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# Joint Linkage and Association of Six Single-Nucleotide Polymorphisms in the Factor XIII-A Subunit Gene Point to V34L As the Main Functional Locus

Marlies de Lange, Toby Andrew, Harold Snieder, Dongliang Ge, T. Simon Futers, Kristina Standeven, Tim D. Spector, Peter J. Grant, Robert A.S. Ariëns

**Objective**—Activated factor XIII (FXIII) crosslinks fibrin to enhance the mechanical strength of a blood clot and increase its resistance to fibrinolysis. The prevalence of a common variant in the FXIII-A gene (V34L) has been reported to be lower in patients with myocardial infarction and ischemic stroke than in controls, suggesting a protective role for this polymorphism in vascular diseases. The current study investigated 6 single-nucleotide polymorphisms (SNPs) within the FXIII A-subunit gene to locate functional polymorphism(s) responsible for variation in FXIII activation.

**Methods and Results**—A total of 201 dizygotic twin pairs were genotyped for 1 promoter and all common nonsynonymous coding polymorphisms in the FXIII A-subunit gene:  $-246G>A$ , V34L, Y204F, P564L, V650I, and E651Q. Tests of linkage, association, and combined linkage and association were performed using QTDT software. Significant linkage to the V34L polymorphism ( $P=5\times 10^{-12}$ ) as well as association ( $P=3\times 10^{-49}$ ) was observed. Adjusting for association while performing linkage made the linkage signal disappear for the V34L polymorphism (from  $\chi^2=47.55$ ,  $P=5\times 10^{-12}$  to  $\chi^2=1.30$ ,  $P=0.25$ ). Only haplotypes containing the 34L allele showed association with FXIII activation.

**Conclusion**—Testing multiple SNPs in the FXIII A-subunit gene indicates that V34L is the main functional polymorphism influencing FXIII activation. (*Arterioscler Thromb Vasc Biol.* 2006;26:1914-1919.)

**Key Words:** joint linkage and association analysis ■ functional locus ■ FXIII/V34L ■ FXIII activation ■ twins

The familial risk for the development of coronary artery disease (CAD) is well recognized. Twin studies have shown that familial risk for CAD is mainly genetic,<sup>1,2</sup> mediated through genetic effects on underlying biological processes involved in atherosclerosis.<sup>3</sup> The complex nature of this genetic component is most likely the cause of the relative minor success that has been achieved in relating polymorphisms directly to CAD outcome.<sup>4</sup>

Factor XIII (FXIII), when activated by thrombin, covalently crosslinks fibrin multimer strands, thereby enhancing the mechanical strength of a clot and increasing the resistance to fibrinolysis.<sup>5</sup> The prevalence of a common variant in the FXIII-A gene (V34L) was lower in patients with myocardial infarction (MI) than those without, suggesting a protective role for this polymorphism.<sup>6,7</sup> Paradoxically, a stepwise increase in FXIII activation with possession of the protective Leu allele has been found, with no differences in FXIII-A and B-subunit levels.<sup>8,9</sup> In vitro studies have shown that 34L is cleaved faster by thrombin than the Val variant,<sup>10</sup> with an increase in proteolytic efficiency by 2- to 3-fold.<sup>10,11</sup> How this relates to the risk of vascular disease remains to be fully elucidated.

We have previously shown moderate to high degree of genetic influence on the variation for FXIII levels, explaining 82% of FXIII activation, 64% and 41% for FXIII-A and B-subunit levels, respectively.<sup>12</sup> It is currently unknown to what degree single-nucleotide polymorphisms (SNPs) in the FXIII genes contribute to the genetic variability. This study aimed to investigate which SNP is the main functional polymorphism influencing FXIII activation among 1 common promoter and all common (allele frequency >1%) nonsynonymous polymorphisms in the FXIII A-subunit gene. To this end, we performed combined linkage and association analysis in a sample of 201 dizygotic (DZ) twin pairs.

