Systematic review and meta-analysis of the genetics of peripheral arterial disease

Cassius Iyad Ochoa Chaar, MD, MS,[a] Tanner Kim, MD,[b] Dana Alameddine, MD,[a] Andrew DeWan, PhD,[c] Raul Guzman, MD,[a] Alan Dardik, MD, PhD,[a] Holly K. Grossetta Nardini, MLS,[d] Joshua D. Wallach, PhD,[e] Iftikhar Kullo, MD,[f] *and* Michael Murray, MD,[g] *New Haven, CT; Honolulu, HI; Atlanta, GA; and Rochester, MN*

ABSTRACT

Background: Peripheral artery disease (PAD) impacts more than 200 million people worldwide. The understanding of the genetics of the disease and its clinical implications continue to evolve. This systematic review provides a comprehensive summary of all DNA variants that have been studied in association with the diagnosis and progression of PAD, with a meta-analysis of the ones replicated in the literature.

Methods: A systematic review of all studies examining DNA variants associated with the diagnosis and progression of PAD was performed. Candidate gene and genome-wide association studies (GWAS) were included. A meta-analysis of 13 variants derived from earlier smaller candidate gene studies of the diagnosis of PAD was performed. The literature on the progression of PAD was limited, and a meta-analysis was not feasible because of the heterogeneity in the criteria used to characterize it.

Results: A total of 231 DNA variants in 112 papers were studied for the association with the diagnosis of PAD. There were significant variations in the definition of PAD and the selection of controls in the various studies. GWAS have established 19 variants associated with the diagnosis of PAD that were replicated in several large patient cohorts. Only variants in intercellular adhesion molecule-1 (rs5498), IL-6 (rs1800795), and hepatic lipase (rs2070895) showed significant association with the diagnosis of PAD. However, these variants were not noted in the published GWAS.

Conclusions: Genetic research in the diagnosis of PAD has significant heterogeneity, but recent GWAS have demonstrated variants consistently associated with the disease. More research focusing on the progression of PAD is needed to identify patients at risk of adverse events and develop strategies that would improve their outcomes. (JVS—Vascular Science 2024;5:100133.)

Keywords: Peripheral artery disease; Single nucleotide polymorphisms; Genetics; Disease progression

Peripheral artery disease (PAD) is a circulatory condition that affects the arteries of the lower extremities and impacts more than 200 million people worldwide.[1] It is a common source of disability and represents a significant financial burden on the U.S. health care system estimated at more than $21 billion annually.[2] PAD is caused by metabolic risk factors, lifestyle choices, and poorly understood genetic factors. Even though numerous studies have suggested that the ankle-brachial index (ABI) exhibits a heritable pattern of disease, association studies with DNA variants have been limited and inconsistent.[3-5]

The current literature describing the genetic associations of PAD in humans is heterogeneous with respect to the definition of the disease, selection of variants, study population, and method of analysis. Although most studies have focused on the presence of a particular single nucleotide polymorphism (SNP) or a group of SNPs with the diagnosis of PAD, other studies have examined the relationship of genetic polymorphisms with markers of disease progression such as severity, prognosis, or outcomes of lower extremity revascularization (LER).[6-8] Moreover, PAD has been studied under the broader umbrella of atherosclerosis, and some papers

From the Division of Vascular Surgery and Endovascular Therapy, Yale University School of Medicine, New Haven[a]; the Department of Surgery, John A. Burns School of Medicine, University of Hawaii, Honolulu[b]; the Department of Chronic Disease Epidemiology, Yale School of Public Health,[c] and Harvey Cushing/John Hay Whitney Medical Library, Yale University,[d] New Haven; the Department of Epidemiology, Rollins School of Public Health, Emory University, Atlanta[e]; the Department of Cardiovascular Medicine, Mayo Clinic, Rochester[f]; and the Department of Genetics, Yale University School of Medicine, New Haven.[g]

Grant support was provided by the US Food and Drug Administration, Arnold Ventures, Johnson & Johnson through Yale University, and the National Institute on Alcohol Abuse and Alcoholism of the National Institutes of Health under award No. 1K01AA028258 (J.D.W.).

Additional material for this article may be found online at www.jvascsurg.org.

Correspondence: Cassius Iyad Ochoa Chaar, MD, MS, Yale University School of Medicine, 330 Cedar St, Boardman 204, New Haven, CT 06510 (e-mail: cassius.chaar@yale.edu).

The editors and reviewers of this article have no relevant financial relationships to disclose per the JVS policy that requires reviewers to decline review of any manuscript for which they may have a conflict of interest.

2666-3503

https://doi.org/10.1016/j.jvssci.2023.100133

analyze the association of SNPs with atherosclerosis grouping patients with PAD together with patients afflicted by cardiovascular disease, cerebrovascular disease, abdominal aortic aneurysms, and even venous thromboembolism.[9-13] The dissemination of high-throughput sequencing accelerated the establishment of large genetic biobanks and made genome-wide association studies (GWAS) of various disease processes possible at a scale unconceivable a decade ago. As such, recent GWAS and targeted sequencing studies in large cohorts have revealed new SNPs associated with the diagnosis of PAD that were not identified in previous smaller case-control studies.[14-17]

Therefore, we conducted a systematic review to provide a comprehensive summary of all DNA variants studied in the literature for an association with PAD. The various polymorphisms are divided into two groups: SNPs associated with the diagnosis of PAD and SNPs associated with disease progression. A limited meta-analysis of variants reported in the literature in two or more case-control candidate gene studies was performed. Variants with a consistent association with PAD are highlighted and put into context with recent major advances made by GWAS. This systematic review constitutes a comprehensive summary for other researchers interested in the genetics of PAD and an essential springboard for future work in that space.

METHODS

Literature search. An experienced medical librarian conducted a comprehensive search across multiple databases from their inception: Embase (Ovid, 1974-July 14, 2020), MEDLINE (Ovid ALL, 1946-July 14, 2020), PubMed for in-process and unindexed material, Scopus (Elsevier, all years), Web of Science SCI-EXPANDED, Social Sciences Citation Index (Clarivate, all years), and Cochrane Central Registry of Controlled Trials. First, known key articles identified by the research team were analyzed to identify Medical Subject Headings (MeSH) terms of relevance and other keywords using the Yale MeSH Analyzer.[18] In each database, scoping searches were run, and an iterative process was used to translate and refine each search strategy. To maximize sensitivity, both controlled vocabulary terms and synonymous free-text terms were used to capture concepts of PAD and genetic markers. The formal search did not include specific SNPs, but rather broad concepts of genetics, and was peer reviewed by a second librarian (Supplementary Table I). Citations from all databases were imported into Covidence after the removal of duplicates.[19] Two independent screeners (C.I.O.C. and T.K.) performed a title abstract review and resolved conflicts by discussion with a third author (A.D.). On June 10, 2022, the searches were updated across all databases. The study was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines and was registered in the International Prospective Register of Systematic Reviews (PROSPERO)—CRD42020214595.

Inclusion criteria. Studies were included in the review if the following two criteria were met: (1) the patient population had a diagnosis of PAD based on clinical assessment or imaging regardless of symptoms and (2) the research studied the association between one or more specific DNA variants with PAD. If multiple studies described the same patient population, only the most recent paper was included. Only studies in the English language were included. Case reports, editorial letters, review articles, animal studies, and studies that did not provide original SNP associations with PAD were excluded. Studies that did not have a clinical diagnosis of PAD or relied on physiological characteristics of the arterial wall such as thickness, stiffness, or calcification were also excluded. Studies examining mitochondrial DNA, epigenetic mechanisms, or molecular associations with PAD with no reference to specific SNPs in the nuclear genome were not included. Lastly, studies that analyzed patients with PAD in combination with patients with other manifestations of atherosclerosis such as cardiovascular and cerebrovascular disease were excluded from this systematic review.

