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Coagulation Factor XIII Val34Leu Polymorphism in the Prediction of Premature Cardiovascular Events—The Results of Two Meta-Analyses

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Abstract: Background: Polymorphisms within the gene that encodes for coagulation factor XIII (FXIII) have been suggested to be involved in the pathogeneses of ischemic stroke (IS) and myocardial infarction (MI). The Val34Leu polymorphism is one of the most commonly analysed FXIII polymorphisms. However, studies on the role of the Val34Leu polymorphism in the aetiology of vascular diseases often show contradictory results. In the present meta-analysis, we aimed to pool data from available articles to assess the relationship between the FXIII Val34Leu polymorphism and the susceptibilities to IS of undetermined source and premature MI in patients aged below 55 years. Methods: We searched databases (PubMed, Embase, Google Scholar, SciELO, and Medline) using specific keywords (the last search was in January 2022). Eventually, 18 studies (627 cases and 1639 controls for IS; 2595 cases and 4255 controls for MI) met the inclusion criteria. Data were analysed using RevMan 5.4 and StatsDirect 3 link software. The relation between Val34Leu polymorphism and disease was analysed in five genetic models, i.e., dominant, recessive, additive, heterozygous, and allelic. Results: No relation between Val34Leu polymorphism and IS in young adults was observed in all analysed genetic models. For premature MI, significant pooled OR was found between the carrier state of the Leu allele (Val/Leu + Leu/Leu vs. Val/Val) and a lack of MI, suggesting its protective role (OR = 0.80 95%CI 0.64–0.99, p = 0.04). A similar finding was observed for the heterozygous model in MI (Val/Leu vs. Val/Val) (OR = 0.77 95%CI 0.61–0.98, p = 0.03). No relation was found for the recessive, additive, and allelic models in MI. Conclusions: In the population of young adults, no positive correlation was found between the FXII Val34Leu polymorphism and IS in any of the analysed genetic models. In turn, the carrier state of the 34Leu allele as well as FXIII heterozygotes themselves were found to play a protective role in relation to premature MI.

Keywords: arterial ischemic stroke; myocardial infarction; young adults; FXIII polymorphism

1. Introduction

The contribution of both genetic predisposition and well-known conventional risk factors to the pathogenesis of the first cardiovascular (CV) event in young people appears to be obvious.

Factor XIII (FXIII) is a multifunctional pro-γ-transglutaminase involved in the formation and stabilisation of a fibrin clot as well as the aggregation and adhesion of platelets [1,2]. Previously, it was suggested that FXIII may act as a modulator of various cell processes, i.e., migration, adhesion, proliferation, and apoptosis [3,4]. The cellular regulation mediated by active FXIII affects, inter alia, monocytes/macrophages, endothelial cells, and...
platelets, which have an impact on inflammatory and atherosclerotic processes in the vascular wall [3–5].

The common FXIII gene polymorphism, G > T transition in the second exon of the F13A1 gene, results in the substitutive exchange of leucine (Leu) for valine (Val) in the A subunit. Research has found higher activity of FXIII in Leu carriers, while Val homozygotes present a decrease in the activity of this factor [6,7]. The Val34Leu polymorphism of FXIII also influences the structure of a fibrin clot, especially in the presence of increased concentrations of fibrinogen, making it much tighter [8]. The role of hemostatic gene variants, e.g., polymorphisms within the gene encoding for coagulation factor XIII, remains of interest in the pathogenesises of ischemic stroke (IS) of undetermined source and premature myocardial infarction (MI) [9–13].

Cryptogenic stroke (CS), possibly embolic, is a common type of IS in the young population, accounting for up to 60% of stroke cases before age 45 and approximately 25% in the 45–49 age range [14]. Its non-atherosclerotic origin (embolic stroke of undetermined source, or ESUS) is supported by the lack of cardiometabolic risk factors or atherosclerotic changes in large proximal arteries and the non-lacunar location of infarcts on neuroimaging found in 65% of cases [15,16]. Undiagnosed episodes of atrial fibrillation with secondary thrombus formation in the left atrial appendage account for up to 30% of IS in the elderly [17] and may result in an ischemic event in the younger population as well. However, in younger people, deep venous thrombosis, including asymptomatic presentations, contributes to CS/ESUS pathogenesis in 10–22% of cases in the presence of cardiac right-to-left shunts, such as patent foramen ovale (PFO) [18]. Accordingly, the prevalence of inherited or acquired hypercoagulable states ranges from 3% to 21% in this type of stroke in groups <50 years old [19,20]. In addition, hypercoagulable conditions that cause arterial thrombosis, including antiphospholipid syndrome, can lead to cerebral embolism from intracardiac sources or cause in situ thrombosis in the cerebral, carotid, and coronary arteries without pre-existent significant stenosis [21].

