Factor XIII Val34Leu variant and the risk of myocardial infarction

A meta-analysis

Mona Shafey1, J osdalyne L. Anderson2, Dimitri Scarvelis1, Steven P. Doucette2, France Gagnon4, Philip S. Wells1,2,3*

1Department of Medicine, University of Ottawa, Ontario, Canada; 2Ottawa Health Research Institute, Ontario, Canada; 3Department of Epidemiology and Community Medicine, University of Ottawa, Ontario, Canada; 4Department of Public Health Sciences, University of Toronto, Ontario, Canada

Summary

Genetic factors are thought to contribute to the pathogenesis of acute myocardial infarction (AMI). A common variant of factor XIII (FXIII), FXIII Val34Leu, may be protective against developing an AMI, but various studies show conflicting results. We performed a meta-analysis to determine whether the FXIII Val34Leu variant is associated with a decreased risk of AMI. One hundred ninety-five articles were reviewed and 12 case-control studies were selected. We included studies involving patients with objectively diagnosed AMIs (WHO criteria), provided that FXIII Val34Leu genotyping data were available. Inclusion decisions, quality assessment, and data extraction were conducted by two reviewers. Hypothesizing that the Leu allele was protective, we performed three analyses with the Val/Val genotype as the reference group. Pooled odds ratios (OR) and their 95% confidence intervals (95% CI) were determined. Prior to pooling, heterogeneity testing was performed using the I² statistic. These studies included a total of 8,743 patients, of which 3,663 were AMI patients and 5,080 were healthy controls. Using the random effects methods, protective effects were seen with the Leu/Val genotype alone (OR 0.79, 95% CI 0.68–0.93) and with Leu/Val and Leu/Leu genotypes combined (OR 0.79, 95% CI 0.66–0.93). There was also a protective effect with the Leu/Leu genotype alone, (not statistically significant: OR 0.83, 95% CI 0.61–1.12), likely due to the low frequency of this genotype. These results suggest that there is an association between the factor XIII Leu allele and a modest protective effect against AMI and may provide useful information in profiling susceptibility to myocardial infarction.

Keywords

Factor XIII, genetic, meta-analysis, myocardial infarction, risk factor

Introduction

Thrombotic events in the coronary arteries resulting in myocardial infarction (MI) are the leading complication of arteriosclerosis (1). Inherited risk factors contribute significantly to the development of coronary artery disease (2); however, individual differences in hemostatic factors may modify an individual’s susceptibility for a MI.

Blood coagulation factor XIII (FXIII) is a tetrameric transglutaminase, consisting of two A-subunits that form the active site, and two B-subunits which protect the A-subunit from proteolytic degradation and non-proteolytic activation (3–5). The active form of FXIII, FXIIIa, is formed via cleavage by thrombin of the peptide bond between Arg-37 and Glu-38 of the A-subunit, and, in the presence of calcium, subsequent dissociation of the A-subunit dimer from the B-subunit, unmasking the catalytic site (5, 6). Once activated, FXIIIa covalently crosslinks fibrin monomers, α-2 antiplasmin, fibronectin, and collagen via γ-glutamyl-ε-lysine peptide bonds (3) resulting in a clot of high mechanical strength and increased resistance to fibrinolysis by plasmin (7).

The FXIII Val34Leu variant is a G-to-T transition in exon two of the gene encoding for FXIIIa, leading to a valine (Val) to leucine (Leu) substitution at amino acid 34 (8). The FXIII Val34Leu variant exhibits a heterogeneous ethnic distribution, being relatively common in Caucasians with an allele frequency of 0.27, less so in Africans (0.17) and rare in Asians (0.01) (9, 10). The biochemical consequences of the FXIII Val34Leu variant are not well understood. The FXIII Val34Leu variant does not result in a change in plasma concentration of FXIII, but the amino acid
change may modify FXIII activity (9, 11, 12). Activation of FXIIIA by thrombin was found to proceed two- to three-fold more rapidly in plasma of FXIII Val34Leu variant carriers (11, 13, 14). This has an effect on clot stability since the catalytic efficiency of thrombin-induced cleavage of FXIIIA alters the structure of the cross-linked fibrin such that fibrin fully cross-linked by FXIII Leu showed a finer structure with thinner fibers and smaller pores. Lateral aggregation of fibrin fibers is impaired (15).

It has already been shown that there is an association between FXIII Val34Leu variant and protection against venous thromboembolism (16). With respect to arterial thrombosis, previous published studies have suggested that this polymorphism is also protective against myocardial infarction (MI). However, this protective effect was not confirmed in other studies. The objective of this meta-analysis was to determine if there is an association between FXIII Val34Leu gene variant and protection against MI.

