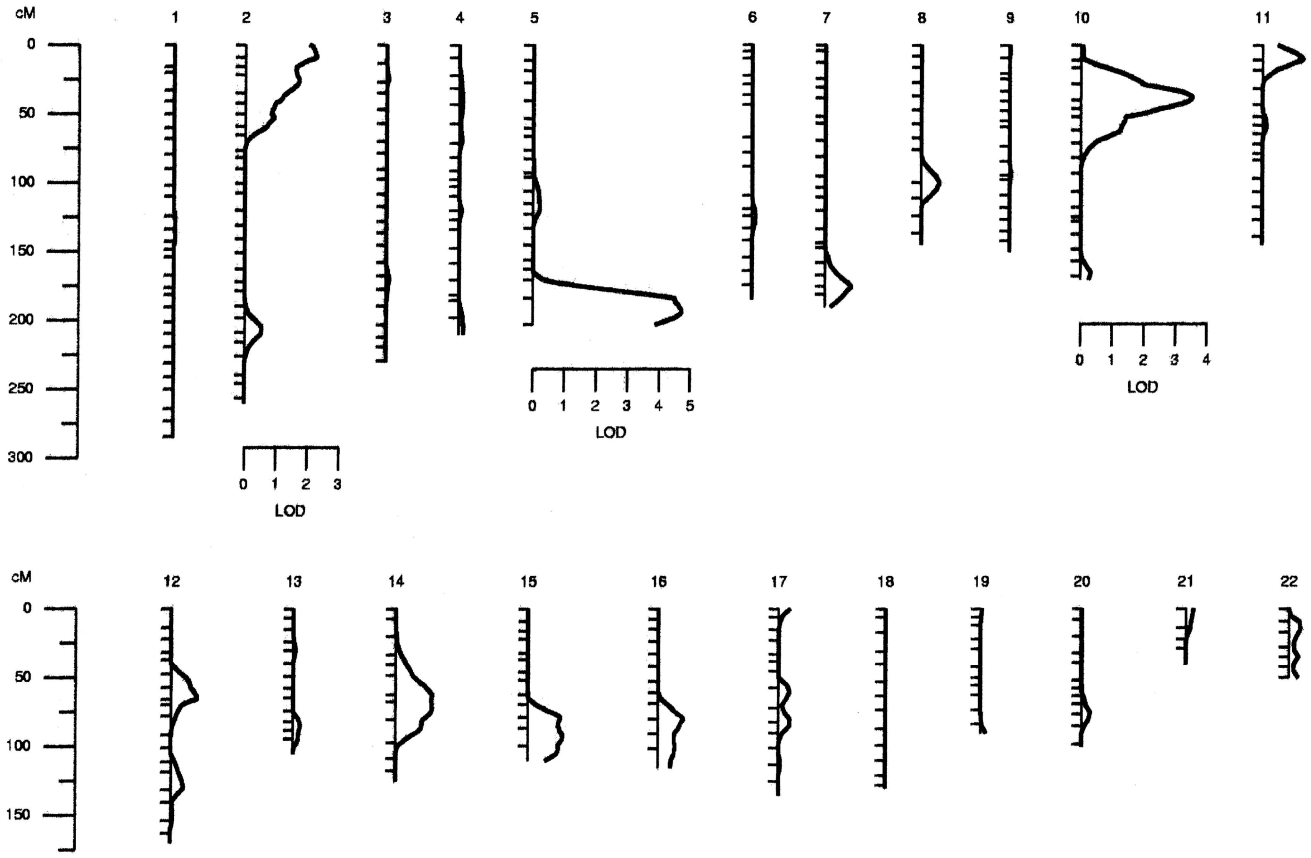

Figure 1 Results of the initial linkage screen

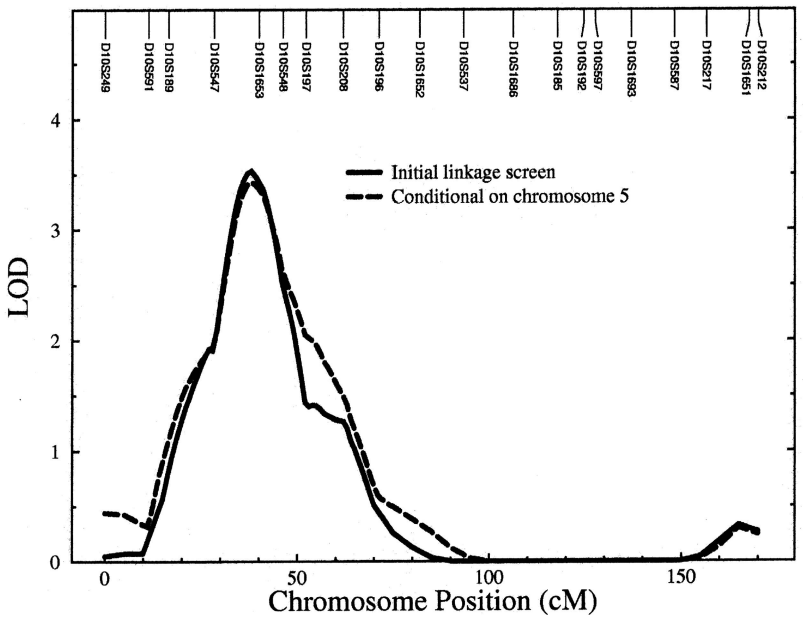

Figure 2 Initial and conditional LOD scores on chromosome 10

was rescreened (table 1). In the second linkage pass, which was conditional on the chromosome 5 QTL that was detected in the initial screen, evidence for a QTL on chromosome 10 remained strong (LOD = 3.43), whereas LOD scores in other regions that previously showed suggestive linkage declined slightly. Conditional on the chromosome 5 and 10 QTLs, little evidence for additional QTLs remained in a third linkage screen (all LOD scores < 1.0).

