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ORIGINAL ARTICLE

Assessing genetic risk factors for early-onset coronary artery disease in Iranians

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Abstract

Background: Coronary artery disease (CAD) is a leading cause of death worldwide. Evidence of genetic predisposition is derived mostly from studies of Europeans and East Asians.

Objectives: To test selected variants for association with early-onset CAD.

Methods: We performed a case-control study of 697 young angiography-defined CAD cases (women aged <55 years, men <45) and 911 age- and sex-matched controls in Iran. We tested 24 variants (12 established CAD loci, 12 candidates in hemostasis genes) using age- and sex-adjusted logistic regression, stratified by ethnicity. A 6-variant unweighted genetic risk score (GRS) was built from the top loci. Additive interaction with cardiovascular risk factors was assessed by relative excess risk due to interaction.

Results: Of 12 established loci, *HCG27–HLA-C*, *CDKN2A/CDKN2B*, and *SORT1* showed nominal or near-nominal replication and 4 were directionally aligned with prior literature, whereas 5 showed no association. Among hemostasis candidates, *F5* rs4524 was nominally associated with increased risk, and *TSPAN15* rs78707713 and *F11* rs2289252 suggested reduced risk. Ethnicity-stratified analyses indicated nominal or suggestive ancestry-specific effects at *SORT1*, *HCG27–HLA-C*, *CDKN2A/CDKN2B*, *LPA*, *VWF*, *MIA3*, and *CXCL12*. The GRS (range, 2-11 alleles/person) yielded a per-allele odds ratio of 1.20 (95% CI, 1.12-1.29), ie, a 5.2-fold higher risk across the observed range. Combining GRS with metabolic comorbidities showed super-additive effects (relative excess risk due to interaction, 6.66; 95% CI, 2.04-11.28).

Conclusion: Ten variants, including 3 previously linked to venous thrombosis, showed nominal or suggestive association with early-onset CAD in Iranians or specific ethnic groups. Genetic burden substantially amplified risk in the presence of metabolic

Ilaria Mancini and Maria Teresa Pagliari contributed equally to this study.

comorbidities. Our findings underscore the need for ancestry-aware studies in multiethnic populations.

KEYWORDS
coronary artery disease, genetic predisposition, genetic risk score, Iran, polymorphism, risk factors, single nucleotide

Essentials

- Most genetic studies of early coronary artery disease have focused on European or East Asian populations, while Middle Eastern populations remain underrepresented.
- We studied 697 young Iranian people with angiography-confirmed coronary artery disease and 911 controls, testing 24 selected genetic variants linked to coronary disease or blood clotting pathways.
- Several known coronary disease variants showed similar or population-specific effects in Iranians, and some clotting-related variants also showed possible links with early coronary disease.
- A simple 6-variant genetic risk score identified groups with higher risk, especially among people with metabolic risk factors, but this score remains exploratory and needs validation in independent cohorts.

1 | INTRODUCTION

Coronary artery disease (CAD), encompassing conditions such as subclinical atherosclerosis, angina pectoris, and myocardial infarction (MI), remains the leading cause of mortality and morbidity worldwide [1–3]. According to the Global Burden of Disease data, ischemic heart disease, a broader clinical category that approximates CAD, was responsible for 15% of all-cause deaths and 7% of disability-adjusted life years worldwide in 2023 [3]. Despite substantial advancements in prevention and treatment, CAD is projected to remain a major global health burden.

CAD arises through a complex interplay of environmental exposures and inherited susceptibility. Classical modifiable factors such as smoking, hyperlipidemia, hypertension, diabetes, and sedentary lifestyle remain pivotal, but twin and family studies consistently attribute about 40% to 60% of overall liability to genetic influences [4,5]. Recent findings from a genome-wide association study (GWAS) and a whole genome sequencing study indicate that genetic variation accounts for at least 30% of CAD susceptibility, with ultra-rare variants contributing to nearly 50% of that heritable component [6,7]. These proportions are likely even higher in early-onset CAD, where cumulative environmental damage is less pronounced. Examining patients with early-onset disease, in whom genetic contributions are less obscured by age-related factors, provides a valuable opportunity to elucidate the genetic underpinnings of CAD.

Genetic discovery has progressed from rare monogenic disorders such as familial hypercholesterolemia [8–10] to polygenic risk uncovered by GWASs [7,11–14]. Since the first landmark reports of the 9p21 locus in 2007 [15–18], more than 300 independent loci have been associated with CAD, implicating lipid metabolism, vascular inflammation, thrombosis, and arterial wall integrity, among other pathways [6,7,14,19–28]. Yet, >80% of these discoveries are derived from cohorts of European or East Asian ancestry, limiting their translational value for most of the world's population [29].

Beyond established CAD loci, hemostasis-related variation represents a biologically plausible source of risk that remains less systematically explored, particularly for early-onset disease, with available evidence again largely confined to populations of European ancestry. The VWF–ADAMTS-13 axis regulates platelet adhesion and thrombus formation, and prior experimental and epidemiological evidence links high VWF levels, low ADAMTS-13 activity, or an imbalance between the 2 to arterial thrombosis and MI [30–32]. In addition, variants in coagulation- and platelet-related genes previously associated with venous thromboembolism (VTE) may be relevant to CAD because thrombin generation, platelet activation, and coagulation pathway activity contribute not only to venous thrombosis but also to arterial thrombosis, particularly at the time of plaque rupture or erosion [33–35].

The ancestry bias in CAD genetic discovery is particularly problematic for the Middle Eastern region, which remains underrepresented in genomic studies and reference databases [6,28,36,37]. Iran illustrates this gap particularly well: a genome-wide population-genetic study and a whole-exome sequencing studies have shown that the Iranian population is genetically heterogeneous, includes multiple ethnic groups, and shows variable levels of consanguinity and admixture, with implications for the design and interpretation of medical genetic studies [36,37]. National burden-of-disease analyses reported 249,000 new cases of ischemic heart disease in 2023, accounting for 22% of all deaths and 8% of disability-adjusted life years, figures that exceed global averages [3]. Nevertheless, few studies have assessed genetic risk factors for CAD in Iranians, and even fewer have focused on early-onset CAD [38–41].

To address this gap, the Tehran Heart Center (Iran) and the Hemophilia and Thrombosis Center of Milan (Italy) initiated the Milano-

Iran (MIran) study in 2004 to investigate environmental and genetic risk factors for early-onset CAD in Iranians. Within this study, we performed targeted genotyping of both established and candidate variants, including polymorphisms in hemostatic genes that have been investigated in arterial disease outside large-scale GWASs or previously linked to venous thrombosis, with the dual goals of replicating associations reported predominantly in European and East Asian populations and evaluating whether selected hemostasis-related variants may contribute to early-onset CAD risk in an underrepresented, high-risk population.

2 | METHODS

2.1 | Study populations

We conducted a case-control genetic association study within the early-onset CAD MIran project, the design and rationale of which have been reported previously [42–44]. Cases were young patients (women aged ≤55 years, men ≤45) who underwent diagnostic coronary angiography at the Tehran Heart Centre (Iran) between June 2004 and July 2011. Indications for coronary angiography comprised unstable angina, stable angina, acute MI, atypical chest pain (with positive exercise test or myocardial nuclear scan), valvular heart disease (candidate for catheterization), peripheral vascular disease (noncardiac arterial disease, renal artery stenosis, carotid artery stenosis) and history of recent MI. CAD was diagnosed when a luminal stenosis ≥50% in ≥1 main coronary artery or their branches was detected. CAD patients with prior MI and with no or poor-quality DNA samples were excluded from this study.

