Received: 26 July 2022 | Revised: 6 December 2022 | Accepted: 18 December 2022

https://doi.org/10.1016/j.rpth.2023.100048

rpth research & practice in thrombosis & haemostasis

ORIGINAL ARTICLE

Factor V Leiden but not the factor II 20210G>A mutation is a risk factor for premature coronary artery disease: a case-control study in Iran

Pasquale Agosti MD, PhD[1] | Ilaria Mancini MSc, PhD[1] | Saeed Sadeghian MD[2] | Maria Teresa Pagliari MSc[3] | Seyed Hesameddin Abbasi MD, PhD[2,4] | Hamidreza Pourhosseini MD[2] | Mohammadali Boroumand MD[2] | Masoumeh Lotfi-Tokaldany MD, PhD[2] | Emanuela Pappalardo MSc[1] | Alberto Maino MD, PhD[5] | Frits R. Rosendaal MD, PhD[6] | Flora Peyvandi MD, PhD[1,3]

[1]Department of Pathophysiology and Transplantation, Università degli Studi di Milano, and Fondazione Luigi Villa, Milan, Italy

[2]Tehran Heart Center, Cardiovascular Diseases Research Institute, Tehran University of Medical Sciences, Tehran, Iran

[3]Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, Milan, Italy

[4]Department of Global Health and Population, Bernard Lown Scholar in Cardiovascular Health, Harvard T.H. Chan School of Public Health, Boston, Massachusetts, USA

[5]Azienda Provinciale per i Servizi Sanitari, Ospedale Santa Chiara, Unit of Internal Medicine, Trento, Italy

[6]Department of Clinical Epidemiology, Leiden University Medical Center, Leiden, The Netherlands

Correspondence
Flora Peyvandi, Department of Pathophysiology and Transplantation, Università degli Studi di Milano, Via Pace 9, 20122, Milan, Italy.
Email: flora.peyvandi@unimi.it

Funding information
This work was partially supported by the

Abstract

Background: Factor V Leiden (FVL) and factor II c.*97G>A (rs1799963) are genetic risk factors for venous thromboembolism. Their contribution to coronary artery disease (CAD) is less clear.

Objectives: This study aimed to investigate the association between FVL, rs1799963, and premature CAD in Iranians.

Methods: We performed a genetic case-control study of 944 cases and 1081 controls from the premature CAD Milano-Iran study, including patients aged 18-55 (female) and 18-45 years (male) who underwent coronary angiography at the Tehran Heart Centre (Iran) in 2004-2011. Cases had luminal stenosis ≥50% in at least 1 main coronary artery or branch. Controls were age- and sex-matched with no CAD history. FVL and rs1799963 were genotyped using TaqMan SNP genotyping assays. Association was tested by logistic regression adjusted for matching factors and ethnicity. Effect modification by sex and cardiovascular risk factors (metabolic [obesity, hypertension, hyperlipidemia, and diabetes], and smoking) was assessed.

Results: The risk of premature CAD was increased by 50% in FVL carriers (adjusted odds ratio [adjOR] 1.54 [95% CI, 0.95-2.48]) and slightly reduced in rs1799963 carriers (adjOR 0.71 [95% CI, 0.40-1.27]). These effects were more pronounced in women than men (FVL, adjOR 1.66 vs 1.25; rs1799963, adjOR 0.60 vs 1.07). The risk of premature CAD was substantially increased in carriers of FVL with at least 1 metabolic risk factor compared with noncarriers without metabolic risk factors (adjOR 25.14 [95% CI, 12.51-50.52]).

P.A. and I.M. contributed equally to this study.

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Italian Ministry of Health - Bando Ricerca Corrente (RC2021) and by the Tehran Heart Center, Tehran University of Medical Sciences.

Handling Editor: L Castellucci

Conclusion: FVL but not FII rs1799963 was associated with an increased risk of CAD in young Iranians. This risk increased considerably when combined with metabolic cardiovascular risk factors.