Data extraction. The following data were extracted: first author last name, journal and year of publication, country of study population, definitions and frequencies of cases, and controls. The reference SNP identification number (rs number), chromosomal gene position, nucleotide base change, the putative mechanism of action of each gene, and the DNA testing methods were captured. Data were complemented using the National Center for Biotechnology Information's SNP database, dbSNP.[20] The eligible studies were subsequently divided into two groups based on the clinical outcomes examined. The first group included studies evaluating the association of a DNA variant with the diagnosis of PAD compared with controls without PAD. The second group included studies evaluating variants associated with the progression of disease defined by more severe manifestation of PAD, incidence of cardiovascular and/or limb adverse outcomes, or failure of LER compared with patients with uncomplicated or less severe forms of PAD.

Trends of genetic research in the diagnosis of PAD. To highlight the changes in genetic research related to PAD, the proportion of specific variables derived from the papers included in this review was calculated over various time periods (5-year increments) based on publication year. The total number of publications in each period was derived, and the corresponding mean number of study subjects (cases and controls combined) was calculated. The mean number of SNPs per study evaluated in each time period was also calculated.

Study characteristics. Studies were reviewed for the definition of PAD cases and criteria of controls. The clinical and imaging characteristics used for defining PAD and whether asymptomatic patients were included in cases were tabulated. The presence of cardiovascular risk factors in controls with no diagnosis of PAD was recorded. For papers evaluating markers of PAD progression, the outcomes representing progression (severity, cardiovascular events, or complications) and the characteristics of the reference patient population with PAD lacking criteria for progression were recorded. The ethnicity of the population studied was abstracted, when available, as well as whether the authors controlled for it in their analysis. The genetic testing methods, the presence of quality control for genotyping, and the assessment of Hardy-Weinberg equilibrium (HWE) in the sample populations were evaluated. In studies where multiple testing was performed, statistical adjustment for multiple testing was noted when performed.

Meta-analysis of case-control studies for the diagnosis of PAD. SNPs examined in more than one case-control candidate gene study (excluding GWAS) were identified, and the genotype counts for cases and controls were extracted when available. Meta-analysis of the SNP reproduced in the literature was performed to provide a summary effect regarding the correlation with PAD. A logistic regression assuming a log additive mode of inheritance was run for each study. This was done to maintain a consistent analysis approach across studies. For each study, we obtained a beta coefficient, standard error, and *P* value, and then meta-analyzed these using METAL to perform a fixed-effect, inverse variance-weighted meta-analysis.[21] All analyses were conducted in R (R.4.3.0) and two-tailed Bonferroni corrected *P* values less than .0038 (0.05/13) were considered statistically significant.

Comparison of SNPs associated with the diagnosis of PAD. All the studies in the literature reporting DNA variant associations were reviewed and the findings summarized. First, SNPs derived from GWAS were compiled as genetic variants supported with highest power and most robust methodology. Next, we focused on the SNPs from case-control studies included in the meta-analysis in this paper and their relevance to more contemporary GWAS. Common pathways of disease and potential effect were highlighted.

Comparison of SNPs associated with the progression of PAD. DNA variants in the literature that were reported to be associated with PAD progression were reviewed. PAD progression had different definitions depending on the cohort of patients studied and the clinical outcomes assessed.

RESULTS

Literature search

The literature search identified 4669 studies. A total of 125 papers were eligible, of which 112 examined the association of a DNA variant with the diagnosis of PAD, and 28 evaluated variants associated with PAD progression. There was overlap between the two groups, with 15 papers reporting on possible associations of variants with the diagnosis of PAD and its progression. Most patients had blood testing for germline DNA variants. Only 27 papers provided sufficient data on SNPs replicated in different case-control studies and were included in the meta-analysis (Fig 1).

Trends of genetic research in the diagnosis of PAD

The number of studies looking at genetic associations with the diagnosis of PAD was stable over the first two decades of the 21st century with a mean of 24 papers every 5 years. The mean number of SNPs studied per paper remained stable at 3 SNPs between 2006 and 2020. On the other hand, the mean number of study subjects progressively increased during the same time with an exponential rise noted after 2015. The mean number of study subjects per paper increased 10-folds from a maximal mean of 2304 before 2015 to 231,270 during 2021-2022 (Fig 2).

Study characteristics

Studies examining DNA variant association with the diagnosis of PAD. The characteristics for all the papers studying the association of specific DNA variants with the diagnosis of PAD are summarized in Supplementary Table II. The definition of patients with PAD was heterogeneous and sometimes inconsistent with current clinical guidelines. In 62 (55%) studies, the diagnosis of PAD was based on a combination of clinical features and imaging findings. However, 27 (24%) studies based the diagnosis of PAD on imaging alone, whereas 20 (17.8%) defined PAD clinically without imaging requirement. In 5 (4.4%) studies, the criterion for diagnosing patients with PAD was not mentioned. On the other hand, 14 (12.5%) papers included patients with asymptomatic PAD who were diagnosed by imaging only. The ABI was the most used testing modality in 74 (66%) studies, followed by ultrasound in 32 (29%). Cross-sectional imaging was used in 31 (28%) of the studies, and the presence of a stenosis ≥50% on computed tomography or magnetic resonance angiography established the diagnosis of PAD. The ABI threshold for inclusion varied between 0.8 and 0.9, whereas some studies even included patients with ABI less than 0.95 or 1.0, which is inconsistent with current clinical guidelines.

As for the control groups, in addition to not having a diagnosis of PAD, 22 (20%) studies limited control subjects to patients without any risk factor for cardiovascular

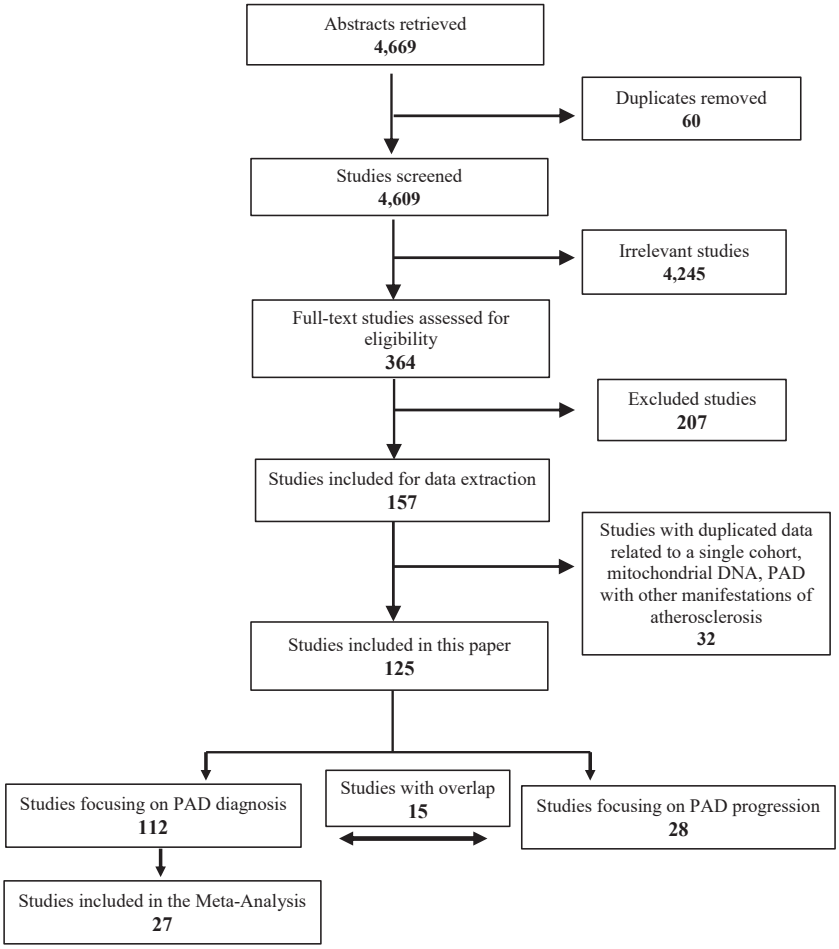


Fig 1. Flowchart of the literature review process. *PAD*, Peripheral artery disease.

disease. In 48 (42.8%) studies, there was no reporting of race or ethnicity of the patient population, but the remaining studies demonstrated populations with very diverse ancestry and/or location of the study population, which included White Europeans, Black/African, Hispanics/Latinos, Chinese, Japanese, Brazilian, and Egyptian. Only 3 (2.7%) studies explicitly stated that they had controlled for race or ethnicity in their analyses.

