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The Genetic Basis of Peripheral Arterial Disease: Current Knowledge, Challenges and Future Directions

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Abstract

Several risk factors for atherosclerotic peripheral arterial disease (PAD) such as dyslipidemia, diabetes 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 due to greater clinical and genetic heterogeneity in PAD. In this review, we a) 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; b) highlight the challenges in investigating the genetic basis of PAD and possible strategies to overcome these challenges; and c) 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.

Keywords

genetic epidemiology; genome wide association studies; genome sequencing; epigenetics; peripheral arterial disease

Introduction

The most common cause of peripheral arterial disease (PAD) is atherosclerotic vascular disease. PAD due to atherosclerosis is relatively highly prevalent, affecting more than 200 million people worldwide[1] including an estimated 8-10 million persons in the United States alone.[2, 3] 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

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associated with considerable morbidity[4] including impaired functional capacity, frailty, poor quality of life, as well as high medical care costs.[5, 6] The Institute of Medicine has listed PAD as a high priority research area to reduce mortality and morbidity from this condition.[7]

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 due to 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 (CHD) or cerebrovascular disease.

Several risk factors for PAD (such as dyslipidemia, diabetes 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 CHD,[14] relatively few genetic variants that influence susceptibility to PAD have been discovered. This may be in part due to 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 non-atherosclerotic 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 **(Fig. 1).** A detailed discussion of the genetic basis of non-atherosclerotic 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 (GWAS).

Ethnic differences in PAD

Ethnic differences in disease prevalence may be in part due to genetic factors as well as differences in socioeconomic status and access to care.[15] In several population-based studies, African American 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 non-white adults (predominantly African Americans) 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, African Americans had approximately 1.5 times as much aortic surface involvement of fatty streaks as did non-Hispanic whites, independent of ante-mortem lipid levels, blood pressure, or

obesity. Analysis of data from National Health and Nutrition Examination Survey (NHANES) revealed that ABI is lower in African Americans than in non-Hispanic whites even among younger individuals without cardiovascular risk factors, raising the possibility that ethnic differences in ABI may not be due to 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 (ACC/AHA) guidelines recommend screening for abdominal aortic aneurysm in patients with family history of abdominal aortic aneurysm.[24] Given that screening for PAD is relatively inexpensive and non-invasive, similar screening in asymptomatic patients with family history of PAD may be useful for early detection and treatment. However, relatively few studies (discussed below) have assessed whether family history of PAD is a risk for factor for PAD.

In the population-based Swedish Twin Registry,[25] the odds ratio of having PAD in persons whose twin had PAD compared with persons whose twin did not have PAD was 17.7 (95% CI, 11.7 to 26.6) for monozygotic twins and 5.7 (95% CI, 4.1 to 7.9) for dizygotic twins (**Fig. 2**). In the San Diego Population study,[26] 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[27] 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% vs. 5.0%, P <0.0001) resulting in a doubling of the odds of the presence of PAD in those with family history of PAD (**Fig. 2**). The association of family history of PAD with prevalent PAD was only modestly attenuated after adjustment for conventional risk factors: OR 1.97 (1.60-2.42). The association was stronger in individuals younger than 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.[28, 29] Genetic factors likely have an important role in premature PAD including those acting through pathways of thrombosis, inflammation, and lipid and homocystine metabolism.[30] Men and women appear to be equally affected, in contrast to early-onset CHD where men are more commonly affected.[31] 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,[32-34] hyperhomocysteinemia [35] and pseudoxanthoma elasticum.[36]

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. Gudmundson and colleagues [37] performed a 10 cM genome-wide scan in 272 patients from 116 extended families who had undergone angiography and/or revascularization procedures for symptomatic PAD.[37] Significant linkage to a region on chromosome 1 between 100 and 110 cM was found (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 ankle brachial index (ABI) as a continuous trait did not reveal any regions of LOD scores ≥3, although several regions with tentative evidence of linkage (multipoint LOD = 1.3-2.0) were detected.[38]

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,[39] apo B,[40] eNOS,[41, 42] MTHFR,[41] G-protein beta unit 3 and alpha-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 al[46] 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 four-fold cross validation. The following associations were significant, replicated, and cross-validated: two SNP main effects in *NOS3*, three SNP-covariate interactions (*ADRB2* Gly 16 – lipoprotein (a) and *SLC4A5* - diabetes interactions), and 25 SNP-SNP interactions (involving SNPs from 29 different genes). The Candidate Gene Association Resource (CARe) consortium performed a meta-analysis of ~ 50,000 SNPs in ~ 2000 cardiovascular candidate genes, but was unable to confidently identify any variants associated with the ABI.[47]

