



## Factor XIII Val34Leu and the risk of myocardial infarction

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### ABSTRACT

**Background and Objectives.** Recent studies have suggested an association between a genetic variation in the coagulation factor XIII (FXIII Val34Leu) and decreased risk of vascular thrombosis.

**Design and Methods.** We investigated the frequency of the FXIII Val34Leu polymorphism in 150 consecutive, unrelated and relatively young (<55 years) survivors of myocardial infarction (MI) with angiographically-proven severe coronary atherosclerosis and in 150 age-, gender- and race-matched controls.

**Results.** FXIII Val34Leu was detected in 54/150 patients and 73/150 controls, yielding an overall odds ratio (OR) for MI of 0.6 (CI95: 0.4-0.9). Homozygosity for FXIII Val34Leu was found in 4/150 patients and in 12/150 controls, yielding an OR for MI of 0.26 (CI95: 0.08-0.9). The OR for heterozygotes was 0.65 (CI95: 0.4-1.1). FXIII Val34Leu carriership decreased the risk of MI related to metabolic risk factors (RF) (hypertension, diabetes, dyslipidemia, and obesity): non-carriers in the presence of a metabolic RF had a 13.9-fold higher risk of MI, whereas in carriers with a metabolic RF the risk was reduced to 6.8. FXIII Val34Leu also attenuated the risk of MI among smokers. Non-carrier smokers had a 6.1-fold higher risk (CI95: 3.1-11.9), whereas the risk among smokers carrying FXIII Val34Leu was 3.9 (CI95: 1.9-8.1).

**Interpretation and Conclusions.** FXIII Val34Leu confers a significant protective effect against the occurrence of MI in relatively young patients. FXIII Val34Leu exhibits a gene dosage effect: the protective effect was particularly strong in homozygous carriers, and heterozygosity conferred moderate protection. Finally, FXIII Val34Leu seems to reduce the risk of MI related to major cardiovascular risk factors.

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Key words: factor XIII, myocardial infarction, thrombosis, risk factor, interaction

Recently a sequence variation in coagulation FXIII was described; a G to T transition in exon 2 of the FXIII A-subunit gene, which results in the substitution of leucine for valine at amino acid position 34 (FXIII Val34Leu).<sup>1</sup> The Leu34 variant influences FXIII transglutaminase activity, and homozygosity for this polymorphism is associated with elevated activity of the enzyme; heterozygous carriers exhibit intermediate enzyme activity.<sup>2-4</sup> Two previous reports have suggested that FXIII Val34Leu is associated with decreased risk of myocardial infarction (MI) and increased predisposition to primary intracerebral hemorrhage.<sup>5,6</sup> More recently, a protective effect against the development of venous thrombotic disease was also found by Catto *et al.* and by our group.<sup>7,8</sup> The protective effect against vascular thrombosis conferred by this polymorphism is not well understood and indeed puzzling, since the higher activity of the Leu34 enzyme would be expected to result in augmented resistance of the fibrin clot to plasmin degradation.

In the current investigation we determined the prevalence of the FXIII Val34Leu polymorphism in a selected group of patients composed of 150 consecutive, unrelated and relatively young survivors of MI with angiographically-proven severe coronary atherosclerosis and in 150 age-, gender- and race-matched healthy controls. Our findings provide further evidence for a role of the FXIII Val34Leu as a genetic factor conferring a protective effect against the occurrence of MI. The polymorphism appears to exhibit a gene dosage effect, homozygosity conferring stronger protection than heterozygosity. In addition, the data suggest that FXIII Val34Leu attenuates the risk of MI conferred by major cardiovascular risk factors.

### Design and Methods

#### Patients and controls

One hundred and fifty consecutive and unrelated individuals (116 males; mean age, 43 years; range 25-55 years; and 34 females; mean age, 45 years; range, 30-55 years) with a diagnosis of acute MI and angiographically-proven coronary atherosclerotic disease (CAD) comprised the patient study group. The inclusion of the patients and blood sampling took place on the day on which each subject was admitted for coronary angiography in the University Hospital of the School of Medicine of Ribeirão Preto, University of São Paulo, Brazil, between June 1996 and December 1997. Included patients were aged less than 55 years, in whom a diagnosis of MI