Quality control for genotyping was reported in 40 (35.7%) studies and HWE in 83 (74.1%); only 26 (23.2%) studies corrected for multiple testing. In a handful of studies, the researchers adjusted their analyses for known clinical risk factors predisposing for PAD such as age, sex, smoking, and hyperlipidemia.[22-26] Table I illustrates only papers that showed the presence of an association between one or more SNP(s) and the diagnosis of PAD and only the papers that reported quality control for genotyping, HWE, and statistically corrected for multiple testing in the methodology.

Studies examining DNA variant association with the progression of PAD. The characteristics of all papers examining various aspects of PAD progression are available in Supplementary Table III. In addition to the heterogeneity in defining cases and controls with respect to the diagnosis of PAD, there were significant variations in the aspects of disease progression studied. A total of 14 (50%) studies looked at severity, 13 (46.4%) assessed prognosis, and 2 (7.1%) looked at both severity and prognosis. Severity of disease was most commonly assessed by comparing patients with intermittent claudication with patients with chronic limb-threatening ischemia (CLTI) as the more advanced form of PAD in 8 (28.6%) studies reviewed. In 2 (7.1%) studies, a specific reduction in ABI (≥0.15 or ≥0.2) was used as a marker of disease progression.[27,28] Various combinations of outcomes including mortality, cardiovascular events, and adverse limb events were used to evaluate prognosis as a marker of disease progression in 14 (50%) the studies. However, none of the studies defined a timeline for the progression of PAD. Failure of LER defined by reintervention or major amputation was specifically included in 6 (21.4%) studies as a marker of disease progression.[29-34]

Only 2 (7.1%) studies restricted control patients to those not having PAD and not having other cardiovascular risk

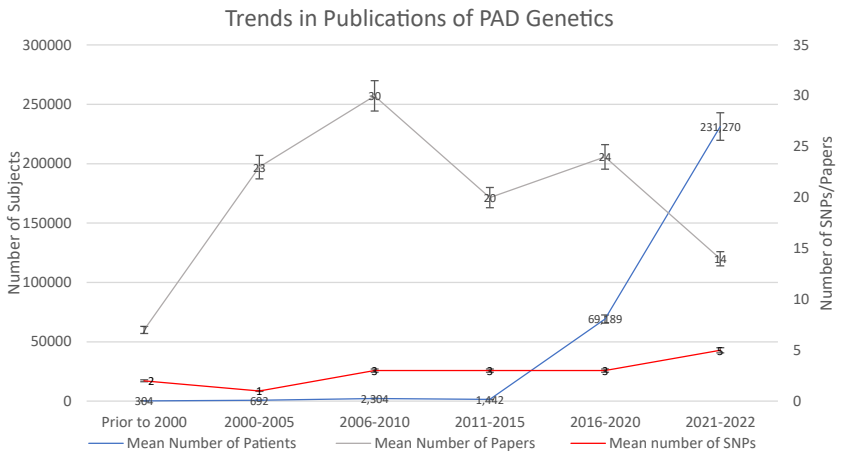


Fig 2. Trends in the mean number of publications studying genetic variant association with peripheral artery disease (*PAD*) as well as mean single nucleotide polymorphisms (*SNP*s) and subjects analyzed per paper during the various periods.

factors. Race and/or ethnicity was reported in 14 (50%) studies analyzing progression, and only one paper controlled for it in the analysis. Quality control was reported in only 7 (25%) studies, but HWE was reported in 17 (60.7%). A total of 8 studies (28.6%) reported statistical correction for multiple testing. Table II displays only papers that showed a correlation of one or more SNP(s) with the progression of PAD and reported in the methodology quality control for genotyping and HWE.

Meta-analysis of studies examining DNA variant association with the diagnosis of PAD. There were 13 SNPs that were studied in 27 papers examining their association with the diagnosis of PAD. The meta-analysis of case-control candidate gene studies revealed three SNPs significantly ($P < .0038$) associated with the diagnosis of PAD based on two or more studies. The SNP that demonstrated the most consistent and strongest association with the diagnosis of PAD based on case-control studies was rs5498, an A>G substitution in the intercellular adhesion molecule-1 (ICAM-1) that is located on chromosome 19p13.3-p13.2. The variant induces a glutamine to lysine amino acid change. It was found to be associated with the diagnosis of PAD in three different studies.[35-37] Based on the current meta-analysis, an individual has a 67% increase in the odds of having a diagnosis of PAD with each G allele compared with a person without this variant (ie, AA genotype) (odds ratio [OR] = 1.667; 95% confidence interval [CI]: [1.419-1.959]).

Another SNP that showed a significant association with the diagnosis of PAD based on four different studies was rs1800795, a G>C mutation in IL-6.[24,36,38,39] Interestingly, this mutation seems to have a protective effect with a reduction in the risk of developing PAD by 38% with OR = 0.622 [CI: 0.523-0.74]. The third SNP involves a mutation in hepatic lipase that breaks triglycerides into fatty acids and has been found to affect the plasma levels of atherogenic lipoproteins. SNP rs2070895 (G>A) on chromosome 15 is a common polymorphism in the promoter region. It was found in two studies to be associated with PAD and when meta-analyzed had an OR = 1.582 [CI: 1.179-2.123].[40,41] There were two additional variants that reached nominal statistical significance ($P < .05$) with the diagnosis of PAD and are mentioned here as plausible associations. They are rs5361 and rs179963 and are annotated to the E-selectin and prothrombin genes, respectively. Although E-selectin is a proinflammatory molecule, prothrombin plays an essential role in the coagulation cascade (Fig 3).

The most common DNA variant evaluated in the literature with the diagnosis of PAD is rs1801133. The C>T mutation in the methylene tetrahydrofolate reductase enzyme at the 677 position is involved in homocysteine metabolism and has been studied in a total of 12 different studies in various countries including the United Kingdom, Austria, Canada, Germany, Brazil, Turkey, Switzerland, Italy, and Czech Republic. The same SNP was associated with elevated plasma levels of homocysteine that predispose to an increased risk of cardiovascular diseases.[42,43] The findings from studies that investigated rs1801133 were mixed. Two studies suggested that the T allele was associated with an increased risk of PAD.[44,45] However, 10 other studies did not find a significant association between rs1801133 and PAD.[42,43,46-53] Meta-analysis of genetic counts available from seven different papers showed no significant association with the diagnosis of PAD[42,43,45-47,49,52] (Supplementary Fig).