Genome-wide association studies (GWAS)

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. Whereas 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 found that the 9p21 locus was associated not only with CHD but also with PAD, abdominal aortic aneurysm (AAA) and intracranial aneurysm.[48] Thorgeirsson et al[49] found a synonymous SNP (rs1051730) within the cholinergic receptor nicotinic alpha 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 al[51] found the *OSBPL10* locus to be associated with PAD in a Japanese cohort. In a meta-analysis[52] of 21 population-based cohort studies that included 41,692 participants of European ancestry among whom 3409 participants had PAD (defined as an ABI <0.90), six SNPs were associated ($P \leq 1 \times 10^{-6}$) 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 efferocytosis (phagocytic clearance) of apoptotic debris in atherosclerotic plaque.[53-55]

In a recent electronic medical record (EMR)-based GWAS of PAD, the allele C of the intronic SNP rs653178 at the *ATXN2-SH2B3* locus on chromosome 12 was present more frequently in PAD cases (52%) than in controls (47%) with a resulting odds ratio (OR) of 1.23 ($P= 5.6\times10^{-4}$) in the discovery cohort.[56] In the replication cohort of 740 PAD cases and 1051 controls, the OR was 1.25 ($P= 8.9\times10^{-4}$) and in the combined sample, the OR was 1.22 ($P=6.5\times10^{-7}$). 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 rs653178 is in strong LD ($r^2=0.99$) with a missense SNP (rs3184504) in *SH2B3*, an adapter protein that plays a key role in immune and inflammatory response pathways and vascular homeostasis.[57, 58] 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 due to natural selection.[59-61]

CHALLENGES

To date, the search for genetic susceptibility variants for PAD has been less successful than for CHD, likely due to 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 so far, 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 EMR systems to conduct genotyping or sequencing studies. Such an approach [71] can reduce the time, effort, and cost involved in conducting genomic association studies. The Electronic Medical Records and Genomics (eMERGE) consortium[72] is leveraging biorepositories linked to the EMR for large-scale genomic research.[73]

Phenotypic heterogeneity appears 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, 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 or not they have diabetes since diabetic PAD is often distal.

FUTURE DIRECTIONS

The field of complex disease genetics has advanced considerably in the last several years, primarily due to assembly of large case control cohorts and availability of newer genomic technologies. In this section we highlight how these advances might be leveraged in 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.[45] Since PAD results from alterations in multiple atherogenic pathways, large single gene effects are unlikely,[75] multiple loci are likely involved and candidate genes may express themselves only through interaction with other genes or with at-risk lifestyles. Identifying the combinations of multilocus genotypes predictive of disease (epistasis) is a daunting task.[76] 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.[77, 78] Exome sequencing in members of three 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 [77] and adenosine treatment of fibroblasts from an affected patient reduced the levels of alkaline phosphatase and calcification. [65] In another study,[66] exome sequencing helped identify the underlying mutation in a family where two siblings had aortic hypoplasia, diffuse atherosclerosis, and PAD. The two siblings were homozygous for a non-synonymous mutation in *IN080D* which leads to disruption in the function of one of the domains of the protein. *INO80D* encodes a key component of the human IN080 complex, a multi-protein complex involved in DNA binding, chromatin modification, organization of chromosome structure, and ATP-dependent nucleosome sliding.[79]

Differential gene expression

Arterial tissue is difficult to obtain and circulating peripheral blood mononuclear cells (PBMCs) have been proposed as ‘reporters’ of arterial wall pathology. Several investigators have examined differentially regulated genes in PBMCs to identify relevant molecular mechanisms for vascular diseases[80-82] Masud et al[84] found genes influencing immune response, inflammation, apoptosis, and various signalling pathways to be differentially expressed in PBMCs from PAD cases and controls. RNA sequencing (RNA-Seq) 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. Valentine et al[83] reported that premature CHD, PAD, and stroke was more common in parents and siblings of individuals with premature PAD or CHD compared to controls, suggesting the presence of shared genetic factors across these subtypes of ASCVD. In the study by Khaleghi et al,[27] 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, e.g., 9p21 and *DAB21P*,[48, 50] that are associated with more than one subtype of atherosclerotic vascular disease.[84, 85] Gretarsdottir et al [50] reported that the A allele of rs7025486 within *DAB2IP*, which encodes an inhibitor of cell growth and survival, was associated with AAA, with an odds ratio (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, $P = 3.1 \times 10^{-5}$) and PAD (OR = 1.14, $P = 3.9 \times 10^{-5}$). The SNP was not associated with risk factors such as smoking, lipid levels, obesity, type 2 diabetes 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 immunological disorders,[86] hematologic traits such as platelet count, mean-platelet volume,[87, 88] and eosinophil count and diabetes.[89] The pleiotropic nature of *SH2B3* may be due to its role in immune and inflammatory signaling pathways including erythropoietin, cytokine receptor-mediated and integrin signaling.[57] The protein also regulates hematopoietic cell lineage and endothelial cells, and influences adhesion and migration of platelets by modulating actin cytoskeleton organization.[58, 87, 88, 90]