In turn, the most common cause of premature MI or IS in people with unfavourable family history and/or conventional atherogenic risk factors is accelerated atherogenesis and atherothrombosis [22]. Atherosclerotic changes appear in the early decades of life (in the coronary vessels in the second decade and the cerebral vessels in the third decade) due to an inflammatory process in the arterial vascular wall. Plaque build-up is secondary to endothelial dysfunction, the proliferation of smooth muscle cells, the synthesis of connective tissue matrix, and the active accumulation of macrophages and lipids under the influence of inflammatory cytokines [23]. The rupture or erosion of unstable atherosclerotic plaque results in an acute thrombotic event [24]. Polymorphic variants of genes related to lipid metabolism, coagulation cascade, the renin–angiotensin pathway, or endothelial nitric oxide synthesis are common in the general population and increase the likelihood of atherosclerotic CV disease [25].

The aim of the present study was to summarise the results of available data regarding the relationship between FXIII Val34Leu polymorphism and premature CV events of atherosclerotic or thrombotic origin in the population of young adults.

2. Materials and Methods

2.1. Search Strategy

We searched five databases (PubMed, MEDLINE, Embase, SciELO, and Google Scholar) to identify available data published before January 2022 with the use of appropriate keywords: (“FXIII polymorphism” or “factor XIII polymorphism” or “Val34Leu polymorphism”) and (“ischemic stroke” or “stroke” or “myocardial infarction”) and (“young adults” or “young” or “premature” or “early”). The identified studies were included in accordance with the population, intervention, comparison, and outcome (PICO) model to select the relevant research question: Is FXIII Val34Leu polymorphism related to IS and MI susceptibility when comparing the prevalence of its alleles and genotypes in young patients with IS and MI against that in controls, according to five genetic models?
2.2. Inclusion/Exclusion Criteria for Analysed Studies

Searched studies were included in the meta-analysis if: (a) there was confirmed ischemic stroke or myocardial infarction, (b) a case–control study methodology was used, (c) the age of the patients was below 55 years, (d) access to genotypes distribution was available, (e) the article was written in a language other than English. Studies were excluded from the meta-analysis for the following reasons: (a) unavailability of genotyping results, (b) lack of reference (control) group, (c) lack of information on the age of the patients, or if the age of the patients was above 55 years, (d) if the material took the form of conference proceedings, review articles, case reports, or meta-analyses, or if the study was an animal study, and (e) if the article was written in a language other than English. When subgroups of patients younger than age 55 were available in the included studies, we used the data regarding those patients.

Finally, 18 case–control studies on young adults analysing FXIII polymorphism with regards to IS and MI met the inclusion criteria (3222 cases and 5894 controls in total), including 6 studies on IS (627 cases with stroke and 1639 controls [9,11,12,26–28]) and 13 studies on MI (2595 cases with premature MI and 4255 controls [10,12,13,29–38]). In the study by Reiner et al. [12], subgroups of patients with both IS and MI were analyzed. Figure 1 displays the flow diagram of the search process and reasons for excluding the studies.

![Flow chart presenting the process of searching for eligible articles.](image)

**Figure 1.** Flow chart presenting the process of searching for eligible articles.

2.3. Data Extraction and Methodological Quality

From each study that was included, the following data were extracted: the first author’s name, the year of the publication, the number of cases and controls, the ages of the cases and control subjects, and the number of the particular genotypes of FXIII Val34Leu polymorphisms in both patients and controls. Allele frequencies were calculated based on
genotype frequencies. Additionally, we used the Hardy–Weinberg equilibrium (HWE) to check the consistency of genotype distribution at the significance level of 0.05 for controls in each study that was included. The Newcastle–Ottawa scale (NOS) for case–control studies was used to evaluate the methodological quality of the included studies [39]. Using the NOS scale, points from 0 to 9 were assigned to each study. A study was considered to be of sufficient quality when the article achieved at least five points. In the case of deviation from HWE, the assumption of Minelli et al. [40] was adopted (to not exclude these studies when no other grounds for doubting the quality of the study were present).

