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Mary M. McDermott, and Donald M. Lloyd-Jones

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The Role of Biomarkers and Genetics in Peripheral Arterial Disease

Mary M. McDermott, MD, Donald M. Lloyd-Jones, MD, ScM

Chicago, Illinois

Men and women with lower extremity peripheral arterial disease (PAD) have higher levels of inflammatory biomarkers than those without PAD. Observational studies link higher levels of several inflammatory biomarkers, including C-reactive protein (CRP), interleukin-6, tumor necrosis factor-alpha, and soluble adhesion molecules, to 1 or more of the following outcomes in people with PAD: more severe PAD, greater lower extremity functional impairment, more adverse calf skeletal muscle characteristics, greater declines in the ankle brachial index, greater declines in lower extremity performance, and higher rates of cardiovascular morbidity and mortality. Higher levels of inflammatory biomarkers are also associated with poorer outcomes after lower extremity revascularization, including graft restenosis and mortality. Increasing levels of CRP are associated with increased mortality and faster functional decline among people with PAD. Statin therapies reduce cardiovascular event rates and may improve walking performance in men and women with PAD, perhaps in part because statins can reduce inflammation. However, no clinical trials have been performed to establish whether therapies that specifically block or lower inflammatory biomarkers improve outcomes in patients with PAD. Family studies show that heritability of PAD ranges from approximately 20% to 45% after adjusting for atherosclerotic risk factors. A genetic marker for PAD has the potential to identify individuals at increased risk for PAD and may also uncover proteins that can help determine mechanisms of development of lower extremity atherosclerosis. However, a genetic marker for PAD has not been identified. (J Am Coll Cardiol 2009;54:1228–37) © 2009 by the American College of Cardiology Foundation

Lower extremity peripheral arterial disease (PAD) affects 8 million men and women in the U.S. and nearly 30% of patients in primary care practice settings who are either age 70 years and older or age 50 to 69 years with risk factors for PAD (1,2). People with PAD have increased cardiovascular event rates, greater functional impairment, and faster rates of functional decline compared with people without PAD (3–5). In a small proportion of patients with PAD, critical limb ischemia will develop or amputation will be required.

Men and women with PAD have higher levels of circulating inflammatory biomarkers compared with people without PAD (6–8). Understanding the significance of elevated inflammatory biomarker levels in PAD can identify prognostic indicators of risk in PAD and improve understanding of adverse outcomes in people with PAD. Similarly, identifying a genetic marker for PAD can help identify people at risk for PAD, improve understanding of mechanisms of lower extremity atherosclerosis, and may ultimately lead to new and better therapies for PAD.

Inflammation and the Pathophysiology of Atherosclerosis

Basic science and animal research studies have identified a fundamental role of inflammation in the development and progression of atherosclerosis (9,10). Early in atherogenesis, circulating monocytes and lymphocytes are recruited into the vascular intima, where they mediate the inflammatory response (Fig. 1). The T-lymphocytes up-regulate plaque-associated cellular responses, including apoptosis, neovascularization, smooth muscle cell migration, and formation of a fibrous cap over the plaque. Monocyte-derived macrophages consume lipoproteins and transform into foam cells that comprise lipid-rich plaque. Release of proteolytic enzymes by macrophages and foam cells can initiate fibrous cap erosion and promote plaque instability or rupture. A ruptured plaque releases its contents to circulating prothrombotic elements, leading to platelet aggregation and thrombus formation (Fig. 1). In the coronary arteries, plaque rupture and subsequent thrombus formation can lead to an acute coronary event (9,10). In the larger lower extremity arteries, supported by numerous and large collateral vessels, acute ischemic events are rare. Therefore, plaque rupture followed by thrombus formation in the lower extremity arteries is more likely to promote silent progression of PAD than an acute clinical event.
Causal associations of inflammatory biomarkers with development or progression of lower extremity atherosclerosis have not been established. However, experimental evidence suggests a role for associations of specific circulating inflammatory biomarkers with atherosclerotic disease progression. For example, C-reactive protein (CRP) is a marker of inflammation produced by hepatocytes in response to interleukin (IL)-6 stimulation. In vitro studies show that CRP stimulates release of endothelial monocyte chemoattractant protein-1, which attracts circulating monocytes to the endothelium. The CRP also up-regulates tissue factor and pro-inflammatory cytokines, induces endothelial adhesion molecules, and inhibits nitric oxide release (11–15).