## Subjects and Methods

### Subjects

A total of 201 DZ healthy white female twin pairs aged 19 to 73 from the TwinsUK Registry<sup>13</sup> were enrolled for this study. Twins from the registry were recruited from the general population through national media campaigns in the United Kingdom. Participating twins were unaware of the specific hypotheses tested and informed consent was obtained from all subjects. The study was approved by the St Thomas' Hospital Research Ethics Committee. Zygosity was deter-

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mined by standardized questionnaire and DNA fingerprinting was used for confirmation.<sup>14</sup> Information on medication use, demographic variables, and fasting time was obtained by standardized nurse administered questionnaire. All DZ twins selected for genotyping were fasting for 8 hours or more and not on oral anticoagulant treatment. For some DZ twins, insufficient DNA was available to genotype all 6 SNPs; for one twin only V34L could be genotyped and for one twin only V34L and -246G>A could be genotyped. For 3, DZ twins insufficient DNA was available to genotype -246G>A, whereas for 2 DZ twins, Y204F and V650I could not be genotyped.

### Blood Sampling and Factor XIII Assays

A venous blood sample was taken between 8:00 and 10:00 AM after an overnight fast. The blood was taken within 5 minutes from the cotwin into 0.13 mol/L trisodium citrate vacutainers (Becton Dickinson, France) and kept on ice for tests of fibrinolysis and at room temperature for tests of coagulation as described previously.<sup>12</sup> Within 1 hour of collection, the samples were centrifuged at 2560g for 20 minutes to obtain platelet-poor plasma, frozen in aliquots in liquid nitrogen (LN<sub>2</sub>) and stored at -40°C until analysis. FXIII activation rate (previously called FXIII activity) was analyzed by a kinetic biotinamido pentylamine-incorporation assay and FXIII A-subunit and B-subunit by sandwich ELISAs as described.<sup>15</sup>

### DNA Extraction

DNA was extracted at the Twin Research Unit using the BACC2 DNA extraction kit (Nucleon Biosciences, Glasgow, UK) from 10 mL of venous blood collected on 1.6 mg/mL EDTA. Extracted DNA was diluted in Tris-EDTA buffer and stored at -20°C until further analysis. Amplification of DNA was carried out by polymerase chain reaction (PCR) in a final volume of 25  $\mu$ L, containing 100 ng of genomic DNA. PCR amplification was carried out using a PTC-100 thermal cycler (MJ Research, Watertown, Mass).

### Genotyping of Six SNPs in the FXIII

#### A-subunit Gene

The twins were genotyped for a common promoter and all common nonsynonymous coding polymorphisms in the FXIII A-subunit gene (references for genome and messenger sequences respectively: NT 034880 and NM 000129) located on chromosome 6. The following 6 SNPs were genotyped: g.-246G>A (rs1024231) in the promoter region (numbered in relation to the transcription start site; -2231 from translation start codon), V34L (c.103G>T; rs5985), Y204F (c.614A>T; rs3024477), P564L (c.1694C>T; rs5982), V650I (c.1951G>A; rs5987), and E651Q (c.1954G>C; rs5988). For the coding SNPs, the amino acid substitutions are numbered for the mature protein and the base substitutions are numbered from the translation start site. The -246G>A promoter polymorphism was determined by PCR amplification and *FokI* restriction analysis. Restriction products were separated on a 4% Metaphore gel (Flowgen, Nottingham, UK). The G allele resulted in a single band of 97 bp, and the A allele resulted in bands of 85 and 12 bp. V34L is a common G-to-T transition in exon 2 coding for a substitution of valine with leucine at residue 34. The V34L polymorphism genotyping by PCR amplification and *DdeI* digestion was as described by Kangsadalampai and Board.<sup>16</sup> The *DdeI* digestion products were resolved on a 3% agarose gel. The presence of the G allele produces a 192-bp fragment and the presence of the T allele fragments of 161 and 31 bp. Y204F was analyzed by PCR amplification followed by restriction digestion with *RsaI*. The phenylalanine allele PCR product is cut into 92- and 20-bp fragments, the tyrosine allele product remains uncut at 112 bp. P564L constitutes a C-to-T transition at +1694 in exon 12 and alters proline 564 to leucine in barrel 1. FXIII E651Q is a G-to-C transition at +1954 in exon 14 and alters glutamic acid 651 to glutamine. P564L and E651Q were genotyped as described previously.<sup>17</sup> V650I was PCR amplified and analyzed by restriction digestion with *HinfI*. The G allele leads to 98- and 26-bp bands, and the C allele remains uncut at 124 bp. All primer sequences and PCR conditions are available on request.