Comparison of DNA variants associated with the diagnosis of PAD. There were 232 variants in 112 studies that were assessed, and 132 (56%) variants demonstrated

Table I. Studies reporting quality control and Hardy-Weinberg equilibrium (HWE) and demonstrated variant association with the diagnosis of peripheral artery disease (*PAD*)

Author	Year	Country	Case (No.)	Clinical PAD definition	Imaging PAD definition	Control (No.)	SNP	Gene	Variant	Chm	Mechanism of action	Association with PAD
Jiang	2010	Taiwan	27	No	US, ABI ≤0.9	345	rs2425895	SLC2A10—Solute carrier family 2 member 10	A>G	20	Glucose homeostasis	No
							rs2143044		T>A			Yes
							rs3092412		A>G			Yes
							rs2235491		G>A			No
							rs2425904		C>A			Yes
							rs2425911		C>A			Yes
							rs3091904		T>C			Yes
							rs1059217		T>A			Yes
							rs6066059		T>C			Yes
							rs2179357		T>C			Yes
Klarin	2019	USA	36,424	Combination of ICD-9/10 codes/ Current Procedural Terminology (CPT) codes, and vascular specialist visits	No	601,044	rs118039278	Lipoprotein A	A>G	6	Lipid pathway	Yes
							rs3130968	HLA-B	C>T	12	Inflammation	Yes
							rs7528419	CELSR2/SORT1	G>A	1		Yes
							rs6025	Factor V Leiden	C>T	1	Coagulation	Yes
							rs2107595	HDAC9	G>A	7		Yes
							rs4722172	IL-6	A>G	7	Inflammation	Yes
							rs322	LPL	C>A	8	Lipid pathway	Yes
							rs505922	ABO	T>C	9		Yes
							rs1537372	CDKN2B-AS1/ 9p21	G>T	9		Yes
							rs7903146	TCF7L2	C>T	10		Yes
							rs566125	MMP-3	C>T	11	Inflammation	Yes
							rs7476	CREB3L1	A>C	11		Yes
							rs11066301	PTPN11	A>G	12		Yes
							rs4842266	RP11-359M6.3	A>G	12		Yes
							rs1975514	COL4A1	T>C	13		Yes
							rs55784307	SMOC1	C>A	14		Yes
							rs10851907	CHRNA3	G>A	15		Yes
							rs62084752	LOC732538	G>C	17		Yes
							rs138294113	LDLR	T>C	19	Lipid pathway	Yes
Kullo	2014	USA	1641	History of LER	ABI ≤ 0.9 or ≥ 1.4	1604	rs653178	ATXN2-SH2B3	C>G	12	Inflammation	Yes
Matsukura	2015	Japan	785	IC, rest pain, tissue loss, or history of PAD therapy	ABI < 0.9	3383	rs9584669	IPO5/RAP2A	T>C	13	Lipid pathway	Yes
							rs6842241	EDNRA	C>A	4	Inflammation	Yes
							rs2074633	HDAC9	T>A	7		Yes
Safarova	2019	USA	1749	ICD-9 code	ABI ≤ 0.9	1855	rs3184504	SH2B Adaptor Protein 3	T>A	12		Yes
							rs616154	ABO	C>T	9		Yes
								ZEB 2	Deletion	2		Yes
Scherer	2010	USA	416	No	ABI < 0.9	416	rs1042602		C>A	11		Yes
							rs9665943		G>A	11		Yes
Thor geirsson	2016	Iceland	3415	IC, rest pain, and tissue loss	ABI ≤ 0.8, US, CTA	332,908	rs56175056	CHRNA4	G>A	20	Anti-inflammatory	Yes

Table I. Continued.

Author	Year	Country	Case (No.)	Clinical PAD definition	Imaging PAD definition	Control (No.)	SNP	Gene	Variant	Chm	Mechanism of action	Association with PAD
Tragante	2013	Netherlands	1751	IC, rest pain, and tissue loss	ABI≤0.9 or postexercise ABI decreasing by 20%	2015	rs4977574	9p21	A>G	9		Yes
							rs964184					Yes
							rs4977574					Yes
Tsai	2012	Taiwan	NS	No	ABI ≤ 0.9	NS	rs2383207	ANRIL	A>G	9		Yes
							rs1333040					No
							rs1333049					No
							rs11066001	BRAP	T>C	12		Yes
Xie	2011	China	455	No	ABI < 0.9	NS	rs4638289		A>C	11	Inflammation	No
							rs12218	Serum Amyloid A1	T>C			Yes
							rs7131332		A>G			No
							rs11603089		A>G			No

ICD, International Classification of Diseases.
All abbreviations included in the Supplementary Appendix.

an association with the diagnosis of PAD. A comprehensive list of the studies, SNPs, genes, and putative mechanisms of action is provided in Supplementary Table IV.

The variants discovered by Klarin et al[17] in the Million Veterans Project were replicated in the UK biobank and in a subsequent meta-analysis with the Genetics of Lower Extremity Arterial Disease consortium at a genome wide level of significance ($P < 5 \times 10^{-8}$).[54] In fact, the meta-analysis performed by Van Zuydam et al[54] analyzed more than 40,000 patients with PAD and more than 600,000 controls derived from numerous North American and European cohorts and is the largest analysis of genetic variants associated with the diagnosis of PAD. On the other hand, the association with SNPs related to HDAC9 (Histone Deacetylase 9) was also discovered in patients of East Asian ancestry in a GWAS performed in the Japanese population suggesting common pathways involved in the disease across patients with different ethnic backgrounds.[14]

Most of the SNPs repeatedly examined in prior smaller candidate genes case-control studies and the focus of the meta-analysis in the current paper were not found to be significant in contemporary GWAS. However, some of the variants were related to the same genes, raising the possibility of common molecules being impacted and playing a role in the development of the disease. Thus, the GWAS by Klarin et al[17] demonstrated that rs4722172 in an intergenic region on chromosome 7 could impact IL-6 expression modulating inflammation. In the current meta-analysis, rs1800795 depicted a variant of IL-6 with a presumptive protective effect on the development of PAD.

The larger case-control studies tested whether SNPs shown in other GWAS to be associated with coronary artery disease were also associated with PAD. Interestingly, rs3184504 that causes a missense mutation in *SH2B3* altering the structure of the protein and its potential interactions has shown a significant association with PAD in the Mayo clinic biorepository and the SMART (Second Manifestations of ARTerial disease) study in the Netherlands.[15,55] *SH2B3* has pleiotropic effects likely through the modulation of inflammation and has been involved in pathogenesis of cardiovascular and immunological diseases as well as the production of platelets.[4]

Comparison of DNA variants associated with the progression of PAD. Meta-analyses of studies looking at progression of PAD were not possible as there were only 28 eligible studies that displayed significant heterogeneity in the surrogate markers of progression as well as the variants studied. Even though a time frame for progression was not provided, patients with CLTI representing more advanced stage of PAD compared with asymptomatic patients or patients with claudication were studied in several papers. In fact, Factor V Leiden mutation, which results in a hypercoagulable state classically associated with an increased incidence of venous thrombosis, was found to be associated with the diagnosis and the severity of PAD in GWAS. The rs6025, G1691 A variant was significantly associated with the diagnosis of CLTI and the likelihood of undergoing amputation.[17] Moreover, another study showed that the G allele for rs6025 is significantly associated with an increased risk of premature PAD diagnosed at age younger than 45 years.[56] However, three smaller studies did not find a significant association between genetic variants in Factor V Leiden and PAD.[43,57,58] In another study, Biscetti et al[7] demonstrated an incremental association of SNPs in the RANK/RANKL/OPG system, which plays a role in

Table II. Studies reporting quality control and Hardy-Weinberg equilibrium (HWE) that examined single nucleotide polymorphism (*SNP*) association with the progression of peripheral artery disease (*PAD*)