Adherence and trans-endothelial migration of circulating leukocytes to the arterial wall are important steps in the initiation and progression of atherosclerosis that are mediated in part by the leukocyte adhesion molecules: vascular cellular adhesion molecule (VCAM)-1 and intracellular adhesion molecule (ICAM)-1 (16). VCAM-1 is a transmembrane glycoprotein that binds β-integrins on leukocytes, drawing them to atherosclerotic plaque sites, thereby facilitating arterial inflammation; ICAM-1 is expressed by endothelial cells and binds to fibroblasts and leukocytes. Circulating levels of soluble vascular cellular adhesion molecule (sVCAM)-1 and soluble intracellular adhesion molecule (sICAM)-1 can be measured in peripheral circulation.

IL-6 amplifies the inflammatory cascade and is the main circulating cytokine linking systemic inflammation with local pathology (17,18). IL-6 stimulates macrophages and promotes proliferation of smooth muscle cells in atherosclerotic plaque (17–20). IL-6 also stimulates coagulation by increasing messenger ribonucleic acid transcription of tissue factor and factor VIII (19).

Metalloproteinases are typically present at the shoulder of the atherosclerotic plaque and promote degradation of the extracellular matrix, thereby promoting plaque instability and rupture. Pregnancy-associated plasma protein-A is a metalloproteinase that promotes inflammatory cell activation and migration in atherosclerotic plaque by inhibiting insulin-like growth factor-1 (21). Myeloperoxidase promotes atherosclerosis in part by increasing reactive oxidants and radical species while reducing nitric oxide availability (22). However, data on associations of these biomarkers with development or progression of lower extremity atherosclerosis are limited.

**Inflammatory Biomarker Levels Are Increased in People With PAD**

Multiple studies have shown elevated levels of inflammatory biomarkers in men and women with PAD (6–8,23,24). For example, in the National Health and Nutrition Examination Survey 1999 to 2002 of 4,787 men and women in the U.S. age ≥40 years who underwent ankle brachial index (ABI) measurement, higher levels of CRP, fibrinogen, and leukocyte counts were associated with lower ABI values (6). After statistical adjustment for traditional cardiovascular disease risk factors, participants in the highest quartile of CRP, fibrinogen, or leukocyte count had a 2.0-fold increased odds of PAD compared with participants in the lowest quartiles (6). In a study of 387 patients with PAD identified from vascular clinics, ABI values were progressively lower across 3 tertiles of CRP: first tertile (CRP ≤1.72 mg/l) ABI = 0.70; second tertile (CRP >1.72 to ≤3.56 mg/l) ABI = 0.65; and third tertile (CRP >3.56 mg/l) ABI = 0.57 (p trend = 0.001) (23). These associations remained after statistical adjustment for traditional cardiovascular disease risk factors. The high prevalence of diabetes mellitus and cigarette smoking in people with PAD may contribute to the higher biomarker levels in PAD because diabetes mellitus and smoking are associated with increased levels of circulating inflammatory biomarkers (25,26).

**Elevated Inflammatory Biomarker Levels Are Associated With Increased Risk of Developing PAD**

In the Physician’s Health Study, higher levels of CRP at baseline were observed among 140 men who later developed PAD, measured by lower extremity revascularization or development of intermittent claudication symptoms, compared with men matched by age and cigarette smoking status who did not develop these PAD outcomes (1.34 mg/l vs. 0.99 mg/l, p = 0.04) (27). In the Women’s Health Study, higher baseline levels of CRP and sICAM-1 were each associated with an increased risk of incident PAD, measured by lower extremity revascularization or development of intermittent claudication symptoms, at 12-year follow-up among 27,935 female health professionals (28). These associations remained statistically significant after adjusting for cardiovascular disease risk factors. The highest hazard ratio was observed for sICAM-1. Participants in the highest tertile of sICAM-1 had a 3.5-fold higher risk of developing PAD compared with participants in the lowest sICAM-1 tertile. In contrast, fibrinogen and homocysteine were not associated with development of PAD in the Women’s Health Study (28). Table 1 summarizes associations of elevated biomarker levels with adverse outcomes in people with PAD.
Inflammation and Progression of PAD

