Thrombosis Research 230 (2023) 74–83

Contents lists available at ScienceDirect

Thrombosis Research

journal homepage: www.elsevier.com/locate/thromres

Full Length Article

Factor V Leiden, prothrombin, MTHFR, and PAI-1 gene polymorphisms in patients with arterial disease: A comprehensive systematic-review and meta-analysis

Emanuele Valeriani [a,b,*], Daniele Pastori [c], Giulia Astorri [c], Angelo Porfidia [d], Danilo Menichelli [a], Pasquale Pignatelli [c]

[a] Department of General Surgery and Surgical Specialty, Sapienza University of Rome, Piazzale Aldo Moro 5, 00185 Rome, Italy
[b] Department of Infectious Disease, Umberto I Hospital, Viale del Policlinico 155, Rome, Italy
[c] Department of Clinical Internal, Anesthesiological and Cardiovascular Sciences, Sapienza University of Rome, Piazzale Aldo Moro 5, 00185 Rome, Italy
[d] Department of Medicine, Fondazione Policlinico Universitario A. Gemelli IRCCS, Università Cattolica del Sacro Cuore, Largo Agostino Gemelli, 00168 Rome, Italy

ARTICLE INFO

Keywords:
Cerebrovascular disorder
Coronary artery disease
Genetic polymorphisms
Peripheral arterial disease
Thrombophilia

ABSTRACT

Introduction: The role of inherited thrombophilia in arterial disease is uncertain. We performed a systematic-review and meta-analysis of inherited thrombophilia in cerebrovascular (CVD), coronary heart (CHD), and peripheral artery disease (PAD) patients.

Materials and methods: MEDLINE and EMBASE were searched up to February 2022. Pooled prevalences (PPs) and odds ratios (ORs) with 95 % confidence intervals (95%CI) were calculated in a random-effects model. Factor V Leiden (G1691A), prothrombin (G20210A), MTHFR C677T/A1298C and PAI-1 4G/5G were evaluated.

Results: 377 studies for 98,186 patients (32,791 CVD, 62,266 CHD, 3129 PAD) and 108,569 controls were included. Overall, 37,249 patients had G1691A, 32,254 G20210A, 42,546 MTHFR C677T, 8889 MTHFR A1298C, and 19,861 PAI-1 4G/5G gene polymorphisms. In CVD patients, PPs were 6.5 % for G1691A, 3.9 % for G20210A, 56.4 % for MTHFR C677T, 51.9 % for MTHFR A1298C, and 77.6 % for PAI-1. In CHD, corresponding PPs were 7.2 %, 3.8 %, 52.3 %, 53.9 %, and 76.4 %. In PAD, PPs were 6.9 %, 4.7 %, 55.1 %, 52.1 %, and 75.0 %, respectively. Strongest ORs in CVD were for homozygous G1691A (2.76; 95 %CI, 1.83–4.18) and for homozygous G20210A (3.96; 95 %CI, 2.05–7.64). Strongest ORs in CHD were for homozygous G1691A (OR 1.68; 95%CI, 1.02–2.77) and G20210A (heterozygous 1.49 95%CI, 1.22–1.82; homozygous 1.54 95%CI, 0.79–2.99). The OR for PAI-1 4G/4G in PAD was 5.44 (95%CI, 1.80–16.43). Specific subgroups with higher PPs and ORs were identified according to age and region.

Conclusions: Patients with arterial disease have an increased prevalence and odds of having some inherited thrombophilia. Some thrombophilia testing may be considered in specific subgroups of patients.

1. Introduction

Cardiovascular disease remains the leading cause of disability and mortality worldwide and hardly contributes to health system costs [1]. The pathogenesis of arterial disease is multifactorial and includes both inherited and environmental variables that promote atherosclerosis and thrombosis [2]. Despite aging, arterial hypertension, diabetes mellitus, dyslipidemia, and smoking are well-established risk factors accounting for a large proportion of arterial thrombotic events, a not negligible proportion of them may be not completely explained by the latter and remain unprovoked [3].

Thrombophilia may be defined as hemostatic abnormalities that predispose to thrombosis. While the association between inherited thrombophilia and venous thromboembolism is better established, its relationship with arterial thrombosis still remains controversial. From a clinical point of view, no sound evidence nor guidelines recommendations were actually available guiding the clinical decision of which type of thrombophilia should be searched, which patients should be tested, and which therapeutic strategies should be taken in patients with positive results [3]. Among several recognized inherited thrombophilia,

* Corresponding author at: Department of General Surgery and Surgical Specialty, Sapienza University of Rome, Italy.
E-mail address: emauele.valeriani@uniroma1.it (E. Valeriani).

https://doi.org/10.1016/j.thromres.2023.08.006
Received 29 May 2023; Received in revised form 4 July 2023; Accepted 9 August 2023
Available online 16 August 2023

factor V Leiden, prothrombin, methylenetetrahydrofolate reductase, and plasminogen activator inhibitor 1 gene polymorphisms have a relatively high prevalence in general population and specific gene abnormality has been identified [2–6].

The aim of our systematic review and meta-analysis is to provide comprehensive values of prevalence and odds of having prespecified inherited thrombophilia in patients with arterial disease including cerebrovascular disease (i.e., acute and recurrent ischemic stroke or transient ischemic attack), coronary heart disease (i.e., acute and recurrent myocardial infarction or stable coronary artery disease), and peripheral artery disease compared to controls.

2. Material and methods

This study-level systematic review and meta-analysis was performed following the Preferred Reporting Items for Systematic reviews and Meta-analysis (PRISMA) guidelines [7]. The protocol was registered in the PROSPERO database (registration number CRD42022308466 -https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42022308466).

2.1. Databases search and study selection

MEDLINE and EMBASE were searched from inception up to February 2022 for studies reporting data on prespecified inherited thrombophilia in arterial disease including cerebrovascular disease (i.e., acute and recurrent ischemic stroke or transient ischemic attack), coronary heart disease (i.e., acute and recurrent myocardial infarction or stable coronary artery disease), and peripheral artery disease. The following gene polymorphisms are the focus of the present review: (i) the G to A substitution at position 1691 of the factor V gene (G1691A), resulting in an arginine to glutamine exchange in codon 506 (commonly referred to as factor V Leiden); (ii) the G to A substitution at position 20210 in the 3′-untranslated region of the prothrombin gene (G20210A); (iii) the C to T point mutation at the position 677 on methylenetetrahydrofolate reductase gene (MTHFR C677T); (iv) the A to C mutation at the position 1298 on methylenetetrahydrofolate reductase gene (MTHFR A1298C); (v) the 4G/5G insertion/deletion in the PAI-1 gene at a position −675 of the promoter region (PAI-1). We decided a-priori to not include other specific inherited thrombophilia (i.e., protein C, protein S, and antithrombin deficiency) as they share lower prevalence values, has been associated with several different gene abnormalities with different degrees of clinical relevance, and their functionality may also depends on transient patients' comorbidities (e.g., sepsis, disseminated intravascular coagulation, liver disease, pregnancy, drugs administration) [2,8–10]. No study design restrictions were applied. The complete search strategy is given in Supplementary Tables 1 and 2.

Two authors (EV and AP) independently reviewed titles and abstracts identified by the databases search to select studies which met the following inclusion criteria: (i) inclusion of patients with prespecified arterial disease; (ii) data available for single allele of each prespecified gene polymorphisms; (iii) mean age of included patients ≥18 years; (iv) sample size ≥20 patients. Finally, full texts were evaluated by two independent authors (EV and AP) to confirm inclusion. References of included studies were also screened by two independent authors (EV and AP) searching additional studies that fit the inclusion criteria. Any disagreement was resolved through discussion or involving a third review author (DP).

2.2. Data extraction and quality assessment

Two review authors (EV and GA) independently extracted data from the included studies onto an electronic database. A consensus between the two review authors or a discussion with a third review author (DP) resolved any disagreement.

The following data were extracted: methodological quality, study design, total number of included patients, number of cases and number of controls, if available, with each allele mutation, patients' characteristics (e.g., age, sex category, belonging region), number of cases and controls with specific risk factors for arterial disease (i.e., arterial hypertension, diabetes mellitus, dyslipidemia, obesity, smoking history). Published supplementary materials were searched for data of interest, if needed and available.

The risk of bias of the included studies were evaluated using the Newcastle-Ottawa scale for observational studies (scores of 7–9, 4–6, and < 4 classified a study as having a low, moderate, or high risk of bias, respectively) and the Cochrane tool for randomized controlled trials [11–13].