### Preliminary Analysis

FXIII activation was log transformed before linkage and association analysis to obtain a distribution that was approximately normal. This log-transformed variable was adjusted for age, date of blood sampling (ie, seasonal variation), and fasting status as described previously.<sup>12</sup> Tests of Hardy-Weinberg equilibrium were carried out in STATA 8 (StatCorp, College Station, Tex). Pairwise linkage disequilibrium (LD) coefficients were reported as the ratio of the nonstandardized coefficient to the maximal value ( $D' = D/D_{\max}$ ).

### Linkage and Association

Tests for linkage, allelic association, and joint linkage and association were carried out using QTDT (Quantitative Transmission Disequilibrium Tests) software.<sup>18</sup> The simplest test for linkage analysis using sib pairs is the method by Haseman and Elston,<sup>19</sup> in which it is postulated that siblings (or twins) who share more alleles identical by descent (IBD) will resemble each other more if that locus (or a locus in close proximity) influences the trait. Using maximum likelihood variance components analysis, the linkage component can be modeled as part of the covariance structure. For this, the estimation of the probability distribution of sharing 0, 1, or 2 alleles IBD for each SNP was accomplished using Genehunter 2 software<sup>20</sup> and used in QTDT software to test for linkage, ie, whether sharing alleles IBD affects phenotypic similarity (or covariance) between the DZ pairs.

Tests of overall allelic association, such as implemented in QTDT, compare mean levels of the hemostatic protein between genotype groups while taking into account the nonindependence of the twin pairs.

Fulker et al<sup>21</sup> developed a method for simultaneous modeling of association and linkage for quantitative traits using sib pair data that also control for population stratification. Combined linkage and association analysis is a powerful tool for pinpointing functional loci responsible for a linkage signal. Assuming there is significant linkage before modeling association, the extent to which evidence for linkage diminishes in the joint test of linkage and association reflects the proximity of the marker to the functional quantitative trait locus (QTL).<sup>21,22</sup> If the linkage signal of a certain locus is entirely explained by modeling association (ie, the linkage signal is no longer significant), that particular locus is either the functional locus or in tight LD with the functional locus.

Apart from close matching of environmental factors, an additional advantage of using family data such as DZ twins is that it is possible to test and control for population stratification as a source of spurious associations by conducting within family association tests such as the quantitative transmission disequilibrium tests implemented in QTDT.<sup>22,23</sup>

A sample size of 201 DZ twin pairs gave us 80% power ( $\alpha=0.05$ ) to detect a QTL explaining 19% of the variance with linkage.<sup>24</sup> For the within-family association and overall association tests, we had 80% power ( $\alpha=0.05$ ) to detect loci explaining 5% and 2% of the variance, respectively.<sup>24</sup>

Details of our approach to test the association of statistically inferred haplotypes with continuous traits have been described previously.<sup>25</sup> In short, we adapted haplotype trend regression from Zaykin et al for the analysis of related subjects such as twins<sup>26</sup> by replacing the linear regression with the GEE procedure using STATA. The probabilities of haplotype pairs were estimated by PHASE 2.0 software.<sup>27</sup>

### Results

All genotypes were in Hardy-Weinberg equilibrium, and no population stratification could be detected. Table 1 presents the general characteristics of the DZ twins used in this study. Table 2 presents the allele frequencies and LD coefficients ( $D'$ ) between the FXIII polymorphisms. Significant LD could be detected between each of the SNPs and their neighboring SNPs, but some LD extended over several SNPs (eg, between

