Human Genome Epidemiology (HuGE) Review

A Field Synopsis and Meta-Analysis of Genetic Association Studies in Peripheral Arterial Disease: The CUMAGAS-PAD Database

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In an electronic search of the literature, the authors systematically retrieved all published studies that investigated genetic susceptibility to peripheral arterial disease (PAD). They created a comprehensive database of all eligible studies, collecting detailed genetic and bioinformatics data on each polymorphism. Data from eligible studies were synthesized using meta-analysis techniques. Gene variants were classified into distinct pathophysiologic pathways, and their potential involvement in PAD pathogenesis was determined. Forty-one publications that examined 44 gene polymorphisms were included. For 37 polymorphisms, the variant form had a functional effect. Twenty-three polymorphisms in 22 potential PAD candidate genes (F2, FGB, MTHFR, ITGB3, ACE, AGT, IL6, CCL2, ICAM1, SELE, MMP9, PPARG, MMP1, ADD1, P2RY12, LPC, PLA2G7, SCARB1, MMP3, MTTP, LPA, CHRNA3; F5 1691 G/A, MTHFR 677C/T, F2 20210 G/A, ITGB3 1565 T/C, ACE I/D, AGT 704C/T, AGT -6G/A, AGT 733C/T, IL6 -174 G/C, MMP9 -1562C/T, ICAM1 1462A/G, CHRNA3 831C/T) showed a significant association in individual studies. Eighty-eight percent of the studies had statistical power of less than 50%, and in 15 studies the genotype distribution in the control group did not conform to Hardy-Weinberg equilibrium. Data on 12 polymorphisms (F5 1691 G/A, MTHFR 677C/T, F2 20210 G/A, ITGB3 1565 T/C, ACE I/D, AGT 704C/T, AGT -6G/A, AGT 733C/T, IL6 -174 G/C, MMP9 -1562C/T, ICAM1 1462A/G, CHRNA3 831C/T) were synthesized, and a positive association was found for 3 (IL6 -174 G/C, ICAM1 1462A/G, CHRNA3 831C/T).

Abbreviations: CI, confidence interval; CUMAGAS, Cumulative Meta-Analysis of Genetic Association Studies; KEGG, Kyoto Encyclopedia of Genes and Genomes; OR, odds ratio; PAD, peripheral arterial disease; SNP, single nucleotide polymorphism.

Editor’s note: This article also appears on the Web site of the Human Genome Epidemiology Network (HuGENet) (http://www.cdc.gov/genomics/hugenet/).

Peripheral arterial disease (PAD) encompasses numerous noncoronary arterial syndromes. It affects approximately 20% of adults aged 55 years or older and an estimated 27 million persons in North America and Europe (1). The term “peripheral arterial disease” includes a diverse group of disorders that lead to progressive stenosis or occlusion, or aneurysmal dilatation, of the aorta and its noncoronary branch arteries, including the carotid, upper extremity, visceral, and lower extremity arterial branches (2). Approximately one-fifth of people with lower-extremity PAD have typical symptoms of intermittent lower-limb claudication, “rest pain,” ulceration, or gangrene; another third have atypical exertional leg symptoms; and half of all patients are asymptomatic (3).

The possible existence of an inherited genetic predisposition to PAD has been investigated in various types of familial aggregation studies (4–8). Heritability estimates have shown that the contribution of genetic factors to overall variation in ankle-brachial index, which is a widely utilized measure for detecting PAD, is 21% (8). One main approach to disentangling the genetic etiology of complex human traits is the use of association studies. Genetic association studies, in particular, are central to efforts to identify and characterize genomic variants (e.g., single nucleotide polymorphisms (SNPs)) underlying susceptibility to PAD. Polymorphisms may
influence gene activity and messenger RNA conformation, alter the binding ability of protein to its substrate, and change its subcellular localization. Therefore, polymorphisms are emerging as possible factors that may predispose people to PAD and correlate with the pathogenesis of the disease (9).

In order to explore the involvement of gene polymorphisms in susceptibility to PAD, we systematically searched the literature and catalogued all published genetic association studies (gene-candidate studies) on PAD. We then retrieved data from bioinformatics and genetics databases (i.e., the International HapMap Project (http://www.hapmap.org), the Genetic Association Database (http://geneticassociationdb.nih.gov/cgi-bin/index.cgi), and the National Center for Biotechnology Information’s SNP database, dbSNP (http://www.ncbi.nlm.nih.gov/SNP/) and created a comprehensive Web-based database and information system with all relevant genetic data called Cumulative Meta-Analysis of Genetic Association Studies (CUMAGAS) (http://biomath.med.uth.gr). CUMAGAS performs meta-analyses for all genetic models (allele contrast, dominant, recessive, and codominant) and provides information on study design and gene polymorphism characteristics. Moreover, we classified the gene variants into pathophysiologic pathways using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (http://www.genome.jp/kegg/) and further characterized the potential involvement of these candidate genes in PAD pathogenesis. Finally, we synthesized the available extracted data with meta-analytic methods (10, 11).