2.3. Study outcomes

The primary outcomes were the prevalence and the odds of having a prespecified gene polymorphism in patients with each type of arterial disease compared to control populations. The secondary outcomes were the prevalence and the odds of having a prespecified gene polymorphism in specific subgroup of patients with arterial disease and controls sorted by age, region, and presence of cardiovascular risk factors.

2.4. Statistical analysis

The logit-transformed prevalence and corresponding sampling variances were calculated. Pooled prevalence with corresponding 95 % confidence intervals (CIs) were calculated in a random-effects model through the inverse variance method and DerSimonian-Laird method was used for τ^2 estimation [14]. Pooled odds ratios (ORs) with corresponding 95 % CIs were calculated in a random-effects model through the Mantel-Haenszel method, Sidik-Jonkman method was used for τ^2 estimation, and Hartung-Knapp method for random effects model adjustment [14]. A continuity correction of 0.5 was applied to studies with zero cells frequencies [14]. Heterogeneity was classified as follow: (i) 0 % to 40 % I^2 values indicate an heterogeneity that might not be important; (ii) 30 % to 60 % I^2 values may represent moderate heterogeneity, (iii) 50 % to 90 % I^2 values may represent substantial heterogeneity, (iv) 75 % to 100 % I^2 values indicate a considerable heterogeneity [14]. However, the importance of the observed I^2 values depends on the magnitude and direction of effects, and on the strength of evidence for heterogeneity [14].

While a dominant model was assumed in the primary analysis, a per-allele (co-dominant) model was assumed in the secondary analysis [15,16]. Furthermore, subgroup analyses were performed sorting patients by age (i.e., mean age of 18 to 55 years and >55 years), region groups (i.e., African, American, Asiatic, European, Oceanic regions), and considering those studies in which cases and controls shared a similar proportion of patients with at least one cardiovascular risk factor (i.e. arterial hypertension, diabetes mellitus, dyslipidemia, obesity, and smoking history).

The presence of publication bias was assessed by funnel plot of logit transformed proportion versus standard error. Funnel plot symmetry was tested by performing the Egger's test.

Statistical analyses were performed using R studio version 1.2.5001, "meta" and "forest" packages [17].

3. Results

Fig. 1 shows the PRISMA flow diagram. A total of 2112 records were identified from the databases search. After removing 168 duplicates, 1532 records were excluded by title and abstract screening. Citation screening of retrieved studies found 153 additional studies of which 132 fit the inclusion criteria. Full-text evaluation allowed the exclusion of 134 studies. Finally, a total of 377 studies were considered in the analysis (the full list of included studies is available on Supplementary

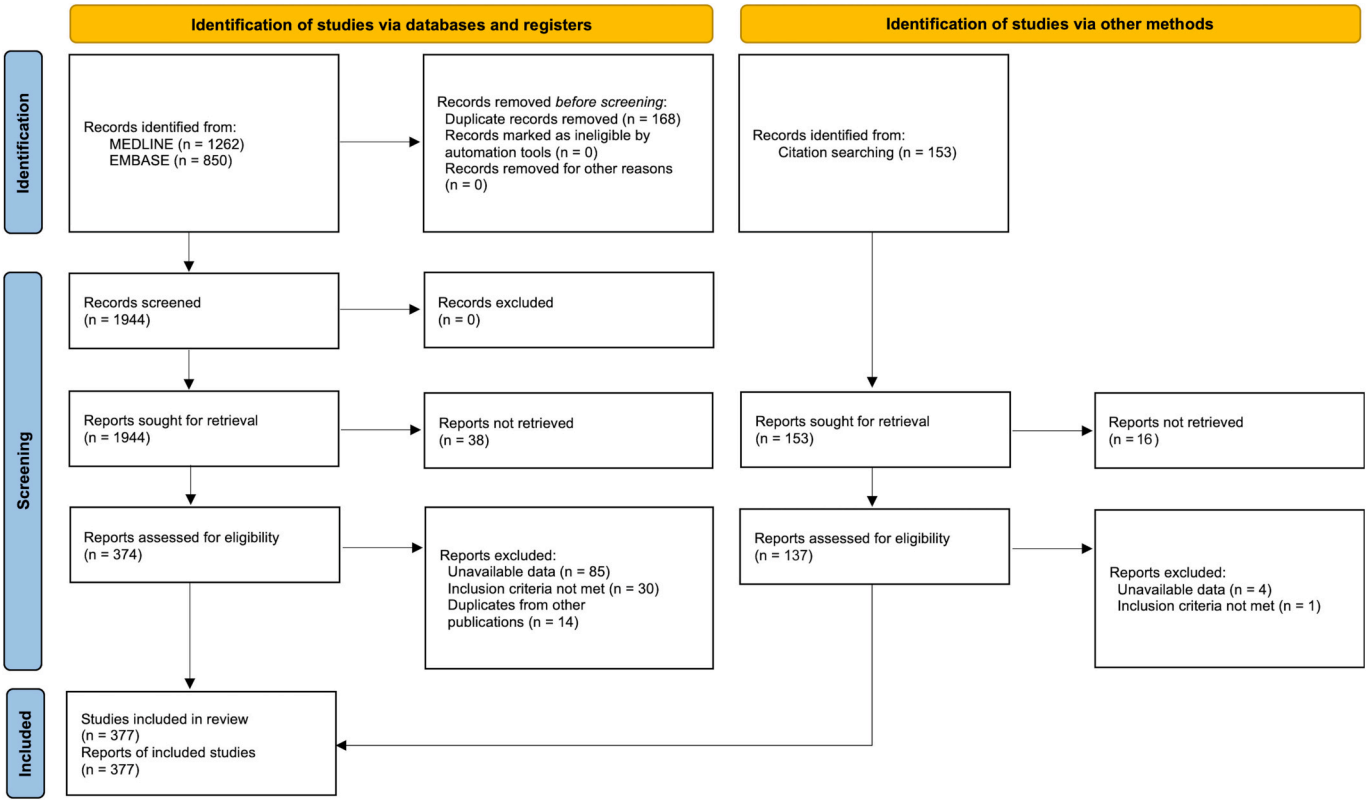

Fig. 1. PRISMA flow diagram.

data). The inter-reviewer agreement was excellent with a kappa statistic of 0.92.

Table 1
Characteristics of included populations of cases and controls according to single gene polymorphisms.