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has been previously established on the basis of clinical, enzymatic and electrocardiographic criteria. Specifically, at least two of the following criteria had to be fulfilled for the diagnosis of MI to be accepted: typical chest pain (longer than 30 minutes), an increase in creatine kinase of more than twice the baseline level, and characteristic electrocardiographic changes in two or more adjacent leads. In addition, we only included patients who had at least 50% obstruction in a major coronary artery, as demonstrated by angiography. At the same time as the patients were enrolled, one hundred and fifty unrelated, asymptomatic and apparently healthy subjects (recruited by active search among blood donor candidates in the local Blood Center) without a personal history of arterial disease or MI were invited to participate in the study as controls. Each case was matched to a control for gender, age ( $\pm 4$  years) and race. All subjects came from the same geographic region, i.e., the city of Ribeirão Preto, State of São Paulo, South-eastern Brazil. All individuals consented to participate in this study, which was approved by the local Ethics Committee.

#### General characteristics and cardiovascular risk factors in patients and controls

All data concerning demographic characteristics and the presence of major risk factors for atherothrombosis were collected on the day of the angiography by completing a standard questionnaire designed to catalog the presence of the characteristics presented in Table 1. The same data were obtained for each control by a physician, who filled out the standard questionnaire. A subject was considered as having arterial hypertension, diabetes or dyslipidemia when he or she was receiving specific medications to treat these conditions and/or when there was an established diagnosis of each disease. Information concerning body mass index (BMI) was also obtained for all patients and controls. Women with BMI  $\geq 27.3$  kg/m<sup>2</sup> and men with BMI  $\geq 30$  kg/m<sup>2</sup> were considered obese. Information concerning previous and current cigarette smoking was also collected, and we included a subject as smoker only when a recent history of regular cigarette consumption was present. Subjects were classified as Whites, Blacks or Mulattos on the basis of phenotypic characteristics.

#### DNA analysis

Genomic DNA from peripheral blood was isolated by standard methods.<sup>9</sup> The following primers were used to amplify exon 2 of the FXIII gene: P1: 5'-GAC-CTTGTAAGTCAAAAATGTC-3' and P2: 5'-GCT-CATACCTTGCAGTTGACGCCCGGGGCATTA-3'.<sup>8</sup> The second primer has two mismatches (underlined) that create a restriction site for the restriction enzyme *MseI* only when the FXIII Val34Leu polymorphism is present. DNA sequencing (Sanger method) confirmed the correct identification of the FXIII Val34Leu genotypes in several samples.

#### Statistics

Odds ratios (OR) as a measure of the relative risk of MI and 95% confidence intervals (CI95) were cal-

culated by standard methods.<sup>10</sup> To assess a possible influence of FXIII Val34Leu on the risk of MI conferred by major cardiovascular risk factors, stratified analyses were also performed.

#### Results

Table 1 summarizes relevant general characteristics of the patient and control groups. As expected, major "classical" risk factors for atherosclerosis were more prevalent among the patients with MI and severe CAD than among controls.

The results of the analysis of the prevalence of the FXIII Val34Leu polymorphism in patients and controls are given in Table 2. FXIII Val34Leu was detected in 54 out of 150 patients (carrier frequency, 36%; allele frequency, 0.193) and in 73 out of 150 controls (carrier frequency, 48.7%; allele frequency, 0.283). These data yielded an OR for MI related to FXIII Val34Leu of 0.6 (CI95: 0.4-0.9). Four homozygotes (2.7%) were observed among the patients and 12 (8%) among the controls, yielding an OR for MI related to the homozygous state for the variant FXIII of 0.26 (CI95: 0.08-0.9). For heterozygotes, the OR was 0.65 (CI95: 0.4-1.1). Separate analysis with only White subjects (who formed the majority of the individuals investigated) did not modify the results. In fact, FXIII Val34Leu was observed with a carrier frequency of 35.5% (allele frequency 0.193) and 50.8%

**Table 1. General characteristics of MI patients with angiographically-demonstrated severe atherosclerosis and of healthy controls.**

Variable	Patients (n=150)	Controls (n=150)
Male	116 (77.3%)	116 (77.3%)
Whites	124 (82.7%)	124 (82.7%)
Blacks and mulattos	26 (17.3%)	26 (17.3%)
Mean age (range)	43 (25-55)	42 (22-55)
Family history of CAD/MI	50 (33.3%)	24 (16%)
Hypertension*	92 (61.3%)	17 (11.3%)
Diabetes mellitus*	22 (14.7%)	3 (2%)
Dyslipidemia*	50 (33.3%)	4 (2.7%)
Obesity <sup>#</sup>	42 (28%)	13 (8.7%)
Smoking	102 (68%)	32 (24%)

\*Physician's diagnosis and/or drug-treatment. <sup>#</sup>Body mass index  $\geq 30$  kg/m<sup>2</sup> (men) or 27.3 kg/m<sup>2</sup> (women).