**TABLE 1. General Characteristics of DZ Twins Used in QTDT Analysis**

Characteristics	DZ (n=201)
Age, y (SD)	49.8 (12.1)
Body mass index, kg/m <sup>2</sup> (SD)	25.7 (4.7)
Postmenopausal, n (%)	237 (61%)
Hormone replacement, n (%)	97 (24%)
Oral contraceptives, n (%)	29 (7%)
Smokers, n (%)	87 (22%)
FXIII activation, % (SD)*	107.3 (34.4)
FXIII A-subunit, units/mL (SD)#	1.08 (0.28)
FXIII B-subunit, units/mL (SD)#	1.01 (0.27)

\*Levels expressed as a percentage of pooled normal plasma. #Levels expressed as units per milliliter of pooled normal plasma.

V34L and P564L [ $D' = -0.59$ ] and between V34L and V650I [ $D' = 0.30$ ]).

Table 3 presents the differences in levels of FXIII activation for the different SNPs. Largest effect is seen for V34L, where twins homozygote for the Leu allele had 72% higher levels of untransformed FXIII activation compared with the Val homozygotes. In regression analysis, the polymorphism explained 39% of the variation in FXIII activation. Smaller, and opposite, effects were seen for the -246G>A and the P564L genotypes, where the homozygotes of the more frequent allele had 38% and 14.5% higher levels compared with the homozygotes of the less frequent allele, respectively. Only 1 twin had 2 alleles of the less frequent Phe allele of Y204F, but twins homozygote for the more frequent Tyr allele had 21.6% higher levels than the heterozygote twins (Table 3). Significant association to levels of FXIII activation was observed for -246G>A, V34L, Y204F, and P564L but not for V650I or E651Q. Permutation tests in QTDT for association of FXIII activation levels using only the within-family information (to account for possible undetected population stratification) calculated the empirical probability values at 0.0003 for -246G>A,  $5 \times 10^{-5}$  for V34L, 0.07 for Y204F, and 0.07 for P564L, with, respectively, 65, 75, 13, and 59 twin pairs informative for analysis.

Table 4 shows haplotype frequencies and results of haplotype association tests. Haplotype analyses confirmed individual SNP associations. Only haplotypes 2 and 7, which contained the 34Leu allele, showed significantly higher levels of FXIII activation ( $P \leq 9 \times 10^{-10}$ ) compared with the most common haplotype 1: 1-1-1-1-1. The  $\beta$  coefficient for

**TABLE 3. Results of Association Tests for FXIII Polymorphisms and Levels of FXIII Activation**

Locus (Genotype)	N	FXIII Activation	P
-246G>A			
G/G	246	112.57 (35.38)	
G/A	136	101.50 (31.50)	
A/A	16	81.63 (22.80)	$4 \times 10^{-6}$
V34L			
V/V	208	87.01 (23.78)	
V/L	163	125.14 (28.41)	
L/L	31	149.32 (33.43)	$3 \times 10^{-49}$
Y204F			
Y/Y	371	108.85 (34.47)	
Y/F	27	89.52 (27.35)	
F/F	1	52	0.0009
P564L			
P/P	241	112.10 (34.19)	
P/L	139	100.38 (33.97)	
L/L	20	97.90 (31.43)	0.001
V650I			
V/V	359	107.01 (34.51)	
V/I	38	111.5 (33.96)	
I/I	2	99 (42.43)	0.92
E651Q			
E/E	249	106.57 (34.56)	
E/Q	128	108.63 (33.88)	
Q/Q	23	108.04 (37.04)	0.72

Data expressed as mean (SD) percentage of pooled normal plasma.

haplotype 2 on FXIII activation was 66.5, meaning that subjects homozygote for haplotype 2 had FXIII activation levels that were 66.5 higher compared with subjects homozygote for the common haplotype 1. The overall probability value of the haplotype test was highly significant ( $P = 1 \times 10^{-41}$ ) with the haplotypes, explaining 31.7% of the variance in FXIII activation levels.

