Published in final edited form as:
*Curr Diab Rep*. 2011 June ; 11(3): 218–225. doi:10.1007/s11892-011-0188-9.

# Peripheral Arterial Disease in Diabetes: Is There a Role for Genetics?

**Arabindra B. Katwal** and
Division of Cardiovascular Medicine, University of Virginia, Charlottesville, VA, USA

**Ayotunde O. Dokun**
Division of Endocrinology, Department of Medicine, University of Virginia, Charlottesville, VA, USA

Ayotunde O. Dokun: DOA3Q@hscmail.mcc.virginia.edu

## Abstract

Atherosclerotic occlusion of vessels outside of the heart is commonly referred to as peripheral arterial disease (PAD). The lower extremity is the most common site of PAD and its development is associated with the same risk factors involved in general atherosclerosis. However, there is emerging evidence that other risk factors may play a key role in the development of PAD. Over the past decade polymorphism in a number of genes has been shown to contribute to the risk of developing PAD. These genes can be classified into proartherosclerosis or proatherothrombosis based on the known gene function. Moreover, they can be categorized as “novel” polymorphism when the function of the genes is not known or when the specific gene within an associated genetic locus is not known. It is intriguing that not only are gene polymorphisms associated with PAD being identified, but more recently studies are now finding gene polymorphisms that may be important in development of this syndrome only in the contest of certain environmental factors such as diabetes. Currently how these gene–environment interactions contribute to the pathogenesis of PAD is poorly understood but will likely play a critical role in future understanding of this complex disease.

### Keywords

Peripheral arterial disease; Angiogenesis; Genetics; Modifier gene; Gene polymorphism; Single nucleotide polymorphism; Linkage disequilibrium; Haplotype; Gene association studies; Genome-wide association studies; Quantitative trait locus; Hind limb ischemia; Type 1 diabetes; Type 2 diabetes; Diabetes; Artherosclerosis; Gene–environment

## Introduction

Peripheral arterial disease (PAD) is characterized by obstruction in arterial beds other than the coronary arteries and is caused by atherosclerosis in the vast majority of patients. The most common site is the lower extremity where occlusive disease leads to impaired perfusion. Although previously under-recognized and under-diagnosed by the medical community and therefore viewed as less important than heart disease, PAD is now



Correspondence to: Ayotunde O. Dokun, DOA3Q@hscmail.mcc.virginia.edu.

**Disclosure** No potential conflicts of interest relevant to this article were reported.

recognized to have a prevalence that is similar to that of ischemic heart disease [1, 2] and affects about 3% to 10% of adults in the world [3, 4].

The major established risk factors for the development of PAD are essentially the same as those recognized as important in generalized atherosclerosis and include increasing age after 40 years, cigarette smoking, diabetes mellitus (DM), hyperlipidemia, and hypertension [5, 6]. Elevated levels of C-reactive protein and homocysteine may also be important risk factors [1, 7, 8]. However, smoking and diabetes account for most of the risk of developing PAD [4, 9]. The unique role of diabetes in the pathogenesis of PAD is discussed in more detail later in this article. Nevertheless, there is now evidence that traditional risk factors can only partially account for the risks of developing PAD [10, 11]. Moreover, results of twin studies looking at genetic influences in PAD show high heritability [12]. Taken together this suggests sequence variations in genes in individuals may play a significant role in their risk of developing PAD. This review provides an overview of the result of recent studies looking at the association of genetic polymorphisms with the risk of developing PAD, with an emphasis on gene polymorphisms that influence development of PAD in diabetes.

## Epidemiology and Clinical Presentations of PAD

Most patients with PAD lack the classic symptoms of PAD and thus are often considered asymptomatic; some estimates suggest as many as 50% of PAD patients fall into this category [3]. It is currently recommended that patients with multiple risk factors, especially smokers and diabetics, with other risk factors, should undergo noninvasive testing such as ankle-brachial index (ABI). ABI measures the ratio of systolic blood pressure in the ankle to that of the brachial vessels; an ABI less than 0.9 is considered diagnostic for PAD [3, 4, 13]. Once the diagnosis of PAD is made, further testing with duplex ultrasonography, segmental Doppler pressure or volume plethysmography, MRA or angiography may be used depending on the clinical situation.