METHODS

Selection of studies

We searched the PubMed biomedical database (US National Library of Medicine, Bethesda, Maryland) for all English-language articles related to PAD and genetic polymorphisms that had been published through December 31, 2008. We supplemented this by hand-searching reference lists and conducting computer-based searches of the Google Scholar (Google, Inc., Mountain View, California), Scopus (Elsevier Science BV, Amsterdam, the Netherlands), and Web of Science (ISI Web of Knowledge; Thomson Reuters, New York, New York) databases. We used the following search criterion: [“peripheral arterial disease” or “peripheral artery disease” or “PAD” or “peripheral arterial occlusive disease” or “lower extremity arterial disease” or “atherosclerotic vascular disease” or “intermittent claudication” or “limb ischemia”] and [“gene polymorphism” or “gene variant” or “polymorphism” or “SNP”]. Bibliographies in the published articles provided further references. Eligible studies fulfilled the following inclusion criteria: 1) they involved cases with clinically diagnosed PAD and healthy controls who were free of PAD; 2) they provided information on genotype frequency or risk estimates; 3) they used DNA-based analysis methods for genotyping; and 4) they included human subjects. In this article, we focus on lower-extremity PAD, which is a chronic obstructive disease of the aortic, iliac, and lower-limb arteries (12). Thus, cases were considered to be patients suffering from lower-extremity PAD, with the diagnosis having been based on both noninvasive and invasive diagnostic tools (see Web Table 1, which appears on the Journal’s website (http://aje.oxfordjournals.org/)).

Studies investigating progression, severity, phenotype modification, response to treatment, or survival were excluded from our analysis. Case reports, editorials, and review articles were also excluded. Finally, family-based studies were excluded because of their different designs (13).

Data extraction

From each article, the following information was extracted (Web Table 1): first author, year of publication, ethnicity of the study population, demographic data, definitions of cases and controls, matching, blindness of genotyping, validity of the genotyping method, and numbers of cases and controls for each genotype. The frequencies of the alleles and the genotypic distributions were extracted or calculated for both cases and controls. The reference SNP identification number (rs number) (14), chromosomal gene position, nucleotide base change, average heterozygosity, amino acid substitution, and functional effect of each polymorphism are additionally presented in Web Table 2 (http://aje.oxfordjournals.org/). According to their involvement in the pathogenesis of PAD, the studied genes were classified into 4 main categories: thrombophilia, hemodynamics, inflammation, and other functions. Furthermore, to examine whether thrombophilia, hemodynamics, and inflammation are causally related to disease risk, we searched for metabolic pathways on which the actions of these risk factors depend. Thus, genes that were assigned to any of the first 3 categories were further grouped into pathophysiologic pathways using the KEGG database. Each of us independently extracted data; we then discussed all disagreements and reached consensus on all items.

Data synthesis and analysis

For each genetic variant, the allele-contrast, dominant, and recessive models were examined (10). When more than 1 study had investigated the same polymorphism, we carried out a meta-analysis of the published results. The associations are indicated as pooled odds ratios with corresponding 95% confidence intervals. Heterogeneity between studies was tested using the Q statistic (10). If \( P_Q \) was less than 0.10, the heterogeneity was considered statistically significant. Heterogeneity was quantified with the \( I^2 \) metric, which is independent of the number of studies in the meta-analysis. \( I^2 \) takes values between 0% and 100%, with higher values denoting a greater degree of heterogeneity (10). The pooled odds ratio was estimated using a random-effects (DerSimonian and Laird) model (15). Random-effects modeling assumes a genuine diversity in the results of various studies, and it incorporates between-study variance into the calculations. When there is a lack of heterogeneity, the random-effects model coincides with the fixed-effects model (10). When
the meta-analysis involved 4 or more studies, the differential magnitude of effect (of variants included in the meta-analysis) in large studies versus small studies was checked with regard to the allele contrast, using the test proposed by Harbord et al. (16). The meta-analysis consisted of the main (overall) analysis, which included all available data; subgroup analyses carried out by ethnicity; and sensitivity analysis, which examined the effect of excluding specific studies (10).

The statistical power of each study with regard to the allele contrast was examined using the CaTS Power Calculator for Genetic Studies (Center for Statistical Genetics, University of Michigan School of Public Health, Ann Arbor, Michigan). In each power calculation, an odds ratio of 1.2 (modest effect), a significance level of 0.05, a 20% disease prevalence, and a disease allele frequency equal to that of the study population were assumed. The distribution of SNP genotypes in the control group was tested for conformity to Hardy-Weinberg equilibrium (17). Studies with a control distribution that deviated from Hardy-Weinberg equilibrium were subjected to sensitivity analysis (10). Sensitivity analysis was performed only when there were more than 2 studies in the meta-analysis. Analyses were performed using the software programs MetaAnalyst (Evidence-Based Practice Centers, Tufts Medical Center, Boston, Massachusetts, 2009), CUMAGAS (http://biomath.med.uth.gr), and Compaq Visual Fortran 90 (Hewlett-Packard Company, Palo Alto, California) with the International Mathematics and Statistics Library (Visual Numerics, Inc., Houston, Texas).

RESULTS

Eligible articles

The literature review identified 185 titles that met the search criteria in PubMed. Each of us independently read the abstracts of these articles and their references to assess their appropriateness for this review. The results were then compared, and disagreements were resolved by consensus. Sixty-four articles remained after abstract selection. The full articles for the remaining studies were evaluated for compliance with the inclusion criteria. Forty-one publications describing 74 polymorphism-disease association tests (studies) fulfilled the inclusion criteria (18–58) (Web Table 1).