The peak LOD score on chromosome 10 occurred between markers D10S189 and D10S1653 in the10p13 region (fig. 2). On chromosome 5, the peak LOD score occurred between markers D5S400 and D5S408 in a region that maps to 5q33-5ter (fig. 3). Because the hu-

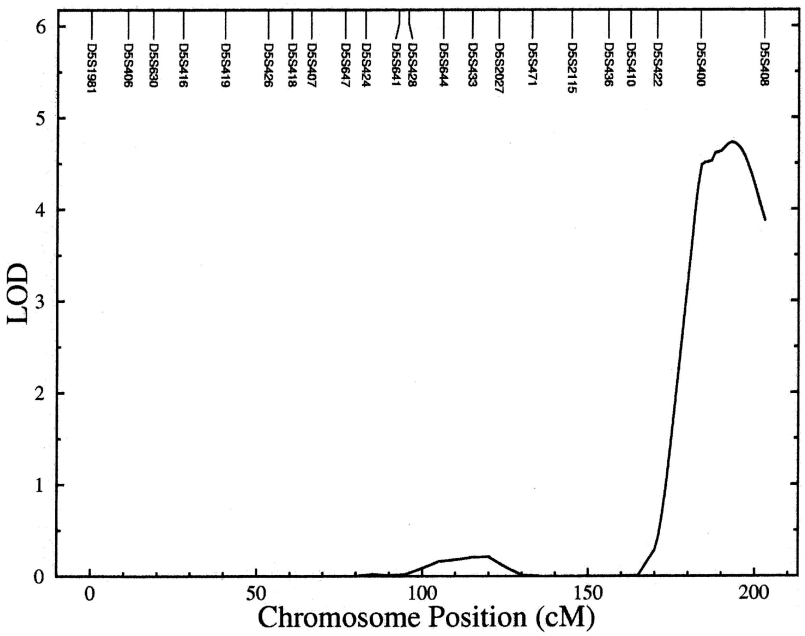

Figure 3 Detailed linkage results for chromosome 5

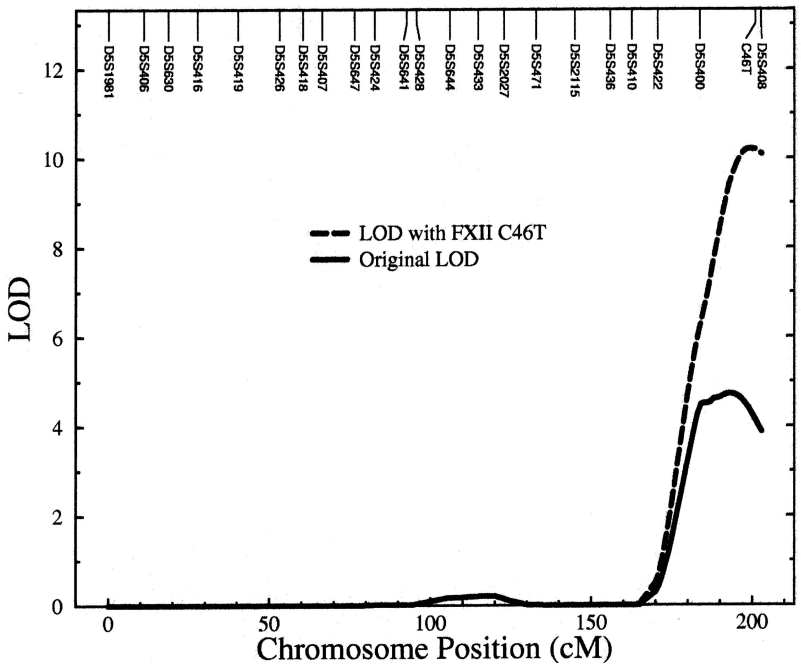

Figure 4 Detailed linkage results for chromosome 5 when the 46C/T variant was added

