The most common cause of peripheral arterial disease (PAD) is atherosclerotic vascular disease. PAD because of atherosclerosis is relatively highly prevalent, affecting >200 million people worldwide, including an estimated 8 to 10 million individuals in the United States alone. Atherosclerotic PAD is typically identified in the clinical setting when patients present with claudication or critical limb ischemia. PAD may also be ascertained on the basis of an abnormal ankle brachial index (ABI) in patients referred to the noninvasive vascular laboratory or based on lower extremity imaging studies (Table 1). PAD is associated with considerable morbidity, including impaired functional capacity, frailty, poor quality of life, and high medical care costs. The Institute of Medicine has listed PAD as a high priority research area to reduce mortality and morbidity from this condition.

**Abstract:** Several risk factors for atherosclerotic peripheral arterial disease (PAD), such as dyslipidemia, diabetes mellitus, and hypertension, are heritable. However, predisposition to PAD may be influenced by genetic variants acting independently of these risk factors. Identification of such genetic variants will provide insights into underlying pathophysiologic mechanisms and facilitate the development of novel diagnostic and therapeutic approaches. In contrast to coronary heart disease, relatively few genetic variants that influence susceptibility to PAD have been discovered. This may be, in part, because of greater clinical and genetic heterogeneity in PAD. In this review, we (1) provide an update on the current state of knowledge about the genetic basis of PAD, including results of family studies and candidate gene, linkage as well as genome-wide association studies; (2) highlight the challenges in investigating the genetic basis of PAD and possible strategies to overcome these challenges; and (3) discuss the potential of genome sequencing, RNA sequencing, differential gene expression, epigenetic profiling, and systems biology in increasing our understanding of the molecular genetics of PAD. 

**Key Words:** epigenomics ■ genome-wide association study ■ molecular epidemiology ■ peripheral arterial disease
PAD is a distinct subtype of atherosclerotic vascular disease that differs from coronary artery and cerebrovascular disease in its clinical presentation. The phenomenon of plaque instability in the coronary or cerebral arterial beds leads to acute events, such as myocardial infarction or ischemic stroke. For reasons that are not fully understood, such acute events are relatively uncommon in PAD and symptoms most often result from progressive arterial narrowing because of ongoing atherogenesis. It is, therefore, likely that risk factors, both genetic and environmental, and the intermediate biochemical pathways through which they act, contribute differently to PAD than to coronary heart disease or cerebrovascular disease.

Several risk factors for PAD, such as dyslipidemia, diabetes mellitus, and hypertension, are heritable. However, predisposition to PAD may be influenced by genetic factors acting independently of these risk factors. Identification of such genetic factors will provide insights into underlying pathophysiologic mechanisms and facilitate the development of novel diagnostic and therapeutic approaches.12,13 In contrast to coronary heart disease (CHD),14 relatively few genetic variants that influence susceptibility to PAD have been discovered. This may be, in part, because of greater clinical and genetic heterogeneity in PAD.12 In this review, we provide an update on the current state of knowledge about the genetic basis of atherosclerotic PAD and discuss challenges and future directions.

The study of the genetic basis of nonatherosclerotic forms of PAD, such as vasculitides and fibromuscular dysplasia may provide insights into the pathogenesis of atherosclerotic PAD, given shared features, such as inflammation, vascular remodeling, aneurysm formation, and smooth muscle cell proliferation (Figure 1). A detailed discussion of the genetic basis of nonatherosclerotic forms of PAD, however, is beyond the scope of this review.

### Current Knowledge

In this section, we provide an update on the current state of knowledge about the genetic basis of PAD, including ethnic differences in the prevalence of PAD, familial clustering, early onset PAD and results of candidate gene, linkage, as well as genome-wide association studies (GWASs).