2.4. Statistical Analyses
Statistical analyses were conducted twice using the Review Manager software (RevMan version 5.4; Cochrane, London, UK) and StatsDirect 3 link software (version 3.3.5; StatsDirect Ltd., Wirral, UK). We calculated the pooled odds ratio (OR) with a 95% confidence interval (CI) to determine the strength of association between the particular genetic model and the disease, that is, IS or MI. We selected the statistical model of the analyses (random or fixed) based on heterogeneity between the included studies; this was assessed using the I² test, which describes the proportion of variance (from 0% to 100%) due to variance in true effect sizes rather than sampling error. I² values of 25%, 50%, and 75% were correlated with low, intermediate, and high inconsistency, respectively. The random effects method (DerSimonian–Laird; REM) was used to calculate the pooled OR with a 95% CI when heterogeneity between the studies was significant; otherwise, the calculation was performed with the fixed-effects method (Mantel–Haenszel; FEM). The strength of the correlation between the FXIII Val34Leu polymorphism and IS and MI was assessed in the following models: dominant (Val/Leu + Leu/Leu vs. Val/Val), recessive (Leu/Leu vs. Val/Val + Val/Leu), additive (Leu/Leu vs. Val/Val), heterozygous (Val/Leu vs. Val/Val), and allelic (Leu allele vs. Val allele). To evaluate the stability of the results, sensitivity analyses were made via the sequential exclusion of each study.

To assess potential publication bias, both Egger’s regression and Begg’s rank correlation tests were performed. The result was considered statistically significant if the p value was below 0.05.

3. Results
3.1. Ischemic Stroke
3.1.1. Characteristics of the Studies Included
Characteristics of the six included studies analysing Val34Leu polymorphism within the FXIII gene and ischemic stroke in young patients are shown in Table 1. The genotype frequencies in control subjects were in agreement with HWE in all included studies. The dominant genotyping method was PCR-RFLP (in three out of six studies) [10,12,27]. The largest groups of both patients and controls were analysed by Pruissen et al. [26] and Shemirani et al. [28], whereas the fewest patients were recruited by Reiner et al. [12] and Ranellou et al. [27]. From the study by Shemirani et al. [28], a subgroup of female patients was extracted since the whole study group was much older and above the age we assumed as an inclusion criterion.

3.1.2. Association between FXIII Val34Leu Polymorphism and IS in Young Patients
Significant heterogeneity was observed for the dominant, heterozygous, and allelic analyses; thus, REM was used to calculate pooled OR. For recessive and additive models, no heterogeneity between the included studies was found; thus, pooled OR was assessed with FEM. In the case of the dominant model analysis of FXIII polymorphism (Val/Leu + Leu/Leu vs. Val/Val), no relation between the carrier state of the Leu allele and IS in young adults was observed. Similar findings were observed for the recessive (Leu/Leu vs. Val/Val + Val/Leu), additive (Leu/Leu vs. Val/Val), and heterozygous model (Val/Leu vs. Val/Val), as well as for the allelic (Leu allele vs. Val allele) models (Figure 2).
Table 1. Characteristics of the studies included to the meta-analysis regarding the relation between FXIII Val34Leu polymorphism and IS in young adults.