	G1691A		G20210A		MTHFR C677T		MTHFR A1298C		PAI-1 4G/5G	
	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls
Cerebrovascular disease[a]										
Number of patients	15,777 (72)	21,399 (60)	12,736 (58)	21,376 (45)	17,087 (90)	18,742 (79)	2646 (15)	3856 (15)	4151 (27)	5891 (25)
Median age, years	50 (58)	49 (47)	50 (31)	45 (34)	55 (78)	56 (59)	57 (14)	55 (13)	53 (21)	52 (20)
Male sex, %	53.5 (53)	45.8 (45)	51.1 (42)	46.3 (32)	56.0 (76)	52.3 (61)	51.6 (12)	50.6 (13)	56.1 (18)	38.3 (17)
Arterial hypertension, %	41.2 (44)	19.9 (30)	39.1 (34)	23.3 (21)	55.3 (58)	27.5 (46)	50.0 (10)	24.2 (10)	48.3 (17)	27.3 (16)
Diabetes mellitus, %	15.8 (42)	5.3 (29)	15.0 (32)	8.2 (20)	18.3 (56)	9.0 (45)	18.9 (10)	7.6 (10)	18.6 (14)	7.8 (13)
Dyslipidemia, %	33.3 (32)	31.5 (20)	33.8 (28)	35.8 (17)	32.4 (33)	25.4 (26)	25.4 (6)	7.8 (6)	40.5 (7)	46.8 (7)
Obesity, %	22.8 (9)	20.8 (7)	19.0 (7)	13.1 (5)	14.1 (3)	9.4 (3)	–	–	11.7 (2)	4.8 (2)
Smoking history, %	43.9 (45)	51.7 (31)	40.7 (34)	29.3 (22)	39.8 (58)	28.6 (46)	32.0 (9)	25.2 (9)	34.6 (15)	15.9 (15)
Coronary heart disease[a]										
Number of patients	19,376 (79)	26,650 (69)	18,193 (63)	25,671 (52)	23,631 (91)	23,746 (79)	5963 (22)	4745 (21)	15,610 (53)	14,319 (45)
Median age, years	53 (44)	50 (37)	51 (37)	48 (29)	53 (66)	51 (54)	54 (18)	51 (17)	53 (29)	48 (19)
Male sex, %	70.1 (40)	43.6 (32)	68.1 (36)	44.0 (27)	76.5 (60)	60.2 (49)	76.1 (18)	62.7 (19)	66.8 (26)	62.5 (17)
Arterial hypertension, %	33.0 (32)	15.0 (23)	34.1 (33)	17.7 (24)	51.0 (49)	26.2 (38)	64.2 (15)	30.0 (14)	49.8 (23)	24.0 (19)
Diabetes mellitus, %	15.5 (26)	3.8 (19)	17.1 (26)	4.6 (19)	22.6 (45)	9.0 (35)	23.5 (12)	7.7 (11)	18.7 (22)	8.0 (17)
Dyslipidemia, %	43.0 (21)	22.5 (15)	41.7 (23)	21.7 (17)	54.1 (26)	9.0 (35)	58.5 (8)	22.9 (7)	51.6 (16)	37.2 (11)
Obesity, %	22.0 (10)	13.3 (8)	22.7 (9)	12.5 (8)	22.4 (8)	30.8 (21)	18.4 (3)	18.6 (3)	21.1 (9)	7.4 (6)
Smoking history, %	68.9 (34)	54.8 (25)	63.6 (31)	32.6 (25)	56.1 (48)	39.2 (41)	55.5 (13)	42.2 (13)	52.6 (23)	30.0 (19)
Peripheral artery disease[a]										
Number of patients	2096 (8)	1855 (6)	1325 (5)	982 (4)	1828 (14)	2493 (14)	280 (2)	150 (2)	100 (1)	60 (1)
Median age, years	66 (7)	65 (6)	68 (5)	67 (4)	64 (14)	60 (12)	63 (1)	57 (2)	64 (1)	61 (1)
Male sex, %	69.2 (7)	52.6 (6)	69.6 (5)	68.8 (4)	70.6 (13)	62.9 (12)	85.0 (1)	62.0 (2)	85.0 (1)	53.3 (1)
Arterial hypertension, %	55.0 (5)	33.6 (4)	55.0 (5)	33.6 (4)	57.5 (11)	33.7 (10)	54.0 (1)	27.3 (2)	54.0 (1)	55.0 (1)
Diabetes mellitus, %	25.5 (5)	16.0 (4)	25.9 (5)	16.0 (4)	27.9 (8)	15.7 (8)	50.0 (1)	24.0 (2)	50.0 (1)	50.0 (1)
Dyslipidemia, %	43.4 (4)	20.4 (3)	43.4 (4)	20.4 (3)	41.9 (5)	20.9 (5)	47.0 (1)	11.3 (2)	47.0 (1)	28.3 (1)
Obesity, %	2.5 (2)	1.9 (1)	2.5 (2)	1.9 (1)	–	–	–	–	–	–
Smoking history, %	47.9 (6)	14.8 (4)	48.6 (5)	14.8 (4)	54.0 (10)	27.0 (9)	67.0 (1)	23.3 (2)	67.0 (1)	26.7 (1)

[a] Number of studies between brackets.

Table 2
Pooled prevalence of thrombophilia in cases and controls.

Arterial disease		G1691A			G20210A	
		Dominant	Heterozygosis	Homozygosis	Dominant	Heterozygosis
Cerebrovascular disease[a]	Cases	6.5 (69)	6.2 (72)	0.6 (71)	3.9 (56)	3.9 (57)
	Control	4.6 (59)	4.5 (60)	0.3 (59)	2.9 (44)	2.9 (45)
Coronary artery disease[a]	Cases	7.2 (77)	6.8 (79)	0.6 (67)	3.8 (61)	3.6 (63)
	Control	5.1 (68)	4.9 (69)	0.4 (67)	2.7 (50)	2.6 (52)
Peripheral artery disease[a]	Cases	6.9 (8)	6.7 (8)	0.4 (8)	4.7 (5)	4.5 (5)
	Control	5.4 (6)	5.4 (6)	0.3 (5)	2.7 (4)	2.7 (4)

aNumber of studies between brackets.

3.1. Characteristic of included studies and populations

Supplementary Table 3 reports the characteristics of included studies. Overall, 320 were case-control, 16 cross-sectional, and 41 cohort studies. Included patients were from African, American, Asiatic, European, and Oceanic regions in 15, 51, 110, 178, and 9 studies, respectively. A mixed population was included in one study while 13 studies did not report this information. A total of 222 studies (62,266 cases and 75,953 controls) reported data for coronary heart disease, 157 studies (32,791 cases and 52,992 controls) for cerebrovascular disease, and 17 studies (3129 cases and 3045 controls) for peripheral artery disease. Controls were included from healthy population in 141 studies (65 matched and 76 unmatched cohorts) or from populations without arterial disease but with risk factors for cardiovascular disease in 156 studies (89 matched and 67 unmatched cohorts). In 25 studies other types of control group were used or no information on control group characteristics were provided.

Data for G1691A, G20210A, MTHFR C677T, MTHFR A1298C, PAI-1 gene polymorphisms were reported, respectively, in 68, 54, 93, 16, and 27 studies for cerebrovascular disease; 80, 64, 95, 23, and 55 studies for coronary heart disease; and 7, 5, 14, 2, and 1 study for peripheral artery disease (Supplementary Table 3). Table 1 reports the characteristics of included populations of cases and controls sorted by the types of arterial event and thrombophilia. Overall, 37,249 patients with G1691A, 32,254 with G20210A, 42,546 with MTHFR C677T, 8889 with MTHFR A1298C, and 19,861 with PAI-1 were included (Table 1). In patients with G1691A gene polymorphism, median age ranged from 50 years in patients with cerebrovascular disease to 66 years in patients with peripheral artery disease. The proportion of male ranged from 53.5 % in cerebrovascular disease to 70.1 % in coronary heart disease patients. Arterial hypertension ranged from 33.0 % in coronary heart disease patients to 55.0 % of peripheral artery disease patients. Diabetes prevalence ranged from 15.5 % in coronary heart disease to 25.5 % of peripheral artery disease patients (Table 1).

In patients with G20210A gene polymorphism, median age ranged from 50 years in cerebrovascular disease to 68 years in peripheral artery disease patients. Proportion of male ranged from 51.1 % in cerebrovascular disease to 69.6 % in peripheral artery disease patients. Arterial hypertension was lowest in coronary heart disease (34.1 %) and highest in peripheral artery disease (55.0 %) patients. Diabetes ranged from 15.0 % in cerebrovascular disease to 25.9 % in peripheral artery disease.

In patients with MTHFR C677T gene polymorphism, median age ranged from 53 years in coronary artery disease to 64 years in peripheral artery disease patients. Nearly 50 % of cerebrovascular disease patients with MTHFR C677T were male, while coronary heart disease or peripheral artery disease patients were men in the majority of cases. Also the prevalence of risk factors was higher in studies investigating MTHFR C677T gene polymorphism.

Studies investigating MTHFR A1298C and PAI-1 gene polymorphisms were most in the setting of cerebrovascular disease and coronary heart disease. Only one study investigated PAI-1 in peripheral artery disease patients (Table 1).

3.2. Risk of bias evaluation and publication bias

As showed in Supplementary Table 4 and 5, the quality of the included studies varied from low to high. Overall, 10.6 %, 65.8 %, and 23.6 % of studies were considered at low, intermediate, and high risk of bias, respectively.

The results of publication bias were reported in Supplementary Figs. 1 to 3.

3.3. Pooled prevalence of evaluated gene polymorphisms in cases and controls

In patients with cerebrovascular disease, the pooled prevalence of heterozygosity for G1691A (6.2 % vs 4.5 %) and G20210A (3.9 % vs 2.9 %) and homozygosity for MTHFR C677T (14.2 % vs 11.1 %) and MTHFR A1298C (12.2 % vs 9.8 %) gene polymorphisms were higher in cases than controls (Table 2 and Supplementary Table 6).

In patients with coronary heart disease, the pooled prevalence of heterozygosity for G1691A (6.8 % vs 4.9 %) and G20210A (3.6 % vs 2.6 %) and homozygosity for MTHFR C677T (11.0 % vs 9.5 %) and MTHFR A1298C (12.2 % vs 9.8 %) gene polymorphisms were higher in cases than controls (Table 2 and Supplementary Table 7).

Finally, in patients with peripheral artery disease, the pooled prevalence of heterozygosity for G1691A (6.7 % vs 5.4 %) and G20210A (4.5 % vs 2.7 %) and homozygosity for MTHFR C677T (14.6 % vs 11.0 %), MTHFR A1298C (17.2 % vs 7.8 %), and PAI-1 (28.0 % vs 6.7 %) gene polymorphisms was higher in cases than controls (Table 2, Supplementary Table 8).