Study characteristics

All studies in this review were conducted in white ethnic groups, with the exception of 4 (40, 48, 54, 58) that were not based only on white populations. The mean age of the participants in the individual studies ranged from 48.8 years to 77.5 years, whereas a wide diversity in diagnosis criteria for PAD was observed.
Hardy-Weinberg equilibrium and genotyping validity. The genotype distribution in controls did not conform to Hardy-Weinberg equilibrium in 15 studies (20, 23, 24, 27, 29, 39, 43, 46, 50, 55), while in 3 articles (28, 45, 47), information was not provided (Web Table 1). The genotyping personnel were reported to be blinded to phenotype in 5 papers (18, 19, 24, 29, 43), and the reliability of the genotyping procedure was controlled in 13 articles (18, 19, 24, 29, 31, 33, 40, 43, 48, 51, 53, 54, 57).

Power analysis. In 34% of the studies, the power to detect a modest genetic effect ranged from 25% to 50%. Statistical power greater than 50% was achieved in only 12% of the studies; the remaining studies proved to be underpowered to test their hypotheses (Web Table 1).

Biologic mechanisms of pathogenesis and candidate genes

PAD is often a consequence of systemic disease processes that affect multiple arterial circulations, although clinically recognized disease in more than 1 organ system is not always present (2). These systemic pathophysiologic processes are diverse and include atherosclerosis, inflammation, and both in situ thrombosis and thromboembolism, among others (2). Atherosclerosis is the most common cause of PAD worldwide; thus, the epidemiology and clinical consequences of PAD are closely associated with classic atherosclerosis risk factors (e.g., hypertension, smoking, diabetes, hyperlipidemia, family history, and the postmenopausal state) (2). Inflammation has also been postulated to play a crucial role in the pathophysiology of PAD (59). It has been recognized that inflammation may contribute to all stages of the atherosclerotic process (60), and considerable evidence indicates that the activated hemostatic system also plays an important role in the pathophysiology of atherosclerotic vascular disease (59).

The high allele frequency of the studied genes (Web Table 2) suggests low genotype relative risk. Such genes may contribute to the development of PAD only in conjunction with exogenous and endogenous exposures (61). Susceptibly genes can be identified by studying the pathways postulated to be involved in PAD. For 37 of the studied polymorphisms, functional implications have been reported in the literature (Web Table 2). In 7 cases, the polymorphisms were of unknown functionality (Web Table 2), but even nonfunctional polymorphisms are likely to be in linkage disequilibrium with causative alleles (62).

Main results and subgroup and sensitivity analyses

In the present study, we focused on the gene variants that are involved in thrombophilia, hemodynamics, and inflammation. Table 1 shows the results of meta-analyses for the polymorphisms investigated in more than 1 study. Web Table 1 shows the studies’ characteristics and provides results for the associations between the different polymorphisms and the risk of PAD in individual studies. Web Table 2 includes the pathways, genes, and polymorphisms (with their respective references) evaluated to date in the field of PAD.

Thrombophilia. Thrombophilia can be mediated through defects in alternative convergent pathways: 1) the coagulation cascade pathway (29, 31, 33, 36, 38, 43, 44), 2) the folate pathway (24, 31, 40, 44, 45, 52, 57, 3) the extracellular matrix receptor interaction pathway (35, 43), and 4) the vitamin K cycle module (42).

The coagulation cascade pathway. The F5 gene encodes coagulation factor V, a large plasma glycoprotein that circulates with little or no activity. A nonsynonymous SNP, designated 1691 G/A, was described as a candidate for increasing the risk of PAD. In the 4 studies that investigated this potential correlation (31, 33, 38, 44), no significant dependence of PAD risk on genotype could be demonstrated. The meta-analysis of the 4 studies showed nonsignificant heterogeneity ($I^2 = 27\%$, $P_Q = 0.25$) among them for the allele contrast, and the association was not significant (odds ratio (OR) = 1.16, 95% confidence interval (CI): 0.77, 1.75). The dominant and recessive models also produced nonsignificant results. The Harbord et al. (16) test indicated that there was a differential magnitude of effect in large studies versus small studies for each polymorphism being investigated ($P = 0.05$).

Factor II (prothrombin) is a coagulation factor that is transformed into thrombin after its activation by prothrombinase complex at the site of vascular injury. A functional SNP in the F2 gene (20210 G/A) was studied in 4 published articles (31, 33, 38, 44), 2 of which showed a significant association for the dominant model and the allele contrast (38, 44). The meta-analysis of the studies showed significant heterogeneity ($I^2 = 72\%$, $P_Q = 0.01$) for the allele contrast, and the association was not significant (OR = 1.80, 95% CI: 0.76, 4.27). The dominant and recessive models also produced nonsignificant results. The Harbord et al. (16) test indicated that there was no differential magnitude of effect in large studies versus small studies for each polymorphism being investigated ($P = 0.38$).

The F7 gene encodes coagulation factor VII, which is a vitamin K-dependent factor essential for hemostasis. Currently, $I289 G/A$ is the only polymorphism in the F7 gene that has been investigated in association with PAD (29). However, there is no evidence of association between $F7 I289 G/A$ polymorphism genotypes and the risk of developing PAD.

$F13A1$ is the gene that encodes the coagulation factor XIII A subunit. As of yet, 1 study (36) has explored the contribution of a functional polymorphism in $F13A1$ (204 G/T) to interindividual differences in susceptibility to PAD. No associations were observed.