Few studies have assessed associations of inflammatory biomarker levels with progression of lower extremity atherosclerosis in nonsurgical cohorts. The Edinburgh Artery Study, a prospective observational study of 1,582 men and women age 55 to 74 years at baseline who were recruited from general medical practices in Edinburgh, showed that higher baseline levels of some inflammatory biomarkers were associated with greater declines in the ABI over time, even after statistical adjustment for cardiovascular disease risk factors, other cardiovascular disease, and physical activity level (29). Among the Edinburgh Artery Study participants, 75% had a normal ABI at baseline. The ABI was repeated at 5-year follow-up in 1,081 participants and at 12-year follow-up in 813 participants. At 5-year follow-up, only higher baseline levels of IL-6 levels, but not CRP, sICAM-1, or sVCAM-1, were associated with greater declines in the ABI adjusting for confounders. At 12-year follow-up, higher baseline levels of IL-6, CRP, and sICAM-1, but not sVCAM-1, were associated with greater declines in the ABI, adjusting for confounders (29). These findings suggest that inflammatory biomarkers may play different mechanistic roles in the progression of lower extremity atherosclerosis, resulting in varying strengths of association with PAD development and progression.

Femoral Artery Atherosclerotic Plaque May Produce CRP

In 14 patients who underwent femoral artery endarterectomy, a reverse-transcriptase polymerase chain reaction was used to measure CRP production in the femoral arteries (30). Four (29%) arterial specimens showed CRP messenger ribonucleic acid production. In the same study, immunohistochemical analyses of femoral atherosclerotic plaque from 20 consecutive patients with PAD showed CRP in all femoral specimens (30). In contrast, brachial artery...
directly and adversely affect lower extremity skeletal muscle functional performance. Second, chronic inflammation may contribute to the progression of lower extremity atherosclerosis, thereby promoting ischemia of lower extremity skeletal muscle and impairing lower extremity function.

At least 2 mechanisms exist by which inflammation may contribute to lower extremity functional impairment and elevated CRP levels in the Physician’s Health Study (26). Elevated CRP levels were associated with a higher risk of developing PAD in the Women’s Health Study (27). Higher CRP levels among community-dwelling men and women in the Edinburgh Artery Study were associated with greater declines in the ABI at 12-year follow-up (28). Elevated CRP levels among PAD participants in the WALCS II cohort are associated with smaller calf muscle area and poorer 6-min walk performance (33,34). Greater average annual increases in CRP levels among PAD participants in the WALCS cohort were associated with greater average annual decline in 6-min walk performance (36). In unadjusted analyses, patients with critical limb ischemia have higher CRP levels compared with patients with intermittent claudication and control subjects (40,41). Among patients with PAD, higher CRP levels are associated with greater all-cause and cardiovascular disease mortality at 4-year follow-up (12). Among participants with history of lower extremity revascularization and elevated CRP levels, statin therapy is associated with significantly lower cardiovascular event rates at 21-month follow-up (48).

In the Walking and Leg Circulation Study, higher sVCAM-1 levels among PAD participants in the WALCS II cohort were associated with a higher rate of death, cardiovascular events, or graft stenosis (41,44). Among participants undergoing lower extremity revascularization, higher baseline levels of CRP are associated with a higher rate of death, cardiovascular events, or graft failure (46).

IL-6 is a pro-inflammatory cytokine. Amplifies the inflammatory cascade, stimulates macrophages, and promotes smooth muscle cell proliferation. The principal pro-coagulant cytokine. Higher IL-6 levels among community-dwelling men and women in the Edinburgh Artery Study were associated with greater declines in the ABI at 5- and 12-year follow-up (28). Higher IL-6 levels among PAD participants in the WALCS II cohort are associated with smaller calf muscle area and greater calf muscle percentage fat (33). Higher IL-6 levels among PAD participants in the WALCS II cohort were associated with poorer 6-min walk performance and slower walking speed (34).

ICAM-1 is a constitutively expressed by endothelial cells and binds cells of hematopoietic origin, including leukocytes. Higher sICAM-1 levels among community-dwelling men and women in the Edinburgh Artery Study were associated with greater declines in the ABI at 12-year follow-up (28). Higher sICAM-1 levels among PAD participants in the WALCS II cohort were associated with slower fast-paced walking velocity (34).