| Study (year) | Patients with Premature Ischemic Stroke | Controls | Genotyping Method | HWE (for Controls) (χ²; p) | QUALITY (Newcastle-Ottawa Scale) |
|--------------|-----------------------------------------|----------|-------------------|-----------------------------|----------------------------------|
|              | Population | Age       | N       | Genotypes of FXIII Val34Leu Polymorphism | Age       | N       | Genotypes of FXIII Val34Leu Polymorphism | Indicated Relation |                                  |
|              |            |           |         | Val/Val | Val/Leu | Leu/Leu |         | Val/Val | Val/Leu | Leu/Leu |                                    |                                  |
| Pruissen et al. [26] | Netherlands | Mean age: 39.8 years | 189 | 121 | 60 | 8 | Mean age: 38.6 years | 747 | 419 | 283 | 45 | 5‘nuclease/TaqMan assay | No | 0.093; 0.95 | 8 |
| Ranellou et al. [27] | Greece | Mean age: 37.8 years | 38 | 18 | 19 | 1 | Mean age: 38 years | 66 | 38 | 22 | 6 | PCR-RFLP method | No | 1.089; 0.58 | 8 |
| Reiner et al. [12] | USA | Mean age: 37.9 years | 36 | 16 | 14 | 6 | Mean age: 37.7 years | 345 | 187 | 138 | 20 | PCR-RFLP method | Yes, between Leu34 homozygotes and IS | 0.693; 0.71 | 8 |
| Salomi et al. [10] | India | Mean range: 32.7 years | 105 | 88 | 15 | 2 | Mean age: 31.8 years | 215 | 192 | 22 | 1 | PCR-RFLP method | No | 0.183; 0.91 | 9 |
| Shemirani et al. [28] | Hungary | Median age: 47 years | 159 | 91 | 61 | 7 | Median age: 47 years | 159 | 83 | 67 | 9 | Real time PCR | No | 0.913; 0.63 | 9 |
| Wypasek et al. [11] | Poland | Mean age: 43.4 years | 100 | 44 | 51 | 5 | Mean age: 43.6 years | 107 | 72 | 30 | 5 | Single nucleotide polymorphism (SNP) analysis | Yes | 0.644; 0.72 | 6 |
| TOTAL |             |           | 627 | 378 | 220 | 29 | TOTAL | 1639 | 1001 | 562 | 86 |                                  |                                  |
3.1.2. Association between FXIII Val34Leu Polymorphism and IS in Young Patients

Significant heterogeneity was observed for the dominant, heterozygous, and allelic analyses; thus, REM was used to calculate pooled OR. For recessive and additive models, no heterogeneity between the included studies was found; thus, pooled OR was assessed with FEM. In the case of the dominant model analysis of \( \text{FXIII} \) polymorphism (Val/Leu + Leu/Leu vs. Val/Val), no relation between the carrier state of the Leu allele and IS in young adults was observed. Similar findings were observed for the recessive (Leu/Leu vs. Val/Val + Val/Leu), additive (Leu/Leu vs. Val/Val), and heterozygous model (Val/Leu vs. Val/Val), as well as for the allelic (Leu allele vs. Val allele) models (Figure 2).

Figure 2. Forest plots for relations between different genetic models of FXIII polymorphism and ischemic stroke in total groups of young patients: (A) Val/Leu + Leu/Leu vs. Val/Val; (B) Leu/Leu vs. Val/Leu + Val/Val; (C) Leu/Leu vs. Val/Val; (D) Val/Leu vs. Val/Val; (E) Leu vs. Val. M-H: Mantel–Haenszel; CI: confidence interval; \( I^2 \): heterogeneity; df: degrees of freedom [10–12,26–28].
3.1.3. Sensitivity Analyses

In the sensitivity analysis, no change in the OR value was demonstrated in the case of all analysed genetic models after excluding subsequent studies. Therefore, these analyses were considered stable.

3.1.4. Publication Bias in the Total Group of Studies Analysing Val34Leu Polymorphism in the FXIII Gene and IS in Young Patients

Regarding the analyses for IS, publication bias was observed for the dominant and heterozygous genetic models. For the remaining models, no publication bias was observed since the shapes of the funnel plots were roughly symmetrical. Table 2 shows the exact results of both Egger’s and Begg’s tests for all genetic models between stroke patients and controls.

Table 2. The results of Egger’s and Begg’s tests for all genetic models between the studies analysing stroke patients and controls.

| Genetic Model     | Egger’s Test Intercept | 95% CI      | p    | Kendall’s Tau | p    |
|-------------------|------------------------|-------------|------|---------------|------|
| Dominant          | 4.543                  | −0.584 to 9.670 | 0.070 | 0.200         | 0.719|
| Recessive         | 0.359                  | −4.509 to 5.227 | 0.848 | 0.200         | 0.719|
| Additive          | 0.975                  | −3.906 to 5.857 | 0.609 | 0.333         | 0.469|
| Heterozygous      | 3.990                  | −1.174 to 9.154 | 0.098 | 0.467         | 0.272|
| Allelic           | 4.508                  | −0.644 to 9.660 | 0.072 | 0.200         | 0.719|

CI: confidence interval.