Controls were sampled from the Tehran Cohort Study, a population-based cohort that enrolled >8000 city residents aged ≥35 years between 2016 and 2019 to study risk factors for cardiovascular disease, trauma, and their related psychological factors [45]. Participants unrelated to cases and without history of CAD, acute MI, 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 to cases by sex and approximate age (women <60 years, men <50 years).

The study was approved by the Tehran Heart Center Ethics Committee, and written informed consent was obtained from every participant.

2.2 | Clinical information and variable definitions

Demographic, clinical, and angiography data were abstracted from patients' medical records and from the Coronary Angiography Data Registry of the Tehran Heart Center, as detailed elsewhere [42,43]. The dataset comprised age, sex, ethnicity, conventional cardiovascular risk factors (family history of CAD, body mass index, smoking status, hypertension, hyperlipidemia, and diabetes mellitus) as well as procedural angiographic findings. Ethnicity was self-declared as Fars, Turk, Mazani, Gilak, Lor, Kurd, Turkmen, or Arab. Obesity was defined as body mass index ≥30 kg/m^2. Smoking status was categorized as current (≥1 cigarette/day or cessation <12 months), former (cessation ≥12 months) or never. Hypertension was diagnosed on the basis of repeated blood pressure measurements ≥140/90 mmHg or current antihypertensive therapy. Hyperlipidemia was defined as hypercholesterolemia (total cholesterol ≥5.2 mmol/L) or hypertriglyceridemia (triglycerides ≥2.3 mmol/L) or chronic use of lipid-lowering drugs. Diabetes mellitus was defined by fasting plasma glucose ≥7.0 mmol/L or blood glucose ≥11.1 mmol/L after oral glucose tolerance test or by a documented clinical diagnosis irrespective of treatment.

2.3 | Variant selection, genetic analysis, and quality control

Between January and June 2019, we compiled a panel of 25 single nucleotide polymorphisms (SNPs) for genotyping. The selection of these variants was conducted as follows: first, we performed a bibliographic search of GWASs and sequencing studies available at that time to identify top SNPs consistently associated with CAD phenotype; second, we ranked these variants based on the power we had to replicate the previously observed associations (≥40% power assuming a 2-tailed alpha error of 0.05 and our MIran population size), their reported effect size estimates, and clinical relevance; and third, we included 12 hemostasis-related candidate variants selected a priori on the basis of biological plausibility and previous literature. Two variants in *ADAMTS13* and *VWF* were selected because they had previously been associated with CAD-related arterial outcomes outside large-scale GWASs, whereas the remaining variants were selected because they had been associated with VTE in prior genetic studies and mapped to genes involved in VWF biology, thrombin generation, platelet activation, or coagulation pathway regulation, mechanisms that may also contribute to arterial thrombosis.

Genotyping was performed in Milan (Italy) using a custom TaqMan OpenArray panel on the QuantStudio 12K Flex Real-time PCR system (Applied Biosystems). Genomic DNA (20 ng per reaction) was amplified per the manufacturer's cycling conditions. Positive, negative, and no-template controls were included on each plate.

Stringent quality control (QC) criteria were applied to both samples and genotyping prior to analysis. Sample QC included verification of DNA concentration and purity for all samples, and, in a random subset of samples, DNA QC by 1% agarose gel electrophoresis and sex verification. DNA purity was assessed by the absorbance ratio at 260 and 280 nm (A260/A280), and samples with an A260/A280 ratio <1.8 were considered to have suboptimal purity and were excluded from genetic analyses. Genotyping QC included visual inspection of allelic discrimination plots to check the quality of amplification. Because this was a small targeted OpenArray panel and DNA quality varied across archived samples, we applied a conservative sample-level genotype completeness threshold to minimize

inclusion of samples with potentially unreliable genotype calls. Individuals with genotype calls for <95% of the assayed SNPs, corresponding to ≥2 missing genotypes of 25, were excluded from the analysis. Among excluded individuals, failed calls were often extensive, with a median of 22 missing calls of 25 assayed SNPs (IQR, 3.5-25), supporting the interpretation of sample-level DNA/genotyping quality issues rather than isolated failure of individual variant assays. Variant-level QC was then performed, and 1 SNP showing significant differential missingness between cases and controls ($P < .00001$) and significant deviation from Hardy–Weinberg equilibrium in controls ($P < .0001$) was excluded from the subsequent statistical analysis.

2.4 | Statistical analysis

Continuous variables were summarized as median and IQR and categorical variables as count and percentage. The association between each variant and early-onset CAD was tested by logistic regression under additive, recessive, and dominant genetic models. All models were adjusted for matching factors (age, sex) and results expressed with odds ratios (ORs) and 95% CIs as measures of relative risk. All association tests were 2-sided. Nominal statistical significance was defined as $P < .05$, whereas findings with $.05 \leq P < .10$ were described as suggestive. For established CAD loci that did not meet nominal statistical significance, effect estimates in the expected direction were described as directionally consistent. Established CAD loci were evaluated as a targeted replication panel based on previously reported associations and known direction of effect. For the exploratory hemostasis candidate variants, Benjamini–Hochberg false discovery rate q values were additionally reported to account for multiple testing across the 12 candidate variants. Exploratory analyses to assess the modifier effect of sex and ethnicity were carried out by stratification.

Next, we performed an exploratory combined variant analysis by constructing an unweighted genetic risk score (GRS) from the variants showing nominal or suggestive association with early-onset CAD in our Iranian cohort. The GRS was calculated by counting the total number of risk-increasing alleles in each individual. An unweighted score was chosen because reliable external effect estimates for early-onset CAD were not available for all included variants, particularly for the hemostasis candidates, and because using internally estimated weights would have further increased the risk of overfitting. The analysis was restricted to the subjects with a valid genotype for all variants included in the score.

Finally, we assessed the combined effect of the unweighted GRS (coded as >7 and ≤7) and the co-occurrence of major cardiovascular risk factors (categorized as current/former smoking or metabolic risk factors [ie, ≥1 risk factor among obesity, hypertension, hyperlipidemia, and diabetes]) by means of stratification and interaction analyses, assuming additive effects [46]. The relative excess risk due to interaction (RERI) was computed as $RERI = OR_{11} - OR_{10} - OR_{01} + 1$, where OR_{11} is the OR for joint exposure to both risk factors, OR_{10} and OR_{01} are the odds ratios for exposure to each factor alone, and 1 represents the baseline (unexposed) risk. The 95% CIs for RERI were derived via the delta method, based on the variance–covariance matrix of the GRS, risk factor, and interaction coefficients from the sex- and age-adjusted logistic regression. ORs were used as approximations of risk ratios.

Statistical analyses were performed using PLINK version 1.07 (http://pngu.mgh.harvard.edu/purcell/plink/) [47], the SPSS statistical software package (IBM SPSS Statistics for Windows, version 27.0, IBM Corp), and R (version 4.5.1, R Foundation for Statistical Computing).

3 | RESULTS

A total of 887 early-onset CAD cases and 950 controls were genotyped for 25 SNPs using a TaqMan OpenArray custom genotyping panel. After QC, 697 cases, 911 controls, and 24 SNPs were available for analysis (Figure 1).