SAA is an inflammatory protein. Marker of inflammation that is synthesized by hepatocytes within hours in response to stimulation by IL-6 and other inflammatory cytokines. Higher levels of SAA are associated with higher all-cause and cardiovascular mortality in people with PAD. High SAA levels are more strongly associated with near- than later-term mortality in people with PAD (42). Greater annual increases in SAA levels are associated with higher cardiovascular mortality during the year after the SAA increase (42). Increasing levels of SAA after lower extremity angioplasty and stent placement are associated with a higher incidence of stent restenosis (46).

VCAM-1 is a transmembrane glycoprotein that binds β-integrins on leukocytes and draws them to atherosclerotic plaque. Higher sVCAM-1 levels among PAD participants in the WALCS II cohort are associated with smaller calf muscle area and greater calf muscle percentage fat (33). Higher sVCAM-1 levels among PAD participants in the WALCS II cohort were associated with poorer 6-min walk performance and slower walking speed (34).

Inflammatory biomarkers are associated with more adverse outcomes in people with PAD (34). Among 423 people with PAD identified from noninvasive vascular laboratories, higher levels of CRP, IL-6, sVCAM-1, and homocysteine were associated with a smaller calf muscle area, lower calf muscle density, and/or higher calf muscle percentage fat as measured by computed tomography (Fig. 2). These findings remained statistically significant after adjusting for the ABI and physical activity levels. However, specimens from 2 healthy individuals showed nearly a complete absence of CRP. The clinical significance of local production of CRP from atherosclerotic plaque is unknown.

Inflammation Is Associated With Functional Impairment and Functional Decline in PAD

At least 2 mechanisms exist by which inflammation may contribute to lower extremity functional impairment and faster functional decline in people with PAD. First, inflammation may contribute to the progression of lower extremity atherosclerosis, thereby promoting ischemia of lower extremity skeletal muscle and impairing lower extremity functional performance. Second, chronic inflammation may directly and adversely affect lower extremity skeletal muscle in PAD, independently of lower extremity ischemia. In support of the second potential causal pathway, animal and in vitro data suggest that inflammatory cytokines may alter skeletal muscle homeostasis by inhibiting repair after tissue injury and by promoting skeletal muscle proteolysis (31–33). In addition, 1 prior study shows that higher levels of inflammatory biomarkers are associated with more adverse calf muscle characteristics in people with PAD (34). Among 423 people with PAD identified from noninvasive vascular laboratories, higher levels of CRP, IL-6, sVCAM-1, and homocysteine were associated with a smaller calf muscle area, lower calf muscle density, and/or higher calf muscle percentage fat as measured by computed tomography (Fig. 2). These findings remained statistically significant after adjusting for the ABI and physical activity levels.
Further prospective studies are needed to determine whether elevated levels of inflammatory biomarkers are associated with greater declines in muscle mass and quality in patients with PAD.

Elevated levels of inflammatory biomarkers are also associated with greater functional impairment and faster functional decline in people with PAD (35,36). Among men and women with PAD, higher levels of IL-6, D-dimer, sVCAM-1, CRP, and homocysteine are associated with slower walking velocity and shorter distances achieved in the 6-min walk test even after adjustment for the ABI, comorbidities, and other potential confounders (35)(Fig. 3). Among people with and without PAD, those with high levels of 3 or more inflammatory biomarkers or D-dimer had a greater decline in 6-min walk performance and other functional performance measures at 3-year follow-up compared with those with uniformly low levels of these biomarkers at baseline (36). Recent data also show that greater annual increases in CRP levels are associated with faster declines in annually measured 6-min walk performance over 3-year follow-up among participants with PAD (37).

In summary, higher and increasing levels of inflammatory biomarkers are associated with adverse functional outcomes in men and women with PAD, even after adjusting for disease severity and other confounders.