3.2. Myocardial Infarction

3.2.1. Characteristics of the Studies

Characteristics of the thirteen studies analysing Val34Leu polymorphism within the FXIII gene and premature MI are shown in Table 3. The genotype frequencies in control subjects agreed with HWE in all included studies except for the study by Hancer et al. [33]. In most included studies, the PCR-RFLP method was used to genotype the FXIII Val34Leu polymorphism (in 7 out of 13 studies) [10,12,30–33]. The largest groups of both patients and controls were analysed by the Atherosclerosis, Thrombosis, and Vascular Biology Italian Study Group [31]; Silvain et al. [10]; and Siegerink et al. [37], whereas the fewest young patients were analysed by Alkhiary et al. [29], Butt et al. [32], and Roldan et al. [36].

3.2.2. Association between FXIII Val34Leu Polymorphism and MI in Young Patients

Significant heterogeneity was observed in all genetic models; thus, REM was used to calculate pooled OR. In the case of dominant model analysis of FXIII polymorphism (Val/Leu + Leu/Leu vs. Val/Val), significant pooled OR was demonstrated between the carrier state of the Leu allele and a lack of MI, suggesting its protective role (OR = 0.80 95%CI 0.64–0.99, p = 0.04). A similar finding was observed for the heterozygous model (Val/Leu vs. Val/Val; OR = 0.77 95%CI 0.61–0.98, p = 0.03; Figure 3). No relation was found for the recessive, additive, and allelic models.
Table 3. Characteristics of the studies included to the meta-analysis regarding relation between FXIII Val34Leu polymorphism and myocardial infarction in young adults.

| Study (year)                          | Patients with Premature Myocardial Infarction | Controls | Genotyping Method | HWE (for Controls) (x²; p) | QUALITY (Newcastle-Ottawa Scale) |
|--------------------------------------|-----------------------------------------------|----------|-------------------|-----------------------------|--------------------------------|
|                                      | Population Age N Genotypes of FXIII Val34Leu Polymorphism | Age N Genotypes of FXIII Val34Leu Polymorphism | Genotyping Method | Indicated Relation |
| Alkhiary et al. [29] Egypt            | Mean age: 34.16 ± 3.65 years 31 Val/Val 24 Val/Leu 7 Leu/Leu | Mean age: 32.25 ± 3.89 years 20 Val/Val 15 Val/Leu 4 Leu/Leu | The CVD Strip Assay method | No 0.930; 0.33 |
| Ambrozak et al. [30] Poland           | Age < 50 years 143 Val/Val 76 Val/Leu 48 Leu/Leu | Age-matched to patients 150 Val/Val 85 Val/Leu 53 Leu/Leu | PCR-RFLP method | No 0.822; 0.26 |
| Atherosclerosis, Thrombosis, and Vascular Biology Italian Study Group [31] | Age < 45 years 1210 Val/Val 779 Val/Leu 375 Leu/Leu | Age-matched to patients 1210 Val/Val 789 Val/Leu 365 Leu/Leu | PCR-RFLP method | No 3.081; 0.06 |
| Butt et al. [32] Canada               | Age < 50 years 46 Val/Val 27 Val/Leu 19 Leu/Leu | Age < 50 years 373 Val/Val 197 Val/Leu 176 Leu/Leu | PCR–RFLP method | No 6 |
| Franco et al. [13] Brazil             | Mean range: 43 years 150 Val/Val 96 Val/Leu 50 Leu/Leu | Mean age: 42 years 150 Val/Val 77 Val/Leu 61 Leu/Leu | PCR–RFLP method | Yes, protective role for carriers of 34Leu allele 0.003; 0.99 |
| Hanor et al. [33] Turkey              | Age range: 18-50 years 95 Val/Val 85 Val/Leu 10 Leu/Leu | Age range: 16-50 years 112 Val/Val 68 Val/Leu 44 Leu/Leu | PCR–RFLP method | Yes, protective role for carriers of 34Leu allele 6.082; 0.01 |
| Mohammed et al. [14] Iraq             | Mean age: 42.6 ± 6.19 years 102 Val/Val 76 Val/Leu 22 Leu/Leu | Mean age: 45.6 ± 7.89 years 77 Val/Val 55 Val/Leu 21 Leu/Leu | The CVD Strip Assay method | No 0.414; 0.52 |
| Rallidas et al. [35] Greece           | Mean age: 32.1 ± 3.6 years 139 Val/Val 111 Val/Leu 43 Leu/Leu | Mean age: 31.6 ± 3.8 years 121 Val/Val 64 Val/Leu 30 Leu/Leu | The CVD Strip Assay method | Yes, protective role was observed 0.467; 0.49 |
| Reiner et al. [12] USA                | Mean age: 39.6 years 68 Val/Val 41 Val/Leu 24 Leu/Leu | Mean age: 37.2 years 345 Val/Val 167 Val/Leu 128 Leu/Leu | PCR–RFLP method | No 0.003; 0.71 |
| Roldan et al. [36] Spain              | Mean age: 44.8 ± 6.7 years 30 Val/Val 19 Val/Leu 6 Leu/Leu | Mean age: 47.6 ± 19.8 years 385 Val/Val 368 Val/Leu 195 Leu/Leu | PCR– allele-specific restriction assay method | Yes 0.376; 0.54 |
| Sagerström et al. [30] Sweden [31]    | Mean age: 42.8 ± 6.9 years 218 Val/Val 124 Val/Leu 80 Leu/Leu | Mean age: 38.6 ± 8.0 years 797 Val/Val 419 Val/Leu 283 Leu/Leu | The 5’ nuclease TaqMan assay | No 0.093; 0.76 |
| Silvain et al. [10] France            | Mean age: 39.1 ± 5.5 years 242 Val/Val 141 Val/Leu 87 Leu/Leu | Mean age: 39.1 ± 5.5 years 242 Val/Val 120 Val/Leu 99 Leu/Leu | PCR–RFLP method | No 0.519; 0.47 |
| Vishwajeet et al. [38] India          | Mean age: 37.1 ± 4.3 years 101 Val/Val 73 Val/Leu 27 Leu/Leu | Mean age: 30.4 ± 3.9 years 103 Val/Val 81 Val/Leu 20 Leu/Leu | Amplification-created restriction enzyme onPCR | No 0.332; 0.56 |
| TOTAL                                | 2595 Val/Val 1672 Val/Leu 779 Leu/Leu | TOTAL 4295 Val/Val 2513 Val/Leu 1331 Leu/Leu | | |