Baseline characteristics of study participants are reported in Table 1. Participants were predominantly women (69%) with a median age of 46 years. The most frequent ethnic groups were Fars (53% of cases and 51% of controls) and Turk (22% vs 32%). Classical cardiovascular risk factors (family history of CAD, obesity, diabetes mellitus, hyperlipidemia, hypertension, and smoking) were more prevalent among cases, with broadly similar distributions across ethnicities (Supplementary Table S1). Table 2 [17,19–25,48–54] summarizes single-variant associations, assuming an additive model of inheritance for the 24 SNPs that passed QC, including 12 SNPs previously reported as associated with early-onset CAD in GWASs and sequencing studies and 12 SNPs mapping to hemostasis genes that were previously associated with CAD outside GWASs/sequencing studies or with venous thrombosis. Among the 12 previously reported CAD loci (Table 2, top), we observed nominal replication for *HCG27–HLA-C* rs3869109 (age- and sex- adjusted OR, 1.18; 95% CI, 1.02-1.38), *CDKN2A/CDKN2B* rs4977574 (OR, 1.16; 95% CI, 1.01-1.34), and a directionally consistent effect proximate to nominal replication for *SORT1* rs599839 (OR, 1.19; 95% CI, 0.99-1.43). Four additional loci (*LPA*, *PCSK9*, *SMARCA4/LDLR*, and *MIA3*) showed effect estimates aligned with the literature, whereas the remaining SNPs did not show evidence of association in this cohort.

Of the 12 hemostasis-related variants (Table 2, bottom), *F5* rs4524 showed a nominal risk-increasing association with early-onset CAD (age- and sex- adjusted OR, 1.21 [95% CI, 1.04-1.41]; age-, sex-, and factor V Leiden [FVL, rs6025]-adjusted OR, 1.20 [95% CI, 1.03-1.40]), whereas *TSPAN15* rs78707713 (OR, 0.79; 95% CI, 0.62-1.01) and *F11* rs2289252 (OR, 0.87; 95% CI, 0.75-1.01) showed suggestive risk-decreasing associations. The *VWF* rs35335161 variant showed an imprecise risk-increasing estimate overall (OR, 1.37; 95% CI ,0.83-2.27), consistent with prior association and functional evidence in the German population [49]. The remaining 8 variants in hemostasis genes did not show evidence of association with early-onset CAD in Iranians.

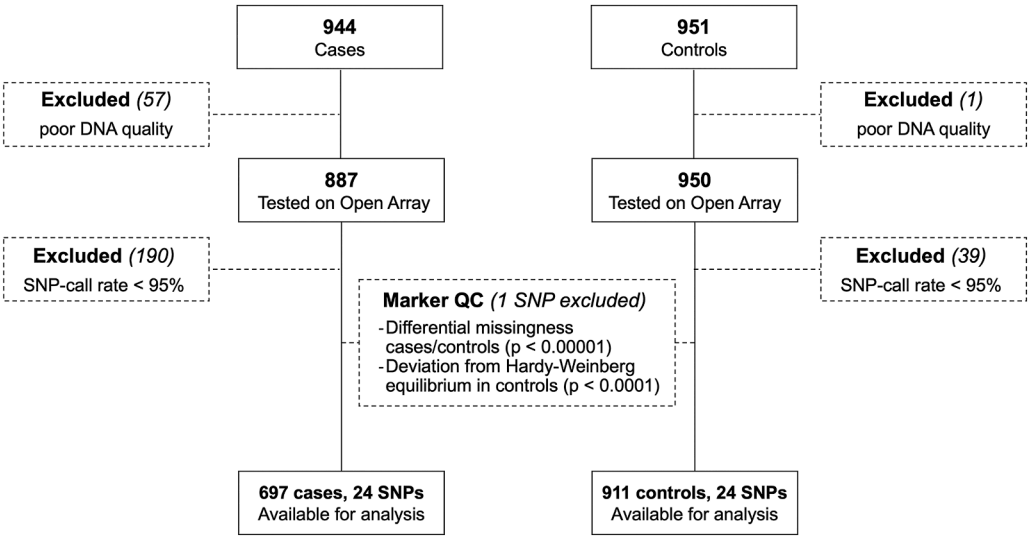

FIGURE 1 Study flow-chart. QC, quality control; SNP, single nucleotide polymorphism.

In ethnicity-stratified analyses (Table 3), the associations at *SORT1*, *HCG27–HLA-C*, and *CDKN2A/CDKN2B* appeared to be primarily driven by Turk, Fars, and Fars/other ethnicities, respectively. Additional nominal ethnicity-specific signals were noted: *LPA* rs3798220 in Fars (OR, 5.61; 95% CI, 1.17-26.8), *CXCL12* rs501120 with opposite effects in Fars versus other minorities (OR, 1.34 [95% CI, 1.06-1.69] and OR, 0.55 [95% CI, 0.38-0.80], respectively), and *MIA3* rs17465637 in Turks (OR, 1.42; 95% CI, 1.04-1.95) (Table 3, top). Regarding the newly investigated hemostasis-related SNPs, the association with *F5* rs4524 appeared primarily driven by the other minorities category (OR, 1.44; 95% CI, 1.02-2.02), while the associations with *TSPAN15* rs78707713 and *F11* rs2289252 appeared primarily driven by Fars (OR, 0.74 [95% CI, 0.53-1.04] and OR, 0.87 [95% CI, 0.70-1.07], respectively). Variant *VWF* rs35335161, which showed a risk-increasing estimate in the overall Iranian population, reached nominal association in the Fars ethnicity (OR, 2.45; 95% CI, 1.11-5.40) (Table 3, bottom).

Sex-stratified results (Table 4) suggested stronger effects among males for *SORT1*, *HCG27–HLA-C*, *CDKN2A/CDKN2B*, *TSPAN15*, and *F11*, whereas the effect of *F5* rs4524 appeared more pronounced in females. *LPA* rs3798220 showed a risk-increasing estimate only in females, and *VWF* rs35335161 showed a stronger trend in females, in line with the aforementioned previous reports in Germans [49].

We next constructed an exploratory unweighted GRS from the 6 variants showing nominal or suggestive association with early-onset CAD in our Iranian cohort: the previously reported *SORT1* rs599839, *HCG27–HLA-C* rs3869109, *CDKN2A/CDKN2B* rs4977574, and the newly identified *F5* rs4524, *TSPAN15* rs78707713, and *F11* rs2289252. Among 1556 individuals with complete genotyping, risk allele counts per person ranged from 2 to 11 (median 7 in both cases and controls). Using the median (7 alleles) as the reference category, the risk for early-onset CAD ranged from an OR of 0.51 (95% CI, 0.29-0.91) for $\leq$4 risk alleles to an OR of 1.32 (95% CI, 0.78-2.24) for $\geq$10 risk alleles (Figure 2). When modeled as an ordinal variable, the average per-allele increase in risk was 1.20 (95% CI, 1.12-1.29), corresponding to an approximately 5.2-fold difference in risk across the observed range in our Iranian population (2-11 alleles).

Finally, we examined joint effects of the unweighted GRS (>7 vs $\leq$7 risk alleles) and conventional cardiovascular risk factors (grouped into current/former smoking and metabolic risk factors) in the overall Iranian population. A GRS >7 increased the risk of CAD from 2.6- to 4.4-fold in smokers and from 13.6- to 21-fold in individuals with $\geq$1 metabolic risk factor, compared with those with GRS $\leq$7 without the respective risk factor (Table 5). On the additive scale, the observed combined effect of GRS >7 plus smoking only slightly exceeded the sum of individual effects, with a RERI of 1.23 (95% CI, −0.69 to 3.15). For GRS >7 plus metabolic risk factors, the departure from additivity was larger and statistically significant, resulting in a RERI of 6.66 (95% CI, 2.04-11.28), consistent with a positive interaction for metabolic risk but not for smoking.