### Ethnic Differences in PAD

Ethnic differences in disease prevalence may be, in part, because of genetic factors, as well as differences in socioeconomic status and access to care.15 In several population-based studies, black ethnicity has been associated with a lower ABI and higher prevalence of PAD in both men and women, independent of age and other conventional risk factors.15–18 Moreover, in nonwhite adults (predominantly blacks) symptomatic PAD was associated with worse clinical outcomes than in non-Hispanic white adults.19 In the Bogalusa Heart Study,20 during adolescence and early adulthood, blacks had 1.5x as much aortic surface involvement of fatty streaks as did non-Hispanic whites, independent of antemortem lipid levels, blood pressure, or obesity. Analysis of data from National Health and Nutrition Examination Survey revealed that ABI is lower in blacks than in non-Hispanic whites even among younger individuals without cardiovascular risk factors, raising the possibility that ethnic differences in ABI may not be because of differences in atherosclerotic burden.21

### Family History

Family history is a simple and inexpensive yet powerful clinical tool for improving risk assessment and, thereby reducing the burden of common chronic diseases.22,23 The American College of Cardiology/American Heart Association guidelines recommend screening for abdominal aortic aneurysm (AAA) in patients with family history of AAA.24 Given that screening for PAD is relatively inexpensive and noninvasive, similar screening in asymptomatic patients with family history of PAD may be useful for early detection.
detection and treatment. However, relatively few studies (discussed below) have assessed whether family history of PAD is a risk factor for PAD.

In the population-based Swedish Twin Registry, the odds ratio (OR) of having PAD in individuals whose twin had PAD compared with individuals whose twin did not have PAD was 17.7 (95% confidence interval, 11.7–26.6) for monozygotic twins and 5.7 (95% confidence interval, 4.1–7.9) for dizygotic twins (Figure 2). In the San Diego Population study, any family history of PAD or parental history of PAD was only marginally associated with presence of PAD and family history of cardiovascular disease was not associated with presence of PAD. This study was likely underpowered to detect significant associations as only 87 patients with PAD were included. In a study that elicited detailed family history in 2296 PAD cases and 4390 controls, prevalence of family history of PAD was significantly higher in patients with PAD than in controls (10.4% versus 5.0%; \( P < 0.0001 \) resulting in a doubling of the odds of the presence of PAD in those with family history of PAD (Figure 2). The association of family history of PAD with prevalent PAD was only modestly attenuated after adjustment for conventional risk factors: OR of 1.97 (1.60–2.42). The association was stronger in individuals <68 years of age and in those with greater number of affected relatives. These results suggest that shared environmental and genetic factors are associated with PAD and motivate the search for genetic susceptibility variants.

**Early Onset PAD**

In the Western world, atherosclerosis is the major cause of occlusive disease of the lower extremities in young adults. Genetic factors probably have an important role in premature PAD, including those acting through pathways of thrombosis, inflammation, and lipid and homocysteine metabolism. Men and women seem to be equally affected, in contrast to early onset CHD where men are more commonly affected. Similar to CHD, several Mendelian disorders are associated with PAD. These include familial lipoprotein disorders, such as chylomicronemia as a result of mutations in the lipoprotein lipase gene and familial hypercholesterolaemia, hyperhomocysteinemia, and pseudoxanthoma elasticum.

**Linkage Studies**

Linkage analyses for complex diseases have the potential to identify new disease susceptibility genes that may have been unsuspected based on a priori knowledge of disease mechanisms. However, such an approach has been largely unsuccessful in identifying specific disease susceptibility variants. Gudmundsson et al performed a 10-cM genome-wide scan in 272 patients from 116 extended families who had undergone angiography or revascularization procedures for symptomatic PAD. Significant linkage to a region on chromosome 1 between 100 and 110 cM was found (logarithm of the odds [LOD] score, 3.93; \( P = 1.04 \times 10^{-5} \)). Several candidate genes (in pathways of inflammation, coagulation, lipid metabolism, blood pressure regulation, and vascular matrix regulation) for atherosclerosis were present under the linkage signals but the causal variants could not be identified. Linkage analyses for ABI as a continuous trait did not reveal any regions of LOD scores \( \geq 3 \), although several regions with tentative evidence of linkage (multipoint LOD, 1.3–2.0) were detected.