KEYWORDS
coronary artery disease, factor V Leiden, factor II, hypercoagulability, myocardial infarction

Essentials

- This study aimed to understand the role of 2 gene mutations in coronary artery disease (CAD).
- We enrolled young Iranians with CAD and young Iranians from the general population.
- Individuals with the factor V Leiden mutation had a higher risk of CAD.
- The co-presence of factor V Leiden mutation and cardiovascular risk factors further increased CAD risk.

1 | INTRODUCTION

Coronary artery disease (CAD) is the major cause of death in developing and developed countries. CAD is a progressive chronic disease characterized by clinically silent periods but which can become unstable at any time. This occurs when the rupture or erosion of an unstable atherosclerotic plaque leads to an acute atherothrombotic occlusion by platelet- and fibrin-rich thrombi [1]. The underlying mechanism consists of atherosclerotic plaque progression in the epicardial arteries. Consistently with this mechanism, atherogenic factors (ie, hypertension, dyslipidemia, type 2 diabetes, and smoking) are well-established risk factors for CAD. CAD and venous thromboembolism (VTE) have been traditionally considered to have distinct pathogenesis. However, factors involved in the hemostasis pathway may play a pathogenic role also in the development of arterial thrombosis, by inducing a state of hypercoagulability. Indeed, different genetic association studies have already reported that variants located in genes encoding coagulation proteins modulate CAD risk [2–5]. Among these, the gain-of-function factor V Leiden (FVL) and factor II (FII) c.*97G>A variant (rs1799963, also known as factor II [FII] or prothrombin G20210A mutation) have been the most well-studied. FVL is a hereditary clotting alteration, with a prevalence of heterozygous carriers of 3% to 8% in Caucasian populations and of homozygous carriers of 1/5000 [6]. This variant consists of guanine to adenine substitution at nucleotide 1601 in the coagulation factor V gene, leading to the substitution of glutamine with arginine (c.1601G>A p.Arg534Gln) in the cleavage site of the natural anticoagulant activated protein C (APC). As result, factor Va becomes resistant to APC with a 10-fold slower inactivation rate than normal [7]. Heterozygous carriers of the FVL variant have a 7-fold increased risk of VTE and this risk grows up to 80-fold in homozygosity [6]. The rs1799963 variant is located in the 3′UTR of the FII gene and is associated with an increase in prothrombin plasma levels. Carriers of the AG genotype have a 2.8-fold increased risk of VTE compared with carriers of the reference GG genotype [8].

Although FVL and FII rs1799963 variants are well-established genetic risk factors for VTE, their contribution to CAD is less clear. Rosendaal and colleagues were the first to show an effect of these variants on acute myocardial infarction (AMI) in men [4] and young women [2,3], with relative risks, an increase ranging from 1.4 [4] to 4.0-fold [2,3]. However, these effects were not always confirmed by subsequent studies in other populations [9,10]. Overall, authors reported a CAD risk increase in carriers of these genetic risk factors of a small-moderate entity or confined only in those with at least 1 concomitant traditional cardiovascular risk factor. With this background, in the present study, we aimed to investigate the association of FVL and FII rs1799963 variant with premature CAD in a population of young Iranians and to assess whether the risk of premature CAD associated with these genetic variants is modified by the co-presence of traditional cardiovascular risk factors.

2 | METHODS

2.1 | Study design and subjects

We performed a genetic case-control association study in the frame of the premature CAD Milano-Iran study. The Milano-Iran study has been set up to investigate phenotypic and genotypic risk factors for CAD in young Iranians [11,12]. It includes consecutive young patients (women aged ≤55 years, men aged ≤45 years) who underwent diagnostic coronary angiography (CA) at the Tehran Heart Centre (Iran) between June 2004 and July 2011 for the following indications: unstable angina, stable angina, AMI, atypical chest pain (with positive exercise test or myocardial nuclear scan), valvular heart disease (candidate for catheterization), peripheral vascular disease (aorta disease, renal artery stenosis, carotid artery stenosis), and history of recent myocardial infarction. Patients with luminal stenosis greater than 50% in at least 1 main coronary artery or their branches were included as CAD cases. Patients with luminal stenosis below 50%

(minimal CAD) were excluded from this study, as well as patients with a history of myocardial infarction and unavailable DNA samples.