4 | DISCUSSION

We conducted a case-control genetic association study within the MIran project to investigate genetic determinants of early-onset CAD in Iranians. Through a targeted analysis, we tested 24 prespecified SNPs (12 established CAD loci and 12 hemostasis variants) in 697 cases and 911 controls for association with angiography-defined CAD, derived a 6-variant unweighted GRS, and evaluated joint effects with smoking and metabolic risk using age- and sex-adjusted logistic models. We observed nominal replication of 2 established loci, *HCG27–HLA-C* rs3869109 and *CDKN2A/CDKN2B* rs4977574, and a directionally consistent effect close to nominal replication at *SORT1* rs599839 variant. Within a targeted hemostasis panel of variants, the major allele of *F5* rs4524 showed nominal association with higher risk of early-onset CAD, consistent with its reported effect in deep vein thrombosis (DVT),

TABLE 1 Baseline characteristics of study subjects.

Characteristic	Cases (*n* = 697)	Controls (*n* = 911)
Female sex, *n* (%)	461 (66)	641 (70)
Age, y, median (IQR)	47 (43-52)	45 (40-51)
Ethnicity[a], *n* (%)		
Fars	364 (53)	463 (51)
Turk	148 (22)	290 (32)
Mazani	54 (8)	13 (1)
Gilak	51 (8)	47 (5)
Lor	32 (5)	43 (5)
Kurd	24 (4)	21 (2)
Other minorities	12 (2)	34 (4)
Family history of coronary artery disease, *n* (%)	299 (43)	207 (23)
Body mass index, kg/m^2, median (IQR)	29.2 (26.4-32.4)	27.7 (24.8-31.1)
Diabetes mellitus, *n* (%)	259 (37)	105 (12)
Hyperlipidemia, *n* (%)	534 (77)	236 (26)
Hypertension, *n* (%)	398 (57)	142 (16)
Cigarette smoking, *n* (%)	185 (27)	115 (13)
Former	48 (7)	5 (1)
Current	137 (20)	110 (12)

Available data in cases/controls: ethnicity 685/all; family history 694/902; diabetes mellitus all/910; hyperlipidemia all/910; smoking all/904.
[a]Geographic distribution of ethnic groups: Fars, all over the Country; Kurd: Kurdistan province, Kermanshah Province, some parts of West Azerbaijan Province (all located in the West of Iran); Lor: Lorestan province (West of Iran); Gilak and Mazani: Gilan and Mazandaran provinces, respectively (North of Iran); Turk: West and East Azerbaijan, Ardabil, and Zanjan provinces in the North-West of Iran.

whereas the DVT-risk alleles *TSPAN15* rs78707713 and *F11* rs2289252 showed suggestive risk-decreasing estimates for CAD. A simple 6-variant, unweighted GRS yielded a risk gradient (per-allele OR, 1.20), corresponding to ~5.2-fold higher odds across the observed range of 2 to 11 risk alleles. Finally, GRS and metabolic risk factors displayed positive interaction on the additive scale (RERI, 6.66; 95% CI, 2.04-11.28), while the additive departure for GRS × smoking was small and not statistically significant (RERI, 1.23; 95% CI, −0.69 to 3.15).

Our data extend to an underrepresented Middle Eastern population the relevance of signals in the HLA region (HCG27–HLA-C) and cell-cycle control affecting vascular smooth muscle cell proliferation (CDKN2A/CDKN2B), and remain consistent with the lipid pathway via SORT1, albeit with limited power for some loci. Indeed, the other 3 lipid-related loci we tested, *LPA* (rs3798220 modulates lipoprotein(a) levels [19]), *PCSK9* (which promotes low-density lipoprotein-receptor degradation, thereby increasing low-density lipoprotein cholesterol levels [9]), and *SMARCA4/LDLR* (again influencing low-density lipoprotein cholesterol levels [20,55]), showed effects aligned with the literature but did not reach nominal significance in the entire cohort. This is unsurprising given both allele frequency differences between Iranians, Iranian ethnic groups, and gnomAD reference populations (Supplementary Table S2) and the modest effect sizes reported for *PCSK9* and *SMARCA4/LDLR* (ORs ~1.10-1.20 [20]). For example, *LPA* rs3798220 was uncommon overall, with a risk allele frequency of 0.008 in our controls, ranging from 0.002 in Fars, in whom nominal replication was observed, to 0.014 in Turks, versus ~0.02 in Europeans. Limited power and ethnicity-specific effects may also explain the directionally consistent but nonsignificant finding at *MIA3*, a locus involved in collagen secretion and extracellular matrix remodeling relevant to plaque biology [17,56]. In keeping with this, exploratory ethnicity-stratified analysis showed nominal replication of *MIA3* rs17465637 variant only in the Turk subgroup.

The remaining 5 established variants did not show evidence of association with CAD in young Iranians. For *BRAP–ALDH2* rs671 (a functional alcohol–aldehyde metabolism variant that is common in East Asians [25] but rare in this cohort) and *BTN2A1* rs6929846 (an immunity/inflammation variant that is several-fold more frequent here than in the Japanese populations in which it was first described [23]), ancestry-related effect heterogeneity is a plausible explanation. For the other 3 loci (*CXCL12* and *MRAS*, both implicated in vascular biology, and *SH2B3*, involved in immune signaling and inflammation), larger samples are needed to clarify their role in early-onset CAD in Iranians. Notably, the 95% CIs for *CXCL12* and *MRAS* overlapped previously reported effect sizes, so modest effects cannot be excluded. Additionally, we observed ethnicity-related heterogeneity for *CXCL12*: the variant appeared to increase risk in Fars, showed no material effect in Turks, and was protective in the aggregated 'other ethnicities' group. Hence, in addition to limited power, an important consideration is that these GWAS index SNPs were originally identified in largely European cohorts and may be suboptimal proxies for the underlying causal variants in Iranians. Differences in local linkage disequilibrium structure and allele frequencies across Iranian ethnic groups can weaken the correlation between the index SNP and the causal allele or even cause the same index variant to tag a different causal allele in this ancestry. Consequently, the observed effect at the index SNP may be attenuated or distorted, even if the underlying causal effect is shared across ancestries. This challenge is inherent to trans-ancestry analyses that rely on index SNPs discovered in other populations rather than on ancestry-specific fine-mapping.

Although VTE and CAD have distinct pathophysiologies, variation in coagulation and platelet pathways may influence arterial thrombosis, particularly at the time of plaque rupture or erosion [33–35]. The nominal risk-increasing association at *F5* rs4524 and the suggestive risk-decreasing estimates at *F11* rs2289252 and *TSPAN15* rs78707713 are coherent with this mechanistic framework. Functionally, *F5* rs4524 modulates activated protein C sensitivity and acts within *F5* haplotypes that influence thrombin generation and VTE risk; its direction can depend on the haplotype background and co-carriage of FVL (rs6025), with evidence for both prothrombotic and protective effects in different contexts [57,58]. Consistent with a

TABLE 2 Association of selected variants with the occurrence of early-onset CAD in Iranians.