man *FXII* gene has been mapped to this region (Royle et al. 1988), we have genotyped a known polymorphism in exon 1 of the *FXII* gene (46C/T). In the present study, we found 264 homozygotes for the common variant (*C/C*), 117 heterozygotes (C/*T*), and 11 homozygotes (*T/T*); the maximum-likelihood estimate of the frequency of the T allele was 0.21. If Hardy-Weinberg equilibrium is assumed, this allele frequency predicts a heterozygote frequency of 0.331.

When this variant was added to the chromosome 5 linkage map, the multipoint LOD score increased dramatically to 10.21 ($P = 3.6 \times 10^{-12}$; fig. 4). The results support the presence of a major QTL in the region of the *FXII* gene. Given this unequivocal evidence for linkage, we performed an association analysis to test for linkage disequilibrium between the QTL and the 46C/T variant. This measured-genotype–association analysis revealed significant differences among the three 46C/T genotypes in their plasma FXII values ($CC = 128.9$; $CT = 92.2$; $TT = 55.6$; $P < 1 \times 10^{-7}$). Under the assumption that 46C/T affects the function of the *FXII* locus, we calculated that this mutation accounts for 40% of the variance in FXII activity levels in this population.

Because our previous studies have suggested that FXII levels are correlated with risk of thrombosis and that this relationship is, in part, a result of genes that influence both traits (Souto et al. 2000*a*), we wished to test whether either of the QTLs influencing FXII levels might also contribute to genetic susceptibility to thrombosis. This was accomplished through bivariate linkage analysis of FXII and thrombosis (Williams et al. 1999). Such bivariate analyses with related phenotypes have been shown to increase power to detect linkage (Soria et al. 2000). In the combined analysis of FXII levels and thrombosis, the LOD score on chromosome 10 remained at its previous level, providing no evidence that this QTL influences thrombosis risk, whereas the LOD score on chromosome 5 rose to 11.73, providing strong evidence that this QTL has a pleiotropic effect on the risk of thrombosis ($P = .004$).

To determine whether the 46C/T marker could be the functional QTL underlying our linkage signal, we performed a conditional linkage analysis (Soria et al. 2000) that simultaneously accounted for association with the 46C/T mutation. Conditional on 46C/T, evidence for linkage to 5q still remained (LOD = 0.9; fig. 4), indicating that, although 46C/T may be functional, there must be one or more additional polymorphisms in the same region that influence FXII levels and thrombosis risk.

Discussion

Our results represent the first genomewide scan undertaken to identify regions containing genes that influence variation in susceptibility to thrombotic disease and their

related phenotypes. Initial and subsequent conditional passes of variance-component linkage analyses revealed two regions, one on chromosome 5 and another on chromosome 10, that showed strong evidence of linkage with levels of plasma FXII, an important intermediate phenotype correlated with thrombosis (Souto et al. 2000*a*).

In the region of the linkage signal on chromosome 10, there are no obvious candidate hemostasis-related genes. In contrast, we have documented the close linkage between a QTL influencing FXII levels and the *FXII* gene (specifically, the 46C/T FXII DNA variant). When we added this genetic variant as a marker, a highly significant LOD score of 10.21 was calculated, providing strong evidence for the existence of a QTL that influences FXII activity.

Recent case-control studies demonstrated association between the 46C/T polymorphism in exon 1 of the *FXII* gene and variation in levels of FXII (Kanaji et al. 1998; Ishii et al. 2000). In our study, under the assumption that 46C/T is itself a functional QTL, we calculated that this mutation accounts for 40% of the variance in FXII activity levels in our population. If 46C/T is not itself functional but is only in disequilibrium with another site, this effect size may be an underestimate. There remains substantial residual genetic variation in FXII activity, even after taking into account the effects of 46C/T. This indicates that there are other QTLs that influence FXII activity level, as is suggested by the highly significant linkage signal on chromosome 10. Moreover, the residual linkage signal (LOD = 0.9) in the combined linkage/disequilibrium analysis (Soria et al. 2000), which simultaneously allowed for association between the 46C/T polymorphism and FXII levels, indicated that, although 46C/T may be functional, there must be one or more additional polymorphisms in the region that influences FXII levels. This result supports the observation we reported elsewhere that multiple QTLs of varying effects will be involved in determining variation in hemostasis-related phenotypes (Souto et al. 2000*a,* 2000*b*).