There are two major clinical manifestations of PAD: intermittent claudication (IC) and critical limb ischemia (CLI). IC manifests as reduced blood flow to the extremities during exercise resulting in pain relieved only by rest, whereas CLI describes pain at rest that may be associated with non-healing leg ulcers or gangrene. Interestingly, although patients with IC have amputation and an annual mortality rate of 1% to 2%, those with CLI have a 6-month amputation risk of 25% to 40% and an annual mortality of 20% [14].

The diagnosis of IC versus CLI is based upon time-tested clinical classification schemes, namely the Rutherford and the Fontaine classifications. In the Rutherford classification, IC encompasses categories 1 to 3 (mild, moderate, and severe claudication, respectively), whereas CLI includes categories 4 to 6 (ischemic rest pain, minor tissue loss, and ulceration or gangrene). The Fontane classification is more commonly used in Europe, with stages IIa and IIb describing IC, whereas stages III to IV are categories of CLI. Although there is evidence of association of some biomarkers with IC and CLI [15–17], there is no biomarker or hemodynamic measure that is pathognomonic for either IC or CLI.

The observation that progressively lower ABIs are associated with worsening symptoms of claudication [18] has made some to assume that IC and CLI are due to a continuum of reduced blood flow. In this line of thought, CLI is simply a worse form of IC. However, the ultimate symptomatic presentation of PAD is heterogeneous with some individuals presenting with claudication while others present de novo with CLI. Moreover, many with IC never progress to CLI and many presenting with CLI do not report antecedent claudication [19–21]; therefore, IC and CLI may actually be distinct.

## Diabetes and PAD

As mentioned earlier, diabetes and smoking account for most of the risk of developing PAD [4, 9, 22]. When PAD is present in diabetes there is a higher likelihood of occurrence within the femoral-popliteal and tibia vessels [23], whereas other risk factors such as smoking tend to be associated with more proximal disease (ie, iliofemoral vessels) [9]. In individuals with diabetes, PAD typically presents at an earlier age and is associated with a more rapid progression than in nondiabetics [23]. Moreover, the incidence of PAD increases significantly the longer an individual has been diabetic. For instance, Melton et al. [24] found that the cumulative incidence of PAD was 15% in those with diabetes duration of 10 years, and this increased to 45% in those with an additional 20 years exposure to diabetes. The true prevalence of PAD among individuals with diabetes has been difficult to assess due to a number of factors, including absence of symptoms, poor reporting of symptoms, blunted pain perception due to peripheral neuropathy, and inadequate screening. Nevertheless, using ABI measures some studies have estimated the prevalence of PAD among diabetics to be three times higher than among nondiabetics (7% vs 20.9%) [25]. The prevalence of PAD among individuals with diabetes was found to be as high as 38% in some cohorts [26–28].

Both type 1 and type 2 diabetes are associated with increased risk of PAD [24–26]. However, whether both type of diabetes are associated with similar prevalence of PAD is not clear because conflicting results have been reported. A United Kingdom study showed type 2 DM is associated with a higher prevalence (23.5%) of PAD than type 1 DM (8.7%) [29]. In contrast, an Australian study showed no difference in prevalence of PAD in type 1 and type 2 DM [26]. Nevertheless, because there are differences in the underlying pathology in type 1 and type 2 DM, it is likely that the molecular mechanisms through which they contribute to pathogenesis of PAD differ.

The role of diabetes in the pathogenesis of PAD can be considered in at least two major ways. First, diabetes may accelerate the development of atherosclerotic vessel occlusion; alternatively, it may negatively impact adaptive processes involved in restoring perfusion following vessel occlusion (eg, angiogenesis and arteriogenesis). It is now known that diabetes does accelerate the development of atherosclerosis through different mechanisms [30]. Moreover, there is emerging evidence from our group and others that diabetes may contribute to development of PAD by negatively impacting adaptive processes involved in restoring perfusion following vessel occlusion [31, 32]. Nevertheless, our understanding of the molecular mechanism by which diabetes contributes to the pathogenesis of PAD is quite limited.