Controls were recruited from the Tehran Cohort Study, a population-based cohort enrolling between 2016 and 2019 more than 8000 voluntary participants over the age of 35 years from the general population of Tehran city with the purpose of detecting risk factors for cardiovascular disease, trauma, and their related psychologic factors [13]. Subjects unrelated with cases and free from a history of CAD, AMI, symptoms of stable or unstable angina, known symptomatic valvular heart disease, coronary angioplasty, or coronary artery bypass surgery were randomly selected from this cohort and frequency matched for sex and age (women aged <60 years, men aged <50 years).

The study was approved by the Ethics committee Board of Tehran Heart Center, and all patients and controls signed informed consent before inclusion in the study.

2.2 | Clinical data

Demographic, clinical, risk factors, and angiography procedural data were obtained from medical records and the CA Data Registry of Tehran Heart Center [11,12]. In this registry data of all the patients who undergo CA are routinely collected by trained physicians in the outpatient and inpatient settings at the time of CA. The collected information included age, sex, ethnicity, cardiovascular risk factors including body mass index (BMI), current or past smoking habits, history of hypertension, hyperlipidemia, and diabetes mellitus. BMI was calculated as weight (kilograms) divided by height squared (meters). Obesity was defined as when BMI was equal to or greater than 30 kg/m^2. Current smokers were those who smoked at least 1 cigarette per day, or who had stopped smoking for less than 1 year; former smokers were those who had stopped smoking for at least 1 year, and never smokers were those who have never smoked. Subjects were considered hypertensive in the presence of a blood pressure repeatedly higher than 140/90 mmHg or in the case of chronic use of antihypertensive medications. Hyperlipidaemia was defined as hypercholesterolemia (total cholesterol greater than 5.2 mmol/L) or hypertriglyceridemia (triglycerides greater than 2.3 mmol/L) or chronic intake of lipid-lowering drugs. Diabetes mellitus was defined if fasting blood glucose was greater than 7.0 mmol/L or blood glucose greater than 11.1 mmol/L after an oral glucose tolerance test, or in the presence of a history of diabetes mellitus, with or without the use of anti-diabetic medications.

2.3 | Genetic analysis

FVL (c.1601G>A; rs6025) and FII rs1799963 variants were genotyped using the TaqMan SNP genotyping assays (Applied Biosystems) in Milan (Italy) and Leiden (The Netherlands), following the manufacturer's instructions. For both FVL and FII rs1799963 variants, subjects were categorized as homozygous for the risk allele (AA), heterozygous (AG), or homozygous for the reference allele (GG). Carriership of the gene variants was defined as being homozygous or heterozygous for the risk allele (AA + AG subjects).

2.4 | Statistical analysis

Continuous variables were reported as the median and IQR, whereas categoric variables were reported as count and percentage.

The association between FVL or rs1799963 and premature CAD was assessed by logistic regression assuming an additive genetic model and adjusting for matching factors (age, sex) and ethnicity to calculate the odds ratios (ORs) and 95% CIs as measures of relative risk. Ethnicity was self-reported as Fars, Tork, Mazani, Gilak, Lor, and Kord. Other minor ethnic groups were grouped as others (eg, Turkmen and Arabs). The modifier effect of sex and major cardiovascular risk factors (smoking and metabolic risk factors [ie, at least 1 risk factor among obesity, hypertension, hyperlipidemia, and diabetes]) was assessed by stratification and interaction analyses, assuming additive effects [14]. Statistical analyses were performed by using the SPSS statistical software package (IBM SPSS Statistics for Windows, Version 27.0; IBM Corp).