The $FGB$ gene encodes the $\beta$ component of fibrinogen. Two polymorphisms in the $FGB$ gene (-455 G/A, $1689 T/G$) have been reported (29, 43), with contradictory results. A positive association with PAD was found only for the -455 G/A polymorphism (29) under the recessive and additive models.

The folate pathway. Methylene tetrahydrofolate reductase is a critical folate-metabolizing enzyme involved in the folate/homocysteine metabolic pathway. $MTHFR 677 C/T$ is a common polymorphism that has been reported for
this enzyme and has been associated with a variety of disorders (63–71). Seven studies (24, 31, 40, 44, 45, 52, 57) have investigated the MTHFR 677 C\text{\textit{C}}/T\text{\textit{C}} polymorphism for susceptibility to PAD, with a positive association being found in 3 (40, 44, 57). The dominant model produced significant results in all 3 studies. The allele contrast showed a positive association in only 2 studies (40, 44) and the recessive model in only 1 (44). The meta-analysis of the 7 studies showed significant heterogeneity ($I^2 = 70\%$, $P_Q = 0.01$) among them for the allele contrast, and the association was not significant (OR = 1.17, 95% CI: 0.92, 1.49). In sensitivity analysis (excluding the study by Fowkes et al. (24), where the controls violated Hardy-Weinberg equilibrium), an association of borderline significance emerged (OR = 1.26, 95% CI: 1.00, 1.60). The recessive model revealed a marginal association that was not confirmed in sensitivity analysis, whereas the dominant model did not produce significant results. The Harbord et al. (16) test indicated that there was no differential magnitude of effect in large studies versus small studies for each polymorphism being investigated ($P = 0.46$).

The extracellular matrix receptor interaction pathway. A genetic variation in the ITGB3 gene that involves a single amino-acid substitution of proline for leucine at position 33 has been related to increased platelet aggregation. This polymorphism in the ITGB3 gene (I565 T/C) has been evaluated in 2 studies (35, 43). Significant results were reported in only 1 study (43), in which I565C carriers had a decreased risk of PAD. When results from the 2 studies were synthesized, the association was not significant (OR = 1.37, 95% CI: 0.84, 2.22). The dominant and recessive models also produced nonsignificant results.

The vitamin K cycle module. The VKORC1 gene encodes the enzyme that is responsible for reducing vitamin K 2,3-epoxide to the enzymatically activated form. Currently, the VKORC1-1693 G/A polymorphism is the only one that has been investigated in relation to PAD (42). No significant association was found.

**Hemodynamics.** Perturbations in local hemodynamics are implicated in the atherogenic process (72) and may confer susceptibility to PAD through diverse pathways, such as the calcium signaling pathway (24, 30), the renin-angiotensin system pathway (37, 46, 48, 53, 54), and the porphyrin and chlorophyll metabolism pathway (32, 56).

Calcium signaling pathway. The NOS3 gene encodes a calcium-regulated endothelial nitric oxide synthase that is capable of producing nitric oxide in blood vessels. To date, only 1 functional 27-base-pair tandem repeat polymorphism in the NOS3 gene has been studied (24). This polymorphism did not influence the risk of PAD.

The GNB3 gene encodes a $\beta$ subunit of a G protein. An SNP (C825T) in this gene has been associated with essential hypertension (73) and obesity (74). Notwithstanding, this polymorphic variant was not found to be significantly associated with PAD (30).

Renin-angiotensin system pathway. Angiotensin-converting enzyme processes the decapetide angiotensin I to the 8-amino-acid peptide angiotensin II, which functions as a strong vasoconstrictor. Five studies (37, 46, 48, 53, 54) to date have addressed whether a functional II/D polymorphism in the ACE gene alters the risk of PAD, with results of borderline significance being produced in 2, for the allele contrast model (54) as well as for the recessive model (48). The meta-analysis of the 5 studies did not reveal significant heterogeneity ($I^2 = 2\%$, $P_Q = 0.40$) among them for the allele contrast, and the association was not significant (OR = 0.94, 95% CI: 0.84, 1.04). In sensitivity analysis (excluding the study by Taute et al. (46), where the controls violated Hardy-Weinberg equilibrium), the association remained nonsignificant. The dominant and recessive models also produced nonsignificant results. The Harbord et al. (16) test indicated that there was no differential magnitude of effect in large studies versus small studies for each polymorphism being investigated ($P = 0.33$).

Angiotensinogen is the precursor of angiotensin II, which is involved in the regulation of blood pressure. Three polymorphisms in the AGT gene (Met235Thr, Thr174Met, and -6 G/A) have been reported in 2 populations (54), with contradictory results. A significant genetic effect was found only for the Met235Thr polymorphism in an African-American population, under the dominant model. When results from the Caucasian and African-American populations were synthesized, no association was observed for any of the aforementioned polymorphisms.

Porphyrin and chlorophyll metabolism pathway. The UGT1A1 gene encodes uridine diphosphate glucuronosyltransferase, which mainly determines bilirubin elimination in humans. One functional TA repeat polymorphism in the promoter of the UGT1A1 gene has been reported, with no evidence of association (32).

Heme oxygenase degrades heme into biliverdin, which is subsequently converted to bilirubin and carbon monoxide. A (G)T$_n$-length polymorphism in the HMOX1 gene that modulates its transcription has been investigated in relation to PAD, but no significant association was found (56).