The pooled prevalence of prespecified thrombophilia in cases and controls sorted by age and region groups, and by the presence or absence of similar proportion of cases and controls with at least one cardiovascular risk factor were reported in Supplementary Tables 6 to 8. The paucity of available data did not allow any subgroup analysis in patients with peripheral artery disease and MTHFR A1298C and PAI-1 gene polymorphisms.

3.4. Primary analysis

Patients with cerebrovascular disease had an increased odds than controls of having the mutant allele of G1691A (OR, 1.50; 95 % CI, 1.30–1.73; I^2 19%; 57 studies), G20210A (OR, 1.60; 95 % CI, 1.31–1.95; I^2 6%; 43 studies), and MTHFR C677T (OR, 2.04; 95 % CI, 1.84–2.27; I^2 75%; 72 studies) and a similar odds of having the mutant allele of MTHFR A1298C (OR, 1.21; 95 % CI, 0.83–1.77; I^2 90%; 15 studies) and PAI-1 (OR, 1.03; 95 % CI, 0.86–1.24; I^2 46%; 24 studies) gene polymorphisms (Fig. 2).

Patients with coronary heart disease had an increased odds than controls of having the mutant allele of G1691A (OR, 1.39; 95 % CI, 1.19–1.63; I^2 35%; 66 studies), G20210A (OR, 2.31; 95 % CI, 1.34–3.99;

G20210A	MTHFR C677T			MTHFR A1298C			PAI-1 4G/5G		
Homozygosis	Dominant	Heterozygosis	Homozygosis	Dominant	Heterozygosis	Homozygosis	Dominant	Heterozygosis	Homozygosis
0.5 (56)	56.4 (84)	41.1 (85)	14.2 (90)	51.9 (15)	37.9 (15)	12.2 (15)	77.6 (26)	45.2 (27)	29.6 (26)
0.2 (44)	52.4 (72)	40.2 (74)	11.1 (79)	47.1 (15)	37.1 (15)	9.8 (15)	77.9 (24)	47.3 (25)	29.2 (24)
0.4 (59)	52.3 (86)	40.6 (86)	11.0 (91)	53.9 (21)	42.1 (21)	12.2 (22)	76.4 (53)	45.2 (53)	29.6 (54)
0.3 (50)	50.3 (75)	40.4 (75)	9.5 (79)	53.9 (20)	43.1 (20)	9.8 (21)	74.0 (45)	47.3(45)	29.2 (45)
0.3 (5)	55.1 (13)	40.9 (13)	14.6 (14)	52.1 (2)	35.4 (2)	17.2 (2)	75.0 (1)	47.0 (1)	28.0 (1)
0.3 (4)	50.4 (13)	40.8 (13)	11.0 (14)	44.1 (2)	36.3 (2)	7.8 (2)	76.7 (1)	70.0 (1)	6.7 (1)

I^2 74%; 50 studies), MTHFR C677T (OR, 1.07; 95 % CI, 1.00 1.15; I^2 38%; 75 studies), and PAI-1 (OR, 1.17; 95 % CI, 1.05–1.31; I^2 49%; 41 studies) and a similar odds of having the mutant allele of MTHFR A1298C (OR, 0.95; 95 % CI, 0.85–1.08; I^2 5%; 20 studies) gene polymorphisms (Fig. 3).

Patients with peripheral artery disease did not appear to have an increased odds than controls of having the mutant allele of G1691A (OR, 1.22; 95 % CI, 0.65–2.29; I^2 40%; 6 studies), G20210A (OR, 2.00; 95 % CI, 0.69–5.80; I^2 18%; 4 studies), MTHFR C677T (OR, 1.15; 95 % CI, 0.89–1.48; I^2 38%; 13 studies), and MTHFR A1298C (OR, 1.07; 95 % CI, 0.18–6.53; I^2 0%; 2 studies), but appeared to have a lower odds than controls of having PAI-1 (OR, 0.21; 95 % CI, 0.07–0.65; I^2 not applicable; 1 study) gene polymorphisms (Fig. 4).

Pooled odds of having a prespecified thrombophilia sorted by age and region groups, and by the presence or absence of similar proportion of cases and controls with at least one cardiovascular risk factor were reported in Figs. 2 to 4 and Supplementary Figs. 4 to 16. The paucity of data did not allow any subgroup analysis in patients with peripheral artery disease and MTHFR A1298C and PAI-1 gene polymorphisms.

3.5. Secondary analysis

The odds being heterozygous for G1691A (OR, 1.48; 95 % CI, 1.29–1.71; I^2 15%; 58 studies), G20210A (OR, 1.53; 95 % CI, 1.27–1.84; I^2 0%; 44 studies), MTHFR C677T (OR, 1.08; 95 % CI, 1.00–1.16; I^2 75%; 73 studies) and the odds being homozygous for G1691A (OR, 2.76; 95 % CI, 1.83–4.18; I^2 0%; 14 studies), G20210A (OR, 3.96; 95 % CI, 2.05–7.64; I^2 0%; 8 studies), MTHFR C677T (OR, 1.40; 95 % CI, 1.23–1.60; I^2 60%; 78 studies) gene polymorphisms were higher in patients with cerebrovascular disease than controls (Fig. 2).

The odds being heterozygous for G1691A (OR, 1.39; 95 % CI, 1.19–1.61; I^2 29%; 66 studies) and G20210A (OR, 1.49; 95 % CI, 1.22–1.82; I^2 24%; 46 studies), and the odds being homozygous for G1691A (OR, 1.68; 95 % CI, 1.02–2.77; I^2 0%; 21 studies), G20210A (OR, 1.54; 95 % CI, 0.79–2.99; I^2 0%; 11 studies), MTHFR C677T (OR, 1.21; 95 % CI, 1.08–1.35; I^2 44%; 78 studies), and PAI-1 (OR, 1.18; 95 % CI, 1.03–1.34; I^2 64%; 45 studies) gene polymorphisms were higher in patients with coronary heart disease than controls (Fig. 3).

Finally, the odds being homozygous for MTHFR C677T (OR, 1.45; 95 % CI, 1.03–2.03; I^2 26%; 13 studies) and PAI-1 (OR, 5.44; 95 % CI, 1.80–16.43; I^2 not applicable; 1 study) gene polymorphisms were higher in patients with peripheral artery disease than controls (Fig. 4).

4. Discussion

Our meta-analysis provides a comprehensive overview of the prevalence of single gene polymorphism in patients with arterial disease. In particular, we found that the prevalence and odds of having G1691A and G20210A gene polymorphisms is higher in cases with arterial disease than controls, mainly in cerebrovascular and coronary heart diseases. Data for MTHFR C677T and A1298C, and PAI-1 gene polymorphisms are weaker than for other types of inherited thrombophilia and no relevant differences between cases and controls were identified with the exception of MTHFR C677T in cerebrovascular disease and of PAI-1 4G in peripheral arterial disease. Furthermore, specific subgroups of patients with higher prevalence and odds of having a specific thrombophilia have been identified according to their age and region groups.

While the presence of G1691A and PAI-1 gene polymorphisms seemed to contribute to an enhanced atherothrombosis, the hyperprothrombinemia due to G20210A gene polymorphism appeared not to affect arterial thrombosis development in mice models [18–21]. Furthermore, it should be acknowledged that MTHFR gene polymorphism is associated with hyper-homocysteinemia and, in cases of very high level of homocysteinemia, it may be responsible of an increased risk of cardiovascular disease [22].

Providing a comprehensive evaluation of several inherited thrombophilia in arterial disease, our systematic-review and meta-analysis confirms and strengthens the results of previous studies, and allow the identification of specific subgroups of patients who may be tested for gene polymorphisms [4,23,24]. For example, patients with coronary heart disease and cerebrovascular disease aged 18–55 years appeared to have higher prevalence and odds of having G1691A and G20210A gene polymorphisms than patients aged >55 years. Similarly, patients with coronary heart disease from Africa, Asia, and Europe seemed to have higher prevalence and odds of having G1691A gene polymorphism, while patients from America and Europe seemed to have higher odds of having G20210A gene polymorphism. Even if limited by the intrinsic characteristics of a study-level meta-analysis our data rise interesting hypothesis that must be confirmed in future studies.