Significant linkage of FXIII activation was shown to V34L ( $P = 5 \times 10^{-12}$ ) and marginally to E651Q ( $P = 0.03$ ) but not to any of the other SNPs (Table 5). Linkage in the presence of association only showed a drop in fit for the V34L polymorphism (from  $\chi^2 = 47.55$ ,  $P = 5 \times 10^{-12}$ , to  $\chi^2 = 1.30$ ,  $P = 0.25$ ), indicating that this locus is either the functional polymorphism or in strong LD with the functional locus

**TABLE 2. Allele Frequency and Pairwise Linkage Disequilibrium Coefficients, or  $D'$ , of FXIII Polymorphisms in All Twins**

Locus	Physical Distance (bp)*	Allele Frequency	-246G>A	V34L	Y204F	P564L	V650I
-246G>A	—	0.79/0.21	—				
V34L	2333	0.72/0.28	-0.87‡	—			
Y204F	67675	0.96/0.04	-0.59	-0.80†	—		
P564L	76254	0.78/0.22	0.02	-0.59‡	0.62‡	—	
V650I	22,726	0.95/0.05	-0.25	0.30‡	-1	-0.99‡	—
E651Q	3	0.78/0.22	0.03	0.04	-1*	-0.10	0.96‡

\*Genomic physical distance in base pairs between adjacent SNPs. † $P < 0.01$ , ‡ $P < 0.001$ .

**TABLE 4. Association of FXIII A-Subunit Gene Haplotypes Levels of FXIII Activation**

Haplotype	Frequency (%)	$\beta$ (SE)	<i>P</i>	Variance Explained
111111*	28.5			
<b>121111</b> †	18.0	66.5 (6.8)	$3 \times 10^{-21}$	
211111	11.7	-7.8(8.7)	0.27	
111211	10.1	-8.3(9.7)	0.41	
111112	6.4	-7.8(10.7)	0.39	
<b>121112</b> †	3.4	84.3 (14.2)	$9 \times 10^{-10}$	
211211	3.3	-7.6(14.2)	0.50	
Overall			$1 \times 10^{-41}$	31.7%

Frequency, >3%. \*The most common haplotype with which the others were compared. 1 indicates major allele; 2, minor allele. †Haplotypes containing the 34Leu allele, represented by a boldface 2.

(Figure and Table 5). No drop in fit was observed for E651Q for which initial linkage was detected ( $P=0.03$ ), indicating that this SNP is a marker linked to the functional polymorphism.

### Discussion

Combined linkage and association analysis of a common promoter and all common nonsynonymous coding polymorphisms in the FXIII A-subunit gene demonstrated that the V34L polymorphism is either the main functional polymorphism influencing FXIII activation or is very close to an alternative functional site. This was confirmed by haplotype analysis, whereby only those haplotypes containing the 34L allele significantly associated with altered FXIII activation levels when compared with the most common haplotype. These findings, combined with our observation that V34L explains over a third of the variation in FXIII activation and results from previous studies,<sup>8,10,11,16,28,29</sup> which demonstrated an effect of V34L on the biochemistry of FXIII cleavage by thrombin, all point to V34L as the functional locus.

Although there was clear evidence for linkage to V34L, no significant linkage could be seen for either of the flanking markers of V34L. For -246G>A, this was especially unexpected given its close proximity to V34L and its equal informative marker heterozygosity (Table 3). One flanking marker further from V34L, E651Q, showed marginal evidence for linkage. When calculating the empirical probability values for the associations robust to population stratification (ie, using only the within family component), evidence for association of V34L and -246G>A were still significant at  $P=5 \times 10^{-5}$  and  $P=0.0003$ , respectively, but evidence for

association to both Y204F and P564L reduced to  $P=0.07$ . This is probably attributable to the lower LD observed between V34L and P564L compared with V34L and -246G>A, and the low informative sample size ( $n=13$ ) for the within family tests for Y204F.