3 | RESULTS

A total of 1000 CAD cases and 1082 controls were genotyped for FVL and FII rs1799963 variants. Genotyping failed for both variants in 56 cases and 1 control likely because of low concentration or poor quality of DNA samples, leaving 944 cases and 1081 controls available for analysis. Table 1 reports the demographic and clinical characteristics of the study participants. The majority of them (65%) were women and had a median age of 47 years. In both cases and controls the most prevalent ethnicity was Persian (52.3% vs 49.5%), followed by Turkish ethnicity (23.9% vs 32.8%). As expected, a higher proportion of traditional cardiovascular risk factors (family history of CAD, obesity, diabetes mellitus, hyperlipidemia, hypertension, and smoking) was observed in cases than in controls.

At the genotyping analysis, FVL and rs1799963 resulted to be low-frequency variants in the Iranian population, with minor allele frequencies in controls of 1.5% and 1.4%, respectively. Table 2 reports the results of the association analysis between FVL and rs1799963 variants with premature CAD. FVL in the heterozygous or homozygous state was found in 4.5% of cases, compared with 3% of controls. Carriership of FVL was associated with a 1.5-fold increased risk of CAD (OR adjusted for matching factors [age and sex], 1.53; 95% CI, 0.95-2.46), with no difference after adjusting for the potential confounding effect of ethnicity (OR, 1.54; 95% CI, 0.95-2.48). At variance with FVL, rs1799963 was less prevalent in cases than controls (2.2% vs 2.9% of carriers, respectively), with a fully adjusted OR (adjOR) of 0.71 (95% CI, 0.40-1.27).

When the analysis was stratified by sex, we found that the relative risk of premature CAD associated with FVL appeared greater in

TABLE 1 Baseline characteristics of study subjects.

Variables	Cases (*n* = 944)	Controls (*n* = 1081)
Female sex, *n* (%)	616 (65)	756 (70)
Age, y, median (IQR)	47 (43-52)	45 (40-51)
Ethnicity, *n* (%)[a]		
Fars	487 (52.3)	535 (49.5)
Tork	213 (23.9)	354 (32.8)
Mazani	73 (7.8)	22 (2.0)
Gilak	68 (7.3)	57 (5.3)
Lor	44 (4.7)	45 (4.2)
Kord	29 (3.1)	30 (2.8)
Other	17 (1.8)	38 (3.5)
Family history of CAD, *n* (%)[b]	411 (44)	242 (23)
BMI, Kg/m^2, median (IQR)[c]	29.2 (26.3-32.2)	27.5 (24.8-31.0)
Diabetes mellitus, *n* (%)[d]	340 (36)	125 (12)
Hyperlipidemia, *n* (%)[e]	707 (75)	278 (26)
Hypertension, *n* (%)	529 (56)	159 (15)
Cigarette smoking, *n* (%)[f]	263 (28)	139 (13)
Former, *n* (%)	69 (7.3)	7 (0.7)
Current, *n* (%)	194 (20.6)	132 (12.3)

BMI, body mass index; CAD, coronary artery disease.
[a]Available for 931 cases/all controls.
[b]Available for 941 cases/1071 controls.
[c]Available for 942 cases/1074 controls.
[d]Available for all cases/1080 controls.
[e]Available for 943 cases/1080 controls.
[f]Available for all cases/1073 controls.

women (adjOR, 1.66; 95% CI, 0.95-2.89) than in men (adjOR, 1.25; 95% CI, 0.48-3.31). The same more pronounced effect in women, but in the opposite direction, was found for the association between rs1799963 and CAD (adjOR women, 0.60; 95% CI, 0.29-1.23; adjOR men, 1.07; 95% CI, 0.39-2.91) (Table 3).