## Genetic Background as a Risk Factor for PAD

As previously mentioned, the major risk factors for the development of PAD are essentially the same as those recognized as important in generalized atherosclerosis and include increasing age after 40 years, cigarette smoking, DM, hyperlipidemia, hypertension, and elevated levels of C-reactive protein and homocysteine. However, not all patients with these risk factors develop PAD. Even in cohorts in which the prevalence of PAD among diabetics is quite high (38%) about 60% of diabetic individuals do not have PAD. Additionally, in patients with similar risk factors, atherosclerotic burden, and similar peripheral hemodynamics, the clinical presentation of PAD in not necessarily identical. For instance, some patients may present with IC whereas others present with CLI. Taken together these observations suggest that there are factors other than the currently known risk factors for PAD that may influence the development of this clinical syndrome.

Interestingly, there is an emerging body of evidence that suggests the genetic background of an individual may be important in the pathogenesis of PAD. In one study the prevalence of PAD in various ethnic backgrounds was evaluated and their results suggest higher prevalence in African Americans even after adjusting for age and other traditional risk factors for PAD [33]. Because individuals of the same ethnicity are likely to share certain ancestral genes, the higher prevalence of PAD in African Americans suggests that there may be gene polymorphisms contributing to PAD in this group. Further evidence supporting the possible role of genetic risk factors in PAD comes from both association and linkage studies. Estimates of the heritability of PAD using ABI as a surrogate have shown that the contribution of genetic factors to the overall variation in ABI ranges from 21% to 48% [12, 34, 35]. Moreover, in a recent twin study of genetic influences on PAD, Wahlgren et al. [36] showed genetic effects could account for about 58% of the phenotypic variance among twins. Therefore, genetic background may be a major determinant in development of PAD. Nevertheless, our understanding of the role of genetics in the pathogenesis of PAD remains poor [37, 38].

## Gene Polymorphisms Contributing to PAD

Sequence variations in a variety of genes have shown statistically significant association with PAD [39•]. These genes can be classified into three different categories: proatherosclerotic, proatherothrombotic, or novel, based on the function of the gene products (Table 1). Below we provide an overview of recently described gene polymorphisms contributing to PAD within each of the three categories stated above and, where known, we will describe gene polymorphisms associated with PAD in the setting of diabetes.

### Proatherosclerotic Gene Polymorphisms in PAD

PAD is a consequence of atherosclerosis in the lower extremities; therefore, it is not unexpected that polymorphisms of genes contributing to the development of atherosclerosis can be found to be associated with PAD. One of the important events in the initiation and progression of artherogenesis is endothelial dysfunction. Nitric oxide (NO) is a key modulator in this process and it contributes to regulation of vascular tone, leukocyte adhesion to vascular endothelium, inhibition of platelet aggregation, and inhibition of smooth muscle cell migration and proliferation [40]. Endothelial-derived nitric oxide synthase (eNOS) is one of three isoforms of NO synthase responsible for NO synthesis. eNOS gene polymorphisms have been shown to cause decreased NO synthesis, thereby reducing NO availability and causing endothelial dysfunction [41]. Polymorphism of the eNOS gene has been shown to be associated with carotid atherosclerosis and abdominal aortic aneurysm [42, 43]. In a recent study, Sticchi et al. [44] showed that in smokers, but not in nonsmokers, the concomitant presence of the eNOS −786 C/4a haplotype was significantly associated with increased predisposition to PAD. The role of eNOS gene polymorphisms in predisposition to PAD among diabetic individuals is not known.

Angiotensin-converting enzyme (ACE) processes the decapeptide angiotensin I to octapeptide angiotensin II, which is a strong vasoconstrictor. It is thought that ACE may affect the atherosclerotic process through bradykinin degradation and reduction in release of NO [44]. An insertion/deletion (I/D) of a 287-bp fragment in the intron 16 of the ACE gene has been described, and the D allele is associated with increased serum levels of the circulating enzyme [45]. Although prior studies did explore possible association of this allele with PAD they showed only borderline significance [46, 47]. However, a more recent study by Fatini et al. [48] studied association of the ACE I/D polymorphism and the −240 A>T polymorphism in the promoter region of the ACE gene to PAD and showed that the ACE D allele and the ACE D/−240T haplotype significantly and independently influenced

the predisposition to PAD. Interestingly, the presence of eNOS −786 C/4a haplotype (previously described above) increased predisposition to PAD in smokers carrying the ACE D allele [44]. These results are consistent with a gene-environment interaction in the modulation of PAD development. Nevertheless, whether polymorphisms in the eNOS or ACE genes play a role in predisposition to PAD in individuals with diabetes is not known.

