Factor XIII Val34Leu variant protects against coronary artery disease

A meta-analysis

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Summary
Several studies suggested that Val34Leu variant of factor XIII (FXIII) might have a protective effect against coronary artery disease (CAD), but studies not supporting these findings have also been published. The authors performed a meta-analysis of 16 studies on 5,346 cases and 7,053 controls that investigated the association between Val34Leu polymorphism and CAD defined as history of myocardial infarction or significant stenosis on a coronary artery assessed by coronary angiography. Because of the heterogeneity of the study-specific results, the pooled effect estimates were calculated by a random-effects empirical Bayes model. The combined odds ratios for CAD were 0.82 (95% confidence interval [95% CI] 0.73, 0.94) for the heterozygotes of the FXIII Val34Leu variant, 0.89 (95% CI 0.69, 1.13) for the homozygotes, and 0.81 (95% CI 0.70, 0.92) for the heterozygotes and homozygotes combined. The results were essentially the same when only myocardial infarction was considered as outcome. The beneficial effect of the polymorphism might be smaller than the effect estimates obtained in this meta-analysis, because the analysis raised the possibility of publication bias. Data published in the literature suggest that gene-gene and gene-environmental interactions might significantly influence the protective effect of FXIII-A Val34Leu polymorphism.

Keywords
Coronary disease, factor XIII, polymorphism, meta-analysis

Introduction
FXIII is a tetrameric zymogen (A2B2) consisting of two potentially active A (FXIII-A) and two inhibitory/carrier B subunits (FXIII-B). It is activated by the concerted action of thrombin and Ca2+ in the terminal phase of the clotting cascade. Thrombin cleaves off an activation peptide of 37 amino acids from the N-terminal end of FXIII-A, then in the presence of Ca2+ FXIII-B dissociates, and the dimer of FXIII-A assumes an active configuration. Activated FXIII (FXIIIa) is a transglutaminase which crosslinks peptide bound glutamine and lysine residues through isopeptide bonds. Its physiological function in hemostasis is the cross-linking of fibrin \( \gamma \)- and \( \alpha \)-chains and the covalent attachment of \( \alpha \)-plasmin inhibitor to fibrin. This way it mechanically stabilizes the fibrin clot and protects it from the prompt elimination by fibrinolysis (1).

There are five common polymorphisms in FXIII-A, one of which, the Val34Leu polymorphism, is located in the activation peptide just three amino acid residues upstream from the thrombin cleavage site (2). This mutation results in a faster liberation of the activation peptide, earlier activation of FXIII, and consequently, in accelerated cross-linking (3–5). The mutation also influences the structure of fibrin (5, 6).

The association of the Val34Leu polymorphism with different venous and arterial thrombotic disorders has been intensively studied with sometimes contradictory results. Recently, a meta-
analysis on the association of this polymorphism and venous thromboembolism has been published (7). Here, we report a quantitative review of studies on the association between Val34Leu variant of factor XIII and coronary artery disease (CAD).

Methods
Identification and selection of studies involved in the analysis
We performed a search on MEDLINE (National Library of Medicine, Bethesda, Maryland) up to September 15, 2006 with the following search key: “Factor XIII AND (Val34Leu or Leu34 or polymorphism)”. The search resulted in 209 papers. From these studies two authors independently selected papers that investigated the relationship between factor XIII Val34Leu polymorphism and CAD (8–26). The two independent selections were identical with the exception of a letter (24). The data presented in the letter was identical with the data presented later in an original paper (22), therefore the letter was excluded. Additionally, we included data from a manuscript of our working group (27).

Studies were included in the analysis if the definition of CAD was based on the occurrence of myocardial infarction (MI) or significant coronary artery stenosis verified by coronary angiogram. In one study patients who underwent coronary angiography were included as cases (8). Only 75% of these had significant (≥ 50%) stenosis on at least one coronary artery. The data was not presented separately for this group in the original report. We included the results of this study in the analysis, but we performed the analysis with the omission of this study, as well.