**Candidate Gene Association Studies**

In contrast to hundreds of candidate gene association studies for CHD, relatively few have been reported for PAD.
The candidate genes studied include β-fibrinogen,30 apolipoprotein B,30 endothelial nitric oxide synthase (eNOS),41,42 methylene tetrahydrofolate reductase,41 G-protein β unit 3 and α-adducin,43 interleukin-6,44 and glutathione S-transferase.45 However, any reported associations between variants in these genes and PAD have not been confirmed in independent cohorts or in GWAS. Kardia et al46 investigated the association of 435 single nucleotide polymorphisms (SNPs) in 112 positional and biological candidate genes with the ABI in 1046 non-Hispanic whites belonging to hypertensive sibships. The contributions of each SNP, as well as SNP-covariate and SNP–SNP interactions, to the overall genetic architecture of ABI were assessed. Significant associations were corrected for multiple testing and replicated by 4-fold cross-validation. The following associations were significant, replicated, and cross-validated: 2 SNP main effects in NOS3, 3 SNP–covariate interactions (ADRB2 Gly 16—lipoprotein (a) and SLC4A5—diabetes mellitus interactions), and 25 SNP–SNP interactions (involving SNPs from 29 different genes). The Candidate Gene Association Resource (CARe) consortium performed a meta-analysis of ≈50000 SNPs in ≈2000 cardiovascular candidate genes, but was unable to confidently identify any variants associated with the ABI.47

**Genome-Wide Association Studies**

The GWAS approach, made possible by knowledge of linkage disequilibrium across the genome as well as the availability of high-density genotyping platforms, is unbiased in nature and has the potential to discover novel disease susceptibility genes. Although multiple genetic loci have been associated with inflammatory forms of arterial disease (Table 2), fewer loci with weaker associations have been implicated in atherosclerotic PAD (Table 3). Helgadottir et al48 found that the 9p21 locus was associated not only with CHD but also with PAD, AAA and intracranial aneurysm. Thorgeirsson et al49 found a synonymous SNP (rs1051730) within the cholesterol receptor nicotinic α 3 gene (CHRNA3) on chromosome 15q24 to affect nicotine dependence, smoking quantity, and the risk of PAD and lung cancer. In a GWAS for AAA, the variant DAB12 was identified as being associated with both AAA and PAD.50 Koriyama et al51 found the OSBPL10 locus to be associated with PAD in a Japanese cohort. In a meta-analysis52 of 21 population-based cohort studies that included 41692 participants of European ancestry among whom 3409 participants had PAD (defined as an ABI <0.90), 6 SNPs were associated (P≤1×10−6) with PAD, but none was significant at a genome-wide significance level. The top SNP associated with PAD was near the PAX gene. In this study, however, a variant at the 9p21 locus was associated with ABI (as a continuous variable) at the genome-wide significance level. Potential mechanisms, by which this locus is thought to promote atherosclerosis include cell proliferation, inflammation, and impaired effectorcytosis (phagocytic clearance) of apoptotic debris in atherosclerotic plaque.53–55

In a recent electronic medical record–based GWAS of PAD, the allele C of the intronic SNP rs653718 at the ATXN2-SH2B3 locus on chromosome 12 was present more frequently in PAD cases (52%) than in controls (47%) with a resulting OR of 1.23 (P=5.6×10−6) in the discovery cohort.56 In the replication cohort of 740 PAD cases and 1051 controls, the OR was 1.25 (P=8.9×10−6) and in the combined sample, the OR was 1.22 (P=6.5×10−5). The strength of association of previous GWAS hits was tested, but neither the 9p21 variant nor the OSBPL10 variants were associated, whereas the CHRNA3 variant was weakly (P=0.001) associated with PAD. The lead SNP rs653718 is in strong linkage disequilibrium (r2=0.99) with a missense SNP (rs3184504) in SH2B3, an adapter protein that plays a key role in immune and inflammatory response pathways and vascular homeostasis.66,67 The SNP results in substitution of tryptophan (aromatic side chain) by arginine (basic side chain) that may result in altered lipid binding and protein–protein interactions. The SNP may have been protective against bacterial infection in the past allowing it to rise to a relatively high frequency because of natural selection.66–70