Inflammation is becoming increasingly recognized as an important factor in the pathogenesis of atherosclerosis, with its development and progression orchestrated by several molecules belonging to different families of inflammatory mediators, such as cytokines, chemokines, adhesion molecules, and proteolytic enzymes [49, 50]. In a study of 157 PAD patients and 206 controls, Flex et al. [50] analyzed gene polymorphisms including interleukin-6 (IL-6;−174 G/C), E-selectin Ser128Arg, intercellular adhesion molecule-1 (ICAM-1; 469 E/K), monocyte chemoattractant protein-1 (MCP-1) −2518 A/G, matrix metalloproteinase (MMP)-1 −1607 1 G/2 G, and MMP-3 −1171 5A/6A. The IL-6, MCP-1, MMP-1, and MMP-3 polymorphisms influence the plasma concentrations of these proteins. They found that IL-6, E-selectin, ICAM-1, MCP-1, MMP-1, and MMP-3 gene polymorphisms were significantly and independently associated with PAD. Interestingly, they also found that polymorphisms of these proinflammatory proteins act synergistically (depending on the number of high-risk genotypes concomitantly present in a given individual) to confer different levels of risk for PAD and CLI. This exemplifies how susceptibility to a disease results from functional interactions between modifier genes [50]. Although 49% of the PAD patients and 29% of the controls were diabetic, the effect of these gene polymorphisms in coffering risk for developing PAD among diabetics is not clear. Of note, a prior study did address the role of one of these gene polymorphisms in promoting the development of PAD among diabetics. The study showed that the GG genotype of the IL-6 (−174 G/C) polymorphism was more common among diabetics with PAD than diabetics without PAD. Moreover, the genotype was associated with higher plasma concentrations of IL-6 [51].

In addition to the above study a recent study also identified proatherosclerotic gene polymorphisms associated with the development of PAD among individuals with diabetes. Connexin 37 is a protein expressed in endothelial cells, monocytes, and macrophages and appears to play a role in atherogenesis [52]. The C to T substitution at nucleotide 1019 in the connexin 37 gene results in a proline to serine substitution. This substitution is thought to contribute to atherosclerosis and its polymorphism has been associated with the development of coronary artery disease. Katakami et al. [53•] investigated association of genetic polymorphisms in the connexin 37 gene with PAD among 2,261 Japanese individuals with PAD. Their data showed a statistically significant higher association of low ABIs among diabetics with the TT genotype than the CC or CT genotypes (7.2% vs 2.0%; $P$=0.0008). They reported no association of these genotypes to sex, age, body mass index, hemoglobin $A_{1c}$, duration of diabetes, hypertension, and dyslipidemia.

Guided by previous genome-wide association studies (GWAS) and subsequent replication studies that demonstrated a strong association of a common variant at chromosome 9p21 (tagged by the rs1333049 or rs10757278 single nucleotide polymorphism [SNP]) with myocardial infarction (MI) [54] and coronary artery disease, Cluett et al. [55] hypothesized that this SNP might also be associated with risk of PAD. Using three different study populations (InCHIANTI [Invecchiare in Chianti, aging in the Chianti area); Health, Aging and Body Composition; and Baltimore Longitudinal Study of Aging studies) they analyzed the association of the 9p21 MI SNP (rs1333049) with PAD and found the C allele is associated with an increased prevalence of PAD and lower mean ABI independent of the presence of previous MI and atherosclerotic risk factors. It is intriguing that the nearest genes to this marker are *CDKN2b* and *CDKN2a*, which are known cell cycle regulators.

These genes may contribute to the development of heart disease (and possibly PAD) via reduced regrowth of arterial intimal cells [56].