HWE: Hardy-Weinberg Equilibrium
3.2.2. Association between FXIII Val34Leu Polymorphism and MI in Young Patients

Significant heterogeneity was observed in all genetic models; thus, REM was used to calculate pooled OR. In the case of dominant model analysis of FXIII polymorphism (Val/Leu + Leu/Leu vs. Val/Val), significant pooled OR was demonstrated between the carrier state of the Leu allele and a lack of MI, suggesting its protective role (OR = 0.80 95%CI 0.64–0.99, \( p = 0.04 \)). A similar finding was observed for the heterozygous model (Val/Leu vs. Val/Val; OR = 0.77 95%CI 0.61–0.98, \( p = 0.03 \); Figure 3). No relation was found for the recessive, additive, and allelic models.

Figure 3. Cont.
were not significant after omitting the data from the studies by Franco et al. [13], Hancer et al. [33], and Rallidis et al. [35]. Thus, these analyses should be treated with caution.

3.2.3. Sensitivity Analyses

During sensitivity analysis, no change in the OR value was demonstrated in the cases of the recessive, additive, and allelic genetic models for MI after excluding subsequent studies. Therefore, these analyses were considered stable. However, in the case of the dominant model, after excluding subsequent studies by Butt et al. [32], Franco et al. [13], Hancer et al. [33], and Silvain et al. [10], the significance of the results was lost in REM analysis. Similarly, in the case of the heterozygous model, the results were not significant after omitting the data from the studies by Franco et al. [13], Hancer et al. [33], and Rallidis et al. [35]. Thus, these analyses should be treated with caution.

3.2.4. Publication Bias in the Total Group of Studies Analysing Val34Leu Polymorphism in the FXIII Gene and MI in Young Patients

For all of the genetic models, no publication bias was observed since the shapes of the funnel plots were roughly symmetrical. Table 4 shows the exact results of both Egger’s and Begg’s tests for all genetic models between MI patients and controls.

Figure 3. Forest plots for relations between different genetic models of FXIII polymorphism and myocardial infarction in total groups of young patients: (A) Val/Leu + Leu/Leu vs. Val/Val; (B) Leu/Leu vs. Val/Leu + Val/Val; (C) Leu/Leu vs. Val/Val; (D) Val/Leu + Val/Val; (E) Leu vs. Val. M-H: Mantel–Haenszel; CI: confidence interval; I²: heterogeneity; df: degrees of freedom [10,12,13,29–38].

Table 4. The results of Egger’s and Begg’s tests for all genetic models between the studies analysing MI patients and controls.