Statin medications reduce inflammation and lower CRP levels. Therefore, statins may mitigate against the association of elevated levels of inflammatory biomarkers with functional impairment and decline in PAD. In support of this hypothesis, 2 small randomized controlled clinical trials show that statin therapy improves treadmill walking performance in people with PAD (38,39). In 1 clinical trial, 86 PAD participants with intermittent claudication and total cholesterol levels >220 mg/dl were randomized to receive 40 mg simvastatin versus placebo. At 6-month follow-up, the simvastatin group had increased their maximal walking distance by 126 m more than the placebo group (p < 0.001) (39). A separate study randomized 60 participants with intermittent claudication and low-density lipoprotein cholesterol levels >125 mg/dl to receive 40 mg simvastatin daily versus placebo. Treadmill time to onset of intermittent claudication symptoms increased significantly in the simvastatin group at 6- and 12-month follow-up, compared with the placebo group (39). However, the largest randomized controlled clinical trial to study statin use and treadmill walking performance in people with PAD yielded mixed results (40). In this study, 354 patients with intermittent claudication were randomized to placebo, 10 mg of atorvastatin daily, or 80 mg of atorvastatin daily. There were no differences in the primary outcome measure of change in maximal treadmill walking distance at 12-month follow-up between the 2 atorvastatin groups and the placebo group (40). However, significant improvement in the secondary outcome measure of pain-free treadmill walking time was observed in the 80-mg atorvastatin group compared with the placebo group (40). In summary, inflammation-reducing statin therapies are associated with inconsistent improvement in treadmill walking performance.

Circulating Biomarker Levels and Critical Limb Ischemia

Circulating inflammatory biomarker levels may be higher in PAD patients with critical limb ischemia compared with those with intermittent claudication (41,42). One study showed that 30 patients with critical limb ischemia had higher CRP levels than 132 patients with intermittent claudication (p = 0.001) and 40 patients without PAD (p < 0.001) (7.17 mg/l for critical limb ischemia vs. 3.4 mg/l for intermittent claudication vs. 1.04 mg/l for control participants, respectively). Another study of 91 patients undergoing lower extremity revascularization showed higher levels of inflammatory biomarkers in the 50 patients with critical limb ischemia compared with those without critical limb ischemia. However, neither study adjusted for confounders,
such as diabetes mellitus and smoking. Thus, differences in CRP levels between participants with versus without critical limb ischemia may be explained by differences in clinical characteristics between these groups. Alternatively, tissue necrosis, which promotes an acute phase response, may be a stimulus for increases in CRP and other inflammatory biomarkers in patients with critical limb ischemia. Further study is needed.

**Biomarkers and Cardiovascular Events in PAD**

Several studies show that elevated biomarker levels are associated with increased cardiovascular event rates and mortality in people with PAD (43–45). In one cohort of 377 men and women with PAD, higher baseline levels of CRP and serum amyloid A (SAA) were significantly associated with higher all-cause and cardiovascular disease mortality, even after adjusting for cardiovascular disease risk factors and history of cardiovascular disease (43). Other studies have also shown that increased CRP levels are associated with higher cardiovascular event rates and mortality (44,45).

**Statin Therapy May Mitigate the Association of Elevated CRP With Cardiovascular Events in PAD Patients**

Showing a causal association of inflammatory biomarkers with mortality requires a clinical trial demonstrating that therapeutic lowering of inflammatory biomarkers lowers cardiovascular and all-cause mortality rates. No such clinical trial has been performed among patients with PAD. Among men and women without PAD, the JUPITER (Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin) trial randomized those with CRP levels >2.0 mg/l and low-density lipoprotein cholesterol levels <130 mg/dl to high-dose rosuvastatin therapy versus placebo (46). Rosuvastatin therapy was associated with a 44% reduction in all-cause and cardiovascular disease mortality and a concomitant reduction in CRP levels.

Although clinical trials such as JUPITER have not been performed in PAD populations, one observational study showed that statin therapy may be more beneficial among PAD patients with elevated CRP levels compared with PAD patients with lower CRP levels (45). Figures 4 and 5 show findings from an observational study of 515 PAD patients undergoing lower extremity revascularization. Those with higher baseline CRP levels experienced greater all-cause and cardiovascular disease mortality rates than PAD patients with lower CRP levels (45). Among all 515 patients with PAD, statin use was associated with lower risk of death or myocardial infarction at follow-up (hazard ratio: 0.48 for statin users vs. nonusers, p = 0.004), adjusting for known and potential confounders (Fig. 4). When PAD patients were stratified by baseline CRP level, the protective association of statins with cardiovascular events was observed primarily among those with elevated CRP levels at baseline (Fig. 5). These observational findings suggest that lowering high levels of inflammation may be a mechanism by which statins reduce cardiovascular and all-cause mortality in patients with PAD. However, clinical trials in participants with PAD are needed to verify this hypothesis.
Elevated Inflammatory Markers Are More Closely Associated With Near-Than Later-Term Mortality in People With PAD