One study was excluded, because cases were not defined by history of MI or by findings on the angiogram (25). Patients were

Table 1: Characteristics of studies included in the meta-analysis. MI: myocardial infarction, GP: general practitioner, BMI: body mass index, CAD: coronary artery disease, ARIC cohort: The Atherosclerosis Risk in Community Study, ATVBISG: Atherosclerosis, Thrombosis and Vascular Biology Italian Study Group. *only 75% of the 398 patients had significant (≥50%) stenosis. †including MI, death of CAD, and coronary revascularization.
identified as having CAD if they had a hospital admission or died of CAD which was defined as codes 410–414 according to the International Classification of Diseases, 9th revision (28). Another study was excluded because only the relationship between Val34Leu variant of factor XIII and the combined outcome of unstable angina and MI was published together with the estimated increased risk of MI as compared to unstable angina in patients with factor XIII Leu34 allele (26).

A further study was excluded from the selection because of the possibility of systematic error (14). In this study the control group did not represent the source population cases were selected from, because it consisted of subjects attending for coronary angiography. This might cause selection bias if the polymorphism under study was related to CAD, since in this case the allele frequencies among the controls might be different from the ones in the source population. In another study two control groups were used, one autopsy series of unselected deaths and a group of patients who had undergone a coronary angiography. We included only the former series in the analysis (9).

**Data extraction**

All but one studies had a case-control design. Based on the number of cases and controls with different genotypes presented in the original publications we calculated crude odds ratios (OR) as an association measure estimating the relative risk of CAD. Subjects with Val/Leu genotype and Leu/Leu genotype were compared to subjects with the wild Val/Val genotype. Although most studies used matching, none of them presented and analysed the data accordingly, therefore we could not take matching into account in the interval estimation. One study which was performed within the framework of The Atherosclerosis Risk in Communities (ARIC) Study used a case-cohort design (15). The estimates of the hazard ratios (HR) corresponding to different genotypes were adjusted for other cardiovascular risk factors. We used these estimates in the analysis. Only HR corresponding to the different genotypes were given, but not for the Leu allele carriers, therefore this study was excluded from the analysis that compared the risk of Leu allele carriers to the risk of subjects with the wild genotype. In the original report confidence intervals of the HR were not given, only p-values. Based on these, we calculated test-based confidence intervals (CI) of the HR and used them in the meta-analysis (29).

From one report we could not extract the data separately for the different genotypes, just for the wild type and for the Leu allele carriers (heterozygotes and homozygotes combined) (11). Although allele frequencies were also reported, and this would have allowed us to calculate the number of subjects with different genotypes, the data presented by the authors led to inconsistencies in the calculations, therefore this report was included only in the analysis when the risk of the Leu carriers was compared to the risk of subjects with the wild genotype.

**Pooling**

Since the range of the study specific OR was wide and the test for heterogeneity was significant ($p = 0.001$) (30), we chose to use empirical random-effects Bayes model to obtain study specific estimates of the OR for each study and to combine these estimates (31). This method gives more conservative estimates than conventional fix-effect methods, furthermore it shrinks the study-specific estimates towards the combined value reducing the influence of studies with extreme results. We used the routine of “meta” of the statistical package STATA for the estimation and for drawing forest plots (32, 33).

Since some studies suggested that the effect of the polymorphism might be larger for early-onset disease, we performed separate analysis for the subgroup of patients under age 55. Further...
In addition, considering CAD as an outcome, we analyzed the data using only MI as outcome.

**Assessing publication bias**

We used the “metabias” routine of the software package STATA to assess the possibility of publication bias by making funnel plots and performing the Egger’s test for publication bias (34, 35).

**Results**

The major characteristics of the 16 studies selected to be involved in the analysis are shown in Table 1. The studies were conducted in Europe, North America, and South America. The vast majority of the enrolled people were Caucasian.
The overall risk of CAD was 18% less (OR 0.82, 95% CI 0.73, 0.94) in subjects with Val/Leu genotype than in subjects with Val/Val genotype (Fig. 1). For the Leu/Leu genotype the combined OR was 0.89, 95% CI 0.69, 1.13 (Fig. 2). When Leu allele carriers were compared to subjects with Val/Val genotype the estimate of the combined OR was 0.81, 95% CI 0.70, 0.92 (Fig. 3). Considering only MI as outcome did not materially change this result (OR 0.84, 95% CI 0.76, 0.94). In subjects under age 55 the effect was more marked (OR 0.72, 95% CI 0.50, 1.04), but because of the fewer number of cases the precision of the estimate was reduced.

Omission of the study in which only 75% of the cases had significant coronary artery stenosis had essentially no effect on the results (8).