Whether patients with positive results of thrombophilia tests may benefit from a more aggressive therapeutic approach have to been yet evaluated. Furthermore, a potential therapeutic challenge should be acknowledge in the management of patients with arterial events and known thrombophilia as an inappropriately lifelong antithrombotic therapy may be administered without further clinical investigations [3]. Possibly due to the inconsistent available data, major guidelines on coronary heart disease and peripheral artery disease do not recommend any testing strategy nor therapeutic approach in patients with known thrombophilia [25–30]. In cerebrovascular disease, thrombophilia screening is suggested only in certain clinical scenarios as in cases of paradoxical emboli caused by venous thrombosis or recurrent venous thromboembolism and no therapeutic recommendation were provided [31]. Interestingly, folate, vitamin B_6, and vitamin B_{12} supplementation appeared not to be effective for preventing a recurrent event in patients with ischemic stroke or transient ischemic attack and hyperhomocysteinemia [31]. Despite a higher risk of recurrent events may be hypothesized in patients with thrombophilia due to the increased thrombotic burden, data are limited to few and small observational studies with contrasting results. Patients with G1691A, G20210A, and MTHFR C677T gene polymorphisms seemed to share a trend towards a higher risk of recurrent arterial events [32–36]. Conversely, no significant differences in the PAI-1 levels appeared to be present between patients with recurrent and non-recurrent stroke [37]. This lack of knowledge in the field makes daily clinical decision challenging and gives high importance to the evaluation of other patients-related variables (e.g., bleeding risk, site of thrombosis, and patient preference) [3].

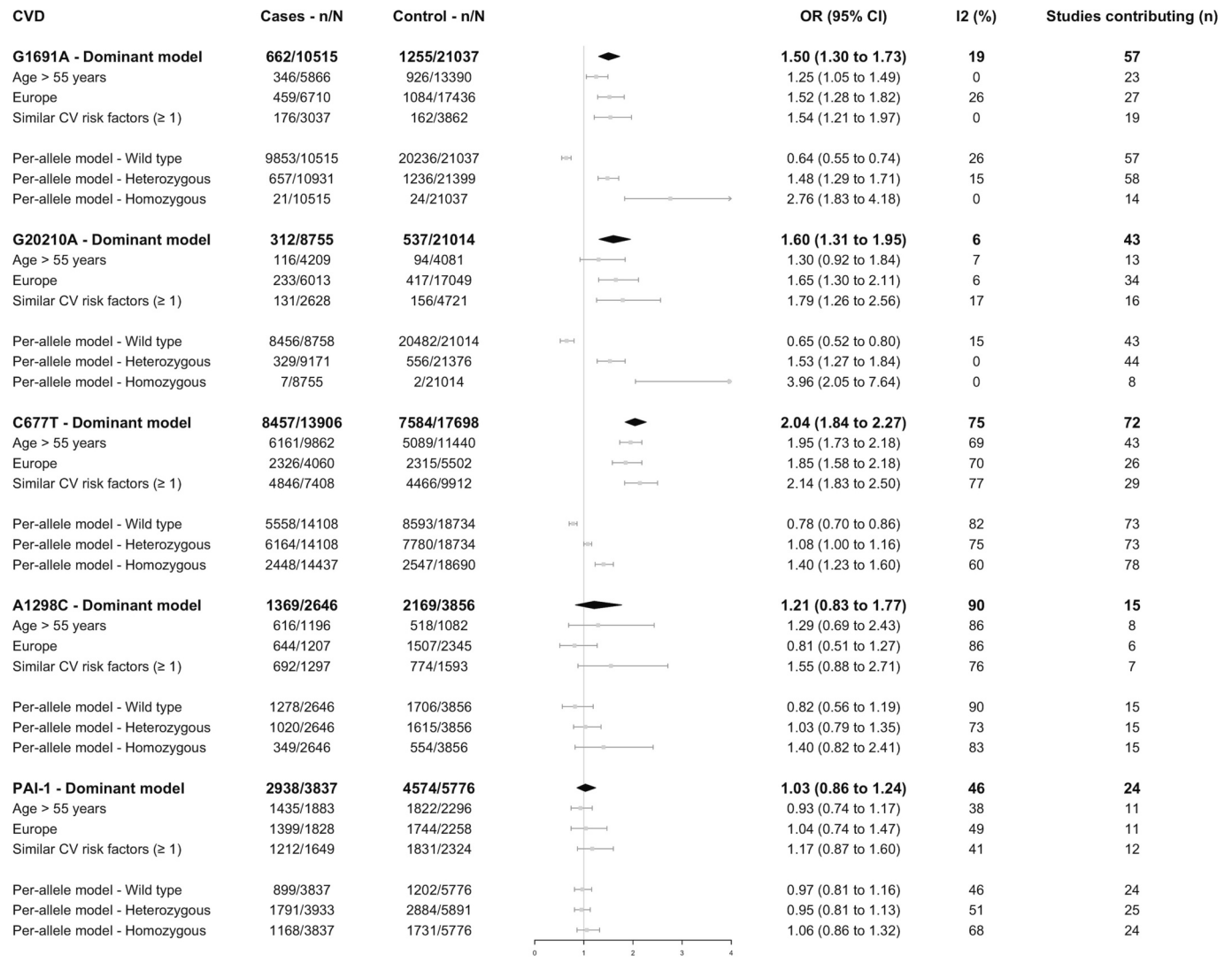

Fig. 2. Inherited thrombophilia in cerebrovascular disease.
Under the dominant model both heterozygous and homozygous patients were included. Under the per-allele model wild-type, heterozygous, and homozygous patients were independently considered. CVD, cerebrovascular disease; OR, odds ratio.

The major strength of our work is the systematic search with rigorous evaluation of study quality according to standard methodological assessment tools. Furthermore, this represents the most up to date meta-analysis that provide comprehensive values of prevalence and odds of having thrombophilia in arterial disease.

However, there are several limitations that warrant discussion. First, the studies included patients who were heterogenous in their underlying characteristics resulting in between-studies heterogeneity and possibly affecting the external validity of the results. The overall size of the included population and the availability of some patients' characteristics allowed the execution of subgroup analysis that partly explained this heterogeneity but leaved the risk for residual confounding. Second, the evaluation of all outcomes on a study-level basis represents an intrinsic design limitation of a study-level meta-analysis and hampered an in-depth analysis of the impact of specific characteristics (e.g., presence of concomitant risk factor for arterial events) on the outcomes of interest. Subgroup analyses including just those studies in which cases and controls shared at least one cardiovascular risk factor (i.e., arterial hypertension, diabetes mellitus, dyslipidemia, obesity, and smoking history) confirmed the overall results. Furthermore, specific subgroup of patients with higher prevalence and odds of having prespecified inherited thrombophilia has been identified according to age and region groups. We also acknowledge the fact that some studies may have included patients without an acute thrombotic-related arterial events, mainly in coronary heart and peripheral artery disease, possibly affecting the results. However, the heterogeneity of data did not allow any other analysis. A further possible bias of our analysis is represented by the lack of data on the modalities of screening in cases and controls; indeed, a higher prevalence of gene polymorphisms may be the result of a more rigorous diagnostic work-up in patients with thrombotic events than controls. Furthermore, the low prevalence of some risk factors such as hypertension may be due to the relatively low median age of patients