Methods

Search strategy

The electronic databases MEDLINE, EMBASE, and Healthstar were searched using the OVID search engine from 1966 to week 1 of April 2005. The search strategy was based on combinations of "myocardial infarction", "coronary artery disease", "coronary heart disease", "ischemic heart disease", "cardiovascular disease", "coronary arteriosclerosis", "coronary artery atherosclerosis", "Factor XIII", "Val34Leu", "V34L", "polymorphism", "mutation", and risk factor. We considered all studies (published in full or in abstract form) that examined the association between FXIII Val34Leu variant and myocardial infarction. In addition to the MEDLINE search, a manual search was performed of reference lists from the retrieved articles, and from abstracts from major thrombosis conferences (American Society of Hematology, December 2003 and 2004; International Society on Thrombosis and Haemostasis, July 2003).

Inclusion criteria

A study was considered eligible for our analysis if it was a case-control or prospective cohort study of patients with objectively diagnosed myocardial infarction (first or recurrent, non-fatal or fatal) consistent with the World Health Organization criteria, i.e. two out of three of typical ischemic chest pain, a rise in serum cardiac enzymes, and typical ECG changes involving the development of Q waves. Autopsy-proven MI was also considered as part of our study population. In addition, genotyping of patients for the FXIII Val34Leu variant must have been performed as part of the study.

Data extraction

Two reviewers (MS and JA) independently extracted data from the studies using a standardized form. Disagreements were resolved by discussion or in consultation with a third reviewer (PW). The following information was sought from each report: authors, journal and year of publication, country of origin, selection and characteristics of MI cases and controls, demographics, ethnic group of the study population, and number of cases and controls for each FXIII genotype. Frequency of alleles and genotypes were also obtained, and if not reported, were calculated from the available data.

Statistical methods

Based on the hypothesis that the Leu allele has a protective effect against MI, we performed three comparisons with the Val/Val genotype as the reference group: Leu/Leu genotype, Leu/Val genotype, and Leu/Leu and Leu/Val genotypes combined. We used the OR with a 95% CI as the metric of risk. Prior to pooling the studies for the meta-analysis, Hardy-Weinberg Equilibrium (HWE) was assessed in the control groups of individual studies by the goodness of fit Chi² statistic with one degree of freedom. A two-sided p-value of >0.05 was considered consistent with HWE. For each of the gene variant comparisons study heterogeneity was assessed using the Breslow-Day chi² statistic (17), but since several of the studies had small sample sizes, which may influence the power of the heterogeneity test, we used a conservative two-sided p-value of <0.10 as our threshold for considering the test significant for heterogeneity. (18). We also used the I² statistic as another test of heterogeneity, as it describes the percentage of variability in point estimates due to sample heterogeneity rather than sampling error (19, 20). I² values between 31% and 56% correspond to low to moderate level of heterogeneity, while those above 56% have “notable” heterogeneity (20). Our data showed consistent heterogeneity throughout all comparisons and thus a random effects model was used as the pooling method (21). We chose to use the DerSimonian-Laird random-effects model to combine the data, because the selection criteria were not entirely identical among the eligible studies and because heterogeneity tests may lack power in some context. This approach incorporates an estimate of the between-study variance and therefore, the confidence intervals tend to be wider when the studies differ among themselves. Again, this leads to more conservative estimates (22). Statistical analysis was performed with SAS software (Version 8.2, SAS Institute Inc., Cary, NC, USA).

Results

This search resulted in retrieval of 124 references from MEDLINE, 71 additional references from EMBASE, and no additional references from Healthstar. Of the 195 articles, 143 were eliminated because they did not involve patients with MI. A further 32 were eliminated because they were not case-control studies, including 25 review articles. One article was eliminated because the FXIII Val34Leu polymorphism was not part of the study. One article did not distinguish between MI patients and other causes of coronary heart disease, thus the available data could not be analyzed and this article was not included (23). Six articles were eliminated because the results reported were from data reanalyzed from a previously published article from the same authors already included in our search. The remaining 12 articles were considered eligible for data extraction based on the inclusion criteria. These were all case-control studies, as we did not identify any prospective cohort study that was eligible for our meta-analysis. Review of abstracts from major thrombosis conferences and symposia proceedings identified one potential article (24), but we were unsuccessful in our attempts to obtain the appropriate data.
The eligible studies summarized in Table 1 included a total of 8,743 subjects, of which 3,663 (41.9%) were acute MI patients and 5,080 (59.1%) were healthy controls (25–36). All patients had suffered a non-fatal MI, as there were no studies involving fatal MI. Three studies explicitly mention that only patients with their first MI were included (31, 33, 36). The remaining studies did not report a past history of MI in the baseline characteristics of their patient populations. The studies varied with respect to the age limits for eligible patients, and as a consequence the mean age was 52.6 in case subjects and 52.5 in control subjects. Five studies deliberately enrolled only patients less than 50 years of age (26, 27, 30, 33, 35). Eight out of 12 studies were carried out in Europe, and all studies involved predominantly Caucasian populations. Two studies contained only female patients (30, 31).
and one contained only male patients (36). Overall, females were less often represented in the studies, accounting for only 25.6% (n = 937) of cases and 40.8% (n = 2,072) of controls. Controls in most studies were age- and gender-matched to the cases, but only three studies mention matching for one or more traditional MI risk factors (27–29). Five studies mention risk factors for MI (e.g., hypertension, diabetes, dyslipidemia, family history of MI, smoking, obesity, male gender) that were more prevalent in the cases than controls (25, 26, 31, 32, 35) The remaining studies do not mention the prevalence of traditional risk factors for MI.