**Inflammation.** The vascular inflammatory process is pivotal in the pathophysiology of PAD, and it can be initiated through various pathways. However, the genes that have been investigated in PAD to date can be classified into 3 distinct pathways: 1) the cytokine-cytokine receptor interaction pathway (20, 22, 26, 27, 50, 55); 2) the leukocyte transendothelial migration pathway (25, 34, 50, 51); and 3) the peroxisome proliferator-activated receptor $\gamma$ signaling pathway (19, 50).

Cytokine-cytokine receptor interaction pathway. Interleukin-6 is a multifunctional cytokine produced by several cell types, including fibroblasts, monocytes, adipocytes, and endothelial cells. Four studies (20, 22, 50, 55) have evaluated the IL6-174G/C polymorphism for its potential role in PAD. The 4 studies produced contradictory results, since significant associations were found in 2 (22, 50). When results from the 4 studies were synthesized, significant heterogeneity was found among them for the allele contrast ($I^2 = 73\%$, $P_Q = 0.01$), and the association was not significant (OR = 1.44, 95% CI: 0.97, 2.13). However, in sensitivity analysis (excluding the studies by Daniellson et al. (20) and Potaczek et al. (55), where the controls violated Hardy-Weinberg equilibrium), significant associations were found for the dominant, recessive, and allele-contrast models; the odds ratios were 2.79 (95% CI: 1.80, 4.32), 2.04

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respectively. The Harbord et al. (16) test indicated that there was no differential magnitude of effect in large studies versus small studies for each polymorphism being investigated (\(P = 0.35\)).

The CX3CR1 gene encodes fractalkine receptor, which is a leukocyte chemotactic/adhesion receptor. Gugl et al. (27) investigated 2 CX3CR1 polymorphisms (\(837\) G/A, \(931\) C/T), but the reported findings provide no evidence that CX3CR1 plays a role in the development of PAD.

The CCR5 gene encodes a member of the \(\beta\) chemokine receptor family. A common 32-base-pair deletion mutation in the CCR5 gene was evaluated in 1 study (26), with no association being observed.

Chemokine (C-C motif) ligand 2 is an important mediator of monocyte recruitment into the vascular wall. Recently, a functional polymorphism in the CCL2 gene (\(-2518\) A/G) was described in 1 article (50) as a candidate for increasing the risk of PAD, providing significant results for the GG genotype and the G allele.

The CYBA subunit of cytochrome \(b\) is encoded by the CYBA gene and is a primary component of nicotinamide adenine dinucleotide/nicotinamide adenine dinucleotide phosphate

### Table 1. Random-Effects Odds Ratios for Peripheral Arterial Disease and Heterogeneity Test Results for the Minor Allele of Several Gene Polymorphisms in Relation to Peripheral Arterial Disease

<table>
<thead>
<tr>
<th>Gene (Polymorphism) and Model</th>
<th>Population</th>
<th>No. of Studies</th>
<th>Odds Ratio</th>
<th>95% Confidence Interval</th>
<th>(I^2,%)</th>
<th>(P) Value From (Q) Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>(F5) (1691 G/A)</td>
<td>Allele contrast: G vs. A</td>
<td>Whites</td>
<td>4</td>
<td>1.16</td>
<td>0.77, 1.75</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>Recessive model</td>
<td>Whites</td>
<td>4</td>
<td>1.19</td>
<td>0.78, 1.81</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>Dominant model</td>
<td>Whites</td>
<td>4</td>
<td>NA(^a)</td>
<td>NA(^a)</td>
<td>NA</td>
</tr>
<tr>
<td>(F2) (20210 G/A)</td>
<td>Allele contrast: A vs. G</td>
<td>Whites</td>
<td>4</td>
<td>1.80</td>
<td>0.76, 4.27</td>
<td>72</td>
</tr>
<tr>
<td></td>
<td>Recessive model</td>
<td>Whites</td>
<td>4</td>
<td>1.71</td>
<td>0.27, 10.88</td>
<td>0</td>
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<tr>
<td></td>
<td>Dominant model</td>
<td>Whites</td>
<td>4</td>
<td>1.77</td>
<td>0.75, 4.17</td>
<td>71</td>
</tr>
<tr>
<td>(MTHFR) (677C/T)</td>
<td>Allele contrast: T vs. C</td>
<td>All subjects</td>
<td>6</td>
<td>1.17</td>
<td>0.92, 1.49</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>All subjects in HWE</td>
<td>All subjects</td>
<td>5</td>
<td>1.26</td>
<td>1.00, 1.60</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td>Whites</td>
<td>Whites</td>
<td>5</td>
<td>1.09</td>
<td>0.89, 1.34</td>
<td>60</td>
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<td></td>
<td>Recessive model</td>
<td>All subjects</td>
<td>7</td>
<td>1.38</td>
<td>1.01, 1.88</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>All subjects in HWE</td>
<td>All subjects</td>
<td>6</td>
<td>1.42</td>
<td>0.99, 2.03</td>
<td>33</td>
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<td>Whites</td>
<td>Whites</td>
<td>6</td>
<td>1.35</td>
<td>1.00, 1.81</td>
<td>18</td>
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<td></td>
<td>Dominant model</td>
<td>All subjects</td>
<td>6</td>
<td>1.20</td>
<td>0.85, 1.70</td>
<td>73</td>
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<td>All subjects in HWE</td>
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<td>1.35</td>
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<td>Whites</td>
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<td>1.08</td>
<td>0.79, 1.46</td>
<td>67</td>
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<td>(ITGB3) (1565 T/C)</td>
<td>Allele contrast: T vs. C</td>
<td>Whites</td>
<td>2</td>
<td>1.37</td>
<td>0.84, 2.22</td>
<td>NA</td>
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<tr>
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<td>Recessive model</td>
<td>Whites</td>
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<td>1.43</td>
<td>0.88, 2.31</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>Dominant model</td>
<td>Whites</td>
<td>2</td>
<td>0.91</td>
<td>0.37, 2.22</td>
<td>NA</td>
</tr>
<tr>
<td>(ACE) I/D</td>
<td>Allele contrast: D vs. I</td>
<td>All subjects</td>
<td>6</td>
<td>0.94</td>
<td>0.84, 1.04</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>All subjects in HWE</td>
<td>All subjects</td>
<td>5</td>
<td>0.93</td>
<td>0.82, 1.06</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>Whites</td>
<td>Whites</td>
<td>4</td>
<td>1.00</td>
<td>0.88, 1.13</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Recessive model</td>
<td>All subjects</td>
<td>6</td>
<td>0.89</td>
<td>0.72, 1.09</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>All subjects in HWE</td>
<td>All subjects</td>
<td>5</td>
<td>0.89</td>
<td>0.70, 1.14</td>
<td>45</td>
</tr>
<tr>
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<td>Whites</td>
<td>Whites</td>
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<td>1.00</td>
<td>0.80, 1.23</td>
<td>16</td>
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<tr>
<td></td>
<td>Dominant model</td>
<td>All subjects</td>
<td>6</td>
<td>0.92</td>
<td>0.77, 1.10</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>All subjects in HWE</td>
<td>All subjects</td>
<td>5</td>
<td>0.90</td>
<td>0.74, 1.10</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Whites</td>
<td>Whites</td>
<td>4</td>
<td>0.97</td>
<td>0.78, 1.20</td>
<td>0</td>
</tr>
</tbody>
</table>