4. Discussion

The results of the present meta-analysis show different relationships for two types of ischemic events with different pathogeneses. In the case of IS with a cryptogenic background, often secondary to thromboembolic processes, we observed no relation with FXIII Val34Leu polymorphism in each genetic model analysed. On the other hand, when we collected patients with premature MI most often caused by accelerated atherosclerotic processes, we demonstrated that carrying the 34Leu allele (i.e., Val/Leu or Leu/Leu genotypes) could have a protective role. The carrier state of the Leu allele was more common in controls compared to young patients with MI (40% vs. 35.6%, respectively). Subjects with Val/Leu genotypes were more frequent in controls than in MI patients (36.3% vs. 32.1%, respectively) in reference to wild-type homozygous Val/Val, which may also suggest a protective role.
effect. However, these results should be treated with caution since there was some loss of significance after omitting subsequent studies. The results for the remaining genetic models did not reveal significance between \textit{FXIII} polymorphism and premature MI.

Coagulation factor polymorphisms, including \textit{FXIII} polymorphisms, have been analysed in the context of premature CV events, including coronary artery disease (CAD) \cite{33,41}, IS \cite{42}, haemorrhagic stroke \cite{43}, and venous thromboembolism \cite{44} in various populations and age ranges. Focusing on the young adult population makes it possible to reveal the influence of genetic factors, which in the population aged $\leq 55$ may still prevail over the influence of environmental factors, undiagnosed atrial tachyarrhythmias, and other major classic CV risk factors.

Numerous data confirmed the protective effect of 34Leu allele carriage on the development of premature MI. This effect was stronger in the 18–50-year-old population than in patients over 50 years of age \cite{11,12,33,35,36,45}. Most of the studies based their observations primarily on the higher frequency of the 34Val allele and the lower frequency of the 34Leu allele in the MI groups \cite{13,33,45}.

Val34Leu polymorphism is characterised by high ethnic variability. The prevalence of the 34Leu allele in Caucasians has been estimated at 37–51\% \cite{12,36,42,44}, while it shows lower prevalence among inhabitants of the Middle East (14–37\%) \cite{29}, South Asia (12\%) \cite{38}, and the Far East (up to 2.5\%) \cite{41,46}. Thus, the comparison of ethnically different groups may give misleading results. It is known that the development of premature MI/IS is influenced by many other genetic and environmental risk factors, including those indirectly related to ethnicity, e.g., the type of diet (protective role of the Mediterranean diet), the percentage of obese people in the population, habit and manner of smoking (pipes, cigars, glass pipes, shishas), the percentage of women using oral contraception, and polymorphisms regarding other genes related to the development of atherothrombosis and hypercoagulability \cite{47–51}.

In 1210 young adult Italian people \cite{31} with a history of MI, the effects of major CV risk factors, including family history (OR = 4.0), smoking (OR = 7.6), hypertension (OR = 4.5), being overweight (OR = 1.6), dyslipidaemia (OR = 1.4), and diabetes (OR = 7.4), were greater than the effects of genes involved in clotting, platelet function, fibrinolysis, or homocysteine metabolism, including the \textit{FXIII} 34Leu variant (OR = 1.1). In a group of 1030 Turkish patients, the protective role of the \textit{FXIII} Val34Leu polymorphism against MI was confirmed (OR = 0.31), but it was not an independent variable when major CV risk factors were taken into account in multivariate analysis \cite{33}.

Franco et al. \cite{13} reported different results and confirmed that the carrier state of the 34Leu allele reduced the risk of MI related to metabolic risk factors. Individuals who did not carry the 34Leu allele had a 13.9-fold higher risk of MI in the presence of hypertension, diabetes, dyslipidaemia, and obesity, while in 34Leu allele carriers, the risk was reduced to 6.8. In addition, the \textit{FXIII} 34Leu variant significantly reduced the risk of MI among smokers (OR = 3.9 in 34Leu allele carriers vs. OR = 6.1 in non-carriers). In the above study, the risk reduction was greater in homozygotes than in heterozygotes for the Leu allele, suggesting a gene dosage effect.