Traditional atherosclerotic disease risk factors, such as diabetes mellitus and hyperlipidemia, are associated with increased cardiovascular event rates over long-term follow-up. In contrast, inflammatory markers may be better predictors of near-term cardiovascular events than traditional cardiovascular disease risk factors. Inflammatory biomarkers, such as CRP, can be rapidly synthesized within hours in response to inflammatory stimuli. Inflammatory biomarkers and cytokines can promote plaque instability and rupture in the near term and/or they may signal the presence of vulnerable, active plaque (9,10). Thus, elevated or increasing levels of these biomarkers may signal increased risk for near-term acute coronary events.

A recent observational study assessed whether elevated levels of CRP and SAA were more closely associated with near-term than with later-term mortality in men and women with PAD. Among 377 men and women with PAD, associations of elevated biomarker levels with mortality were studied during the following time intervals: 1) within 1 year after the biomarker measure; 2) between 1 and 2 years after the biomarker measure; and 3) between 2 and 3 years after the biomarker measure (43). In fully adjusted analyses, higher levels of each biomarker were associated significantly with greater all-cause and cardiovascular mortality during the year immediately after each biomarker measurement and during the time interval between 1 and 2 years after biomarker measurement (Fig. 6). However, higher levels of the biomarkers were not associated with all-cause or cardiovascular mortality during the interval between 2 and 3 years after the biomarker measurement (43).
Elevated CRP Levels Are Associated With Poorer Patency After Lower Extremity Revascularization

Higher levels of CRP are associated with increased adverse event rates after lower extremity revascularization. Among 91 patients undergoing lower extremity bypass surgery (including 55% with critical limb ischemia), patients with a baseline CRP level >5.0 mg/l had significantly higher rates of death, cardiovascular events, and graft-related events compared with patients with a baseline CRP level <5.0 mg/l during a mean follow-up of 342 days (42). Patients with higher baseline levels of fibrinogen and SAA did not have increased event rates. Other patient characteristics, including ABI, statin use, and demographics, did not predict events after revascularization (42).

A separate study of 172 consecutive patients with PAD who underwent percutaneous angioplasty of the superficial femoral or popliteal arteries for Fontaine stages IIa, IIb, or III showed that greater increases in levels of CRP and SAA during the first 48 h after angioplasty were associated with higher rates of restenosis and lower ABI levels at 6-month follow-up (47). Mechanisms of these associations may relate to a higher perivascular inflammatory response, perhaps related to intimal and medial artery injury after balloon angioplasty, that increases circulating levels of inflammatory biomarkers and culminates in restenosis (47). In summary, increasing levels of CRP and SAA during the hours after lower extremity angioplasty are associated with higher restenosis rates.

Proteomic Profiling and PAD

Recently, proteomic profiling has been performed in an attempt to find a biomarker to aid in the diagnosis of PAD (48). This study used surface-enhanced laser desorption/ionization time-of-flight mass spectrometry and found that 11 of 1,619 protein peaks were stronger in 45 participants with PAD compared with 43 without PAD (48). Six of these protein peaks represented beta-2 microglobulin (B2M), a 12-kDa protein. Higher levels of B2M in PAD compared with non-PAD people were established in 2 confirmatory studies involving 40 and 237 participants, respectively (48). However, further study is needed in larger cohorts to determine whether B2M or other unidentified biomarkers are sufficiently sensitive and specific for diagnosing PAD.

Genetic Approaches to PAD

Several family studies have assessed the heritability of PAD. The National Heart, Lung, and Blood Institute Twin Study assessed the heritability of PAD in 94 monozygotic and 90 dizygotic white male twin pairs (49). Concordance rates for twin-pair similarity for low ABI were 33% for monozygotic pairs and 31% for dizygotic pairs. Given that the overall prevalence of PAD in the study population was 8.2%, findings indicate that the twin of a participant with PAD was 4 times more likely to have PAD than a randomly selected individual. Results suggested that genetic factors determined approximately 48% of the variability in ABI after adjustment for cardiovascular disease risk factors. However, twin-pair similarity for PAD was not higher in monozygotic than in dizygotic twins, suggesting a more limited role for heritability than the overall high relative risk for PAD associated with twin status (49).