Variants			Literature data		Entire population (*N* = 1608)		
SNP	Gene	Pathway	RAF (allele)	OR (95% CI)	RAF in controls	OR (95% CI), *P*	FDR *q*
GWAS-established loci							
rs3798220	*LPA*	Lipoprotein(a)/lipid metabolism	0.02 (C)	1.92 (1.48-2.49) [19]	0.008	1.44 (0.68-3.02), .34	-
rs501120	*CXCL12*	Chemokine signaling/ vascular biology	0.87 (T)	1.33 (1.20-1.48) [17]	0.75	1.03 (0.88-1.22), .68	-
rs599839	*SORT1*	LDL/VLDL metabolism (hepatic secretion)	0.77 (A)	1.29 (1.18-1.40) [17]	0.80	1.19 (0.99-1.43), .06	-
rs11206510	*PCSK9*	LDLR metabolism	0.81 (T)	1.15 (1.10-1.21) [20]	0.87	1.14 (0.91-1.41), .25	-
rs3869109	*HCG27–HLA-C*	Immune/inflammation (HLA region)	0.56 (G)	1.14 [24]	0.65	1.18 (1.02-1.38), .03	-
rs1122608	*SMARCA4/ LDLR*	LDLR/lipid regulation *(chromatin remodeling contributory)*	0.77 (G)	1.14 (1.09-1.19) [20]	0.73	1.06 (0.91-1.25), .45	-
rs671	*BRAP–ALDH2*	Aldehyde/alcohol metabolism & oxidative stress	0.28 (A)	1.43 (1.35-1.51) [25]	0.003	0.29 (0.03-2.50), .26	-
rs3184504	*SH2B3*	Immune signaling/blood pressure regulation	0.41 (T)	1.19 (1.13-1.25) [21]	0.39	0.89 (0.77-1.03), .12	-
rs6929846	*BTN2A1*	Costimulatory immunity/ inflammation	0.06 (T)	1.51 (1.28-1.77) [23]	0.24	0.95 (0.81-1.12), .56	-
rs4977574	*CDKN2A/ CDKN2B*	Cell cycle control/vascular remodeling	0.56 (G)	1.29 (1.25-1.34) [20]	0.55	1.16 (1.01-1.34), .03	-
rs17465637	*MIA3*	Extracellular matrix/collagen secretion	0.71 (C)	1.20 (1.12-1.30) [17]	0.72	1.08 (0.93-1.26), .31	-
rs2306374	*MRAS*	Signal transduction (MAPK/ PI3K)/vascular biology	0.18 (C)	1.15 (1.11-1.19) [22]	0.13	1.02 (0.83-1.25), .83	-
Newly investigated hemostasis variants							
rs685523	*ADAMTS13*	VWF proteolysis	0.09 (T)	Death from cardiac cause in CAD, 2.67 (1.59-4.49) [48]	0.06	0.80 (0.58-1.10), .16	0.47
rs35335161	*VWF*	VWF–platelet adhesion/ arterial thrombosis	0.05 (T)	≥1 MI in young, 2.53 (1.07-5.99); early-onset CAD, 1.62 (0.80-3.26) [49]	0.017	1.37 (0.83-2.27), .22	0.51
rs1063856	*VWF*	VWF levels	0.37 (C)	VTE, 1.23 (1.13-1.34) [50]	0.27	1.01 (0.86-1.18), .90	0.91
rs4524	*F5*	Coagulation (factor V, APC resistance)	0.73 (T)	DVT, 1.33 (1.19-1.48) [51]	0.69	1.21 (1.04-1.41), .02	0.23
rs78707713	*TSPAN15*	Platelet /ADAM10–Notch axis	0.89 (T)	DVT, 1.42 (1.24-1.62) [52]	0.92	0.79 (0.62-1.01), .06	0.36
rs5937	*PC*	Anticoagulant Protein C pathway	0.32 (C)	DVT, 1.37 (1.15-1.64) [50]	0.36	0.96 (0.83-1.12), .62	0.83
rs2289252	*F11*	Coagulation (intrinsic pathway)	0.41 (T)	DVT, 1.35 (1.27-1.44) [53]	0.32	0.87 (0.75-1.01), .07	0.30
rs2036914	*F11*	Coagulation (intrinsic pathway)	0.52 (C)	DVT, 1.20 (1.11-1.49) [53]	0.45	0.99 (0.86-1.13), .83	0.83
rs2227589	*SERPINC1*	Anticoagulant antithrombin pathway	0.10 (T)	DVT, 1.29 (1.11-1.49) [51]	0.15	0.92 (0.74-1.13), .40	0.53
rs2288904	*SLC44A2*	Leukocyte–platelet interaction/neutrophil biology	0.76 (G)	DVT, 1.28 (1.16-1.40) [52]	0.70	1.10 (0.94-1.28), .23	0.47

(Continues)

TABLE 2 (Continued)

Variants			Literature data		Entire population (*N* = 1608)		
SNP	Gene	Pathway	RAF (allele)	OR (95% CI)	RAF in controls	OR (95% CI), *P*	FDR *q*
rs1613662	*GP6*	Platelet collagen receptor signaling	0.82 (A)	DVT, 1.15 (1.01-1.30) [51]	0.80	0.91 (0.77-1.08), .27	0.51
rs1039084	*STXBP5*	Endothelial Weibel–Palade body/VWF secretion	0.54 (A)	VTE, 1.23 (1.14-1.35) [54]	0.49	0.98 (0.85-1.14), .82	0.83

For each variant, ORs and 95% CIs from the corresponding cited study are reported when available. The literature estimates for the GWAS-established loci were derived from European-ancestry CAD or MI cohorts, except for rs671, derived from a Japanese CAD GWAS, and rs6929846, derived from East Asian populations. The literature estimates for the newly investigated hemostasis variants were derived from Brazilian CAD patients (rs685523), a German early-MI cohort (rs35335161), US population-based VTE cohorts (rs1063856, rs5937, rs1039084), Dutch DVT cohorts or mainly European VTE studies (rs4524, rs2227589, rs1613662, rs2289252, rs2036914, rs78707713, rs2288904). Risk alleles are defined based on prior literature. ORs adjusted for matching factors (sex, age) from logistic regression assuming an additive model of inheritance are presented.

ADAM10, a disintegrin and metalloproteinase domain-containing protein 10; APC, activated protein C; CAD, coronary artery disease; DVT, deep vein thrombosis; FDR, false discovery rate; GWAS, genome-wide association study; HLA, human leukocyte antigen; LDL, low-density lipoprotein; LDLR, low-density lipoprotein receptor; MAPK, mitogen-activated protein kinase; MI, myocardial infarction; OR, odds ratio; PI3K, phosphoinositide 3-kinase; RAF, risk allele frequency; SNP, single nucleotide polymorphism; VLDL, very low-density lipoprotein; VTE, venous thromboembolism; VWF, von Willebrand factor.

prothrombotic contribution, our group previously showed that FVL (rs6025) was associated with early-onset CAD in this same MIran cohort [44]. Notably, adjusting for age, sex, and FVL did not materially change the *F5* rs4524 association with CAD in Iranians, suggesting that rs4524 is not simply a proxy for FVL in this population. *F11* rs2289252 associates with factor XI activity levels and DVT risk, consistent with a role of intrinsic pathway activation in thrombosis, although arterial endpoints show less consistent genetic evidence [53,59,60]. *TSPAN15* rs78707713 marks a robust VTE susceptibility locus; TSPAN15 interacts with ADAM10, a sheddase that modulates Notch and other substrates relevant to platelet/vascular biology, offering a plausible (albeit indirect) mechanistic link to thrombosis [52,61].