Importantly, the meta-analysis by Jung et al. \cite{52} found that the Val/Val genotype was associated with CAD in MI only and not in chronic coronary syndrome. Additionally, in a smaller group of Greek patients, the protective effect of the 34Leu allele carrier was limited to those with significant atherosclerotic lesions in the coronary arteries \cite{35}. It cannot be ruled out that increased thrombogenicity has clinical significance and results in the development of CV events, especially in the presence of genetically induced atherosclerotic plaques susceptible to rupture \cite{24}. However, some clinical studies on the involvement of FXIII in inflammatory processes \cite{4,53} may support the hypothesis about the atherogenic influence of this factor’s polymorphisms, alone or in association with other genes, in the development of premature atherosclerotic lesions. The identification of groups with increased CV risk may contribute each time to the earlier introduction of pharmacological prophylaxis, e.g., statins and acetylsalicylic acid, in the primary prevention of atherosclerotic events.
Conversely, most researchers did not confirm the protective effect of the Val34Leu polymorphism in relation to IS [26,27,54–57] or describe a higher percentage of 34Leu allele carriers in groups of patients who experienced cerebrovascular events, particularly in the presence of a PFO [9,11,12,58].

Elbaz et al. [59] described the protective effect of the 34Leu allele in a group of 456 patients aged 69 (20–85) years with IS (OR 0.58); this effect was independent of traditional CV risk factors and even exceeded the effect of smoking. However, in the population with IS, most researchers found no correlation between age, gender, the presence of traditional CV risk factors, the type of acute cerebrovascular event, and the Val34Leu genotype [11,26,42,54,57,59]. It is worth mentioning that Undas et al. [60] demonstrated higher anti-aggregation effectiveness from a low dose of aspirin in 34Leu allele carriers than in patients with the Val/Val genotype, and that the effect was also more significant for smokers.

An additional problem that makes it difficult to confidently assess the contribution of gene polymorphisms in CS patients is the presence of undetected, asymptomatic atrial tachyarrhythmias such as atrial fibrillation and atrial flutter. Large clinical trials [61,62] have confirmed the effectiveness of long-term ECG monitoring in verifying the causes of IS, with 9–10% of confirmed FA episodes in the ESUS patient groups. However, in these subgroups of young people at low risk, as assessed by the CHA2DS2-VASc score (0–1 points), refining the thromboembolic risk by assessing genetic predisposition may lead to earlier initiation of anticoagulant treatment for the primary prevention of IS, regardless of confirmation of atrial fibrillation.

It has also been proven that the interactions between various genetic factors and gene polymorphisms involved in the coagulation and fibrinolysis processes are important in the pathogenesis of premature atherosclerosis and hypercoagulability, and that their synergistic effect may be crucial in the development of CAD/MI [47,63] and IS [64]. Butt et al. [32] reported a 12-fold increase in MI risk in 500 Newfoundland inhabitants in whom the FXIII Leu34 allele and prothrombin 20210G > A (FII 20210A) coexisted. In turn, a decrease in the risk of MI was found in 34Leu carriers with high fibrinogen levels [45,51]. Reiner et al. [12] reported an increased risk of IS associated with the Leu34/Leu34 genotype, though only among young women who carried the alpha2 807T integrin allele, which was previously described as a risk factor for IS at a young age. It is worth mentioning that in the study by González-Conejero et al. [65], the efficacy and safety of fibrinolytic therapy in acute IS varied depending on the type of Val34Leu polymorphism and fibrinogen concentration. Carriers of the 34Leu variant with a high concentration of fibrinogen (>3.6 g/L) were less responsive to fibrinolysis. Moreover, patients with the 34Leu allele and patients with high fibrinogen concentration had a higher risk of severe haemorrhagic infarction and death following such therapy. These reports are consistent with the results of authors describing the synergistic effect of other hemostatic gene polymorphisms in increasing the risk of MI [47] and IS [66,67].

The present meta-analysis has some limitations. No additional data on other factors that could interact with the analysed FXIII polymorphism in the development of CV were available. Meta-analyses of some specific interactions between particular genes and factors that are simultaneously present in patients would be more accurate for understanding the role of the analysed polymorphism and the disease. In the case of FXIII Val34Leu polymorphism, the level of factor XIII should be especially considered.

5. Conclusions

The assessment of gene polymorphisms involved in the processes of coagulation and fibrinolysis may be important in the primary prevention of cardiovascular events in a group of young adults without classic risk factors. In young adults, no positive correlation was found between the FXIII Val34Leu polymorphism and IS in any of the analysed genetic models. In the case of premature MI, our meta-analysis demonstrated that the carrier state of the 34Leu allele might play a protective role in premature MI. In both cases, the influence
of gene–gene and gene–environment interactions on disease development should be taken into account.

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