Epigenetics

Epigenetics is the study of factors that modify gene expression, exclusive of changes in the DNA sequence.[91] 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 as they 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.[92] Availability of RNAseq, 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 non-coding RNAs is now recognized as another major epigenetic regulator of gene expression.[93-95] 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 (reviewed in[96]) as well as angiogenesis in experimental animals. [97] Additionally, miR-503 has been implicated as a putative regulator of diabetic PAD and limb ischemia human tissue samples.[98, 99] A panel of 12 miRs measured in the peripheral blood was recently correlated with the presence of PAD.[100] Long noncoding RNAs (IncRNAs) 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 lncRNAs in PAD. The 9p21 locus, which is associated with several vascular disease phenotypes, harbors polymorphisms within a lncRNA known as *ANRIL* (antisense non-coding RNA in the INK4 locus). *ANRIL* has been shown to recruit polycomb repressive complexes to the promoter of *CDKN2B,*[101] and directly silence the expression of this atheroprotective and anti-aneurysmal gene.[55, 102] The association of 9p21 locus with atherosclerotic vascular disease could be mediated by this indirect epigenetic pathway involving *ANRIL*, through trans-regulation 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.[103] The common disease-rare variant concept has been illustrated by several reports, including the association of uncommon *PCSK9* variants with CHD susceptibility[104] and of rare *CFH* variants with macular degeneration.[105] 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.[106]

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.[107] The Gene Ontology[108] resource provides annotation of the biology and function of gene and protein sequences based on their homology across multiple organisms, using a common vocabulary. KEGG (Kyoto Encyclopedia of Genes and Genomes),[109] 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[110] 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 PCR, Koren et al[111] 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 circulating metabolites and angiographic coronary artery disease.[112, 113] Wang et al[112] have shown that gut microbiome derived metabolites choline, betaine, and trimethylamine-N-oxide (TMAO) are associated with the presence of coronary artery disease. Furthermore, higher levels of TMAO 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 likely 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 in predisposing to PAD. Thus much larger sample sizes will 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 and smoking. Results of the genomic association studies so far 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 RNAseq 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Nonstandard Abbreviations and Acronyms

AAA	abdominal aortic aneurysm
ABI	ankle brachial index
CHD	coronary heart disease
eQTL	expression quantitative trait loci
GWAS	genome-wide association studies
lncRNA	long noncoding RNA
KEGG	Kyoto Encyclopedia of Genes and Genomes
miR	microRNA
OR	odds ratio
PAD	peripheral arterial disease
RNAseq	RNA sequencing
SNP	single-nucleotide polymorphism