Author	Year	Country	Cases with PAD progression (No.)	Cases without PAD progression (No.)	Definition of PAD progression	Definition of PAD without progression	SNP	Gene	Variant	Chm	Mechanism of action	Association with PAD progression
Biscetti	2017	Italy	255	268	CLTI	IC	rs3134069	Osteoprotegerin	T>G, T245 G	8	Inflammation	Yes
							rs2073617	Osteoprotegerin	T>C, T950 C			Yes
							rs2073618	Osteoprotegerin	G>C, G1181 C			Yes
							rs9533156	Receptor activator of nuclear factor kappa-B ligand	C>T	13	Inflammation	Yes
							rs2277438	RANKL	A>G			No
							rs1805034	RANK	T>C		Inflammation	Yes
Cluett	2009	Italy	NS	NS	ABI change	Stable ABI	rs1333049		G>A	9		No
							rs10757278		A>C	9		No
Klarin	2019	USA	NS	They separated counts based on Factor V Leiden only	CLTI/major amputation/ lower ABI	IC/no amputation/ higher ABI	rs118039278	Lipoprotein A	G>A	6	Lipid pathway	Yes
							rs3130968	HLA-B	T>C	12	Inflammation	No
							rs7528419	CELSR2/SORT1	A>G	1		No
							rs6025	Factor V Leiden	T>C	1	Coagulation	Yes
							rs2107595	HDAC9	A>G	7		Yes
							rs4722172	IL-6	G>A	7	Inflammation	Yes
							rs322	LPL	A>C	8	Lipid pathway	No
							rs505922	ABO	C>T	9		No
							rs1537372	CDKN2B-AS1/ 9p21	T>G	9		Yes
							rs7903146	TCF7L2	T>C	10		No
							rs566125	MMP-3	T>C	11	Inflammation	No
							rs7476	CAMP responsive element binding protein 3 like 1	C>A	11		Yes
							rs11066301	PTPN11	G>A	12		No
							rs4842266	RP11-359M6.3	G>A	12		No
							rs1975514	COL4A1	C>T	13		No
							rs55784307	SMOC1	A>C	14		No
							rs10851907	CHRNA3	A>G	15		Yes
							rs62084752	LOC732538	C>G	17		No
							rs138294113	LDLR	C>T	19		No

All abbreviations included in the Supplementary Appendix.

atherosclerosis and arterial calcification, with the increasing risk of CLTI in an Italian cohort of patients with PAD (Table II). All the DNA variants studied in association with progression of PAD as well as the associated genes, and putative mechanisms of action are summarized in Supplementary Table V.

DISCUSSION

This systematic review provides a comprehensive summary of the literature evaluating the genetics of PAD. We found 125 papers focusing on the diagnosis and the progression of PAD with significant heterogeneity in definitions and outcomes. GWAS have established 19 variants consistently associated with the diagnosis of PAD that were replicated in large biobanks. Meta-analysis of smaller case-control studies showed that only three variants were significantly associated with diagnosis of PAD and only rs1800795 variant of IL-6 could be correlated with findings from GWAS. There was limited literature looking at the progression of PAD with one GWAS demonstrating that rs6025 in Factor V Leiden was associated with CLTI and major amputation.

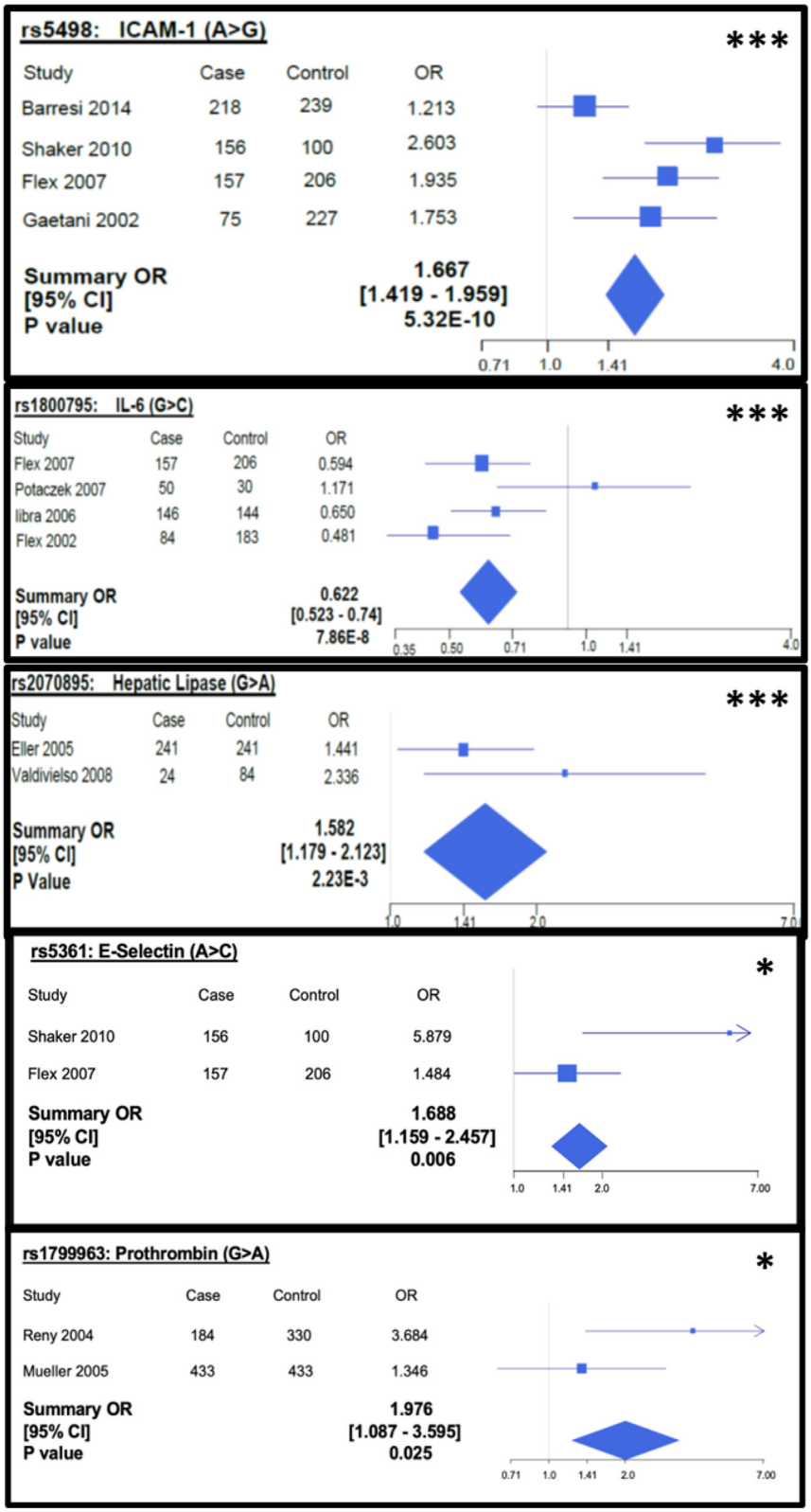


Fig 3. Forrest plots of meta-analysis of statistically significant variants (***) and nominally significant variants (*) associated with the diagnosis of peripheral artery disease (PAD). *CI*, Confidence interval; *ICAM-1*, intercellular adhesion molecule-1; *OR*, odds ratio.