In addition to our analyses, there is biochemical evidence of a functional role of the 46C/T in the phenotypic variability of FXII levels. Kanaji et al. (1998) reported that this mutation decreased the translation efficiency and led to low plasma levels of FXII activity and antigen, probably as a result of the creation of another ATG codon and/or the impairment of the consensus sequence for the translation-initiation scanning model. On the basis of these biochemical results and our linkage analyses, we expect that 46C/T is a functional polymorphisms in *FXII*. To identify the other variant(s), the next step is to catalog, by use of DNA resequencing, the complete menu of DNA sequence variation within the *FXII* gene. Because variation within noncoding regions may influence the regulation of transcription and other genetic functions, DNA resequencing should not be limited to the exons of the gene, but should include 5′ and 3′ regulatory regions, as well as introns. Because *FXII* consists of 13 introns and 14 exons that cover 12 kb (Cool and MacGillivray 1987), this task may be attainable.

It is important to note that this study confirms and extends our previous observation that FXII levels are correlated with risk of thrombosis and that this relationship is due in part to genes that influence both traits (Souto et al. 2000*a*). This was accomplished through bivariate linkage analysis of FXII and thrombosis (Williams et al. 1999). Such bivariate analyses with related phenotypes increase the power to detect linkage (Soria et al. 2000). The combined analysis of FXII levels and thrombosis risk substantially enhanced the linkage signal on chromosome 5, providing strong evidence that this QTL has a pleiotropic effect on the risk of thrombosis. However, the LOD score on chromosome 10 remained at its previous level, providing no evidence that this QTL influences thrombosis risk. Our results confirm the valuable potential of this statistical approach as a basic tool for mapping the genes that affect complex diseases.

Despite the important putative role of FXII—which is a serine protease precursor involved in the initiation of the intrinsic coagulation pathway, in fibrinolysis, in the generation of bradykinin, and in the complement system (Kaplan and Silverberg 1987; Kluft et al. 1987)—its biologic role is not fully understood. Furthermore, there is controversy over the clinical significance of a decreased or increased FXII concentration on venous and arterial thrombosis (Halbmayer et al. 1992; Helft et al. 2000). Further investigation of *FXII* and other genes may enhance our understanding of the factors influencing thrombosis, especially the chromosome 10p13 region, which should be targeted for fine mapping, and gene-identification studies.

In conclusion, our study represents the first direct genetic evidence that at least one QTL in the *FXII* gene influences both FXII levels and susceptibility to thrombosis. Our results also support the conclusion that the 46C/T polymorphism, in addition to at least one other unknown functional variant in the *FXII* gene, is likely to be one of these QTLs. Therefore, variations in the *FXII* gene should be considered as potential genetic risk factors for thrombosis. However, exhaustive enumeration of all potentially functional variants will be required to completely document the allelic architecture of such thrombosis risk–related variation.

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Electronic-Database Information

The URLs for data in this article are as follows:

ABI-Prism, http://www.appliedbiosystems.com/ (for genetic markers)
Centre National de Genotypage, http://www.cng.fr/ (for genetic markers)
Genome Database, http://www.gdb.org (for genetic markers)
Center for Medical Genetics, Marshfield Medical Research Foundation http://research.marshfieldclinic.org/genetics/ (for genetic markers)
Southwest Foundation for Biomedical Research, http://www.sfbr.org/ (for SOLAR program package)

References

Allison DB, Neale MC, Zannolli R, Schork NJ, Amos CI, Blangero J (1999) Testing the robustness of the likelihood-ratio test in a variance-component quantitative-trait loci–mapping procedure. Am J Hum Genet 65:531–544

Almasy L, Blangero JC (1998) Multipoint quantitative trait linkage analysis in general pedigrees. Am J Hum Genet 62: 1198–1211

Amos CI (1994) Robust variance-components approach for assessing genetic linkage in pedigrees. Am J Hum Genet 54: 535–543

Anderson FA, Wheeler HB, Goldberg RJ, Hosmer DW, Forcier A, Patwardhan NA (1991) A population-based perspective of the hospital incidence and case-fatality rates of deep vein thrombosis and pulmonary embolism: the Worcester DVT Study. Arch Intern Med 151:933–938

Blangero J, Williams JT, Almasy L (2000) Robust LOD scores for variance component-based linkage analysis. Genet Epidemiol Suppl 19:S8–S14