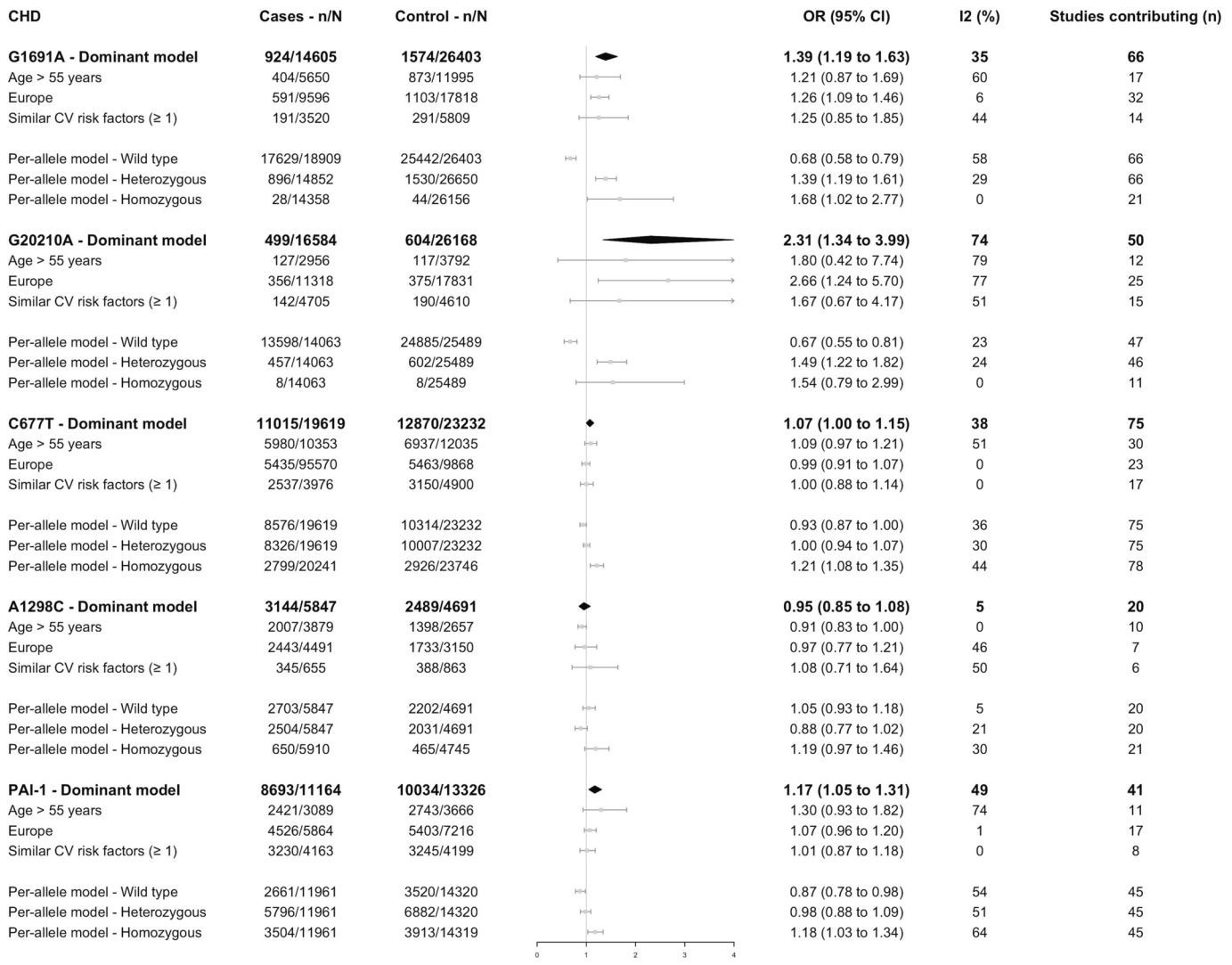

Fig. 3. Inherited thrombophilia in coronary heart disease.
Under the dominant model both heterozygous and homozygous patients were included. Under the per-allele model wild-type, heterozygous, and homozygous patients were independently considered. CHD, Coronary heart disease; OR odds ratio.

included in the studies as well as the higher prevalence and odds of specific thrombophilia in patients with peripheral arterial disease may be related to the low number of available studies. Third, patients with multiple gene polymorphisms may have an even greater risk of arterial disease than patients with a single gene polymorphism. Data were sparce and mainly regard G1691A, G20210A, and MTHFR polymorphisms combinations [38–41]. However, the paucity of data and the heterogeneity of included populations and evaluated gene polymorphisms did not allow any additional analysis and further studies will be needed. Fourth, all included studies were at some risk of bias, which potentially limits the external validity of the results and emphasizes the urgent need for high-level evidence in this field. Finally, there was evidence of significant publication bias for the role of thrombophilia in coronary heart disease and cerebrovascular disease. This finding is consistent with the possibility that small studies with large effect size were not published. However, it is unlikely that the latter were missed by our comprehensive and systematic databases' search.

What should be clear is that despite the higher prevalence of some gene polymorphisms in cases than controls, our analysis does not establish any cause-effect relationship between the prevalence of inherited thrombophilia and risk of arterial disease. It should be considered that large scale testing for thrombophilia in patients with arterial disease is not cost-effective and not likely to provide a real clinical benefit. However, these results should be considered as hypothesis generating, and may help increase the awareness among physicians that thrombophilia may be suspected in patients with arterial disease in whom clear risk factors may not be identified.

For instance, in young patients with myocardial infarction or stroke, or in case of positive family history of premature arterial disease, acquired or inherited thrombophilia may be tested. At this regard, a previous meta-analysis showed a higher risk of acquired thrombophilia (i.e., antiphospholipid antibodies) in patients with coronary heart disease, especially in those with <50 years [42].

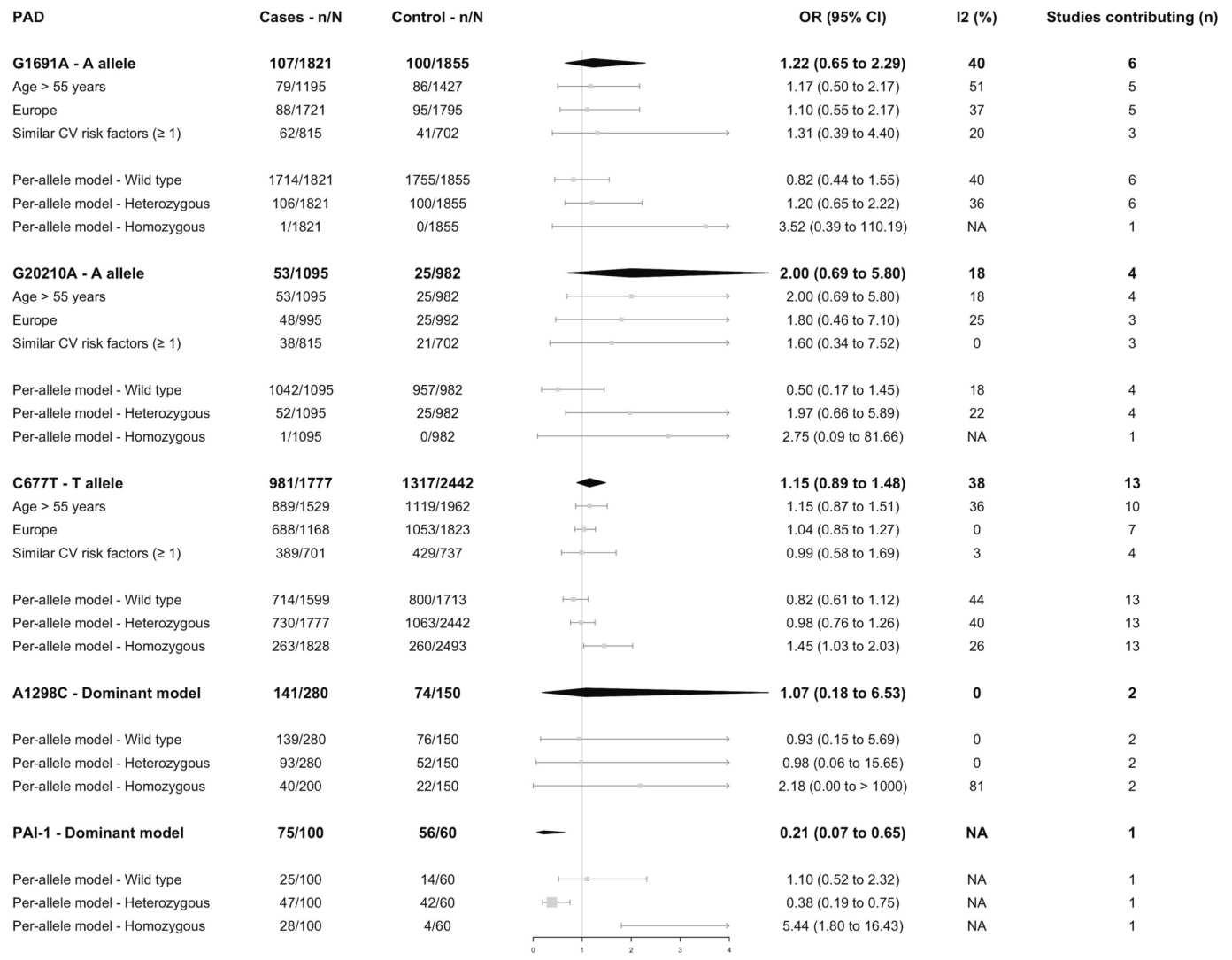

Fig. 4. Inherited thrombophilia in peripheral artery disease.
Under the dominant model both heterozygous and homozygous patients were included. Under the per-allele model wild-type, heterozygous, and homozygous patients were independently considered. OR, odds ratio; PAD, peripheral artery disease.

5. Conclusions

In conclusion, the prevalence and odds of having some inherited thrombophilia appeared to be higher in patients with arterial disease than controls, mainly in specific subgroups of patients according to their age and belonging region. Some thrombophilia testing may be considered in specific subgroups of patients.