These findings illustrate both consistencies and discrepancies in the direction of effect compared with the VTE literature (consistency for *F5* rs4524 and opposite directions for *F11* rs2289252 and *TSPAN15* rs78707713). As noted above, differences in linkage disequilibrium patterns and allele frequencies across Iranian ancestries can weaken the tag–causal correlation at GWAS index SNPs and/or even cause the index SNP to tag a different causal allele in this ancestry. In addition, arterial and venous thrombosis involve overlapping but not identical biology (eg, plaque milieu, shear stress, platelet dominance), so the direction of effect at a given variant may differ by vascular bed. Taken together, these hemostasis signals, selected a priori from VTE literature, should be viewed as hypothesis-generating, pending replication and functional dissection.

The *VWF* rs35335161 estimate was risk-increasing overall and reached nominal association in Fars, aligning with prior biological plausibility and indicating a candidate for replication and functional follow-up. Importantly, rs35335161 (p.Phe2561Tyr) has been characterized as a gain-of-function *VWF* variant that enhances platelet aggregation and increases the risk of early MI, with stronger effects in young women (OR, 5.93; 95% CI, 1.12-31.24 in German young women experiencing >1 MI) [49], a pattern that mirrors our sex-stratified trend.

In stratified analyses, as mentioned above, effect estimates differed in magnitude across Fars, Turk, and other ethnicities, and several variants (eg, *SORT1*, *HCG27–HLA-C*, *CDKN2A/CDKN2B*) appeared stronger in men, whereas the *F5* rs4524 effect appeared more pronounced in women. These observations are exploratory, underpowered, and not adjusted for multiplicity across strata; nonetheless, they are consistent with the view that allele frequencies, linkage disequilibrium, and contextual exposures differ between groups and may modulate penetrance. Larger, ancestry-aware studies are needed to substantiate these patterns.

Finally, we defined an exploratory GRS that counted the total number of risk-increasing alleles per person in our Iranian cohort. The unweighted 6-SNP GRS yielded a monotonic risk gradient (per-allele OR, 1.20) and a separation of risk at the extremes, despite the small number of loci. This finding suggests that a concise allele count score may capture combined genetic burden in early-onset presentations. However, because the score was derived and evaluated in the same cohort, and because some included variants showed only nominal or suggestive association, its predictive performance is likely overestimated and should not be interpreted as externally validated. The use of an unweighted score was intended to avoid assigning internally derived weights, which would have further increased overfitting, and because reliable external effect estimates for early-onset CAD were not available for all selected variants, particularly the hemostasis candidates derived from VTE- or CAD-related literature. Thus, the GRS should be interpreted as an exploratory allele count measure of combined genetic burden rather than as a validated predictive polygenic risk score. Importantly, combining the GRS with the presence of any metabolic risk factor among obesity, hyperlipidemia, hypertension, and diabetes showed super-additive joint risk

TABLE 3 Association of selected variants with the occurrence of early-onset coronary artery disease in Iranians before and after stratifying by ethnicity.

		Entire population (*N* = 1608)		Fars (*n* = 827)		Turk (*n* = 438)		Other (*n* = 331)	
SNP	**Gene**	**RAF in controls**	**OR (95% CI), *P***	**RAF in controls**	**OR (95% CI), *P***	**RAF in controls**	**OR (95% CI), *P***	**RAF in controls**	**OR (95% CI), *P***
GWAS-established loci									
rs3798220	*LPA*	0.008	1.44 (0.68-3.02), 0.34	0.002	5.61 (1.17-26.80), 0.03	0.014	0.75 (0.19-2.92), .68	0.013	0.91 (0.22-3.75), .89
rs501120	*CXCL12*	0.75	1.03 (0.88-1.22), 0.68	0.73	1.34 (1.06-1.69), 0.01	0.74	0.99 (0.72-1.37), .97	0.80	0.55 (0.38-0.80), .001
rs599839	*SORT1*	0.80	1.19 (0.99-1.43), 0.06	0.80	1.16 (0.90-1.49), 0.25	0.79	1.52 (1.03-2.24), .03	0.82	1.02 (0.69-1.51), .93
rs11206510	*PCSK9*	0.87	1.14 (0.91-1.41), 0.25	0.86	1.10 (0.82-1.48), 0.51	0.88	1.13 (0.71-1.78), .61	0.89	1.18 (0.73-1.91), .50
rs3869109	*HCG27–HLA-C*	0.65	1.18 (1.02-1.38), 0.03	0.65	1.27 (1.02-1.56), 0.03	0.64	1.13 (0.84-1.53), .42	0.68	1.01 (0.73-1.38), .97
rs1122608	*SMARCA4/LDLR*	0.73	1.06 (0.91-1.25), 0.45	0.74	0.97 (0.78-1.22), 0.81	0.72	1.13 (0.82-1.55), .46	0.71	1.22 (0.85-1.74), .29
rs671	*BRAP–ALDH2*	0.003	0.29 (0.03-2.50), 0.26	0.003	0.00 (0-inf), 1.00	0.002	2.68 (0.16-44.21), .49	0.003	0.00 (0-inf), 1.00
rs3184504	*SH2B3*	0.39	0.89 (0.77-1.03), 0.12	0.38	0.96 (0.79-1.17), 0.70	0.41	0.89 (0.67-1.20), .44	0.36	0.83 (0.60-1.14), .24
rs6929846	*BTN2A1*	0.24	0.95 (0.81-1.12), 0.56	0.24	0.90 (0.72-1.13), 0.37	0.25	1.01 (0.73-1.40), .96	0.25	0.98 (0.67-1.43), .90
rs4977574	*CDKN2A/CDKN2B*	0.55	1.16 (1.01-1.34), 0.03	0.54	1.23 (1.01-1.49), 0.04	0.59	0.97 (0.73-1.30), .86	0.51	1.34 (0.98-1.83), .07
rs17465637	*MIA3*	0.72	1.08 (0.93-1.26), 0.31	0.74	1.00 (0.81-1.23), 0.97	0.69	1.42 (1.04-1.95), .03	0.74	0.96 (0.67-1.36), .81
rs2306374	*MRAS*	0.13	1.02 (0.83-1.25), 0.83	0.12	1.07 (0.80-1.44), 0.65	0.16	0.96 (0.67-1.40), .85	0.13	1.13 (0.70-1.82), .62
Newly investigated hemostasis variants									
rs685523	*ADAMTS13*	0.06	0.80 (0.58-1.10), 0.16	0.05	0.91 (0.58-1.42), 0.68	0.07	0.75 (0.40-1.40), .36	0.06	0.65 (0.31-1.34), .24
rs35335161	*VWF*	0.017	1.37 (0.83-2.27), 0.22	0.011	2.45 (1.11-5.40), 0.03	0.017	1.35 (0.50-3.66), .56	0.035	0.59 (0.22-1.57), .29
rs1063856	*VWF*	0.27	1.01 (0.86-1.18), 0.90	0.26	1.10 (0.89-1.37), 0.37	0.27	0.77 (0.55-1.06), .11	0.27	0.98 (0.69-1.38), .90
rs4524	*F5*	0.69	1.21 (1.04-1.41), 0.02	0.70	1.15 (0.93-1.42), 0.21	0.69	1.15 (0.84-1.58), .38	0.68	1.44 (1.02-2.02), .04
rs78707713	*TSPAN15*	0.92	0.79 (0.62-1.01), 0.06	0.92	0.74 (0.53-1.04), 0.09	0.92	0.82 (0.49-1.36), .44	0.91	0.95 (0.56-1.61), .84
rs5937	*PC*	0.36	0.96 (0.83-1.12), 0.62	0.36	0.93 (0.76-1.14), 0.46	0.34	1.26 (0.93-1.72), .14	0.38	0.80 (0.58-1.10), .18
rs2289252	*F11*	0.32	0.87 (0.75-1.01), 0.07	0.30	0.87 (0.70-1.07), 0.17	0.35	0.89 (0.66-1.21), .47	0.30	0.97 (0.69-1.37), .87
rs2036914	*F11*	0.45	0.99 (0.86-1.13), 0.83	0.41	1.03 (0.85-1.25), 0.73	0.55	0.90 (0.68-1.19), .44	0.40	1.19 (0.87-1.63), .28
rs2227589	*SERPINC1*	0.15	0.92 (0.74-1.13), 0.40	0.16	0.78 (0.58-1.04), 0.09	0.13	1.08 (0.71-1.64), .71	0.15	0.99 (0.65-1.51), .97
rs2288904	*SLC44A2*	0.70	1.10 (0.94-1.28), 0.23	0.70	1.05 (0.84-1.30), 0.68	0.69	1.02 (0.76-1.39), .88	0.69	1.18 (0.84-1.65), .34
rs1613662	*GP6*	0.80	0.91 (0.77-1.08), 0.27	0.79	0.96 (0.76-1.21), 0.71	0.82	0.89 (0.62-1.28), .53	0.80	0.92 (0.64-1.31), .63
rs1039084	*STXBP5*	0.49	0.98 (0.85-1.14), 0.82	0.49	0.97 (0.79-1.18), 0.75	0.49	1.16 (0.87-1.54), .31	0.52	0.87 (0.64-1.19), .38