Smoking and the presence of at least 1 metabolic risk factor among obesity, hyperlipidemia, hypertension, or diabetes mellitus were strong risk factors for premature CAD. Among cases, 21% were current smokers and 92% had at least 1 metabolic risk factor, compared with 12% and 52% of controls, respectively. Table 4 shows the separate and combined effect of these major cardiovascular risk factors and the carriership of FVL or rs1799963 variants on the risk of premature CAD. Being a carrier of FVL increased the risk of CAD from 2.9- to 4.4-fold in smokers and from 12.2- to 25.1-fold in patients with at least 1 metabolic risk factor, compared with noncarriers without the cardiovascular risk factor. The risk increase estimate for the combination of FVL and smoking was similar to that expected by the sum of the 2 separate relative risks, suggesting the lack of any biological interaction between these 2 variables (OR for the combination 4.35 [95% CI, 1.14-16.56] vs expected OR assuming additive effects 1 + [1.56 − 1] + [2.92 − 1] = 3.48, being 1 the baseline risk). Conversely, the risk increase estimated by combining FVL with a metabolic risk factor was higher than expected by the sum of the 2 separate relative risks, making a positive interaction plausible (OR for the combination 25.14 [95% CI, 12.51-50.52] vs expected OR assuming additive effects 1 + [1.70 − 1] + [12.19 − 1] = 12.89). At variance with FVL, being a carrier of rs1799963 reduced the risk of CAD from 3- to 1.4-fold in smokers and from 11.8- to 8.2-fold in patients with at least 1 metabolic risk factor, compared with noncarriers without the corresponding cardiovascular risk factor. The risk estimated by the combination of rs1799963 with smoking or the presence of a metabolic risk factor was only slightly lower than expected based on the separate relative risks (OR for the combination with smoking 1.40 [95% CI, 0.44-4.40] vs expected OR assuming additive effects 1 + [0.75 − 1] + [2.96 − 1] = 2.71; OR for the combination with metabolic risk factors 8.16 [95% CI, 3.94-16.91] vs expected OR assuming additive effects 1 + [1.04 − 1] + [11.80 − 1] = 11.74).

TABLE 2 Association of FVL and rs1799963 with the occurrence of premature CAD.

FVL genotype	Cases (*n* = 911)	Controls (*n* = 1081)	OR (95% CI)	OR_1 (95% CI)
GG	870 (95.5)	1049 (97.0)	Reference	Reference
AG	39 (4.3)	31 (2.9)	1.49 (0.92-2.42)	1.49 (0.92-2.43)
AA	2 (0.2)	1 (0.1)	2.80 (0.25-31.79)	3.05 (0.27-34.66)
Carrier (AG + AA)	41 (4.5)	32 (3.0)	1.53 (0.95-2.46)	1.54 (0.95-2.48)
rs1799963 genotype	**Cases (*n* = 944)**	**Controls (*n* = 1081)**	**OR (95% CI)**	**OR_1 (95% CI)**
GG	923 (97.8)	1050 (97.1)	Reference	Reference
AG	19 (2.0)	31 (2.9)	0.67 (0.37-1.20)	0.64 (0.35-1.15)
AA	2 (0.2)	0 (0.0)	-	-
Carrier (AG + AA)	21 (2.2)	31 (2.9)	0.75 (0.42-1.31)	0.71 (0.40-1.27)

OR adjusted for the matching factors (age, sex).
CAD, coronary artery disease; FVL, factor V Leiden; OR, odds ratio; OR_1, OR adjusted for the matching factors (age, sex) and the possible confounder ethnicity.

TABLE 3 Association of FVL and rs1799963 and the risk of premature CAD in men and women subjects.