The current study highlights the evolution in the power and quality of genetic research occurring over the past decade with the introduction of large biobanks and the exponential increase in sample size. GWAS have transformed the field and dwarfed prior studies with the statistical power and ability to identify DNA variants with possible clinical relevance using a hypothesis-free approach that does not rely on selecting SNPs based on prior biological knowledge. Thus, the mean sample size in 2021-2022 was 231,270 for studies examining the diagnosis of PAD. This power is further amplified by genetic meta-analysis of several large cohorts as performed by Van Zuydam et al,[54] achieving a total sample of more than 640,000 patients. In the current meta-analysis, we demonstrated that three SNPs were significantly associated with the diagnosis of PAD based on smaller case-control candidate gene studies from various investigators. These findings ought to be tested in large biobanks to confirm the suggested associations. To put things in perspective, the total number of study subjects included in our meta-analysis was 1741 for ICAM-1 (rs5498), 1020 for IL-6 (rs1800795), and only 590 for hepatic lipase (rs2070895). On the other hand, studies with smaller sample size can sometimes provide more detailed characterization of subjects and compliment contemporary attempts for deep phenotyping in large biobanks. In fact, genotype-based recall has been increasingly used to study genetic factors contributing to disease by focusing on groups of patients with the genotype and phenotype of interest derived from biobanks. Subsequently, deeper phenotyping and whole genome analysis could be performed to elucidate pathways contributing to disease.[59]

Vascular inflammation is crucial in the pathophysiology of PAD as suggested by GWAS showing variants related to human leukocyte antigen B, IL-6, and matrix metalloproteinase-3 associated with the diagnosis of PAD.[17] On the other hand, ICAM-1 and IL-6 found in our meta-analysis to be associated with the diagnosis of PAD are central to this process. During inflammation, the endothelial barrier function is lost and P-selectin is relocated to the apical surface of endothelial cells. That is followed by increased expression and release of ICAM-1 and E-selectin that regulate the attachment and transendothelial migration of leukocytes.[35] Interestingly, E-selectin variant rs5361 also showed nominal significance with the diagnosis of PAD in the current meta-analysis.

The heterogeneity in the definition of PAD in various studies is important to highlight. Even though contemporary research relies on a combination of clinical assessment and imaging to identify patients with PAD, this combination has been inconsistent across studies as shown in Supplementary Table II. ABI measurement is suggested as the primary imaging modality to establish the diagnosis of PAD by most guidelines, but its interpretation and usefulness in clinical practice has been questioned specially in patients with calcified arteries.[60,61] The GWAS performed in the Million Veterans Project relied on diagnostic and procedural codes but performed validation of the phenotype using median ABI that was available in only 57% of the sample.[17] The selection of control subjects without the diagnosis of PAD is just as problematic because the majority of patients with PAD are asymptomatic but carry a two- to threefold risk of mortality compared with patients without PAD.[62] Thus, without a dedicated evaluation by a vascular specialist coupled with imaging, which is often not clinically indicated, there is a risk of misclassification of asymptomatic patients with PAD and control subjects.

Defining the progression of disease seems more elusive because PAD has traditionally been treated as a clinical marker of generalized atherosclerosis. In fact, 50% of the studies used various combinations of angina, myocardial infarction, coronary revascularization, cardiac arrest, stroke, carotid revascularization, and mortality to define progression of PAD based on overall patient prognosis as illustrated in Supplementary Table III. Cardiovascular outcomes are important in that patient population but are not specific to the disease. On one hand, major amputation and repeated LER to prevent amputation are disease specific and should be used as the primary surrogates of progression of PAD. Limb outcomes have been examined in only a limited number of publications.[17,30,32,33] On the other hand, major amputation has been shown in the Vascular Quality Initiative to be independently associated with premature PAD (LER at age $\leq$50) regardless of comorbidities and strategies of LER. These findings suggest that adverse limb events may be significantly driven by genetic factors in patients with early onset of disease.[63]

This study has several limitations. First, even though we conducted a meta-analysis of case-control studies, the meta-analysis did not include larger GWAS studies that were recently meta-analyzed.[54] Also, consistent with the general findings of heterogeneity in the definition and the diagnosis of PAD, the papers that were meta-analyzed used a variety of definitions and diagnostic modalities to identify patients with PAD that could impact the results of the analysis. Second, the significance of the variants meta-analyzed in the current study and other variants included in summary tables has not been tested in larger contemporary biobanks, and clinical implication in diagnosis and therapy has not been assessed. A future direction would be to assess the significance of those variants in current biobanks and possibly perform concomitant deep phenotyping. Third, we did not conduct any formal assessment of bias as there was significant heterogeneity in the definitions of the cases and controls as well as the various outcomes selected to report progression of PAD, which was highlighted in the study characteristics. Clinically, 80%-90%

of patients with PAD are asymptomatic, and focusing on more aggressive phenotypes such as premature PAD, surgical patients who undergo multiple reinterventions or patients who suffer major amputations could provide a higher yield patient population to identify targets of aggressive behavior that could affect clinical management.

CONCLUSIONS

This study provides a comprehensive review of the literature on the genetics of the diagnosis of PAD and its progression highlighting significant heterogeneity. Future research should focus on patient populations at risk of adverse limb outcomes that could benefit from genetic testing.

The authors would like to thank Vermetha Polite and the Document Delivery Team from the Yale Cushing/Whitney Medical Library for technical support, and Thomas Mead, Jan Glover, Alexandria Brackett, and Alyssa Grimshaw for assistance with the search strategy.

AUTHOR CONTRIBUTIONS

Conception and design: CC, TK, DA, ADe, RG, ADa, JW, IK, MM
Analysis and interpretation: CC, ADe, JW, IK, MM
Data collection: CC, TK, DA, HN
Writing the article: CC, DA
Critical revision of the article: CC, TK, DA, ADe, RG, ADa, HN, JW, IK, MM
Final approval of the article: CC, TK, DA, ADe, RG, ADa, HN, JW, IK, MM
Statistical analysis: Not applicable
Obtained funding: Not applicable
Overall responsibility: CC

DISCLOSURES

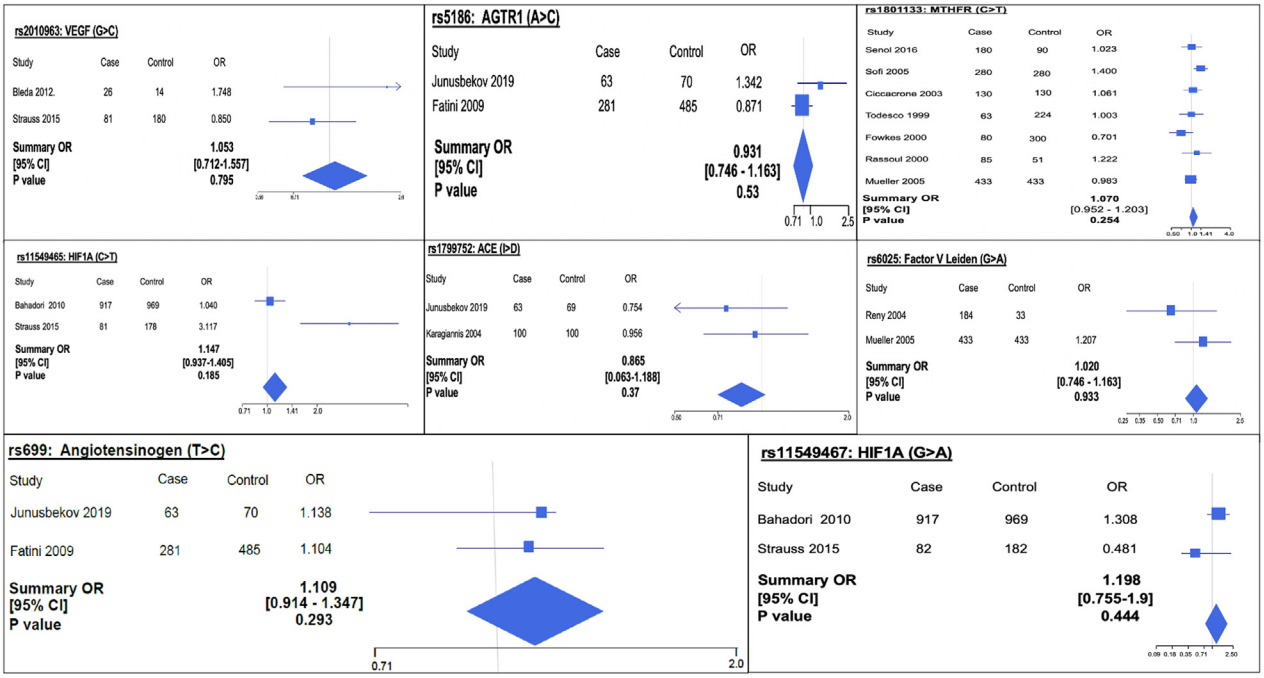
C.I.O.C. has intellectual property related to a device for inferior vena cava filter retrieval. J.D.W. reported receiving grant support by the US Food and Drug Administration, Arnold Ventures, Johnson & Johnson through Yale University, and the National Institute on Alcohol Abuse and Alcoholism of the National Institutes of Health under award No. 1K01AA028258; and reported serving as a consultant for Hagens Berman Sobol Shapiro LLP and Dugan Law Firm APLC. All other authors have no competing interests.