**Challenges**

To date, the search for genetic susceptibility variants for PAD has been less successful than for CHD, probably because of multiple reasons, including a potentially stronger environmental contribution to PAD, for example from smoking. Additional challenges in investigating the genetic basis of PAD are summarized in Table 4. Given the results of GWAS to date, it is clear that large numbers of PAD cases and controls are needed to identify genetic susceptibility variants. Investigators will need to collaborate to conduct meta-analyses of GWAS for PAD, similar to those for CHD. Another option is to leverage repositories of DNA linked to electronic medical record systems to conduct genotyping or sequencing studies. Such an approach71 can reduce the time, effort, and cost involved in conducting genomic association studies. The Electronic Medical Records and Genomics (eMERGE) consortium72 is leveraging biorepositories linked to the electronic medical record for large-scale genomic research.73

Phenotypic heterogeneity seems to be a major challenge in investigating the genetic basis for PAD. PAD is complex and heterogeneous and not a uniform entity. Two broad subtypes of PAD, proximal and distal, are associated with distinct risk factor and comorbidity profiles.74 Female sex, smoking,
hypertension, and dyslipidemia are more significantly associated with proximal disease, whereas older age, male sex, and diabetes mellitus are more significantly associated with distal disease. Subtyping of PAD based on location is possible using noninvasive arterial Doppler; an alternative is to stratify patients based on whether they have diabetes mellitus because diabetic PAD is often distal.

**Future Directions**

The field of complex disease genetics has advanced considerably in the last several years, primarily because of assembly of large case–control cohorts and availability of newer genomic technologies. In this section, we highlight how these advances might be leveraged to increase our understanding of the genetic basis of PAD and where possible give examples of early illustrative studies.

**Gene–Environment and Gene–Gene Interactions**

Smoking is the major environmental risk factor for PAD but variability in the susceptibility of smokers to PAD suggests that novel genetic factors may interact with smoking to influence the development and progression of PAD. As PAD results from alterations in multiple atherogenic pathways, large single gene effects are unlikely, multiple loci are probably involved and candidate genes may express themselves only through interaction with other genes or with at-risk lifestyles. Identifying the combinations of multifocal genotypes predictive of disease (epistasis) is a daunting task. Several statistical methods have been proposed to assess gene–gene and gene–environment interactions but few such interactions have been identified or replicated.

**Whole Genome/Exome Sequencing**

Genome/exome sequencing may help in identifying causal mutations when PAD clusters in families. Exome sequencing in members of 3 families with symptomatic PAD and arterial and joint calcifications implicated mutations in NT5E, a gene encoding a protein that converts adenosine monophosphate to adenosine. Adenosine inhibits ectopic tissue calcification and adenosine treatment of fibroblasts from an affected patient reduced the levels of alkaline phosphatase and calcification. In another study, exome sequencing helped to identify the underlying mutation in a family where 2 siblings had aortic hypoplasia, diffuse atherosclerosis, and PAD. The 2 siblings were homozygous for a nonsynonymous mutation in IN080D, which leads to disruption in the function of 1 of the domains of the protein. IN080D encodes a protein that converts adenosine monophosphate to adenosine, a gene

**Differential Gene Expression**

Arterial tissue is difficult to obtain and circulating peripheral blood mononuclear cells have been proposed as reporters of arterial wall pathology. Several investigators have examined differentially regulated genes in peripheral blood mononuclear cells to identify relevant molecular mechanisms for vascular diseases. Masud et al found genes influencing immune response, inflammation, apoptosis, and various signaling pathways to be differentially expressed in peripheral blood mononuclear cells from PAD cases and controls. RNA sequencing has emerged as a tool for investigating known and novel transcripts affecting disease mechanisms and...
progression. In addition to differential expression and differential splicing, it offers researchers the ability to gain greater insight into changes in gene expression during disease initiation, progression, and response to treatment.