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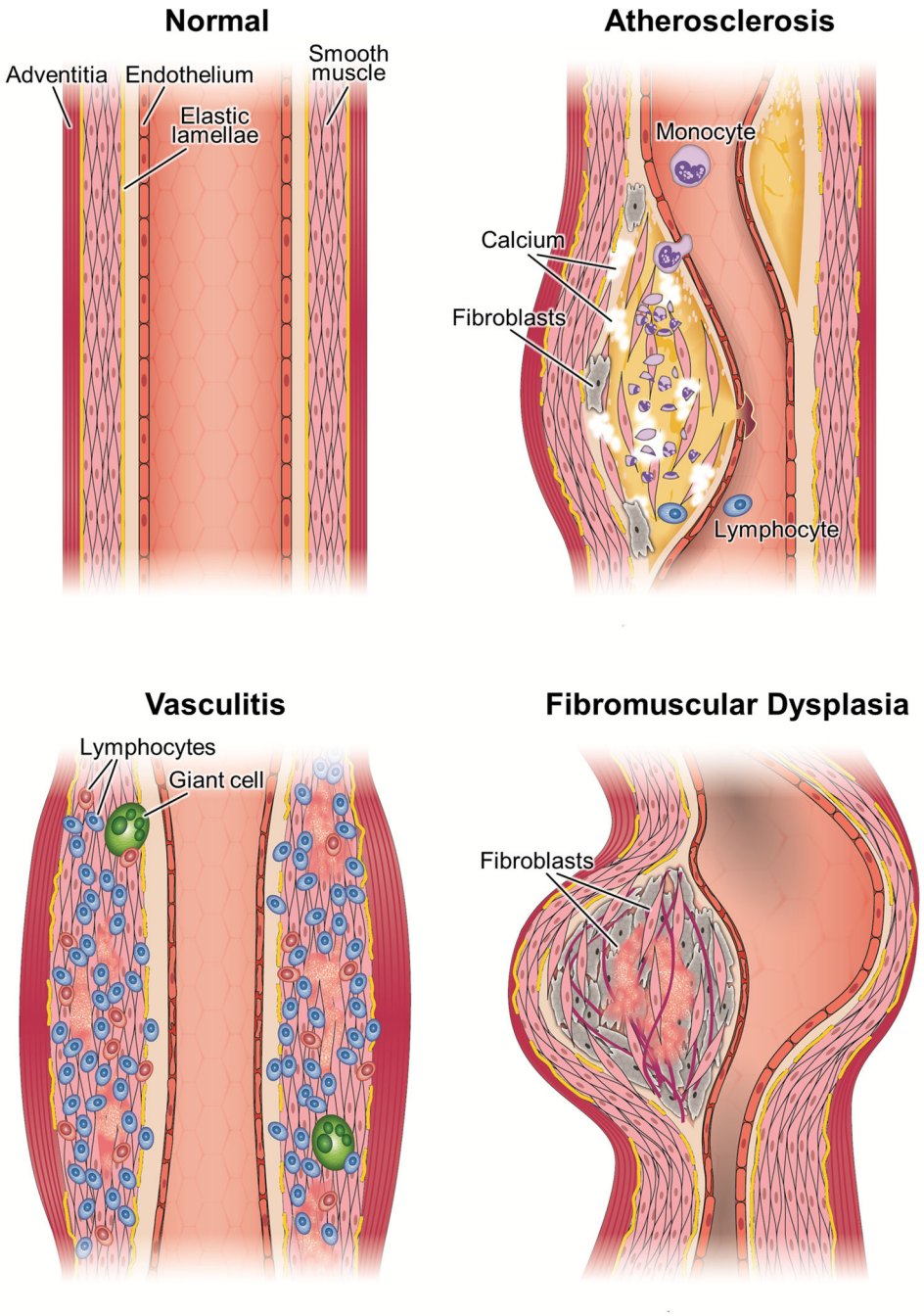

Fig. 1.

Arterial pathology in atherosclerotic, inflammatory, and non-atherosclerotic non-inflammatory arteriopathies. Atherosclerosis is characterized by plaques with varying amounts of inflammatory cells, lipid deposition, fibrosis, calcification, cellular necrosis, smooth muscle proliferation and necrosis, disruption of internal elastic lamina. Inflammatory arterial diseases are characterized by marked inflammatory cell infiltration and in the case of large vessel vasculitis, by giant cell formation. Features of non-inflammatory non-atherosclerotic arterial disease vary. In fibromuscular dysplasia, the

prominent features are excessive fibroblasts, deposition of increased amounts of extra cellular matrix and relative paucity of inflammatory cells.

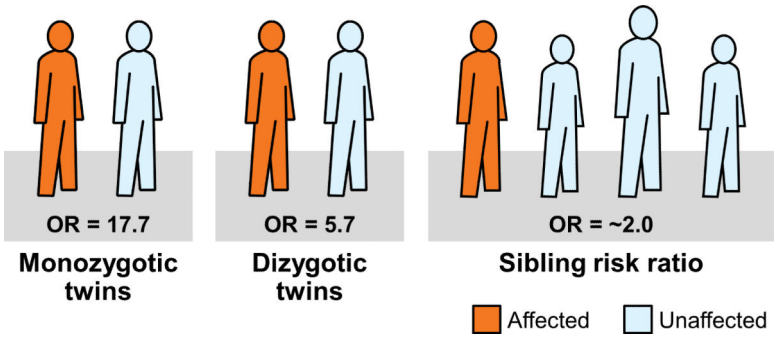

Fig. 2.