REFERENCES

1. Kullo IJ, Leeper NJ. The genetic basis of peripheral arterial disease: current knowledge, challenges, and future directions. *Circ Res*. 2015;116:1551–1560.
2. Mahoney EM, Wang K, Cohen DJ, et al. One-year costs in patients with a history of or at risk for atherothrombosis in the United States. *Circ Cardiovasc Qual Outcomes*. 2008;1:38–45.
3. Carmelli D, Fabsitz RR, Swan GE, et al. Contribution of genetic and environmental influences to ankle-brachial blood pressure index in the NHLBI Twin Study. National Heart, Lung, and Blood Institute. *Am J Epidemiol*. 2000;151:452–458.
4. Kullo IJ, Turner ST, Kardia SL, et al. A genome-wide linkage scan for ankle-brachial index in African American and non-Hispanic white subjects participating in the GENOA study. *Atherosclerosis*. 2006;187:433–438.
5. Murabito JM, Guo CY, Fox CS, et al. Heritability of the ankle-brachial index: the Framingham Offspring study. *Am J Epidemiol*. 2006;164:963–968.
6. Barresi V, Signorelli SS, Musso N, et al. ICAM-1 and SRD5A1 gene polymorphisms in symptomatic peripheral artery disease. *Vasc Med*. 2014;19:175–181.
7. Biscetti F, Straface G, Angelini F, Pitocco D, Landolfi R, Flex A. Association of RANK/RANKL/OPG gene polymorphisms with risk of peripheral arterial disease (PAD) and critical limb ischemia in the general Italian population. *Meta Gene*. 2017;11:85–90.
8. van de Woestijne AP, van der Graaf Y, de Bakker PI, et al. Rs964184 (APOA5-A4-C3-A1) is related to elevated plasma triglyceride levels, but not to an increased risk for vascular events in patients with clinically manifest vascular disease. *PLoS One*. 2014;9:e101082.
9. Bick AG, Akwo E, Robinson-Cohen C, et al. Association of APOL1 risk alleles with cardiovascular disease in blacks in the Million veteran program. *Circulation*. 2019;140:1031–1040.
10. Kardys I, Uitterlinden AG, Hofman A, et al. Fibrinogen gene haplotypes in relation to risk of coronary events and coronary and extracoronary atherosclerosis: the Rotterdam Study. *Thromb Haemost*. 2007;97:288–295.
11. Kardys I, van Tiel CM, de Vries CJ, et al. Haplotypes of the NR4A2/NURR1 gene and cardiovascular disease: the Rotterdam Study. *Hum Mutat*. 2009;30:417–423.
12. Gemmati D, Serino ML, Trivellato C, et al. C677T substitution in the methylenetetrahydrofolate reductase gene as a risk factor for venous thrombosis and arterial disease in selected patients. *Haematologica*. 1999;84:824–828.
13. Strauss E, Waliszewski K, Oszkinis G, et al. Polymorphisms of genes involved in the hypoxia signaling pathway and the development of abdominal aortic aneurysms or large-artery atherosclerosis. *J Vasc Surg*. 2015;61:1105–1113 e1103.
14. Matsukura M, Ozaki K, Takahashi A, et al. Genome-wide association study of peripheral arterial disease in a Japanese population. *PLoS One*. 2015;10:e0139262.
15. Safarova MS, Fan X, Austin EE, et al. Targeted sequencing study to uuncover shared genetic susceptibility between peripheral artery disease and coronary heart disease-brief report. *Arterioscler Thromb Vasc Biol*. 2019;39:1227–1233.
16. Thorgeirsson TE, Steinberg S, Reginsson GW, et al. A rare missense mutation in CHRNA4 associates with smoking behavior and its consequences. *Mol Psychiatry*. 2016;21:594–600.
17. Klarin D, Lynch J, Aragam K, et al. Genome-wide association study of peripheral artery disease in the Million Veteran Program. *Nat Med*. 2019;25:1274–1279.
18. Grossetta Nardini HK, Wang L, The Yale MeSH Analyzer https://mesh.med.yale.edu. Accessed December 19, 2023.
19. NIH, Covidence systematic review software https://www.ncbi.nlm.nih.gov/snp/. Accessed December 19, 2023.
20. Sayers EW, Beck J, Bolton EE, et al. Database resources of the national Center for Biotechnology information. *Nucleic Acids Res*. 2021;49:D10–D17.
21. Willer CJ, Li Y, Abecasis GR. METAL: fast and efficient meta-analysis of genomewide association scans. *Bioinformatics*. 2010;26:2190–2191.
22. Kawasaki I, Tahara H, Emoto M, et al. Relationship between TaqIB cholesteryl ester transfer protein gene polymorphism and macrovascular complications in Japanese patients with type 2 diabetes. *Diabetes*. 2002;51:871–874.
23. Morrison AC, Bray MS, Folsom AR, et al. ADD1 460W allele associated with cardiovascular disease in hypertensive individuals. *Hypertension*. 2002;39:1053–1057.
24. Libra M, Signorelli SS, Bevelacqua Y, et al. Analysis of G(-174)C IL-6 polymorphism and plasma concentrations of inflammatory markers in patients with type 2 diabetes and peripheral arterial disease. *J Clin Pathol*. 2006;59:211–215.
25. Papaoikonomou ST, Tousoulis D, Tentolouris N, et al. Genetic variant of the C-reactive protein gene and prevalence of peripheral arterial disease in patients with type 2 diabetes mellitus. *J Diabetes Metabol*. 2015;6:1–5.
26. Ku EJ, Won GW, Lee YH, et al. Genetic variation in TCF7L2 rs7903146 correlating with peripheral arterial disease in long-standing type 2 diabetes. *Diab Vasc Dis Res*. 2020;17:1479164119888475.