Boehnke M, Lange K (1984) Ascertainment and goodness of fit of variance component models for pedigree data. Prog Clin Biol Res 147:173–192

Cool DE, MacGillivray RT (1987) Characterization of the human blood coagulation factor XII gene: intron/exon gene organization and analysis of the 5′-flanking region. J Biol Chem 262:13662–13673

Coon WW, Park WW, Keller JB (1973) Venous thromboemolism and other venous disease in the Tecumseh Community Health Study. Circulation 48:839–846

Dyke B (1995) PEDSYS: a pedigree data management system user's manual. PGL Tech rep 2. Population Laboratory, Department of Genetics, Southwest Foundation for Biomedical Research, San Antonio

Feingold E, Brown PO, Siegmund D (1993) Gaussian models for genetic linkage analysis using complete high-resolution maps of identity by descent. Am J Hum Genet 53:234–251

Halbmayer W-M, Mannhalter C, Feichtinger C, Rubi K, Fischer M (1992) The prevalence of factor XII deficiency in 103 orally anticoagulated outpatients suffering from recurrent venous and/or arterial thromboembolism. Thromb Haemost 68:285–290

Helft G, Le Feuvre C, Metzger JP, Vacheron A, Monsuez JJ, Lachurie ML, Ahlenc-Gelas M (2000) Factor XII deficiency associated with coronary stent thrombosis. Am J Hematol 64:322–323

Hopper JL, Mathews JD (1982) Extensions to multivariate normal models for pedigree analysis. Ann Hum Genet 46:373–383

Ishii K, Oguchi S, Murata M, Mitsuyoshi Y, Takeshita E, Ito D, Tanahashi N, Fukuuchi Y, Oosumi K, Matsumoto K, Kitajima M, Yamamoto M, Watanabe G, Ikeda Y, Watanabe K (2000) Activated factor XII levels are dependent on factor XII 46C/T genotypes and factor XII zymogen levels, and are associated with vascular risk factors in patients and healthy subjects. Blood Coagul Fibrinolysis 11:277–284

Kanaji T, Okamura T, Osaki K, Kuroiwa M, Shimoda K, Hamasaki N, Niho Y (1998) A common genetic polymorphism (46 C to T substitution) in the 5′-untranslated region of the coagulation factor XII gene is associated with low translation efficiency and decrease in plasma factor XII level. Blood 91:2010–2014

Kaplan AP, Silverberg M (1987) The coagulation-kinin pathway of human plasma. Blood 70:1–15

Kluft C, Dooijewaard G, Emeis JJ (1987) Role of the contact system in fibrinolysis. Semin Thromb Hemost 13:50–68

Lane DA, Mannucci PM, Bauer KA, Bertina RM, Bochkov NP, Boulyjenkov V, Chandy M, Dahlback B, Ginter EK, Miletich JP, Rosendaal FR, Seligsohn U (1996) Inherited thrombophilia: part 1. Thromb Haemost 76:651–662

Miller SA, Dykes DD, Polesky HF (1988) A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acid Res 16:1215

Nordstrom M, Lindblad B, Bergqvist D, Kjellstrom T (1992) A prospective study of the incidence of deep-vein thrombosis within a defined urban population. J Intern Med 232:155–160

Royle NJ, Nigli M, Cool D, MacGillivray RT, Hamerton JL (1988) Structural gene encoding human factor XII is located at 5q33-qter. Somat Cell Mol Genet 14:217–221

Soria JM, Almasy L, Souto JC, Tirado I, Borrell M, Mateo J, Slifer S, Stone W, Blangero J, Fontcuberta J (2000) Linkage analysis demonstrated than the prothrombin G20210A mutation jointly influences plasma prothrombin levels and risk of thrombosis. Blood 95:2780–2785

Souto JC, Almasy L, Borrell M, Blanco-Vaca F, Mateo J, Soria JM, Coll I, Felices R, Stone W, Fontcuberta J, Blangero J (2000*a*) Genetic susceptibility to thrombosis and its relationship to physiological risk factors: the GAIT study. Am J Hum Genet 67:1452–1459

Souto JC, Almasy L, Borrell M, Garí M, Martínez E, Mateo J, Stone W, Blangero J, Fontcuberta J (2000*b*) Genetic determinants of hemostasis phenotypes in Spanish families. Circulation 101:1546–1551

Williams JT, VanEerdewegh P, Almasy L, Blangero J (1999) Joint multipoint linkage analysis of multivariate qualitative and quantitative traits. I. Likelihood formulation and simulation results. Am J Hum Genet 65:1134–1147