Table continues

(95% CI: 1.22, 3.39), and 1.93 (95% CI: 1.47, 2.52), respectively. The Harbord et al. (16) test indicated that there was no differential magnitude of effect in large studies versus small studies for each polymorphism being investigated (\(P = 0.35\)).

The CX3CR1 gene encodes fractalkine receptor, which is a leukocyte chemotactic/adhesion receptor. Gugl et al. (27) investigated 2 CX3CR1 polymorphisms (\(837\) G/A, \(931\) C/T), but the reported findings provide no evidence that CX3CR1 plays a role in the development of PAD.

The CCR5 gene encodes a member of the \(\beta\) chemokine receptor family. A common 32-base-pair deletion mutation in the CCR5 gene was evaluated in 1 study (26), with no association being observed.

Chemokine (C-C motif) ligand 2 is an important mediator of monocyte recruitment into the vascular wall. Recently, a functional polymorphism in the CCL2 gene (\(-2518\) A/G) was described in 1 article (50) as a candidate for increasing the risk of PAD, providing significant results for the GG genotype and the G allele.

Leukocyte transendothelial migration pathway. The \(\alpha\) subunit of cytochrome \(b\) is encoded by the CYBA gene and is a primary component of nicotinamide adenine dinucleotide/nicotinamide adenine dinucleotide phosphate

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oxidase. The influence of a functional polymorphic variant of the CYBA gene (242 C/T) on the predisposition to develop PAD has been assessed in 1 study (34), but no association was found.

Intercellular adhesion molecule 1 is a member of the cytokine-inducible immunoglobulin gene superfamily and binds leukocyte integrins. A nonsynonymous functional SNP in the ICAM1 gene, designated Lys469Glu, was genotyped in 2 studies (25, 50). The Lys469Glu SNP emerged as a potential risk factor for PAD; significant results under the allele-contrast and recessive models were reported in both studies. Meta-analysis of results from the 2 studies showed a lack of heterogeneity for the allele-contrast model ($P_Q = 0.71$) and a positive association (OR = 1.76, 95% CI: 1.39, 2.22). There were also significant associations under the dominant and recessive models, the respective odds ratios being 1.84 (95% CI: 1.25, 2.72) and 2.71 (95% CI: 1.81, 4.05).

E-selectin is expressed by cytokine-stimulated endothelial cells and mediates the adhesion of leukocytes to the vascular lining. To date, only 1 polymorphism in the SELE gene has been studied in relation to PAD (561 A/C), with positive associations being demonstrated for the recessive and allele-contrast models (50).

