Using sophisticated tools of mouse genetics and bioinformatics, a multidisciplinary group of scientists, Dokun et al., have shown that a region of chromosome 7 of a C57BL/6 mouse strain contributes a dominant protective allele (or alleles) that increases limb perfusion and reduces limb necrosis after induction of experimental hindlimb ischemia. The investigators hope that the application of these methods will lead to the identification of genes that might predict the risk of critical limb ischemia in patients with peripheral arterial disease (PAD). Beyond providing the potential to learn more about the genetic and molecular basis of PAD, the genetic and bioinformatics approaches taken by this multidisciplinary team of scientists provide a pathway that could have broader applications to advance the study of many aspects of cardiovascular diseases.

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The mouse remains the most powerful experimental model to study the genetic basis of human disease.2,3 The techniques used in this study represent an evolution from those that were first used in the early 1900s with the development of inbred strains of mice. Subsequently, the ability to analyze the function of single-gene mutations by knockout or knock-in techniques and by regulatable site-specific gene expression in inbred strains of mice has allowed many major advances in understanding the molecular basis of human disease. Despite their power, these techniques have remained limited to studies that analyzed the function of a single gene. In contrast, quantitative trait loci analysis permits the study of phenotypes that vary within a population and are influenced by multiple genes. Since most common human diseases result from such quantitative traits, the phenotype observed often results from the compound effects of multiple genes, the interactions of these genes with other genes, and then the effect of the environment on their collective expression. Thus, it is likely that most human diseases, including cardiovascular diseases, will require complex quantitative trait analysis if we are to reach a deeper understanding of the genetic, molecular, and cellular basis of these diseases and how they vary from normal physiological processes.2,3 A recent review provides a comprehensive overview of the available resources and tools in mouse genetics, particularly those for quantitative trait analysis.2

By attempting to identify genes that might influence the clinical course of PAD, the study by Dokun et al. addresses what is arguably the largest gap in our understanding of any cardiovascular disease. PAD, chronic atherosclerotic occlusive disease of the arteries exclusive of the intracerebral and coronary arteries, affects approximately 12 million people in the United States. Surprisingly, 75% of individuals diagnosed with PAD are asymptomatic. Consequently, PAD is likely the most underdiagnosed and undertreated cardiovascular disease. The age-adjusted prevalence of PAD is 12% in the United States and approaches 20% by 70 years of age.4 The Framingham Heart Study identified age, family history, male gender, serum cholesterol level, hypertension, tobacco use, diabetes mellitus, and coronary artery disease as risk factors for PAD.5 Recently, C-reactive protein and the ratio between total cholesterol and high-density lipoprotein cholesterol are the strongest independent predictors of symptomatic PAD.6

PAD is often defined clinically as an ankle-brachial index (ABI) of ≤0.90. Interestingly, both symptomatic and asymptomatic forms of PAD show equally poor prognosis. These findings of high morbidity and mortality in symptomatic and asymptomatic patients are consistent across many studies.7-9 In addition, a linear relationship has been identified between a 0.1 decrement in the ABI measurement and the associated increased risk of adverse cardiovascular events and death. It is worth noting that the majority of patients in such studies are asymptomatic.

Despite the substantially increased risk of cardiovascular morbidity and mortality, the risk for loss of the affected limb in patients with PAD is quite low. Less than 10% of patients with PAD have symptoms of critical limb ischemia (ie, threatened limb loss due to the presence of gangrene, ischemic ulceration, or rest pain). However, the overall mortality of patients with intermittent claudication is 30%, 50%, and 70% at 5, 10, and 15 years, respectively. Worse, in patients with critical limb ischemia, 20% die within 6 months of the diagnosis, and the annual mortality rate thereafter is ≈25%.4 Finally, in the most recent results of the Resource Utilization Among Congestive Heart Failure Patients (REACH) study, patients with PAD had 1-year event rates for cardiovascular death, myocardial infarction, and stroke that were 30% higher than those for patients with coronary artery disease or carotid artery disease. Thus, PAD poses a much greater risk to life than to limb.10
Recently, Knowles and colleagues reviewed what is known of the genetic susceptibility to PAD and concluded that our understanding of the genetic basis of PAD is quite limited.11 Neither linkage analysis nor linkage associations studies have convincingly uncovered any genes that either identify patients at risk for PAD or predict their clinical course. The major weakness of these published studies is the small number of patients studied. Nonetheless, it is likely that there exists a set of variants that specifically alter susceptibility to PAD. A study by Valentine and colleagues showed that both premature coronary artery disease and asymptomatic lower-extremity vascular disease are more common in probands with premature PAD, indicating a strong familial aggregation of vascular disease.12 No doubt, susceptibility to PAD is likely the result of alleles in multiple genes interacting with a variety of environmental influences. An illustrative example is the Apolipoprotein E polymorphisms and diabetes patients, who are susceptible to developing PAD.13 Among ever smokers, no association existed between Apolipoprotein E polymorphisms and diabetes mellitus. However, among never smokers, both Apolipoprotein E and diabetes mellitus affected PAD prevalence.