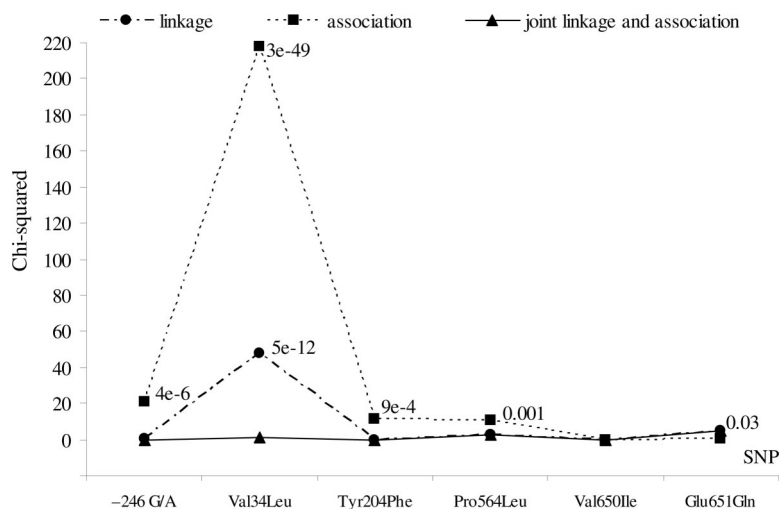
The National Center for Biotechnology Information (NCBI) database (<http://www.ncbi.nlm.nih.gov/SNP>) lists more SNPs (10 in total) in the FXIII-A gene (F13A1) than those included in our study. The reasons for not including some of these SNPs in our study were that either the allele frequency was smaller than 0.01 (ie, heterozygosity as reported on NCBI is smaller than  $[0.99 \times 0.01] \times 2 = 0.0198$ ) or that there were no population data available for the calculation of allele frequency. It is likely that the SNPs listed on NCBI without population data have an allele frequency of <0.01 in our population from Northwest Europe, as we have extensively analyzed the FXIII-A gene by heteroduplex analysis in this population and have found no other common nonsynonymous SNP than those investigated in our current study (T.S. Futers and P.J. Grant, unpublished data). We made 2 exceptions to these SNP selection criteria: (1) we did not choose to analyze rs3024472, because its NCBI heterozygosity estimate of 0.023 was based on an unreliably small population sample size, and we have not observed this SNP in our population by heteroduplex analysis in the past; and (2) we included promoter polymorphism rs1024231, which is the only promoter variant for which previous studies had indicated a potential effect on FXIII gene expression.<sup>30</sup> As changes in gene expression could have influenced our analysis of FXIII activation, this was an important variant to control for. However, our study and methodology was not designed to specifically investigate levels of gene expression, and hence no further noncoding variants were included.

Although we genotyped a promoter polymorphism and all common nonsynonymous coding polymorphisms with an allele frequency >0.01 in the FXIII A-subunit gene in our white population, this does not rule out that other as yet unknown regulatory variants are present elsewhere in the genome. For example, the V34L polymorphism explains 39% of the variation in FXIII activation. If we compare this to the estimated heritability of 82% (the phenotypic variation that can be explained by genetic factors) for FXIII activation, it represents a relatively large contribution of a single SNP to the overall genetic influence. One consequence of this large contribution is that it enabled us to detect linkage to this locus using a relatively modest sample size of 201 DZ twin pairs. However,  $\approx 50\%$  of the genetic variation remains unexplained and may be accounted for by other (regulatory) genes.

**TABLE 5. Results From Linkage, Association, and Combined Tests of Linkage and Association for the Six SNPs in the FXIII A-Subunit Gene**

FXIII Activation	-246 G>A	V34L	Y204F	P564L	V650I	E651Q
Linkage ( <i>P</i> )	0.58 (NS)	47.55 ( $5 \times 10^{-12}$ )	0.00 (NS)	2.81 (NS)	0.03 (NS)	4.79 (0.03)
Association ( <i>P</i> )	21.17 ( $4 \times 10^{-6}$ )	217.8 ( $3 \times 10^{-49}$ )	11.02 (0.0009)	10.91 (0.001)	0.01 (NS)	0.13 (NS)
Linkage and association ( <i>P</i> )	0.02 (NS)	1.30 (NS)	0.00 (NS)	2.42 (NS)	0.03 (NS)	4.61 (0.03)