**Table 2. FXIII Val34Leu mutation in patients with MI and in healthy controls.**

Genotype	Patients (n=150)	Controls (n=150)	OR (CI 95%)
Val/Val	96 (64%)	77 (51.3%)	1.0 <sup>#</sup>
Val/Leu	50 (33.3%)	61 (40.7%)	0.65 (0.4-1.1)
Leu/Leu	4 (2.7%)	12 (8%)	0.26 (0.08-0.9)
Val/Leu + Leu/Leu	54 (36%)	73 (48.7%)	0.60 (0.37-0.9)

<sup>#</sup>Reference category (OR=1.0). Val/Val refers to the wild-type genotype, Val/Leu to heterozygosity and Leu/Leu to homozygosity for FXIII Val34Leu.

**Table 3. FXIII Val34Leu mutation in patients with MI and severe CAD and in healthy controls: interaction with major acquired cardiovascular risk factors.**

Risk factor	FXIII genotype	Patients (n=150)	Controls (n=150)	OR (CI 95%)
Metab. RF (-)*	Non-carrier	22 (14.7%)	62 (41.3%)	1.0 <sup>#</sup>
	Carrier	13 (8.7%)	56 (37.3%)	0.7 (0.3-1.4)
Metab. RF (+)*	Non-carrier	74 (49.3%)	15 (10%)	13.9 (6.7-29.1)
	Carrier	41 (27.3%)	17 (11.3%)	6.8 (3.2-14.3)
Non-smokers	Non-carrier	32 (21.3%)	58 (38.7%)	1.0 <sup>#</sup>
	Carrier	17 (11.3%)	56 (37.3%)	0.6 (0.3-1.1)
Smokers	Non-carrier	64 (42.7%)	19 (12.7%)	6.1 (3.1-11.9)
	Carrier	37 (24.7%)	17 (11.3%)	3.9 (1.9-8.1)

<sup>#</sup>Reference category (OR=1.0). \*Metabolic risk factors (RF): obesity, arterial hypertension, dyslipidemia, and diabetes. (-) indicates absence of these risk factors and (+) indicates the presence of at least one risk factor. "Non-carrier" refers to the FXIII Val34/Val34 (wild-type) genotype and "carrier" refers to both homozygotes and heterozygotes for the FXIII Val34Leu mutation.

**Table 4. FXIII Val34Leu mutation in patients with MI and severe CAD and in healthy controls: interaction with metabolic risk factors.**

Risk factor	FXIII genotype	Patients (n=150)	Controls (n=150)	OR (CI 95%)
Hypertension (-)	Non-carrier	36 (24%)	70 (46.7%)	1.0 <sup>#</sup>
	Carrier	22 (14.7%)	63 (42%)	0.7 (0.4-1.2)
Hypertension (+)	Non-carrier	60 (40%)	7 (4.7%)	16.7 (6.9-40.2)
	Carrier	32 (21.3%)	10 (6.7%)	6.2 (2.7-14.1)
Diabetes (-)	Non-carrier	81 (54%)	75 (50%)	1.0 <sup>#</sup>
	Carrier	47 (31.3%)	72 (48%)	0.6 (0.4-0.9)
Diabetes (+)	Non-carrier	15 (10%)	1 (0.7%)	13.9 (1.8-107.7)
	Carrier	7 (4.7%)	2 (1.3%)	3.2 (0.7-16.1)
Dyslipidemia (-)	Non-carrier	59 (39.3%)	74 (49.3%)	1.0 <sup>#</sup>
	Carrier	41 (27.3%)	72 (48%)	0.7 (0.4-1.2)
Dyslipidemia (+)	Non-carrier	37 (24.7%)	2 (1.3%)	23.2 (5.4-100.2)
	Carrier	13 (8.7%)	2 (1.3%)	8.2 (1.8-37.5)
Obesity (-)	Non-carrier	65 (43.3%)	70 (46.7%)	1.0 <sup>#</sup>
	Carrier	43 (28.7%)	67 (44.7%)	0.7 (0.4-1.1)
Obesity (+)	Non-carrier	31 (20.7%)	6 (4%)	5.6 (2.2-14.2)
	Carrier	11 (7.3%)	7 (4.7%)	1.7 (0.6-4.6)

<sup>#</sup>Reference category (OR=1.0). (-) indicates absence of the risk factor and (+) indicates presence of the risk factor. "Non-carrier" refers to the FXIII Val34/Val34 (wild-type) genotype and "carrier" refers to both homozygotes and heterozygotes for the FXIII Val34Leu mutation.