	Men			Women		
FVL genotype	**Cases (*n* = 314)**	**Controls (*n* = 325)**	**OR (95% CI)**	**Cases (*n* = 597)**	**Controls (*n* = 756)**	**OR (95% CI)**
GG	304 (96.8)	317 (97.5)	Reference	566 (94.8)	732 (96.8)	Reference
AG	10 (3.2)	8 (2.5)	1.25 (0.48-3.31)	29 (4.9)	23 (3.0)	1.60 (0.91-2.82)
AA	0 (0)	0 (0)	-	2 (0.3)	1 (0.1)	3.32 (0.28-39.00)
Carrier (AG + AA)	10 (3.2)	8 (2.5)	1.25 (0.48-3.31)	31 (5.2)	24 (3.2)	1.66 (0.95-2.89)
	Men			**Women**		
rs1799963 genotype	**Cases (*n* = 328)**	**Controls (*n* = 325)**	**OR (95% CI)**	**Cases (*n* = 616)**	**Controls (*n* = 756)**	**OR (95% CI)**
GG	320 (97.6)	317 (97.5)	Reference	603 (97.9)	733 (97.0)	Reference
AG	8 (2.4)	8 (2.5)	1.07 (0.39-2.91)	11 (1.8)	23 (3.0)	0.49 (0.23-1.05)
AA	0 (0)	0 (0)	-	2 (0.3)	0 (0)	-
Carrier (AG + AA)	8 (2.4)	8 (2.5)	1.07 (0.39-2.91)	13 (2.1)	23 (3.0)	0.60 (0.29-1.23)

OR adjusted for age (control matching factor) and ethnicity (possible confounder).

CAD, coronary artery disease; FVL, factor V Leiden; OR, odds ratio.

4 | DISCUSSION

The present study aimed to investigate the association between FVL and FII rs1799963 variant with premature CAD in a population of young Iranians and to assess whether or not this association was influenced by the concomitance of traditional cardiovascular risk factors. We found that FVL, but not the rs1799963 variant, was associated with an increased risk of premature CAD. Our findings are consistent with studies carried out in several populations belonging to a wide variety of ethnic groups (Caucasian, Asian, and African

TABLE 4 Combination of FVL and rs1799963 and major cardiovascular risk factors on the risk of premature CAD.

Cardiovascular risk factor	**FVL**	**Cases**	**Controls**	**OR (95% CI)**
Non-smokers, *n* (%)	GG	626 (95.1)	905 (96.9)	Reference
	AG + AA	32 (4.9)	29 (3.1)	1.56 (0.93-2.62)
Smokers, *n* (%)	GG	244 (96.4)	136 (97.8)	2.92 (2.23-3.82)
	AG + AA	9 (3.6)	3 (2.2)	4.35 (1.14-16.56)
No metabolic risk factor, *n* (%)	GG	68 (94)	496 (96)	Reference
	AG + AA	4 (6)	19 (4)	1.70 (0.56-5.18)
Metabolic risk factor, *n* (%)	GG	802 (96)	549 (98)	12.19 (9.10-16.32)
	AG + AA	37 (4)	13 (2)	25.14 (12.51-50.52)
Cardiovascular risk factor	**FII 20210**	**Cases**	**Controls**	**OR (95% CI)**
Non-smokers, *n* (%)	GG	666 (97.8)	909 (97.3)	Reference
	AG + AA	15 (2.2)	25 (2.7)	0.75 (0.38-1.46)
Smokers, *n* (%)	GG	257 (97.7)	133 (95.7)	2.96 (2.27-3.87)
	AG + AA	6 (2.3)	6 (4.3)	1.40 (0.44-4.40)
No metabolic risk factor, *n* (%)	GG	75 (97.4)	500 (97.1)	Reference
	AG + AA	2 (2.6)	15 (2.9)	1.04 (0.23-4.67)
Metabolic risk factor, *n* (%)	GG	847 (97.8)	546 (97.2)	11.80 (8.90-15.64)
	AG + AA	19 (2.2)	16 (2.9)	8.16 (3.94-16.91)

Metabolic risk factor: at least 1 among obesity, hyperlipidemia, hypertension, and diabetes.