**Table 1. Continued**

<table>
<thead>
<tr>
<th>Gene (Polymorphism) and Model</th>
<th>Population</th>
<th>No. of Studies</th>
<th>Odds Ratio</th>
<th>95% Confidence Interval</th>
<th>$I^2,%$</th>
<th>$P$ Value From $Q$ Test</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AGT (704 C/T)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allele contrast: T vs. C All subjects</td>
<td>2</td>
<td>0.99</td>
<td>0.83, 1.20</td>
<td>NA</td>
<td>0.83</td>
<td></td>
</tr>
<tr>
<td>Recessive model All subjects</td>
<td>2</td>
<td>0.92</td>
<td>0.71, 1.18</td>
<td>NA</td>
<td>0.71</td>
<td></td>
</tr>
<tr>
<td>Dominant model All subjects</td>
<td>2</td>
<td>1.50</td>
<td>0.50, 4.47</td>
<td>NA</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td><strong>AGT (-6 G/A)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allele contrast: A vs. G All subjects</td>
<td>2</td>
<td>1.01</td>
<td>0.84, 1.22</td>
<td>NA</td>
<td>0.85</td>
<td></td>
</tr>
<tr>
<td>Recessive model All subjects</td>
<td>2</td>
<td>0.93</td>
<td>0.72, 1.20</td>
<td>NA</td>
<td>0.93</td>
<td></td>
</tr>
<tr>
<td>Dominant model All subjects</td>
<td>2</td>
<td>1.55</td>
<td>0.53, 4.53</td>
<td>NA</td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td><strong>AGT (733 C/T)</strong></td>
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<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Allele contrast: T vs. C All subjects</td>
<td>2</td>
<td>1.00</td>
<td>0.76, 1.33</td>
<td>NA</td>
<td>0.84</td>
<td></td>
</tr>
<tr>
<td>Recessive model All subjects</td>
<td>2</td>
<td>1.61</td>
<td>0.55, 4.76</td>
<td>NA</td>
<td>0.29</td>
<td></td>
</tr>
<tr>
<td>Dominant model All subjects</td>
<td>2</td>
<td>0.97</td>
<td>0.71, 1.32</td>
<td>NA</td>
<td>0.54</td>
<td></td>
</tr>
<tr>
<td><strong>IL6 (174 G/C)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allele contrast: G vs. C Whites</td>
<td>4</td>
<td>1.44</td>
<td>0.97, 2.13</td>
<td>73</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Whites in HWE</td>
<td>2</td>
<td>1.93</td>
<td>1.47, 2.52</td>
<td>NA</td>
<td>0.26</td>
<td></td>
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<tr>
<td>Recessive model Whites</td>
<td>4</td>
<td>1.39</td>
<td>0.80, 2.39</td>
<td>72</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Whites in HWE</td>
<td>2</td>
<td>2.04</td>
<td>1.22, 3.39</td>
<td>NA</td>
<td>0.15</td>
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<tr>
<td>Dominant model Whites</td>
<td>4</td>
<td>1.86</td>
<td>1.06, 3.27</td>
<td>61</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>Whites in HWE</td>
<td>2</td>
<td>2.79</td>
<td>1.80, 4.32</td>
<td>NA</td>
<td>0.99</td>
<td></td>
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<tr>
<td><strong>ICAM1 (1462A/G)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allele contrast: G vs. A Whites</td>
<td>2</td>
<td>1.76</td>
<td>1.39, 2.22</td>
<td>NA</td>
<td>0.71</td>
<td></td>
</tr>
<tr>
<td>Recessive model Whites</td>
<td>2</td>
<td>2.71</td>
<td>1.81, 4.05</td>
<td>NA</td>
<td>0.43</td>
<td></td>
</tr>
<tr>
<td>Dominant model Whites</td>
<td>2</td>
<td>1.84</td>
<td>1.25, 2.72</td>
<td>NA</td>
<td>0.98</td>
<td></td>
</tr>
<tr>
<td><strong>MMP9 (-1562 C/T)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allele contrast: T vs. C Whites</td>
<td>2</td>
<td>0.99</td>
<td>0.42, 2.33</td>
<td>NA</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>Recessive model Whites</td>
<td>2</td>
<td>0.64</td>
<td>0.01, 80.17</td>
<td>NA</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>Dominant model Whites</td>
<td>2</td>
<td>1.00</td>
<td>0.52, 1.92</td>
<td>NA</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td><strong>CHRNA3 (831C/T)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allele contrast: T vs. C Whites</td>
<td>5</td>
<td>1.19</td>
<td>1.12, 1.27</td>
<td>0</td>
<td>0.79</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: ACE, angiotensin-converting enzyme; AGT, angiotensinogen; CHRNA3, cholinergic receptor, nicotinic, α3; F2, factor II; F5, factor V; HWE, Hardy-Weinberg equilibrium; ICAM1, intercellular adhesion molecule 1; IL6, interleukin-6; ITGB3, integrin, β3; MMP9, matrix metalloproteinase 9; MTHFR, methylenetetrahydrofolate reductase; NA, not applicable.

* Odds ratios and 95% confidence intervals could not be calculated because no subjects were homozygous for allele A.
The enzyme encoded by the matrix metalloproteinase 9 gene degrades type IV and V collagens and has been implicated in the pathogenesis of atherosclerosis. As of yet, 2 studies (50, 51) have explored associations between a functional polymorphism in the MMP9 gene (-1562 C/T) and PAD, with a positive association being observed in both. However, when results from these 2 studies were synthesized, no evidence of association was observed.

Peroxisome proliferator-activated receptor γ signaling pathway. The protein encoded by the PPARG gene is a regulator of adipocyte differentiation. Recently, PPARG was reported as a candidate gene for PAD (19), since a putative functional SNP in the PPARG gene (Pro12Ala) demonstrated significant associations under the allele-contrast and dominant models.

Matrix metalloproteinase 1 appears to play a significant role in atherosclerotic plaque disruption by contributing to the degradation of interstitial collagens. In 1 study (50), a common insertion polymorphism in the MMP1 gene promoter (-16071G/2G) was investigated in relation to PAD and showed a positive association for all of the genetic models examined.