The distribution of the FXIII genotypes and Leu allele frequencies among MI cases and controls of the eligible studies are presented in Table 2. The distribution of genotypes in the control groups was consistent with Hardy-Weinberg equilibrium in all studies. In one study, the genotype frequencies for Leu/Val and Leu/Leu were not reported but were calculated based on the available allele frequencies (25, 30, 31, 33).

<table>
<thead>
<tr>
<th>Study</th>
<th>Val/Val Cases % (N)</th>
<th>Controls % (N)</th>
<th>Val/Leu Cases % (N)</th>
<th>Controls % (N)</th>
<th>Leu/Leu Cases % (N)</th>
<th>Controls % (N)</th>
<th>Leu allele frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kohler et al., 1998</td>
<td>68 (134)</td>
<td>52 (102)</td>
<td>27 (54)</td>
<td>41 (80)</td>
<td>5 (9)</td>
<td>7 (14)</td>
<td>0.183*</td>
</tr>
<tr>
<td>Franco et al., 2000</td>
<td>64 (96)</td>
<td>51.3 (77)</td>
<td>33.3 (50)</td>
<td>40.7 (61)</td>
<td>2.7 (4)</td>
<td>8 (12)</td>
<td>0.193</td>
</tr>
<tr>
<td>Cana et al., 2000</td>
<td>61.7 (124)</td>
<td>52.9 (129)</td>
<td>34.8 (70)*</td>
<td>42.2 (103)*</td>
<td>3.5 (7)*</td>
<td>4.9 (12)*</td>
<td>0.21</td>
</tr>
<tr>
<td>Corral et al., 2000</td>
<td>67.3 (68)</td>
<td>67.3 (68)</td>
<td>31.7 (32)</td>
<td>30.7 (31)</td>
<td>1.0 (1)</td>
<td>2.0 (2)</td>
<td>0.17</td>
</tr>
<tr>
<td>Gemmati et al., 2001</td>
<td>77.5 (93)</td>
<td>56.6 (136)</td>
<td>19.1 (23)</td>
<td>36.2 (87)</td>
<td>3.3 (4)</td>
<td>7.0 (17)</td>
<td>0.129</td>
</tr>
<tr>
<td>Reiner et al., 2002</td>
<td>60.3 (41)</td>
<td>54.2 (187)</td>
<td>35.3 (24)</td>
<td>40.0 (138)</td>
<td>4.3 (3)</td>
<td>5.8 (20)</td>
<td>0.221*</td>
</tr>
<tr>
<td>Kakko et al., 2002</td>
<td>69.0 (98)</td>
<td>62.0 (88)</td>
<td>26.8 (38)</td>
<td>31.0 (44)</td>
<td>4.2 (6)</td>
<td>7.0 (10)</td>
<td>0.176</td>
</tr>
<tr>
<td>ATVBISG, 2003</td>
<td>64.4 (779)</td>
<td>65.2 (789)</td>
<td>31.0 (375)</td>
<td>30.0 (363)</td>
<td>4.6 (56)</td>
<td>4.8 (58)</td>
<td>0.201*</td>
</tr>
<tr>
<td>Butt et al., 2003</td>
<td>53.0 (265)</td>
<td>52.2 (261)</td>
<td>38.6 (193)</td>
<td>41.4 (207)</td>
<td>8.4 (42)</td>
<td>6.4 (32)</td>
<td>0.277</td>
</tr>
<tr>
<td>Roldan et al., 2003</td>
<td>60.0 (108)</td>
<td>62.9 (268)</td>
<td>32.8 (59)</td>
<td>33.3 (195)</td>
<td>7.2 (13)</td>
<td>3.8 (22)</td>
<td>0.236</td>
</tr>
<tr>
<td>Reiner et al., 2003</td>
<td>65.0 (152)</td>
<td>56.3 (406)</td>
<td>29.1 (68)</td>
<td>36.1 (260)</td>
<td>6.0 (14)</td>
<td>7.6 (55)</td>
<td>0.205*</td>
</tr>
<tr>
<td>Doggen et al., 2003</td>
<td>55.9 (313)</td>
<td>55.4 (358)</td>
<td>36.3 (203)</td>
<td>38.4 (248)</td>
<td>7.9 (44)</td>
<td>6.2 (40)</td>
<td>0.26</td>
</tr>
</tbody>
</table>