Role of funding source

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

CRediT authorship contribution statement

Study conception and design: E. Valeriani, D. Pastori, P. Pignatelli; Data acquisition: E. Valeriani, G. Astorri; Statistical analysis: E. Valeriani; Interpretation of the data: E. Valeriani, D. Pastori, P. Pignatelli; Drafting of the manuscript: E. Valeriani, D. Pastori, P. Pignatelli; Critical revision of the manuscript for important intellectual content: All authors; Final approval of the manuscript: All authors.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: E. Valeriani, D. Pastori, G. Astorri, D. Menichelli, and P. Pignatelli have nothing to disclose. A. Porfidia reports personal fees from Bayer, Boehringer Inghelheim, Daiichi Sankyo, BMS-Pfizer, Novartis and Aspen, outside the submitted work.

Data availability

All data were available upon request at emanuele.valeriani@uniroma1.it.

Acknowledgement

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.thromres.2023.08.006.

References

[1] M. Vaduganathan, G.A. Mensah, J.V. Turco, V. Fuster, G.A. Roth, The global burden of cardiovascular diseases and risk: a compass for future health, J. Am. Coll. Cardiol. 80 (25) (2022) 2361–2371.

[2] P. de Moerloose, F. Boehlen, Inherited thrombophilia in arterial disease: a selective review, Semin. Hematol. 44 (2) (2007) 106–113.

[3] J.E. May, S. Moll, How I treat unexplained arterial thrombosis, Blood 136 (13) (2020) 1487–1498.

[4] Z. Ye, E.H. Liu, J.P. Higgins, B.D. Keavney, G.D. Lowe, R. Collins, J. Danesh, Seven haemostatic gene polymorphisms in coronary disease: meta-analysis of 66,155 cases and 91,307 controls, Lancet 367 (9511) (2006) 651–658.

[5] R.J. Kim, R.C. Becker, Association between factor V Leiden, prothrombin G20210A, and methylenetetrahydrofolate reductase C677T mutations and events of the arterial circulatory system: a meta-analysis of published studies, Am. Heart J. 146 (6) (2003) 948–957.

[6] J.P. Casas, A.D. Hingorani, L.E. Bautista, P. Sharma, Meta-analysis of genetic studies in ischemic stroke: thirty-two genes involving approximately 18,000 cases and 58,000 controls, Arch. Neurol. 61 (11) (2004) 1652–1661.

[7] M.J. Page, D. Moher, P.M. Bossuyt, I. Boutron, T.C. Hoffmann, C.D. Mulrow, L. Shamseer, J.M. Tetzlaff, E.A. Akl, S.E. Brennan, R. Chou, J. Glanville, J. M. Grimshaw, A. Hrobjartsson, M.M. Lalu, T. Li, E.W. Loder, E. Mayo-Wilson, S. McDonald, L.A. McGuinness, L.A. Stewart, J. Thomas, A.C. Tricco, V.A. Welch, P. Whiting, J.E. McKenzie, PRISMA 2020 explanation and elaboration: updated guidance and exemplars for reporting systematic reviews, BMJ 372 (2021), n160.

[8] W. Faught, P. Garner, G. Jones, B. Ivey, Changes in protein C and protein S levels in normal pregnancy, Am. J. Obstet. Gynecol. 172 (1 Pt 1) (1995) 147–150.

[9] A.F. Shorr, G.R. Bernard, J.F. Dhainaut, J.R. Russell, W.L. Macias, D.R. Nelson, D. P. Sundin, Protein C concentrations in severe sepsis: an early directional change in plasma levels predicts outcome, Crit. Care 10 (3) (2006) R92.

[10] B. Lipe, D.L. Ornstein, Deficiencies of natural anticoagulants, protein C, protein S, and antithrombin, Circulation 124 (14) (2011) e365–e368.

[11] J.A.C. Sterne, J. Savovic, M.J. Page, R.G. Elbers, N.S. Blencowe, I. Boutron, C. J. Cates, H.Y. Cheng, M.S. Corbett, S.M. Eldridge, J.R. Emberson, M.A. Hernan, S. Hopewell, A. Hrobjartsson, D.R. Junqueira, P. Juni, J.J. Kirkham, T. Lasserson, T. Li, A. McAleenan, B.C. Reeves, S. Shepperd, I. Shrier, L.A. Stewart, K. Tilling, I. R. White, P.F. Whiting, J.P.T. Higgins, RoB 2: a revised tool for assessing risk of bias in randomised trials, BMJ 366 (2019), l4898.

[12] A. Stang, Critical evaluation of the Newcastle-Ottawa scale for the assessment of the quality of nonrandomized studies in meta-analyses, Eur. J. Epidemiol. 25 (9) (2010) 603–605.

[13] G.A. Wells, B. Shea, D. O'Connel, J. Peterson, V. Welch, M. Losos, The Newcastle-Ottawa Scale (NOS) for Assessing the Quality of Nonrandomised Studies in Meta-Analyses, Available online: http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp, 2012.

[14] J. Deeks, J. Higgins, n.D. Altma, Chapter 10: analysing data and undertaking meta-analyses, in: Higgins JPT, J. Thomas, J. Chandler, M. Cumpston, T. Li, M.J. Page, V. A. Welch (Eds.), Cochrane Handbook for Systematic Reviews of Interventions Version 6.1 (Updated September 2020), Cochrane, 2020. Available from, www.training.cochrane.org/handbook (Last accessed January 2023).

[15] H.M. Liu, J.P. Zheng, D. Yang, Z.F. Liu, Z. Li, Z.Z. Hu, Z.N. Li, Recessive/dominant model: alternative choice in case-control-based genome-wide association studies, PLoS One 16 (7) (2021), e0254947.

[16] C. Minelli, J.R. Thompson, K.R. Abrams, A. Thakkinstian, J. Attia, The choice of a genetic model in the meta-analysis of molecular association studies, Int. J. Epidemiol. 34 (6) (2005) 1319–1328.

[17] R.C.T., R: A Language and Environment for Statistical Computing, R Foundation for Statistical Computing, Vienna, Austria, 2019. URL, https://www.R-project.org/2019.

[18] D.T. Eitzman, R.J. Westrick, Y. Shen, P.F. Bodary, S. Gu, S.L. Manning, S.L. Dobies, D. Ginsburg, Homozygosity for factor V Leiden leads to enhanced thrombosis and atherosclerosis in mice, Circulation 111 (14) (2005) 1822–1825.

[19] M.M. Aleman, B.L. Walton, J.R. Byrnes, J.G. Wang, M.J. Heisler, K.R. Machlus, B. C. Cooley, A.S. Wolberg, Elevated prothrombin promotes venous, but not arterial, thrombosis in mice, Arterioscler. Thromb. Vasc. Biol. 33 (8) (2013) 1829–1836.

[20] Z. Chen, A.C. Karaplis, S.L. Ackerman, I.P. Pogribny, S. Melnyk, S. Lussier-Cacan, M.F. Chen, A. Pai, S.W. John, R.S. Smith, T. Bottiglieri, P. Bagley, J. Selhub, M. A. Rudnicki, S.J. James, R. Rozen, Mice deficient in methylenetetrahydrofolate reductase exhibit hyperhomocysteinemia and decreased methylation capacity, with neuropathology and aortic lipid deposition, Hum. Mol. Genet. 10 (5) (2001) 433–443.

[21] A. Luttun, F. Lupu, E. Storkebaum, M.F. Hoylaerts, L. Moons, J. Crawley, F. Bono, A.R. Poole, P. Tipping, J.M. Herbert, D. Collen, P. Carmeliet, Lack of plasminogen activator inhibitor-1 promotes growth and abnormal matrix remodeling of advanced atherosclerotic plaques in apolipoprotein E-deficient mice, Arterioscler. Thromb. Vasc. Biol. 22 (3) (2002) 499–505.

[22] P. Ganguly, S.F. Alam, Role of homocysteine in the development of cardiovascular disease, Nutr. J. 14 (2015) 6.

[23] C. Li, H. Ren, H. Chen, J. Song, S. Li, C. Lee, J. Liu, Y. Cui, Prothrombin G20210A (rs1799963) polymorphism increases myocardial infarction risk in an age-related manner: a systematic review and meta-analysis, Sci. Rep. 7 (1) (2017) 13550.

[24] T. Chiasakul, E. De Jesus, J. Tong, Y. Chen, M. Crowther, D. Garcia, C. Chai-Adisaksopha, S.R. Messe, A. Cuker, Inherited thrombophilia and the risk of arterial ischemic stroke: a systematic review and Meta-analysis, J. Am. Heart Assoc. 8 (19) (2019), e012877.