A subsequent study assessed the heritability of low ABI among participants in the Framingham Offspring Study. Participants were 1,097 men and 1,189 women from 999 families. Heritability of the ABI was determined by calculating intracl class correlation coefficients for sibling pairs using the family correlations procedure in the Statistical Analysis for Genetic Epidemiology (SAGE) (version 3.1, Case Western Reserve University, Cleveland, Ohio) and by using variance-components methods according to the Sequential Oligogenic Linkage Analysis Routines (SOLAR)
computer package (version 1.4.1., Southwest Foundation for Biomedical Research, San Antonio, Texas). Using the SAGE method, the estimated heritability of the ABI after adjusting for cardiovascular disease risk factors was 22%. Results of the SOLAR analyses showed that approximately 21% of the interindividual variability in the ABI was attributable to genetic determinants. Overall, genetic determinants contributed to 21% of the variability in the ABI, a modest heritable effect, and cardiovascular disease risk factors contributed to 14% of the variability. Results also indicated that 65% of the interindividual variability in the ABI could not be explained by genetic or environmental determinants (50).

A third family study, the GENOA (Genetic Epidemiology Network of Arteriopathy) study, assessed heritability of the ABI in 1,310 African Americans and 796 non-Hispanic white subjects participating in a hypertensive sibling study (51). Unadjusted results identified heritability for the ABI of 35.1% in African Americans and 35.7% in non-Hispanic white subjects. After adjustment for cardiovascular disease risk factors, these estimates of heritability were reduced to 19.5% (p = 0.002) and 21.2% (p = 0.006), respectively (51). Together, these 3 family studies suggest a moderate, significant heritability for PAD.

A genetic marker for PAD could identify individuals at increased risk for PAD who may benefit from targeted therapies to delay or prevent development of lower extremity atherosclerosis and its sequelae. Genetic determinants of PAD may also uncover proteins implicated in the pathophysiology of lower extremity atherosclerosis, thereby identifying mechanisms for the development and progression of lower extremity atherosclerosis. A better understanding of mechanisms of development and progression of lower extremity atherosclerosis may ultimately help identify new therapies for prevention and treatment of PAD.

To date, no definitive genetic markers have been identified for PAD. A relatively small number of case-control studies, recently summarized (52), have assessed associations of specific gene polymorphisms with the presence of PAD. However, results have not established a consistent genetic marker for PAD. Genome-wide linkage studies of PAD have been performed in the GENOA trial and in a study of Icelandic patients with PAD (51,53). The GENOA study did not identify any gene linkages that met the criterion for a significant genetic marker of PAD. The study of Iceland patients identified a linkage on chromosome 1p in association with PAD that met the criterion for a significant genetic marker (53). However, this finding has yet to be confirmed in a separate population. A recent genome-wide analysis scan of 10,995 Icelandic smokers identified a genetic variant on chromosome 15q24 in the nicotine acetylcholine receptor gene cluster that was associated significantly with both quantity of cigarette smoking and presence of PAD (54). This finding represents a potential example of a gene–environment interaction in the development of PAD.

**Summary and Recommendations for Clinicians**

Inflammation is an integral component of the development and progression of atherosclerosis. Both higher and increasing levels of inflammation are associated with more adverse outcomes in men and women with PAD. Associations between distinct inflammatory biomarkers with outcomes in PAD differ, suggesting that inflammatory biomarkers play different roles in the pathophysiology of lower extremity and cardiovascular outcomes in patients with PAD. Higher levels of inflammatory biomarkers in patients with PAD compared with those without PAD may contribute to the higher rate of cardiovascular events in people with PAD that has been observed independent of history of other cardiovascular disease and cardiovascular disease risk factors. Despite the consistent associations of elevated inflammatory biomarkers with adverse outcomes in PAD, there are insufficient data to conclude that inflammatory biomarkers are causally related to adverse outcomes in PAD. Similarly, insufficient data exist to support targeting PAD patients who have higher levels of inflammation with more intensive secondary prevention therapies, such as antiplatelet therapies or statins. Although available data suggest a moderate degree of heritability in PAD, a consistent genetic marker for PAD has not been identified.

**Reprint requests and correspondence:** Dr. Mary M. McDermott, Northwestern University Feinberg School of Medicine, 750 North Lake Shore Drive, 10th Floor, Chicago, Illinois 60611. E-mail: mdm608@northwestern.edu.

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