Percentages are calculated within each stratum of cardiovascular risk factor (ie, among the non-smoker controls, 3% were carriers of FVL or rs1799963).

OR adjusted for sex, age (control matching factors), and ethnicity (possible confounder). Unadjusted OR yielded similar ORs.

CAD, coronary artery disease; FVL, factor V Leiden; OR, odds ratio.

American), wherein FVL resulted to be an independent risk factor for CAD, not only at early ages [15–21].

Our data confirmed also previous findings stemming from a large Italian case-control study of 1880 cases, wherein FVL but not rs1799963 was associated with an increased risk of AMI before the age of 45 years, also after adjusting for traditional cardiovascular risk factors [5]. However, unlike our study, the reported association of rs1799963 with AMI, albeit not statistically significant, was slightly positive (OR, 1.32; 95% CI, 0.96-1.80).

Other authors failed to find an association between FVL and CAD [9,10,22–34] and others reported an association between rs1799963 and a moderate increase in CAD risk [21,35]. The lack of consistency among studies may reflect differences in the study population characteristics, regarding the prevalence of cardiovascular risk factors which may act synergistically with FVL, as well as potential gene-gene interactions. Moreover, the differences in study design, genotyping procedures, sample sizes, and definitions used for the CAD endpoint and thus for cases and controls may have contributed to these varied findings. In particular, in a large proportion of studies, CAD was defined only on the basis of the clinical presentation, troponin alterations, and electrocardiographic ischemic changes and was not documented by CA. Therefore, a risk of misclassification as healthy subjects of patients with clinically silent CAD had to be considered.

In our study, the association between FVL and premature CAD was more pronounced in women than men, and this observation may be explained by considering the low incidence of AMI in young women. Indeed, even a small increase in the number of cardiovascular events may lead to high relative risks in a population characterized by low cardiovascular risk. Another possible explanation could be found in the different CAD etiology between men and women. For example, estrogens have been demonstrated to increase APC resistance. Therefore, FVL may play a more relevant role in AMI occurrence in young women [36]. This observation was previously supported by the findings in a large Dutch cohort (n = 1891) that the association of 5 hereditary thrombophilic defects (including FVL) with arterial thromboembolism was stronger in women younger than 55 years [37]. Consistently, in a study of 1653 AMI patients and 909 unrelated subjects from southern Italy, the allelic frequency of FVL was significantly higher in young AMI women (OR, 3.67) [38].

In the present study, the risk of CAD in FVL carriers was further increased and reached statistical significance when a cardiovascular risk factor was also present, with an overall OR of 4.35 or 25.14 for smoking and at least a metabolic risk factor, respectively. This result is consistent with previous studies showing a gene-environmental interaction between FVL and traditional cardiovascular risk factors on the occurrence of AMI. In a population-based case-control study of 84 young women with first AMI and 388 age-matched women controls, the risk of AMI in FVL carriers was increased 4-fold when adjusted for major cardiovascular risk factors. Smoking women who carried FVL had a 32-fold increased risk of AMI compared with nonsmoking noncarriers [2]. In another population-based case-control study among 560 men with a first AMI before the age of 70 years and 646 men control subjects, the AMI risk in FVL carriers was substantially increased when a major cardiovascular risk factor (among smoking, hypertension, diabetes mellitus, or obesity) was present [4].

We found a positive interaction between FVL and the presence of at least 1 metabolic risk factor. This positive interaction may be explained considering that both factors are proatherogenic and prothrombotic and thus their activities may be enhanced in a sort of synergism. Indeed, the FVL-induced increase in thrombin generation may lead to greater atherosclerosis development and progression, as shown by experimental models. In particular, homozygosity for FVL leads to enhanced atherosclerosis in mice [39–41]. This proatherogenic effect may be further accelerated when traditional atherogenic risk factors are present. Moreover, the FVL-related hypercoagulability may induce a higher risk of clot formation on the unstable plaque [42–50]. However, the interaction analyses rely on a lower number of subjects because of the combination of multiple risk factors, hence, their results should be interpreted with caution.