*calculated value; N, number of subjects; Val, valine; Leu, leucine.

and one contained only male patients (36). Overall, females were less often represented in the studies, accounting for only 25.6% (n = 937) of cases and 40.8% (n = 2,072) of controls. Controls in most studies were age- and gender-matched to the cases, but only three studies mention matching for one or more traditional MI risk factors (27–29). Five studies mention risk factors for MI (e.g., hypertension, diabetes, dyslipidemia, family history of MI, smoking, obesity, male gender) that were more prevalent in the cases than controls (25, 26, 31, 32, 35) The remaining studies do not mention the prevalence of traditional risk factors for MI.

The distribution of the FXIII genotypes and Leu allele frequencies among MI cases and controls of the eligible studies are presented in Table 2. The distribution of genotypes in the control groups was consistent with Hardy-Weinberg equilibrium in all studies. In one study, the genotype frequencies for Leu/Val and Leu/Leu were not reported but were calculated based on the available allele frequencies (27). The allele frequencies were not reported in four studies but were calculated from the available genotype frequencies (25, 30, 31, 33).

The presence of the FXIII Val34Leu variant appears to confer protection against MI (Fig. 1). Using the random effects methods, the Val/Val genotype being the reference group, the combined OR for MI were 0.79 (95% CI 0.68–0.93) for the heterozygotes (Leu/Val vs Val/Val), 0.83 (95% CI 0.61–1.12) for the homozygotes (Leu/Leu vs Val/Val), and 0.79 (95% CI 0.66–0.93) for the combined Val/Val and Leu/Leu vs Val/Val. However, heterogeneity using the Breslow-Day statistic was considered significant for all comparisons (p values of 0.008, 0.03 and 0.03, respectively). The I² statistic revealed low to moderate heterogeneity in all comparison groups (Fig. 1).

Removing the earliest study (25) because of potential bias as suggested by Ioannidis et al. did not change the results (37). Analysing only those studies that enrolled patients less than 50 years old (26, 27, 30, 33, 35) also did not result in much change to the OR, but the 95% CI did cross one due to smaller numbers of patients (results not shown).

The association between the FXIII Val34Leu variant and protection against MI was first described by Kohler et al. in 1998. Subsequent studies published confirmed the protective association, while others did not find any significant association between FXIII Leu34 allele and MI. There are no previous meta-analyses in the literature that address the association between the variant and MI, and our meta-analysis is the first to demonstrate the inverse relationship of this variant with MI. We have also demonstrated the protective effect of the FXIII Val34Leu variant against venous thromboembolism which suggests a common mechanism in protecting patients from thrombotic events (16).

Table 2: Distribution of the factor XIII Val34Leu genotypes and allele frequencies among cases and controls in the included studies.

Discussion

In this meta-analysis, based on the results of 12 published case-control studies involving over 8,000 subjects, the presence of one or two copies of the FXIII Val34Leu variant is associated with lower odds of developing a MI. The protective effect was strongest in comparisons that included the heterozygote Leu/Val genotype. The protective effect was also seen with the homozygote Leu/Leu genotype, but this did not reach statistical significance, which may be due to the relative low frequency of this genotype in the general population (2–8% in controls). An analysis of the data with removal of the first publication (as suggested by Ioannidis et al.) did not change the results (37). Analysing only those studies that enrolled patients less than 50 years old (26, 27, 30, 33, 35) also did not result in much change to the OR, but the 95% CI did cross one due to smaller numbers of patients (results not shown).