**Pleiotropic Genetic Effects**

Several lines of evidence suggest shared genetic susceptibility variants between subtypes of ASCVD. Valiente et al reported that premature CHD, PAD, and stroke was more common in parents and siblings of individuals with premature PAD or CHD compared with controls, suggesting the presence of shared genetic factors across these subtypes of ASCVD. In the study by Khaleghi et al, family history of CHD was also associated with presence of PAD, suggesting the presence of genetic susceptibility variants shared between PAD and CHD. Several GWAS have reported variants, for example, 9p21 and DAB2IP, that are associated with >1 subtype of atherosclerotic vascular disease. Gretarsdottir et al reported that the A allele of rs7025486 within DAB2IP, which encodes an inhibitor of cell growth and survival, was associated with AAA, with an OR of 1.21 and \( P = 4.6 \times 10^{-10} \). In tests for association with other vascular diseases, the investigators found this allele to be associated with early onset myocardial infarction (OR, 1.18; \( P = 3.1 \times 10^{-4} \)) and PAD (OR, 1.14; \( P = 3.9 \times 10^{-4} \)). The SNP was not associated with risk factors, such as smoking, lipid levels, obesity, type 2 diabetes mellitus, and hypertension. Thus, variants found to be associated with CHD, AAA, and carotid artery disease should be tested for association with PAD.

The SNP rs3184504 at the ATXN2-SH2B3 locus identified as being associated with PAD is a particularly interesting example of pleiotropy. Not only has it been associated with myocardial infarction but also with immunologic disorders, hematologic traits, such as platelet count, mean-platelet volume, and eosinophil count and diabetes mellitus. The pleiotropic nature of SH2B3 may be because of its role in immune and inflammatory signaling pathways, including erythropoietin, cytokine receptor–mediated, and integrin signaling. The protein also regulates hematopoietic cell lineage and endothelial cells, and influences adhesion and migration of platelets by modulating actin cytoskeleton organization.

**Epigenetics**

Epigenetics is the study of factors that modify gene expression, exclusive of changes in the DNA sequence. Epigenetic factors include structural modifications to the DNA and its surrounding proteins which alter the accessibility of promoters to transcriptional machinery; as well as soluble factors, which interfere with mRNA transcription and translation. Classical epigenetic changes, such as chromatin remodeling, histone modification, and DNA methylation are of great interest because they not only can be long-lived and even inherited but also may be modifiable. Efforts to evaluate the methylome of individuals with PAD could prove insights into how environmental exposures or other risk factors regulate genes important for disease progression. For example, childhood smoking is associated with an increased risk of PAD even after controlling for lifetime tobacco exposure, raising the possibility that early smoking-related epigenetic changes may potentiate risk for disease decades later, as has been shown for other tobacco-related conditions. Availability of RNA sequencing, bisulfite sequencing, and ChIP technology is likely to shed more light into the role of the epigenome in atherosclerosis and PAD. The latter allows mapping of histone modifications across the genome, thereby providing insights into repressive/activating changes in the chromatin surrounding genes implicated in atherosclerotic vascular disease.

A growing family of noncoding RNAs is now recognized as another major epigenetic regulator of gene expression. MicroRNAs (miRs) are small (22 nucleotides) single-stranded RNAs that inhibit mRNA translation after binding to the 3’ untranslated region of a target gene. Because they do not require perfect base pairing to repress translation, each miR can regulate dozens or hundreds of genes. miRs regulate endothelial cell function and tube formation, SMC plasticity, lipid metabolism, and macrophage biology, as well as angiogenesis in experimental animals. In addition, miR-503 has been implicated as a putative regulator of diabetic PAD and limb ischemia human tissue samples. A panel of 12 miRs measured in the peripheral blood was recently correlated with the presence of PAD. Long noncoding RNAs guide chromatin modifiers to transcriptional promoters and are thought to regulate more than two thirds of all protein coding genes. Little is currently known about the role of long noncoding RNAs in PAD. The 9p21 locus, which is associated with several vascular disease phenotypes, harbors polymorphisms within an long noncoding RNA known as ANRIL (antisense noncoding RNA in the INK4 locus). ANRIL has been shown to recruit polycomb repressive complexes to the promoter of CDKN2B, and directly silence the expression of this atheroprotective and antianeurysmal gene. The association of 9p21 locus with atherosclerotic vascular disease could be mediated by this indirect epigenetic pathway involving ANRIL, through transregulation of CDKN2B.