(allele frequency 0.286) in White patients and in White controls, respectively.

In male patients (n=116), 43 heterozygotes and 2 homozygotes for FXIII Val34Leu were found; 46 heterozygotes and 9 homozygotes were found among 116 male controls. These data yielded an overall OR of 0.7 (CI95: 0.4-1.2). The OR for heterozygotes was 0.8 (CI95: 0.5-1.4) and for homozygotes was 0.2 (0.04-0.9). In females, 8 heterozygotes and 2 homozygotes were found among 34 patients, and 15 heterozygotes and 3 mutant homozygotes were observed among 34 controls. The overall OR for MI in females was 0.4 (CI95: 0.1-1); the OR was also 0.4 for heterozygotes (CI95: 0.1-1) and 0.4 for homozygotes (CI95: 0.06-2.9).

Detailed information concerning the presence of major risk factors for MI (arterial hypertension, dyslipidemia, diabetes, obesity, and smoking) was available for patients and controls. This allows us to assess the possibility that FXIII Val34Leu influences the risk of MI associated with these risk factors. In these analyses (shown in Tables 3 and 4), the OR for each category of subjects was calculated in relation to the category in which both the mutation and the risk factor were absent (i.e., the reference category included non-carriers without major risk factors). In Table 3, hypertension, dyslipidemia, diabetes and obesity were analyzed in combination, as "metabolic" risk factors, and smoking was considered as a separate variable. In non-carriers of the FXIII polymorphism in

which a metabolic risk factor was present, the OR for MI was 13.9 (CI95: 6.7-29.1). Among subjects in whom metabolic risk factors were present, but who carried FXIII Val34Leu, a two-fold decrease in the risk of MI was observed (OR 6.8, CI95: 3.2-14.3). An interaction between FXIII Val34Leu and smoking was also observed. Smoking among non-carriers was associated with an OR for MI of 6.1 (CI95: 3.1-11.9). This OR decreased to 3.9 (CI95: 1.9-8.1) among carrier smokers, pointing to an attenuating effect of FXIII Val34Leu on the risk of MI associated with smoking.

Table 4 lists the OR for MI for each metabolic risk factor separately, in the presence and in the absence of FXIII Val34Leu. Some of these results did not reach statistical significance (CI95 includes 1.0) because of the small number of subjects in each subgroup. However, the findings suggest that FXIII Val34Leu carriership resulted in a decrease in the specific risk of MI conferred by each risk factor, i.e. hypertension (2.7-fold decrease), diabetes (4.3 fold decrease), dyslipidemia (2.8-fold decrease), and obesity (3.3-fold decrease).

## Discussion

In the current investigation the FXIII Val34Leu polymorphism was significantly more prevalent among healthy controls than among patients with MI. This observation indicates that FXIII Val34Leu is a genetic factor that confers a protective effect against the occurrence of MI, a finding that agrees with recent data concerning the role of this polymorphism in arterial disease.<sup>5,11</sup> In the present study, the OR for MI related to the homozygous state was lower than the OR related to the heterozygous state. This finding suggests that FXIII Val34Leu exhibits a gene dosage effect, i.e. the protective effect for MI is stronger in homozygous than in heterozygous carriers. Interestingly, we have recently shown that the Leu34 allele is a strong protective factor for the occurrence of deep venous thrombosis, and we specifically observed the protective effect in homozygous carriers.<sup>8</sup> This observation does not exclude a protective effect for venous thrombosis related to the heterozygous state. Indeed, Catto *et al.* observed a protective effect for venous thromboembolism also among heterozygotes.<sup>7</sup> However, the data from the present study and from our previous study analyzing the FXIII Val34Leu polymorphism in venous thrombosis accord in the sense that both investigations indicate that homozygous carriers bear a stronger protection against vascular thrombosis than heterozygotes. Future studies are needed to confirm this.