As concern the prothrombin gene variant, in our study it seemed to be protective on the risk of premature CAD. In agreement with previous studies, our findings indicate that prothrombin has no pathogenic role in CAD development. However, to our knowledge, there is no biological mechanism that could explain a protective effect. Indeed, higher prothrombin levels induce an increased thrombin generation and thus a state of hypercoagulability. Therefore, the unexpected inverse trend of association we observed has to be interpreted with caution. The present study has limitations. The case-control design has led to the exclusion of patients who died before reaching the coronary care units. However, the mortality rate is very low in young subjects, and it is unlikely that patients who died during the acute phase of AMI had a different prevalence of FVL or rs1799963 variants. Therefore, it is unlikely that this led to biased results. Moreover, data stemming from an Iranian young population do not necessarily apply to other populations. It has also to be noted that CIs around the ORs point estimates are wide, limiting the interpretation of our findings. However, this may be explained considering the rarity of the studied genetic variants and their combination with other risk factors, despite the aforementioned limitations, this study provided an opportunity to evaluate the association of FVL and prothrombin mutations with premature CAD, because young subjects are an optimal sample to investigate no atherosclerosis-related hereditary causes of CAD, considering their lower prevalence of traditional cardiovascular risk factors. Moreover, unlike other studies, our definition of CAD and thus of cases and controls was documented by CA. Last, the main interaction analyses with traditional cardiovascular risk factors have been accounted for.

5 | CONCLUSION

In conclusion, we found that FVL, but not the FII rs1799963 variant, was associated with an increased risk of premature CAD in young Iranians. This risk was further increased when combined with other cardiovascular risk factors. CAD has a complex etiology, resulting from the combined effects of genes and environment. Therefore, the contribution of a single prothrombotic mutation as FVL is likely to be

small and might be clinically relevant only in specific groups of patients. The lack of consistency among the published data in this field supports this consideration.

Our data do not justify a routine screening for FVL in cardiovascular prevention or genetic counseling for this mutation in family members of CAD patients. Further investigation is needed to better understand the role of FVL or rs1799963 on the occurrence of CAD and their gene-environment interactions, with the final goal to define the categories of patients who would benefit more from a thrombophilia screening in cardiovascular prevention and thus from a personalized preventive approach.

ACKNOWLEDGMENTS

The authors gratefully acknowledge Professor P. M. Mannucci for his critical revision.

FUNDING

This work was partially supported by the Italian Ministry of Health - Bando Ricerca Corrente (RC2021) and by the Tehran Heart Center, Tehran University of Medical Sciences.

AUTHOR CONTRIBUTIONS

I.M., M.T.P., S.H.A., F.R.R., and F.P. designed the study. S.S., S.H.A., H.P., M.B., and M.L.T. collected data. P.A., I.M., and M.T.P. analyzed the data. P.A., I.M., S.S., M.T.P., S.H.A., H.P., M.B., M.L.-T., E.P., A.M., F.R.R., and F.P. interpreted the data and carefully revised the manuscript. P.A. and I.M. wrote the manuscript.

RELATIONSHIP DISCLOSURE

I.M. received honoraria for participating as a speaker at educational meetings organized by Instrumentation Laboratory and Sanofi. F.P. has received honoraria for participating as a speaker in education meetings organized by Grifols and Roche, and she is a member of the scientific advisory boards of Biomarin, Roche, Sanofi, Sobi, and Takeda. The other authors do not have any conflict of interest to disclose.

TWITTER

Frits R. Rosendaal @FritsRosendaal

Flora Peyvandi @flora_peyvandi

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