[25] J.P. Collet, H. Thiele, E. Barbato, O. Barthelemy, J. Bauersachs, D.L. Bhatt, P. Dendale, M. Dorobantu, T. Edvardsen, T. Folliguet, C.P. Gale, M. Gilard, A. Jobs, P. Juni, E. Lambrinou, B.S. Lewis, J. Mehilli, E. Meliga, B. Merkely, C. Mueller, M. Roffi, F.H. Rutten, D. Sibbing, G.C.M. Siontis, E.S.C.S.D. Group, 2020 ESC Guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation, Eur. Heart J. 42 (14) (2021) 1289–1367.

[26] J. Knuuti, W. Wijns, A. Saraste, D. Capodanno, E. Barbato, C. Funck-Brentano, E. Prescott, R.F. Storey, C. Deaton, T. Cuisset, S. Agewall, K. Dickstein, T. Edvardsen, J. Escaned, B.J. Gersh, P. Svitil, M. Gilard, D. Hasdai, R. Hatala, F. Mahfoud, J. Masip, C. Muneretto, M. Valgimigli, S. Achenbach, J.J. Bax, E.S.D. Group, 2019 ESC guidelines for the diagnosis and management of chronic coronary syndromes, Eur. Heart J. 41 (3) (2020) 407–477.

[27] B. Ibanez, S. James, S. Agewall, M.J. Antunes, C. Bucciarelli-Ducci, H. Bueno, A.L. P. Caforio, F. Crea, J.A. Goudevenos, S. Halvorsen, G. Hindricks, A. Kastrati, M. J. Lenzen, E. Prescott, M. Roffi, M. Valgimigli, C. Varenhorst, P. Vranckx, P. Widimsky, E.S.C.S.D. Group, 2017 ESC guidelines for the management of acute myocardial infarction in patients presenting with ST-segment elevation: the task force for the management of acute myocardial infarction in patients presenting with ST-segment elevation of the European Society of Cardiology (ESC), Eur. Heart J. 39 (2) (2018) 119–177.

[28] V. Aboyans, J.B. Ricco, M.E.L. Bartelink, M. Björck, M. Brodmann, T. Cohnert, J. P. Collet, M. Czerny, M. De Carlo, S. Debus, C. Espinola-Klein, T. Kahan, S. Kownator, L. Mazzolai, A.R. Naylor, M. Roffi, J. Röther, M. Sprynger, M. Tendera, G. Tepe, M. Venermo, C. Vlachopoulos, I. Desormais, E.S.D. Group, 2017 ESC guidelines on the diagnosis and treatment of peripheral arterial diseases, in collaboration with the European Society for Vascular Surgery (ESVS): document covering atherosclerotic disease of extracranial carotid and vertebral, mesenteric, renal, upper and lower extremity arteriesEndorsed by: the European Stroke Organization (ESO) The Task Force for the Diagnosis and Treatment of Peripheral Arterial Diseases of the European Society of Cardiology (ESC) and of the European Society for Vascular Surgery (ESVS), Eur. Heart J. 39 (9) (2018) 763–816.

[29] G. Hindricks, T. Potpara, N. Dagres, E. Arbelo, J.J. Bax, C. Blomstrom-Lundqvist, G. Boriani, M. Castella, G.A. Dan, P.E. Dilaveris, L. Fauchier, G. Filippatos, J. M. Kalman, M. La Meir, D.A. Lane, J.P. Lebeau, M. Lettino, G.Y.H. Lip, F.J. Pinto, G.N. Thomas, M. Valgimigli, I.C. Van Gelder, B.P. Van Putte, C.L. Watkins, E.S.C.S. D. Group, 2020 ESC Guidelines for the diagnosis and management of atrial fibrillation developed in collaboration with the European Association for Cardio-Thoracic Surgery (EACTS): The Task Force for the diagnosis and management of atrial fibrillation of the European Society of Cardiology (ESC) Developed with the special contribution of the European Heart Rhythm Association (EHRA) of the ESC, Eur. Heart J. 42 (5) (2021) 373–498.

[30] D.J. Arachchillage, L. Mackillop, A. Chandratheva, J. Motawani, P. MacCallum, M. Laffan, Thrombophilia testing: a British Society for Haematology guideline, Br. J. Haematol. 198 (3) (2022) 443–458.

[31] D.O. Kleindorfer, A. Towfighi, S. Chaturvedi, K.M. Cockroft, J. Gutierrez, D. Lombardi-Hill, H. Kamel, W.N. Kernan, S.J. Kittner, E.C. Leira, O. Lennon, J. F. Meschia, T.N. Nguyen, P.M. Pollak, P. Santangeli, A.Z. Sharrief, S.C. Smith, T. N. Turan, L.S. Williams, 2021 guideline for the prevention of stroke in patients with stroke and transient ischemic attack: a guideline from the American Heart Association/American Stroke Association, Stroke 52 (7) (2021) e364–e467.

[32] E. Berge, K.B. Haug, E.C. Sandset, K.K. Haugbro, M. Turkovic, P.M. Sandset, The factor V Leiden, prothrombin gene 20210GA, methylenetetrahydrofolate reductase 677CT and platelet glycoprotein IIIa 1565TC mutations in patients with acute ischemic stroke and atrial fibrillation, Stroke 38 (3) (2007) 1069–1071.

[33] S. Garakanidze, E. Costa, E. Bronze-Rocha, A. Santos-Silva, G. Nikolaishvili, I. Nakashidze, N. Kakauridze, S. Glonti, R. Khukhunaishvili, M. Koridze, S. Ahmad, Characterization of a cohort of patients with arterial thrombosis from the Georgian Adjarian population, Curr. Trends Biotechnol. Pharm. 13 (3) (2019) 259–269.

[34] A. Pezzini, M. Grassi, E. Del Zotto, C. Lodigiani, P. Ferrazzi, A. Spalloni, R. Patella, A. Giossi, I. Volonghi, L. Iacoviello, M. Magoni, L.L. Rota, M. Rasura, A. Padovani, Common genetic markers and prediction of recurrent events after ischemic stroke in young adults, Neurology 73 (9) (2009) 717–723.

[35] M.D. van der Krabben, F.R. Rosendaal, J.G. van der Bom, C.J. Doggen, Polymorphisms in coagulation factors and the risk of recurrent cardiovascular events in men after a first myocardial infarction, J. Thromb. Haemost. 6 (5) (2008) 720–725.

[36] R. Vulapalli, C. Liang, W. Zareba, A.J. Moss, Recurrent coronary events are not increased in postinfarction patients with methylenetetrahydrofolate reductase gene C677T polymorphism, Am. J. Cardiol. 87 (11) (2001) 1289–1292.

[37] M.S. Akhter, A. Biswas, S.M. Abdullah, M. Behari, R. Saxena, The role of PAI-1 4G/5G promoter polymorphism and its levels in the development of ischemic stroke in young Indian population, Clin. Appl. Thromb. Hemost. 23 (8) (2017) 1071–1076.

[38] W.Y. Almawi, G. Ameen, H. Tamim, R.R. Finan, N. Irani-Hakime, Factor V G1691A, prothrombin G20210A, and methylenetetrahydrofolate reductase [MTHFR] C677T gene polymorphism in angiographically documented coronary artery disease, J. Thromb. Thrombolysis 17 (3) (2004) 199–205.
[39] G.I. Forte, L. Vaccarino, M. Palmeri, A. Branzi, C.M. Caldarera, L. Scola, C. Caruso, F. Licastro, D. Lio, Analysis of polymorphisms Leiden Factor V G1691A and prothrombin G20210A as risk factors for acute myocardial infarction, Biogerontology 12 (5) (2011) 485–490.
[40] L. M'Barek, S. Sakka, F. Meghdiche, D. Turki, K. Maalla, M. Dammak, C. Kallel, C. Mhiri, MTHFR (C677T, A1298C), FV Leiden polymorphisms, and the prothrombin G20210A mutation in arterial ischemic stroke among young tunisian adults, Metab. Brain Dis. 36 (3) (2021) 421–428.
[41] B.F. Sode, K.H. Allin, M. Dahl, F. Gyntelberg, B.G. Nordestgaard, Risk of venous thromboembolism and myocardial infarction associated with factor V Leiden and prothrombin mutations and blood type, CMAJ 185 (5) (2013) E229–E237.
[42] D. Pastori, T. Bucci, M. Triggiani, P.R.J. Ames, S. Parrotto, F. Violi, P. Pignatelli, A. Farcomeni, Immunoglobulin G (IgG) anticardiolipin antibodies and recurrent cardiovascular events. A systematic review and Bayesian meta-regression analysis, Autoimmun. Rev. 18 (5) (2019) 519–525.