27. Archetti S, Martini M, Botteri E, et al. Influence of genetic and environmental factors in peripheral arterial disease natural history: analysis from six years follow up. *Int J Appl Basic Med Res*. 2012;2:117–122.
28. Taute BM, Gläser C, Taute R, et al. Progression of atherosclerosis in patients with peripheral arterial disease as a function of angiotensin-converting enzyme gene insertion/deletion polymorphism. *Angiology*. 2002;53:375–382.
29. Boc V, Božic Mijovski M, Pohar Perme M, et al. Diabetes and smoking are more important for prognosis of patients with peripheral arterial disease than some genetic polymorphisms. *Vasa*. 2019;48:229–235.
30. Le Hello C, Fradin S, Morello R, et al. Contribution of deletion in angiotensin-converting enzyme but not A1166C angiotensin II type-1 receptor gene polymorphisms to clinical outcomes in atherothrombotic disease. *Arch Med Res*. 2011;42:202–210.
31. Lin JF, Wu S, Juang JJ, et al. IL1RL1 single nucleotide polymorphism predicts sST2 level and mortality in coronary and peripheral artery disease. *Atherosclerosis*. 2017;257:71–77.
32. Piatti PM, Marone E, Mantero M, et al. Effect of normalization of fasting glucose by intensified insulin therapy and influence of eNOS polymorphisms on the incidence of restenosis after peripheral angioplasty in patients with type 2 diabetes: a randomized, open-label clinical trial. *Acta Diabetol*. 2013;50:373–382.
33. Sartori M, Conti E, Favaretto E, et al. Thrombotic risk factors and cardiovascular events after endovascular intervention for peripheral arterial disease. *Eur J Vasc Endovasc Surg*. 2011;42:817–823.
34. Lee J, Cheng N, Tai H, et al. CYP2C19 polymorphism is associated with amputation rrates in patients ttaking clopidogrel after endovascular intervention for critical limb iischaemia. *Eur J Vasc Endovasc Surg*. 2019;58:373–382.
35. Shaker O, Zahra A, Sayed A, et al. Role of ICAM-1 and E-selectin gene polymorphisms in pathogenesis of PAOD in Egyptian patients. *Vasc Health Risk Manag*. 2010;6:9–15.
36. Flex A, Gaetani E, Angelini F, et al. Pro-inflammatory genetic profiles in subjects with peripheral arterial occlusive disease and critical limb ischemia. *J Intern Med*. 2007;262:124–130.
37. Gaetani E, Flex A, Pola R, et al. The K469E polymorphism of the ICAM-1 gene is a risk factor for peripheral arterial occlusive disease. *Blood Coagul Fibrinolysis*. 2002;13:483–488.
38. Flex A, Gaetani E, Pola R, et al. The -174 G/C polymorphism of the interleukin-6 gene promoter is associated with peripheral artery occlusive disease. *Eur J Vasc Endovasc Surg*. 2002;24:264–268.
39. Potaczek DP, Undas A, Nowakowski T, et al. Association between inflammatory markers and the interleukin-6 -174 G/C polymorphism is abolished in peripheral arterial occlusive disease. *Int Angiol*. 2007;26:318–323.
40. Eller P, Schgoer W, Mueller T, et al. Hepatic lipase polymorphism and increased risk of peripheral arterial disease. *J Intern Med*. 2005;258:344–348.
41. Valdivielso P, Ariza MJ, de la Vega-Román C, et al. Association of the -250G/A promoter polymorphism of the hepatic lipase gene with the risk of peripheral arterial disease in type 2 diabetic patients. *J Diabetes Complications*. 2008;22:273–277.
42. Senol S M, Yuksel A, Es MU. Adducin and methylenetetrahydrofolate reductase gene polymorphisms in patients with peripheral arterial disease. *Acta Medica Mediterr*. 2016;32:1773–1778.
43. Mueller T, Marschon R, Dieplinger B, et al. Factor V Leiden, prothrombin G20210A, and methylenetetrahydrofolate reductase C677T mutations are not associated with chronic limb ischemia: the Linz Peripheral Arterial Disease (LIPAD) study. *J Vasc Surg*. 2005;41:808–815.
44. Pollex RL, Mamakeesick M, Zinman B, et al. Methylenetetrahydrofolate reductase polymorphism 677C>T is associated with peripheral arterial disease in type 2 diabetes. *Cardiovasc Diabetol*. 2005;4:17.
45. Rassoul F, Richter V, Janke C, et al. Plasma homocysteine and lipoprotein profile in patients with peripheral arterial occlusive disease. *Angiology*. 2000;51:189–196.
46. Ciccarone E, Di Castelnuovo A, Assanelli D, et al. Homocysteine levels are associated with the severity of peripheral arterial disease in Type 2 diabetic patients. *J Thromb Haemost*. 2003;1:2540–2547.
47. Fowkes FG, Lee AJ, Hau CM, et al. Methylene tetrahydrofolate reductase (MTHFR) and nitric oxide synthase (ecNOS) genes and risks of peripheral arterial disease and coronary heart disease: Edinburgh Artery Study. *Atherosclerosis*. 2000;150:179–185.
48. Santos ME, das CLESF, Gomes KB, et al. Mutations in methylenetetrahydrofolate reductase and in cysthationine beta synthase: is there a link to homocysteine levels in peripheral arterial disease? *Mol Biol Rep*. 2011;38:3361–3366.
49. Sofi F, Lari B, Rogolino A, et al. Thrombophilic risk factors for symptomatic peripheral arterial disease. *J Vasc Surg*. 2005;41:255–260.
50. Stricker H, Soldati G, Balmelli T, et al. Homocysteine, vitamins and gene mutations in peripheral arterial disease. *Blood Coagul Fibrinolysis*. 2001;12:469–475.
51. Svatos J, Orendác M, Musková B, et al. Statistical approaches for evaluation of genetic risk factor of peripheral arterial occlusive disease. *Stud Health Technol Inform*. 1999;68:419–422.
52. Todesco L, Angst C, Litynski P, et al. Methylenetetrahydrofolate reductase polymorphism, plasma homocysteine and age. *Eur J Clin Invest*. 1999;29:1003–1009.
53. Zsóri KS, Csiki Z, Katona É, et al. Vitamin B12 level in peripheral arterial disease. *J Thromb Thrombolysis*. 2013;36:77–83.
54. van Zuydam NR, Stiby A, Abdalla M, et al. Genome-wide association study of peripheral artery disease. *Circ Genom Precis Med*. 2021;14:e002862.
55. Tragante V, Doevendans PA, Nathoe HM, et al. The impact of susceptibility loci for coronary artery disease on other vascular domains and recurrence risk. *Eur Heart J*. 2013;34:2896–2904.
56. Bérard AM, Bedel A, Le Trequesser R, et al. Novel risk factors for premature peripheral arterial occlusive disease in non-diabetic patients: a case-control study. *PLoS One*. 2013;8:e37882.
57. Renner W, Köppel H, Brodmann M, et al. Factor II G20210A and factor V G1691A gene mutations and peripheral arterial occlusive disease. *Thromb Haemost*. 2000;83:20–22.
58. Reny JL, Alhenc-Gelas M, Fontana P, et al. The factor II G20210A gene polymorphism, but not factor V Arg506Gln, is associated with peripheral arterial disease: results of a case-control study. *J Thromb Haemost*. 2004;2:1334–1340.
59. Franks PW, Timpson NJ. Genotype-based recall studies in ccomplex cardiometabolic traits. *Circ Genom Precis Med*. 2018;11:e001947.
60. Conte MS, Pomposelli FB, Clair DG, et al. Society for Vascular Surgery practice guidelines for atherosclerotic occlusive disease of the lower extremities: management of asymptomatic disease and claudication. *J Vasc Surg*. 2015;61:2s–41s.
61. Gerhard-Herman MD, Gornik HL, Barrett C, et al. 2016 AHA/ACC guideline on the management of patients with lower extremity peripheral artery disease: executive summary: a report of the American college of cardiology/American Heart Association task force on clinical practice guidelines. *Circulation*. 2017;135:e686–e725.
62. Criqui MH, Langer RD, Fronek A, et al. Mortality over a period of 10 years in patients with peripheral arterial disease. *N Engl J Med*. 1992;326:381–386.
63. Kim TI, Loh S, DeWan A, et al. Major adverse limb events among patients with premature peripheral artery disease compared with those at the common age undergoing revascularization in the vascular quality iinitiative. *Ann Vasc Surg*. 2022;87:188–197.

Submitted Jul 17, 2023; accepted Sep 27, 2023.



Supplementary Fig. Forrest plots of variants in the meta-analysis that did not reach statistical significance with the diagnosis of peripheral artery disease (PAD). *ACE*, Angiotensin I-converting enzyme; *CI*, confidence interval; *HIF1A*, hypoxia-inducible factor 1 A; *MTHFR*, methylene tetrahydrofolate reductase enzyme; *OR*, odds ratio; *VEGF*, vascular endothelial growth factor.