Family history as a risk factor for PAD. Shown are odds ratios when the affected family member is a monozygotic twin, a dizygotic twin or a sibling.[25,27]

Table 1

Ascertaining PAD in the clinical setting

Symptomatic PAD. The classical symptom of PAD is intermittent claudication but less than one third of patients with abnormal ankle brachial index (ABI) (< 0.9) have claudication. Additional clinical presentations include atypical leg discomfort, critical limb ischemia (including rest pain, gangrene) and history of amputation or revascularization for limb ischemia.
Abnormal ankle brachial index (ABI).[8] ABI – the ratio of systolic BP at the ankle to the systolic BP in the arm – is an established noninvasive measure of PAD that is inversely related to disease severity. When segmental blood pressures are combined with Doppler, disease location can also be ascertained. An ABI of < 0.90 is 95% sensitive in detecting stenosis of 50% or greater (determined angiographically) involving the lower extremities.[9], [10] There are two main limitations of the ABI as a phenotype of PAD. Since the ABI becomes abnormal only with hemodynamically significant lesions, disease of lesser severity may be missed. Use of post-exercise ABIs increases the sensitivity and partially addresses this problem. ABI may be falsely elevated due to medial calcification that typically occurs in the elderly and diabetic subjects and leads to poorly compressible arteries. [11]
Atherosclerotic plaque on imaging. Several imaging modalities can detect atherosclerotic plaque in lower extremity arteries: Ultrasound- Arterial ultrasound is a noninvasive imaging modality that is more sensitive than the ABI in identifying subclinical PAD. In addition to visualizing plaque burden, hemodynamic assessment with Doppler is also possible. Methods for quantifying plaque burden are not yet available. Intravascular ultrasound provides more accurate estimates of plaque burden but is invasive in nature. Angiography- Conventional angiography is the gold standard for assessment of luminal narrowing of the peripheral arteries but is invasive and expensive. Furthermore it may not provide an accurate measure of the true burden of atherosclerosis much of which could be accommodated in the arterial wall due to remodeling. Angiography can also be performed by computed tomography or by magnetic resonance imaging.

Table 2

Genetic loci associated with inflammatory arterial diseases in genome-wide associated studies

Disease	Involvement of peripheral arteries	Genes implicated
ANCA-associated vasculitis[62]	Inflammation of small-sized blood vessels	*SERPINA1, HLA-DP, COL11A2*
Kawasaki's disease [63-66]	Inflammation of medium- and small-sized blood vessels	*FAM167A – ELK, CD40, HLA-DQB2–HLA-DOB FCGR2A, ZFHX3, LNX1, CAMK2D, CSMD1, TCP1*
Behcet's disease[67-70]	Inflammation of large, medium-and small-sized blood vessels	*GIMAP, STAT4, ERAP1, IL23R - IL12RB2, IL10, CCR1-CCR3, KLRC4*

ANCA=Anti neutrophil cytoplasmic antibody

Table 3

SNPs associated with PAD in genome-wide associated studies

SNP	Locus	Cases (n)	Controls (n)	OR (95% CI)	*P-value*	RAF*	Nearest gene
rs10757278-G	9p21	2599	15012	1.14 (1.07, 1.22	6.1×10^{-5}	0.42-0.51	*CDKN2A/CDKN2B*
rs1051730-C	15q24	2738	29,964	1.19 (1.12, 1.27)	1.4×10^{-7}	0.27-0.37	*CHRNA*
rs7025486-A	9q33	3690	12,271	1.14 (1.07, 1.21)	3.9×10^{-5}	0.23	*DAB12*
rs1902341-G	3p23	195 699	1358 1540	1.31 (1.18, 1.46)	5×10^{-7}	0.397	*OSBPL*
rs6584389-C	10	3409	68,002	1.17(1.10, 1.25)	2.3×10^{-6}	0.50	*PAX2*
rs653178-C	12q24	1641 740	1604 1051	1.22 (1.13, 1.32)	6.46×10^{-7}	0.49	*SH2B3-ATXN*

* in controls. OR= odds ratio; RAF= risk allele frequency in controls

Table 4

Challenges in identifying genetic determinants of peripheral arterial disease

Challenge	Comment
Phenotypic heterogeneity	Genomic association analyses stratifying by subtypes of PAD could address phenotypic heterogeneity.
Genetic heterogeneity	Increasing sample size in case-control association studies may help in addressing genetic heterogeneity.
Modest effect sizes of genetic variants	Uncovering variants of small effects requires large sample sizes, and recognition of this fact has motivated assembly of genetic consortia for common diseases.
Gene-gene and gene-environment interactions	Identifying such interactions will require large sample sizes and precise measures of environmental factors e.g. pack years in the case of smoking.
Rare variants	Both common and rare variants likely influence PAD susceptibility. To identify rare variants that influence susceptibility to PAD, very large sample sizes will be required.
Structural variants	Additional studies are needed to investigate the association of structural genetic variants with complex diseases such as PAD.

Abbreviations: PAD, peripheral arterial disease; ABI, ankle brachial index.