Values presented in the table are  $\chi^2$  with 1 *df* and *P* (in parentheses). Linkage results obtained using single-point IBDs. FXIII activation levels were as measured by pentylamine incorporation.  $\chi^2$  values can be converted into LOD (logarithm of the odds) scores with the formula  $\text{LOD} = \chi^2/4.605$



Graphical representation of linkage, association, and joint linkage and association of FXIII SNPs to FXIII activation levels. Analysis was carried out using QTDT. Of the 6 common FXIII polymorphisms, V34L was the main functional site that influenced FXIII activation.

The V34L polymorphism has been associated with decreased cardiovascular risk in a case control study of patients undergoing coronary angiography, stratified by history of MI.<sup>6</sup> This finding was confirmed in some, but not all, studies,<sup>31</sup> and protection against thrombosis was additionally described in relation to venous thrombosis and ischemic cerebrovascular disease.<sup>31</sup> In another study, only the V34L polymorphism (not P564L or E651Q) showed a difference in genotype prevalence in patients with coronary artery disease who had an MI compared with either patients without MI and controls.<sup>17</sup> The results from that study supported *in vitro* studies that indicated that V34L had a direct role in influencing FXIII function, although the conundrum remained that the protective allele (34Leu) was associated with marked increases in FXIII activation,<sup>8</sup> a finding apparently at odds with the molecular epidemiology. Subsequently, other studies confirmed that 34Leu enhances FXIII activation.<sup>10,11,29</sup> It has been proposed that as FXIII 34Leu has more extensive contact with the binding region for the FXIII activation peptide on thrombin compared with 34Val, which may lead to 34Leu being more susceptible to wasteful conversion to the activated enzyme to provide a protective effect against cardiovascular disease,<sup>11</sup> but other mechanisms likely play a role.

Initial studies of fibrin structure and function showed that individuals possessing the protective Leu allele produced fibrin with thinner fibrin fibers and smaller pores<sup>10</sup>; however, this finding is more in support of a hypothesis of increased CAD risk as observed earlier for these kind of fibrin structures by Fatah et al.<sup>32</sup> It was also observed that individuals possessing the protective Leu allele, but who did experience MI, had higher levels of plasminogen activator inhibitor (PAI)-1 and were more insulin resistant, suggesting that additional risk factors for CAD may negate the protective effect of the Leu allele.<sup>33</sup> In another study, we observed that effects of 34Leu on fibrin structure were reversed in the presence of high concentrations of fibrinogen, indicating that protection against thrombosis only occurs under conditions of raised fibrinogen.<sup>34</sup> These gene–environment interactions will make univariate (linkage and association) studies difficult to interpret and may represent a clear example of the

general reason why there has been relatively little success in finding hemostatic genetic polymorphisms that link to CAD outcome, even if they explain a relatively large proportion of the intermediate (hemostatic) phenotypes (see Lane and Grant<sup>4</sup>). It is likely that both environmental and genetic risk factors act in concert, and taking these interactions into account may improve gene discovery<sup>35,36</sup> and their applied value in future prediction and treatment of common disease. For example, specific genes may have relatively large effects on intermediate phenotypes but, because of interaction with a number of other factors, may have low predictive value on disease outcome. Investigating these genes (or intermediate phenotypes) in isolation may give poor results at both statistical and practical level. Nonetheless, studies into the associations between polymorphisms and phenotype may provide crucial insights into the underlying mechanisms and enhance understanding of disease processes.

Our study indicates that of 6 SNPs in the FXIII A-subunit, V34L is the main functional polymorphism that consistently associates with FXIII activation. The clear effect of this polymorphism on activation and the reports of its cardioprotective effect are an incentive to study the function of FXIII V34L more closely. These studies could provide new opportunities for the prediction and treatment of atherothrombotic coronary artery disease.

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### Disclosures

None.

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