**Rare Variant Association Studies**

Common genetic susceptibility variants do not fully explain the heritability of complex diseases and the extent to which rare variants contribute to disease susceptibility is not known. The common disease-rare variant concept has been illustrated by several reports, including the association of uncommon PCSK9 variants with CHD susceptibility and of rare CFH variants with macular degeneration. Association studies of rare variants in gene coding regions are a logical next step to complement genome-wide analysis of common variants. New genotyping arrays allow testing the association of rare (defined as minor allele frequency <1%) functional variants with traits of interest. Such an approach has been successful in identifying rare genetic variants associated with complex traits, such as insulin resistance.

**Network and Pathway Analyses**

Identifying disease susceptibility genes/variants by itself may not provide insights into the relevant pathophysiologic pathways. Genes often act in networks to influence susceptibility to complex diseases and such effects are unlikely to be identified by SNP-level analyses. Knowledge-based approaches, such as enrichment analysis, and network and pathway analysis may
provide insights into how genes and proteins interact to influence disease susceptibility. The Gene Ontology resource provides annotation of the biology and function of gene and protein sequences based on their homology across multiple organisms, using a common vocabulary. Kyoto Encyclopedia of Genes and Genomes (KEGG), a database of biological systems that integrates genomic, chemical, and systemic functional information, provides a link between genes and higher order processes, such as pathways. Reactome is a curated, peer-reviewed database of reactions and pathways that allows representation of intermediary metabolism, regulatory pathways, and signal transduction, and high-level processes, such as the cell cycle.

Microbiome

Periodontal disease has been associated with atherosclerosis, suggesting that bacteria from the oral cavity may contribute to the development of atherosclerosis and cardiovascular disease. Using quantitative polymerase chain reaction, Koren et al111 found bacterial DNA in atherosclerotic plaque and then used 454 pyrosequencing of 16S rRNA genes to demonstrate that several bacterial phylotypes were common to the atherosclerotic plaque and oral or gut samples of the same individual. Several recent studies suggest an association between gut-derived metabolites and angiographic coronary artery disease. Wang et al12 have shown that gut microbiome–derived metabolites choline, betaine, and trimethylamine-N-oxide are associated with the presence of coronary artery disease. Furthermore, higher levels of trimethylamine-N-oxide were associated with increased risk of adverse cardiovascular events over in patients referred for coronary angiography.113 These reports motivate additional studies in carefully phenotyped PAD cohorts to identify new pathophysiologic pathways associated with PAD, novel metabolomic markers of disease initiation and progression, and new targets for therapy, such as altering gut/dental flora by dietary intervention and probiotics.

Summary

In spite of relatively large sample sizes, GWAS for PAD have not been as successful as those for CHD. This is probably because susceptibility variants have modest effects, PAD is phenotypically heterogenous and genetic susceptibility variants differ across the subtypes of PAD. Genetic variants may also interact with risk factors, such as smoking and diabetes mellitus, in predisposing to PAD. Thus, much larger sample sizes are necessary to identify susceptibility variants with small effects sizes and genomic association analyses will need to be stratified based on the presence versus absence of risk factors, such as diabetes mellitus and smoking. Results of the genomic association studies, to date, confirm the presence of pleiotropy across the various forms of atherosclerotic vascular disease and variants found to be associated with CHD, AAA, and carotid artery disease should be tested for association with PAD. New directions include the use of whole genome/exome sequencing to uncover the genetic basis of rare forms of PAD, deploying new technologies, such as RNA sequencing to identify genes that are differentially expressed in the initiation and progression of PAD, investigating the role of rare variants and structural variation as susceptibility factors and the study of oral and gut microbiomes in the pathogenesis of PAD.

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