### Proatherothrombotic Gene Polymorphisms in PAD

It is fairly well established that thrombosis plays an important role in the pathogenesis of atherosclerosis [57]. Thrombin is not only important in fibrin formation and platelet aggregation but thrombin is also important in endothelial activation, platelet and leukocyte recruitment [58]. Consequently, various groups have hypothesized that polymorphisms in genes encoding hemostatic proteins may contribute to development of atherosclerosis and PAD. Studies have shown gene polymorphisms associated with PAD in hemostatic proteins including fibrinogen [59], factor II and V [60].

The importance of platelet aggregation in arterial thrombosis is also well known. The platelet ADP receptor P2Y12 is a seven-transmembrane receptor that upon activation promotes platelet aggregation [61, 62]. Blockade of P2Y12 by thienopyridines has been shown to be beneficial in patients with cardiovascular disease. Polymorphism in the *P2Y12* gene has been described and one of the alleles (H2) results in a gain-of-function haplotype on ADP-induced platelet aggregation [63]. Thus, it was hypothesized that this allele may be associated with increased risk of PAD. The H2 allele was found associated with PAD even after adjusting for traditional PAD risk factors [64].

Defects in the folate pathway may contribute to the development of a prothrombotic state [65]. Methylenetetrahydrofolate reductase (MTHFR) is an important folate-metabolizing enzyme involved in metabolism of homocysteine. The MTHFR 677 C/T is a well-described polymorphism of MTHFR enzyme and elevated homocysteine has been described among carriers of the 677 C>T allele [66]. Elevated plasma homocysteine levels may promote vascular disease through endothelial injury predisposing vessels to atherosclerosis [67]. Several studies found a significant positive association between MTHFR 677 C/T polymorphism and PAD [65, 68, 69].

Out of all the genetic polymorphisms described in this section only one has been associated with increased risk of PAD among individuals with diabetes. In a study of PAD among aboriginal Canadians, Pollex et al. [70] showed that individuals with diabetes who also carried the MTHFR 677 T allele had a modest but increased risk of PAD (odds ratio, 3.54; 95% CI, 1.01, 12.4; $P$=0.049) even after adjusting for traditional risk factors such as age, hypertension, duration of diabetes, current smoking habit, and method of diabetes treatment. Therefore, much less is known about the role of prothrombotic gene polymorphisms and the risk of PAD among individuals with diabetes.

## Novel Gene Polymorphisms Involved in PAD

Human studies of the genetics of PAD are quite limited outside of genes contributing to atherosclerosis or thrombosis. Despite an extensive review, we were able to identify only a few studies in which polymorphisms associated with PAD were not of genes contributing to atherosclerosis or thrombosis. One of these was a family-based linkage study that identified a genetic locus conferring susceptibility to PAD. This study of Icelandic families with multiple family members exhibiting PAD identified a locus termed *PAOD1*, which mapped to human chromosome 1p31 [71]. Interestingly, other risk factors for PAD such as hypertension, hyperlipidemia, and diabetes did not contribute to the positive linkage. Despite these strong and convincing genetic data, the genes responsible for *PAOD1* have not been identified. This is not surprising in light of the difficulties involved in studying genetics of a complex disease such as PAD in humans.

More recently, investigators have used GWAS to identify gene polymorphisms associated with susceptibility to PAD. One study found a significant association between nicotine dependence and a SNP (rs1051730) on chromosome 15q2. This SNP is located within the *CHRNA3* gene, which is in a linkage disequilibrium block that contains genes encoding nicotinic acetylcholine receptors. Interestingly, this study also demonstrated a significant association of this SNP with PAD and lung cancer, suggesting a gene–environment interaction [72•].