Risk alleles are defined based on previous literature. ORs adjusted for matching factors (sex, age) from logistic regression assuming an additive model of inheritance are presented.

OR, odds ratio; RAF, risk allele frequency; SNP, single nucleotide polymorphism.

TABLE 4 Association of selected variants with the occurrence of early-onset coronary artery disease in Iranians before and after stratifying by sex.

		Entire population (*N* = 1608)		Males (*n* = 506)		Females (*n* = 1102)	
SNP	Gene	RAF in controls	OR (95% CI), *P*	RAF in controls	OR (95% CI), *P*	RAF in controls	OR (95% CI), *P*
GWAS-established loci							
rs3798220	*LPA*	0.008	1.44 (0.68-3.02), 0.34	0.011	0.77 (0.21-2.77), .69	0.006	2.09 (0.82-5.32), .12
rs501120	*CXCL12*	0.75	1.03 (0.88-1.22), 0.68	0.73	1.13 (0.84-1.52), .41	0.75	1.01 (0.83-1.23), .91
rs599839	*SORT1*	0.80	1.19 (0.99-1.43), 0.06	0.81	1.31 (0.95-1.81), .10	0.80	1.15 (0.92-1.43), .23
rs11206510	*PCSK9*	0.87	1.14 (0.91-1.41), 0.25	0.88	1.04 (0.71-1.52), .84	0.87	1.19 (0.91-1.55), .21
rs3869109	*HCG27–HLA-C*	0.65	1.18 (1.02-1.38), 0.03	0.65	1.28 (0.99-1.66), .06	0.65	1.13 (0.94-1.36), .19
rs1122608	*SMARCA4/LDLR*	0.73	1.06 (0.91-1.25), 0.45	0.71	1.17 (0.88-1.55), .29	0.73	1.03 (0.85-1.25), .78
rs671	*BRAP–ALDH2*	0.003	0.29 (0.03-2.50), 0.26	0.004	0.00 (0-inf), 1.00	0.002	0.58 (0.06-5.61), .64
rs3184504	*SH2B3*	0.39	0.89 (0.77-1.03), 0.12	0.39	0.85 (0.66-1.09), .19	0.38	0.93 (0.78-1.12), .45
rs6929846	*BTN2A1*	0.24	0.95 (0.81-1.12), 0.56	0.26	0.85 (0.64-1.12), .25	0.24	1.03 (0.84-1.26), .79
rs4977574	*CDKN2A/CDKN2B*	0.55	1.16 (1.01-1.34), 0.03	0.55	1.33 (1.03-1.71), .03	0.55	1.10 (0.93-1.30), .28
rs17465637	*MIA3*	0.72	1.08 (0.93-1.26), 0.31	0.72	1.01 (0.77-1.32), .95	0.72	1.14 (0.94-1.38), .18
rs2306374	*MRAS*	0.13	1.02 (0.83-1.25), 0.83	0.12	1.19 (0.83-1.70), .34	0.14	0.97 (0.76-1.25), .84
Newly investigated hemostasis variants							
rs685523	*ADAMTS13*	0.06	0.80 (0.58-1.10), 0.16	0.05	1.00 (0.59-1.70), .99	0.06	0.69 (0.46-1.04), .08
rs35335161	*VWF*	0.017	1.37 (0.83-2.27), 0.22	0.013	1.16 (0.40-3.37), .78	0.019	1.48 (0.83-2.63), .18
rs1063856	*VWF*	0.27	1.01 (0.86-1.18), 0.90	0.27	0.97 (0.73-1.29), .84	0.26	1.03 (0.85-1.24), .78
rs4524	*F5*	0.69	1.21 (1.04-1.41), 0.02	0.67	1.13 (0.86-1.49), .37	0.70	1.24 (1.02-1.49), .03
rs78707713	*TSPAN15*	0.92	0.79 (0.62-1.01), 0.06	0.93	0.65 (0.41-1.02), .06	0.91	0.87 (0.65-1.17), .36
rs5937	*PC*	0.36	0.96 (0.83-1.12), 0.62	0.34	0.92 (0.71-1.19), .51	0.37	1.00 (0.84-1.20), .99
rs2289252	*F11*	0.32	0.87 (0.75-1.01), 0.07	0.32	0.76 (0.58-1.00), .05	0.31	0.93 (0.78-1.12), .46
rs2036914	*F11*	0.45	0.99 (0.86-1.13), 0.83	0.44	1.05 (0.82-1.34), .72	0.46	0.98 (0.83-1.16), .84
rs2227589	*SERPINC1*	0.15	0.92 (0.74-1.13), 0.40	0.14	1.17 (0.83-1.65), .37	0.15	0.80 (0.61-1.03), .09
rs2288904	*SLC44A2*	0.70	1.10 (0.94-1.28), 0.23	0.71	1.25 (0.93-1.67), .14	0.69	1.04 (0.87-1.26), .65
rs1613662	*GP6*	0.80	0.91 (0.77-1.08), 0.27	0.79	1.03 (0.76-1.40), .84	0.81	0.86 (0.70-1.05), .14
rs1039084	*STXBP5*	0.49	0.98 (0.85-1.14), 0.82	0.53	0.94 (0.74-1.22), .67	0.48	0.99 (0.83-1.19), .95

Risk alleles are defined based on prior literature. ORs adjusted for matching factors (sex, age) from logistic regression assuming an additive model of inheritance are presented.

OR, odds ratio; RAF, risk allele frequency; SNP, single nucleotide polymorphism.

effects, as quantified by RERI, suggesting convergent biology (namely, lipid handling leading to more atherogenic lipids, vascular wall processes leading to a more reactive arterial wall, and a prothrombotic milieu lowering the threshold for thrombosis, consistent with the loci included in the score) that amplifies disease susceptibility in genetically predisposed individuals with metabolic comorbidity. By contrast, combining the GRS with smoking showed only a small, nonsignificant departure from additivity. These results underscore the value of examining additive interaction, which is often more relevant for public health and absolute risk, alongside conventional single-exposure analyses.