The association between the FXIII Val34Leu variant and protection against MI was first described by Kohler et al. in 1998. Subsequent studies published confirmed the protective association, while others did not find any significant association between FXIII Leu34 allele and MI. There are no previous meta-analyses in the literature that address the association between the variant and MI, and our meta-analysis is the first to demonstrate the inverse relationship of this variant with MI. We have also demonstrated the protective effect of the FXIII Val34Leu variant against venous thromboembolism which suggests a common mechanism in protecting patients from thrombotic events (16).
Figure 1: Meta-analysis for the effect of the FXIII Val34Leu variant on the risk of myocardial infarction. Each comparison is presented by the name of the first author and year of publication. For each comparison, the point estimate of the odds ratio and the accompanying 95% confidence interval (CI) are shown. “Combined” represents the summary random-effects estimate for the comparison along with the respective 95% CI. The three comparisons with the Val/Val genotype as the reference group versus Leu/Leu genotype, Leu/Val genotype, and Leu/Leu and Leu/Val genotypes combined are shown.
meta-analysis involving patients with ischemic stroke did not find a statistically significant association with the FXIII Val34Leu variant (OR 0.97, 95% CI 0.75–1.25) (38). However, the authors assumed a recessive pattern of expression of the Leu variant, comparing those with Leu/Leu versus all others, rather than not presenting a specific inheritance model, the strategy we used in our analysis. As it is the case for most genes involved in complex traits, the appropriate genetic model is unknown for FXIII Val34Leu variant. Thus it is difficult to rule out the possibility that the Leu variant is protective in ischemic stroke without repeating the analysis using a strategy like the one we used. Our results are limited by the demonstrated heterogeneity. There are several possible explanations, most of which are illustrated in Table 1. First, only four studies clearly indicated that the cases selected were consecutive. Second, six studies clearly indicated a selection bias by using age criteria for study eligibility (<45 years in three studies). Two studies enrolled only women. Finally, only six studies attempted to match controls, and in these cases it was by age, sex and ethnicity, not other cardiac risk factors. Publication bias is also a concern, given that most of the studies that showed positive results (i.e. an association between the FXIII Val34Leu variant and MI) were small case-control studies. Interaction of this polymorphism with other traditional risk factors for MI is not known, and without individual patient data we were unable to test for associations between the FXIII Val34Leu variant and these factors. Two studies demonstrated a greater risk reduction for the occurrence of MI among smokers with the Leu34 allele (26, 32). In contrast, patients with insulin resistance who possess the Leu34 allele may lose the protective effect through interaction with insulin resistance and inhibition of fibrinolysis (39). The magnitude of risk attributed to the polymorphism in comparison to traditional risk factors has yet to be determined. The heterogeneity also led us to perform the analysis with a random effects approach. This point is widely debated (18) and is often advocated if there are small studies but in practice most authors use fixed effects only when there is limited or no heterogeneity.

Analysis on a per allele basis is sometimes performed in genetic association studies. We chose to follow recommendations based on a methodologic review of meta-analyses of molecular association studies (18) and suggested guidelines from an expert panel (40). Analysis on a per allele basis does not enable the assessment of the magnitude of effect of specific genotype. It is important to note that since we did not know the genetic model (i.e. dominant, recessive, etc), we did not pre-select one model over the other. We could have also chosen to test all of the possible models and combinations but this would have significantly increased the number of tests and would have required stringent multiple testing adjustment. Our choice of comparisons was based on our prior hypothesis that the Leu allele is either protective or has no effect. This was based on the literature which never reported a risk effect of the Leu allele (it was either a protective effect or no effect).

In summary, MI, as other complex and multi-factorial diseases, is likely to be under the influence of several genes, as well as environmental factors. The implication of an association between the FXIII Val34Leu variant and protection against MI is biologically plausible. It has been demonstrated that carriers of the FXIII Val34Leu variant have a more rapid activation and depletion of the FXIII A subunits. This results in less stable clots and indeed may result in a decrease in FXIII A available for stabilization of the clot and thus, could provide a protective effect against MI. The impact of the association between the FXIII Val34Leu variant and protection against MI from a clinical and public health perspective is unknown, and its applicability to the non-Caucasian population cannot be determined based on our study. Further research is required to determine if this protection is selective by age, gender or smoking status. Finally, it is important to note that FXIII is a gene with a very large number of polymorphisms (see http://pga.gs.washington.edu/data/f13al) and in the future haplotype analysis may provide a better approach to understanding the contribution of this gene to thromboembolic disease. Further research would be ideal before testing for this polymorphism in combination with testing for traditional cardiovascular risk factors in profiling individual susceptibility for MI.

Abbreviations

CI, confidence interval; FXIII, factor XIII; FXIII Val34Leu, factor XIII valine-34-leucine; Leu/Leu, leucine/leucine; OR, odds ratio; 95% CI, 95% confidence interval; Leu/Val, leucine/valine; Val/Val, valine/valine; MI, myocardial infarction.

References