Unlike the above two studies, a recent study by Jiang et al. [73••] directly explored genetic polymorphisms in the *SLC2A10* gene and development of PAD among individuals with diabetes [11]. Recent studies have shown that loss-of-function mutation in the gene encoding the facilitative glucose transporter GLUT10 (*SLC2A10*) causes arterial tortuosity syndrome via upregulation of the transforming growth factor-β (TGF-β). Moreover, TGF-β signaling has been implicated in microangiopathics changes in diabetes; the authors hypothesized that the *SLC2A10* gene is a candidate gene for vascular complications in type 2 diabetes [74, 75, 73••]. In a prospective cohort study of 372 diabetic patients, several common SNPs of the *SLC2A10* gene were significantly associated with PAD, with the strongest association shown by the T allele at rs2179357. By combining all 11 markers tested they identified a common haplotype termed H4 that conferred a strong risk of PAD among type 2 diabetic individuals at baseline (OR, 14.5; 95% CI, 1.3–160; $P$=0.03). Furthermore, over an average follow-up period of 5.7 years carriers of the H4 haplotype were more likely to develop PAD than those with other haplotypes (hazard ratio, 6.78; 95% CI, 1.66–27.6; $P$=0.007) [11]. Of note, the mechanism by which the H4 haplotype and other genetic polymorphisms of the *SLC2A10* gene contributes to PAD in diabetes is not known.

Further evidence for novel gene polymorphisms that may be important in PAD comes from preclinical studies of PAD. In a mouse preclinical model of PAD in which the mouse femoral artery is ligated and excised to introduce ischemic stress (hind limb ischemia [HLI]), our group and others observed that recovery is strain-dependent [76•, 77] with C57BL/6 mice showing robust perfusion recovery and rare necrosis compared with the BALB/C or A/J mice after HLI. Because the ischemic stress in this model is independent of atherosclerosis and yet recovery is strain-dependent, this suggests a role for polymorphism in genes other than those contributing to atherosclerosis and is more likely involved in skeletal muscle function and adaptation to ischemic stress.

We took advantage of the strain-determined differences in recovery following HLI described above to identify a single quantitative trait locus (LSq-1) on chromosome 7 spanning approximately 31 Mb and centered at the SNP marker rs13479513 that showed significant linkage to the phenotypes of tissue necrosis and perfusion following HLI (logarithm of the odds score of 7.96 and 3.71, respectively). Moreover, we further refined the locus and identified candidate genes that may prevent the development of necrosis or confer improved perfusion recovery in some mouse strains [76•].

## Conclusions

There is sufficient evidence to support a significant role for genetic background in an individual’s risk of developing PAD. Similar to other complex diseases the risk of developing PAD is likely the result of the interaction of several genes that act collectively [39•]. Additionally, the interaction between gene polymorphisms and the environment (eg, diabetes) is likely a key factor in the pathogenesis of PAD. Although a few gene polymorphisms associated with PAD among diabetics have been identified (described earlier in this review), there is much less known about the mechanisms by which this gene–environment interaction contributes to PAD and should be the subject of future studies.

Identification of gene polymorphisms important in PAD in the setting of diabetes and other major risk factors such as smoking may result in the creation of personalized screening tests for predicting individual risk of disease and/or development of individualized treatment approaches.

## Acknowledgments

This manuscript was supported by the Robert Wood Johnson Foundation, Harold Amos Medical Faculty Development Program Training Grant Award # 65874 to A.O. Dokun.

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**Table 1**

Gene polymorphisms associated with PAD

| Gene polymorphisms contributing to atherothrombosis and PAD | Gene polymorphisms contributing to atherosclerosis and PAD | Novel gene polymorphisms contributing to PAD |
|---|---|---|
| Factor II (FII G20210A) | Connexin 37[a] | *SLC2A10*[a] |
| P2Y12 (H2 allele) | APO E and APO B | *PAOD1* |
| Fibrinogen (β) | IL-6 promoter (−174 G/C)[a] | LSq-1 |
| MTHFR 677T[a] | E-Selectin Ser128Arg<br>ICAM-1 (469E/K) | *CHRNA3* (rs1051730) |
| | MCP-1 (−2518 A/G) | |
| | MMP-1 and MMP-3 | |
| | eNOS (−786 C) | |
| | ACE D | |
| | *CDKN2b* and *CDKN2a* rs1333049 | |

*ACE* angiotensin-converting enzyme, *APO* apolipoprotein, *eNOS* endothelial nitric oxide synthase, *ICAM-1* intercellular adhesion molecule-1, *IL-6* interleukin-6, *MCP-1* monocyte chemoattractant protein-1, *MMP* matrix metalloproteinase, *MTHFR* methylenete-trahydrofolate reductase, *PAD* peripheral arterial disease

[a]Gene polymorphisms associated with PAD in individuals with diabetes