This study has several limitations, including (i) the sample size, which constrains power for low-frequency alleles and subgroup analyses, such as those in different ethnic groups; (ii) the candidate variant design rather than genome-wide coverage relying on GWAS index SNPs largely discovered in European/East-Asian cohorts, which, given potential differences in linkage disequilibrium and allele frequencies across Iranian ethnic groups, may provide imperfect tagging of the causal variant(s) or even tag different causal alleles (allelic heterogeneity), thereby attenuating or altering observed effects; (iii) potential differences between cases (2004-2011) and controls (2016-2019). Although cases were angiography-defined and controls underwent a comprehensive and

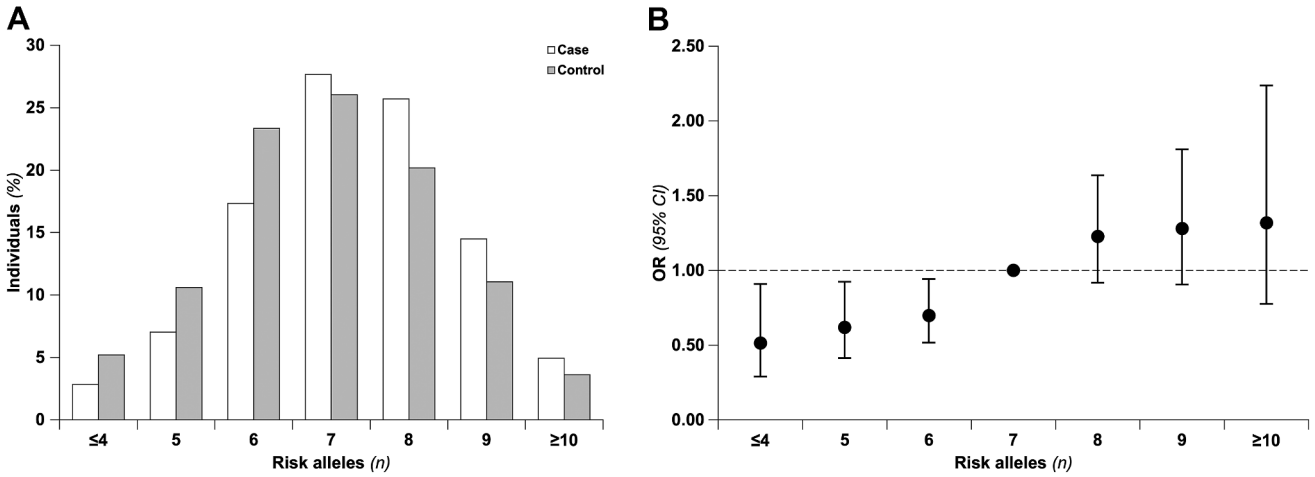

FIGURE 2 Genetic risk score from variants associated with early-onset CAD in Iranians. The 6-SNP risk allele distribution in patients with early-onset CAD and controls and corresponding ORs are represented in panels A and B, respectively. The genetic risk score was calculated by counting the total number of risk alleles in cases and controls (panel A). Age- and sex- adjusted ORs and 95% CI for early-onset CAD were calculated relative to the median number of risk alleles (7 alleles, panel B). CAD, coronary artery disease; OR, odds ratio; SNP, single nucleotide polymorphism.

standardized cardiovascular assessment, reducing outcome misclassification, this time gap may have influenced the prevalence and management of classical cardiovascular risk factors, making comparisons of risk factor distributions and gene–environment interactions more difficult to interpret. Germline allele frequencies, however, are expected to remain essentially stable over this time span within the same underlying population, so major bias in the primary genetic association estimates is unlikely. (iv) In addition, none of the hemostasis variants met statistical significance after false discovery rate control at 5%, reinforcing the exploratory nature of these signals and possibly reflecting limited power given our sample size. (v) Finally, the GRS was derived and evaluated in the same cohort using variants with nominal or suggestive association in this dataset. Therefore, the observed risk gradient may be affected by overfitting and should be interpreted as exploratory rather than predictive. Given the modest sample size, we did not split the cohort into derivation and validation subsets, as this would have produced unstable estimates; independent validation in larger Middle Eastern cohorts is required.

In summary, 10 SNPs showed nominal or suggestive association with early-onset CAD in Iranians or specific ethnic groups. Six SNPs showed nominal or suggestive association in the overall Iranian population: 3 mapping to immune-inflammatory, vascular smooth muscle cell proliferation, and lipid pathways, previously established in European and East Asian populations, and 3 in hemostatic genes previously implicated in venous, but not arterial, thrombosis. Four additional SNPs, 3 previously established and 1 hemostasis-related, showed nominal association in specific ethnic groups. Our findings support the generalizability of selected CAD loci to Iranians, highlight hemostasis-related variation as a plausible contributor to early-onset risk, and show that a concise GRS can identify subgroups in whom metabolic

TABLE 5 Combination of the GRS and major cardiovascular risk factors on the risk of early-onset coronary artery disease.

Cardiovascular risk factor	GRS	Cases, *n* (%)	Controls, *n* (%)	OR (95% CI)
Nonsmokers	≤7	267 (54)	495 (65)	Reference
	>7	224 (46)	272 (35)	1.55 (1.23-1.95)
Smokers	≤7	100 (56)	78 (68)	2.62 (1.82-3.77)
	>7	78 (44)	36 (32)	4.40 (2.83-6.85)
No metabolic risk factor	≤7	24 (53)	271 (65)	Reference
	>7	21 (47)	144 (35)	1.68 (0.90-3.13)
Metabolic risk factor	≤7	343 (55)	304 (65)	13.63 (8.66-21.44)
	>7	281 (45)	164 (35)	20.97 (13.12-33.50)

Metabolic risk factor: at least one among obesity, hyperlipidemia, hypertension, and diabetes. Percentages are calculated within each stratum of cardiovascular risk factor (ie, among the nonsmokers, 54% of cases and 65% of controls had a GRS ≤7). OR adjusted for sex and age (control matching factors). The unadjusted logistic regression and models adjusted for potential confounders as ethnicity, other cardiovascular risk factors (metabolic or smoking) and factor V Leiden yielded similar ORs (data not shown).

GRS, genetic risk score; OR, odds ratio.

burden confers super-additive risk. Furthermore, ethnicity-specific associations underscore the importance of studying the genetic determinants of CAD in genetically diverse, underrepresented populations. Further work is needed to replicate these findings in larger Middle Eastern cohorts and to evaluate the newly identified hemostasis signals in other populations.

Ultimately, because a 'one-size-fits-all' approach is inadequate, a weighted composite score integrating genetic variants, clinical factors, biomarkers, geographic/ancestry modifiers, and lifestyle, together with predicted drug response and an individual's thrombotic–bleeding propensity, should be developed to guide personalized treatment.

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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.A.B., and M.L.-T. collected clinical data. M.T.P. and E.P. performed genotyping experiments. I.M. and M.T.P. analyzed the data. I.M. wrote the manuscript. All authors interpreted the data, carefully revised the manuscript, and approved the final version of the article.

RELATIONSHIP DISCLOSURE

I.M. received honoraria for participating as a speaker at educational meetings organized by Werfen and Sanofi. P.A. received honoraria for participating as a speaker at educational meetings organized by Sanofi. F.P. received honoraria for participation in symposia and educational events organized by Sanofi, Sobi, Biomarin, Kedrion, and Bayer, and she participated to scientific advisory committees organized by Sanofi, Sobi, Roche, CSL Behring, Novo Nordisk, Biomarin, Regeneron, Metagenomi, Star-Therapeutics, Bayer, Pfizer, Takeda, and TargED. The other authors do not have any conflict of interests to disclose.

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SUPPLEMENTARY MATERIAL

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