Other genes. The genes in the “other” category could not be assigned to any of the 3 main groups (i.e., thrombophilia, hemodynamics, and inflammation). Therefore, we did not classify them further by means of the KEGG database, although the evidence for these genes was assessed in a consistent way and their associations with PAD are depicted in detail in Web Table 1. In brief, 9 genetic variants that had been investigated in only 1 study each (SCARB1 55C/T, SCARB1 1050 C/T, MTPP -493 G/T, LPA 93 C/T, ADD1 1566 G/T, P2RY12 H1/H2, LIPC -250 G/A, PLA2G7 994 G/T, MMP3 -1171 6A/5A) showed significant associations with PAD risk (18, 23, 30, 39, 41, 49, 50, 58). Moreover, a polymorphism in the CHRNA3 gene (831C/T) was evaluated in 5 distinct Caucasian populations, with significant results being reported in 2 (47). When results from the 5 studies of the CHRNA3 (831C/T) variant were synthesized, the association for the allele contrast was significant (OR = 1.19, 95% CI: 1.12, 1.27). The Harbord et al. (16) test indicated that there was no differential magnitude of effect in large studies versus small studies, we employed a recently published test by Harbord et al. (16) rather than the test of Egger et al. (75), since it is powerful when the number of studies is relatively small. Most of the studies we analyzed included small numbers of cases and controls and consequently did not have adequate statistical power to detect a modest genetic effect. Apart from the need for larger sample sizes, selecting cases that are genetically loaded may also improve power. Through selection of cases with early-onset disease and a strong family history, cases will be weighted toward those persons whose disease has a strong genetic etiology (76).

Stratification in genetic association studies might blur the genetic effect. The possibility cannot be excluded that some of the results were biased because of undetected population stratification in the original case-control samples (10, 77). The role of gene polymorphisms in the field of PAD has been mainly examined in Caucasian populations. Given the lack of concordance between risks of PAD in white, South Asian, and black ethnic groups (78), it would appear likely that unequal genetic admixture in the control and patient populations can result in spurious associations (10, 77). Sampling variability in the case-control study design can be a possible confounding factor in assessing the role of genetic markers. Application of strict selection criteria would ensure clear case and control definitions for meta-analysis, since the possibility of a case’s being considered a control is minimized and the estimation of risk is unbiased. The cases in this review were not defined with similar inclusion criteria, and they covered a wide spectrum of disease in terms of classification and diagnostic methods.

In the studies with controls that did not conform to Hardy-Weinberg equilibrium, the lack of Hardy-Weinberg equilibrium indicates genotyping errors, population stratification, and selection bias. Additionally, deviation from Hardy-Weinberg equilibrium in a population implies continued selection, migration, and mutation and the absence of random mating (17). Hence, the validity of the genotyping method and the selection of controls are questionable (17, 79). Moreover, the absence of reporting of blinding to phenotype among genotyping laboratory personnel in 36
articles and the possible lack of a controlled genotyping procedure in 28 papers could have caused potential bias.

As with other complex diseases, the development of PAD is probably determined by several genes that act collectively, and allelic variants in different genes may have either additive or contrasting effects (62). Illuminating the pathogenesis of the disorder would demand investigation of association for many variants of genes that participate in distinct pathophysiologic pathways. In addition, there are several possible interactions between gene polymorphisms and environmental modifiers, such as age, sex, smoking, diabetes, hypertension, and hyperlipidemia, which have been implicated in the alteration of the natural history of PAD (72). PAD is more common in older populations, which means that recruiting the large numbers of affected sibling pairs or family trios needed for genome-wide scans and family-based association studies might be problematic. Thus, elucidating the genetics of PAD largely relies upon designing and undertaking rigorous genetic candidate-gene association studies or genome-wide association studies in unrelated subjects. Genome-wide association studies not only appear to be a powerful new tool for identifying genes influencing common diseases but also represent a valuable instrument for examining genomic function and clarifying pathophysiologic mechanisms (80). In the field of PAD, only ankle-brachial index has been investigated in a genome-wide association study (81), although no association results meeting criteria for genome-wide significance were reported.

Long-term prospective and case-control studies (82, 83) designed to incorporate gene-gene and gene-environment interactions and utilizing the vast amount of data generated by genomic studies might produce more conclusive claims about the genetics of PAD (10). In addition, future studies should be planned with the prospect of their results’ being combined with those of other, similar studies in a meta-analysis. The potential benefits offered by a meta-analysis are the enhancement of power; the ability to place each study in the context of all others, particularly early spurious results; and the possibility of examining why investigators in different studies reach different conclusions (10). Nevertheless, each result must be interpreted with caution and considered preliminary, until the putative disease-modifying effects have been confirmed in sufficiently powered analyses and until plausible molecular mechanisms are documented for the observed statistical associations (10).

In conclusion, in this evaluation of possible genetic susceptibility to PAD, individual studies and meta-analyses provided no supportive evidence of a strong association. Nevertheless, we identified 3 gene variants (IL6-174 G/C, ICAM1 1462A/G, and CHRNA3 831C/T) that appear to be particularly promising and warrant follow-up. By utilizing the linkage disequilibrium data from the HapMap Project, researchers could investigate these polymorphisms in the context of disease-associated haplotypes, to obtain further insights about the role of genetic variation in these genes. Moreover, interactions with other candidate genes involved in inflammatory pathways or with environmental factors should be investigated. Given that PAD is a complex disease with multifactorial etiology, a minor contributing pathogenic role of the investigated gene polymorphisms in specific cases and in cooperation with other factors cannot be excluded.

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