Recent data demonstrated an increased FXIII transglutaminase activity among FXIII Val34Leu carriers.<sup>2-4</sup> In principle, higher activity of the Leu34 enzyme could be expected to lead to increased resistance of the fibrin clot to plasmin degradation. Some authors have suggested that FXIII Val34Leu might influence thrombin activation of the FXIII A-subunit to produce a fibrin clot less resistant to fibrinolysis or, alternatively, affect cross-linking of other coagulation proteins.<sup>5,6</sup> However, the protective role of FXIII Val34Leu against arterial and venous thrombosis is not understood,

pointing to the need for additional studies to explore the exact mechanisms by which this mutation exerts an anti-thrombotic effect. In this respect, the possibility that FXIII Val34Leu may be in linkage disequilibrium with another (unknown) polymorphism that could explain its protective effect in vascular thrombosis should be considered.

The present investigation focused on the possibility that the FXIII Val34Leu polymorphism influences the risk of MI associated with major cardiovascular risk factors such as arterial hypertension, dyslipidemia, diabetes, obesity and smoking. The data indicate that there is indeed an interaction between FXIII Val34Leu and major risk factors for MI. Firstly, we observed that carriership of this polymorphism results in a moderate reduction of the risk of MI among subjects with a *metabolic* risk factor. Specifically, non-carrier subjects in the presence of a metabolic risk factor presented a 13.9-fold higher risk of MI, whereas in subjects with a metabolic risk factor who also carried FXIII Val34Leu this risk was reduced to 6.8. Secondly, a decreased risk (ranging from 2.7 to 4.3-fold) was also observed when each metabolic risk factor was analyzed separately (Table 4). Thirdly, it seems that the mutation also attenuates the risk of MI among smokers. Non-carrier smokers had a 6.1-fold higher risk of MI, whereas the risk among smokers carrying FXIII Val34Leu was 3.9.

We designed this investigation in order to minimize several potential biases: cases and controls were matched for sex-, age- and race, all subjects came from the same geographic area, cases were consecutively included by one medical service according to strict selection criteria, a standard questionnaire was employed for all subjects enrolled, and each inclusion was checked by two of the investigators. In spite of these precautions, we believe that the findings of this study should be interpreted with the prudence that a case-control study deserves. For instance, the prevalence data should not be directly extrapolated to other populations. Additionally, the results may be representative for young MI patients and not for older patients. Finally, patients were included after the acute event and after angiography, and therefore cases represent only survivors of MI. These findings should encourage further exploration of the interactions between genetic and classical factors here described in other populations, especially in the context of large prospective studies.

Over the last years, several coagulation variables have been investigated as candidate risk factors for arterial thrombotic disease. For instance, previous reports have pointed to an association of high plasma fibrinogen, factor VII, factor VIII and PAI-1 with arterial thrombosis.<sup>12</sup> An increased predisposition to MI in young women was also linked to factor V Leiden (FVL), a frequent genetic variation causally related to venous thrombosis.<sup>13</sup> More recently, a role for a common sequence variation in the 3'-untranslated region of the factor II gene (FII 20210 G→A) as a risk factor for arterial disease was also claimed.<sup>14-16</sup> In some studies, synergism between the genetic abnormalities FVL and FII 20210 G→A and major risk factors for MI has also been observed.<sup>13,14,16</sup> Our data concerning the possible interaction between FXIII

Val34Leu and classical cardiovascular risk factors are, therefore, an additional demonstration of interactions between genetic and acquired factors determining the risk of MI, in this case the inherited variable playing a protective role against the occurrence of the clinical event. These results should stimulate future investigations to identify genetic variations in clotting factor genes that play a role in the risk of arterial thrombotic disease.

### Contributions and Acknowledgments

RFF was the principal investigator involved in the design of the study, analysis of the data and interpretation. He wrote the paper with MAZ, and both were responsible for general supervision and acquisition of funding for this research. All of the other authors played a part in the design and execution of the study, revised the manuscript and contributed to its intellectual content. APF was the main investigator involved in the selection of the patient study group. The order of authorship is based on the time, work and intellectual and scientific contribution of the authors.

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### Disclosures

Conflict of interest: none.

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### Manuscript processing

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### Potential implications for clinical practice

- ◆ FXIII Val34Leu confers a significant protective effect against the occurrence of myocardial infarction in young patients.
- ◆ In this respect FXIII Val34Leu exhibits a gene dosage effect.

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