Dokun and colleagues began with the well-established observation that different mouse strains vary widely in their capacity to respond to the induction of hindlimb ischemia.14,15 Helisch and colleagues have shown that C57BL/6 mice have larger and more abundant preexisting collateral arteries in the hindlimb than do BALB/c mice and thus respond more robustly to experimental hindlimb ischemia.15 On the basis of these known strain differences, they hypothesized that sequence variations in specific genes expressed in C57BL/6 and BALB/c mice may account for the observed phenotypic differences. In order to test their hypothesis, they attempted to identify the quantitative trait locus for measurable traits responsible for phenotypic differences in recovery of perfusion and the development of necrosis after induction of hindlimb ischemia.

The F1 offspring of the cross of the C57BL/6 and BALB/c mice showed no difference in recovery of limb perfusion or in the incidence of necrosis from that seen in the C57BL/6 mice. Thus, it was clear that a dominant allele was responsible for the lack of tissue loss and increased perfusion. N2 progeny were derived from 1-way mating of F1 males and BALB/c females. Genome-wide linkage scans for necrosis and perfusion 21 days after induction of hindlimb ischemia were then examined for the 239 SNP that differed between the C57BL/6 and BALB/c. Linkage analysis showed that all linkage profiles were flat for necrosis and perfusion at day 21 except for chromosome 7. The study identified a segment spanning approximately 31 Mb with a logarithm of odds score of 7.96 for necrosis and a logarithm of odds score of 3.71 for perfusion ratios. A detailed internal map of chromosome 7 identified a single quantitative trait locus at SNP marker RS1347951.

The authors hypothesized that the variation of chromosome 7 recovery locus was most likely due to genes that matched within the ancestral haplotype blocks, which are shared between the BALB/c and AJ strains but differ from the C57BL/6 strains. Using a dense microsatellite and SNP map of the broad chromosome 7 locus, they identified 2 primary areas of interest in haplotype blocks of approximately 1.7 Mb and 1.9 Mb, which contain 21 and 16 known or predicted genes, respectively. Subsequent to this, a chromosome substitution strain was created from the A/J mouse strain. This resulted in a C57BL6J-Chr7^A/J chromosome substitution strain. The A/J strain had been shown to have a perfusion recovery pattern and incidence of necrosis nearly identical to that of the BALB/c. Proving the authors’ hypothesis, the studies of the chromosome substitution strain showed that it had decreased perfusion and increased necrosis in response to hindlimb ischemia similar to that seen in the BALB/c strain.

The conclusive findings of the study by Dokun et al.1 that a quantitative trait locus, Lsq-1, on chromosome 7 of the C57BL6 mouse strain contributes a protective allele to prevent limb necrosis after induction of hindlimb ischemia, is potentially of substantial clinical significance. This finding supports the hypothesis that inherited genetic variations may influence the phenotypic outcome after femoral artery occlusion. Future studies identifying the specific genes responsible for the impaired recovery of BALB/c mice should prove highly instructive. It may also be possible to study the human orthologs once these genes are identified in the mouse and perform linkage association studies to investigate whether these genes can predict the severity of disease in patients with PAD.

Finally, future experimental studies that show the way in which the Lsq-1 locus influences the known mechanisms that regulate collateral artery enlargement should prove to be valuable. These mechanisms include mechanical factors such as increases in shear and wall stresses, the inflammatory response, particularly monocyte infiltration into the region of collateral arteries, and finally, the homing and infiltration of bone marrow–derived cells such as the hemangiocyte. All of these cells exert paracrine effects, secreting many proteins of which endothelial nitric oxide synthase and vascular endothelial growth factor have been the best studied.16–18

One question about this study from the standpoint of the applicability to mechanisms determining the development of symptoms in patients with PAD is the known lack of a direct correlation between the degree of limb perfusion as defined by an ABI and the presence or absence of symptoms. As mentioned above, the vast majority of patients who have an ABI <0.4 (ie, in the range within which critical limb ischemia occurs) have no symptoms. Thus, no direct correlation exists between perfusion and symptoms or specifically between the level of perfusion and the risk of necrosis as was shown in this study.

None of these questions should obscure the substantial importance of this study performed by a multidisciplinary team of scientists who successfully identified a new complex trait analysis that predicted the severity of disease outcome in a mouse model of hindlimb ischemia. This is an important first step toward understanding the genetic basis of a highly lethal disease that is increasing in prevalence.

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References

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