The result of the Egger test for publication bias was statistically significant (p = 0.002). In accordance with this, the funnel plot indicates that some small studies that did not find beneficial effect in the Leu allele carriers might be missing in the published literature (Fig. 4).

**Discussion**

Our meta-analysis of the results of sixteen studies that involved 5,346 cases and 7,053 control subjects showed that Val34Leu variant of factor XIII provides some protection against CAD.

The individual study results pooled in this analysis showed considerable heterogeneity; the range of the study specific OR without the shrinkage was 0.31–1.11. Based on the published information we could not identify any potential determinants that could explain this heterogeneity. The strength of the effect of a risk factor in a population depends on the prevalence of its causal complement (36). Although the biological mechanism in which a genetic factor causes a disease remains unchanged in the different populations, the actual strength of the association may vary considerably as the prevalence of the relevant environmental factors vary. It should be noted that some of the negative studies came from Mediterranean countries – Italy, Spain, and Southern France (10, 11, 20), in which the prevalence of environmental risk factors is lower than in other populations, and in these cases presumably no further protection could be provided by a relatively moderate genetic protection factor. On the other hands, the Hungarian study with negative outcome involved a population at high risk of CAD, one of the highest in Europe (37), and in this case the protective effect of Val34Leu polymorphism might have been overcome by strong environmental and other genetic risk factors.

It has already been suggested that gene-environmental and gene-gene interactions might be responsible for the conflicting results on the effect of FXIII-A Val34Leu polymorphism (6, 38). For instance, it was found that in the presence of insulin resistance the cardio-protective effect of Leu34 allele was lost (39). A high prevalence of insulin resistance has been presumed to be associated with the lack of protection by the Leu34 allele in the UK Asian population (14). The interaction between fibrinogen level and FXIII-A Val34Leu polymorphism has also been demonstrated (25). Decrease in clot permeability with increasing fibrinogen concentration was diminished by the Leu34 FXIII variant and at high fibrinogen concentration plasma samples homogenous for the Leu allele form clots with looser structures and thicker fibers more prone to fibrinolysis (6). We have demonstrated that in the Hungarian population the protective effect of Leu34 allele prevails only at high fibrinogen concentrations (27).

The biochemical mechanism behind the protective effect of the Leu34 allele is not clear. One possibility is the down-regulation of the adhesion of platelets to the fibrin network by cross-linking fibrin, a mechanism that operates at high shear rate (40, 41). Faster activation of Leu34 FXIII and earlier cross-linking of fibrin in the thrombus could result in the limitation of the growth of the thrombus by inhibiting the incorporation of further platelets into the thrombus. The reduced mass/length ratio of fibrin fibres and reduced porosity of fibrin in the presence of Leu34 FXIII could also contribute to the protective effect (5).

The parameters chosen to be of interest in a meta-analysis are often not reported as such but have to be estimated based on the original reports. In this regard the current meta-analysis is no exception, we estimated OR based on the published numbers of cases and controls by different genotypes. In doing so, we had to disregard that in many studies controls were matched to cases. This should have been taken into account in the estimation of the study-specific OR. The way the data was reported did not allow us to calculate the appropriate Mantel-Haenszel estimator of the OR, just the crude OR, which are usually expected to be biased towards the null result (OR of 1.0) in matched case-control studies. However, this occurs only if the matching factors are closely related to the exposure under study (42). This is unlikely in the case of FXIII Val34Leu polymorphism, therefore it does not limit the interpretation of our results.

Because of the considerable heterogeneity, we used empirical Bayes model in our study. Compared to conventional random-eff-
fects meta-analysis this reduces the influence of studies with extreme results. This should be taken into account in the interpretation of our results.

The analysis raises the possibility of publication bias. Because of the large sampling error, the results of small studies tend to vary around the true value of the parameter of interest to a larger extent than the results of large studies. However, there was only one small study that found less beneficial effect than our pooled estimate (11). If these findings exist but remained unpublished, then our estimate on the beneficial effect of FXIII Val34Leu polymorphism might be somewhat more optimistic than the true effect.

Abbreviations

FXIII, factor XIII; FXIIia, activated factor XIII; FXIII-A, factor XIII A subunit; FXIII-B, factor XIII B subunit; Val34Leu, Valine34Leucine.

References