Atherosclerotic cardiovascular disease (ASCVD) frequently clusters in families. Although environmental factors such as diet, exercise, and smoking play a role in ASCVD, genetic factors are a major determinant of ASCVD risk. For example, in a study of Swedish twins, males with a dizygotic twin who died of premature coronary heart disease had a 4.3-fold increased risk of also dying from coronary heart disease, and if the twin was monozygotic, this risk increased to 14.9-fold. The genetic risk of ASCVD is conferred in part through known metabolic risk factors such as hypertension, dyslipidemia, and diabetes mellitus. However, these risk factors alone do not account for the entire genetic contribution to risk of ASCVD, and there remains substantial interest in the identification of additional genetic cardiovascular risk factors.

Like humans, mouse strains differ considerably in their genetic predisposition to atherosclerosis. Most mouse strains are resistant to atherosclerosis even when fed an atherogenic diet; the C3H strain is a prototypical-resistant mouse strain. In contrast, C57BL/6J mice have been extensively studied as the mouse strain that is most susceptible to atherosclerosis when fed an atherogenic high-fat, high-cholesterol, cholic acid-containing diet. Initially, the focus on C57BL/6J mice centered on differences in plasma lipid levels, especially reduced HDL cholesterol levels, in response to the atherogenic diet and attempts to map gene loci linked to the phenotype of low HDL cholesterol. However, previous studies did not distinguish whether these differences in lipid profiles were restricted to particular dietary challenges or reflect an underlying genetic factor that contributes to atherosclerosis susceptibility. Furthermore, recent studies have suggested that C57BL/6J mice differ from less-susceptible strains in their response to inflammatory stimuli. Given the interest in atherosclerosis as an inflammatory disease, this has led to the hypotheses that C57BL/6J mice may be genetically susceptible to certain inflammatory responses that promote atherosclerosis.

There are a variety of cell types involved in atherosclerosis, including endothelial cells, vascular smooth muscle cells, monocytes and macrophages, and T lymphocytes. Genetic variation that affects any one of these cell types (including inflammatory responses) could potentially influence susceptibility to atherosclerosis. If the major genetic differences leading to susceptibility to atherosclerosis between C57BL/6J mice and other strains could be attributed to one or more defined cell type, it would be a major advance in the search for genes influencing atherosclerosis. The study by Shi et al in this issue of Circulation Research represents an important advance in defining the cellular compartments that confer genetic susceptibility to atherosclerosis in mice. They demonstrate in bone marrow chimeras that the genotype of nonhematopoietic cells dominates over the genotype of the cells of the hematopoietic compartment in determining the extent of atherosclerosis. In this study, the authors addressed whether differences between susceptible C57BL/6J mice and resistant C3H mice could be attributed to genetically determined differences in plasma lipoproteins, cells of hematopoietic origin (including inflammatory cells such as monocytes and macrophages and T cells), or nonhematopoietic cells, including cells indigenous to the vessel wall.

To exclude differences in plasma lipids as the sole basis for the difference in atherosclerosis between these 2 strains, Shi et al used apoE-deficient mice, a well-known and extensively studied mouse model of atherosclerosis that develops atherosclerosis on a chow diet. The authors bred apoE-deficient mice that had either 100% C57BL/6J or 96% C3H/HeJ genetic background. Importantly, the studies demonstrated that in mice with an apoE-deficient background that were fed a chow diet, there were not major differences in plasma lipids; specifically, HDL cholesterol levels were comparable and total cholesterol levels were higher in mice with the C3H/HeJ background. Nevertheless, the studies found that under these conditions, mice with the C57BL/6J background developed markedly more atherosclerosis than mice with the C3H/HeJ background, confirming major strain differences in atherosclerosis susceptibility, and indicating that these differences do not reside in differences in plasma lipid levels.

In a second series of experiments, Shi et al addressed the question of whether the genetic differences in susceptibility to atherosclerosis reside within cells of hematopoietic origin, nonhematopoietic cells, or both. In these studies, the authors used mice that were not from the apoE-deficient background and therefore required feeding of the atherogenic diet. They generated chimeric mice by performing bone marrow transplantation of C57BL/6J bone marrow into C3H/HeJ mice and C3H/HeJ marrow into C57BL/6J mice (along with the appropriate same-strain controls). To circumvent the incompatibility at the MHC loci between C3H/HeJ (H2k) and C57BL/6J
(H2b) mice, the chimeras were generated using H2b MHC congenic C3H/SW mice, thus eliminating MHC as an important genetic factor in this model. C57BL/6J mice that received C3H/SW bone marrow were not protected from atherosclerosis, and C3H/SW mice that received C57BL/6J bone marrow did not develop increased atherosclerosis. Therefore, a key finding in this study was that the extent of atherosclerosis in the chimeric animals was determined by the genotype of the host rather than the genotype of the marrow donor, indicating that under these conditions the dominant genetic differences in atherosclerosis susceptibility between these 2 strains of mice are not manifested by the hematopoietic cells. However, because these studies were carried out with wild-type instead of apoE-deficient mice, genetic differences in response to the atherogenic diet were not completely eliminated. In fact, C57BL/6J host mice had lower HDL cholesterol levels than C3H/SW host mice. Furthermore, other metabolic differences could exist between these strains that are not detected by simple measurement of plasma lipids. Therefore, it is possible that genetic differences in nonhematopoietic tissues that are important for systemic metabolism, such as adipose, skeletal muscle, kidney, and liver, could be responsible for some of the difference in atherosclerosis susceptibility between these 2 strains. Nevertheless, the findings are also consistent with the hypothesis that genetic differences in vessel wall cells account for some of the variation in atherosclerosis.

To test this hypothesis, Shi et al9 turned to ex vivo studies of endothelial cells isolated from C57BL/6J mice and C3H/HeJ mice. Interestingly, they found that endothelial cells from C57BL/6J mice exhibited substantial induction of a limited panel of proinflammatory genes (MCP-1, M-CSF, VCAM-1, and heme oxygenase-1) in response to minimally modified native LDL had little effect on gene expression in endothelial cells from either strain, whereas LPS induced substantial but specific inflammatory stimulus, namely MM-LDL. Interestingly, native LDL had little effect on gene expression in endothelial cells to modified LDL could be one source of differences in genetic susceptibility to atherosclerosis. Defining the molecular mechanism of this phenomenon will be a critical next step in unraveling this fascinating story.

There are a number of important implications of this study9 for additional research in the genetics of atherosclerosis. One immediate question is whether other nonsusceptible strains demonstrate the same differences with C57BL/6J mice in the endothelial response to MM-LDL. In addition, microarray studies of endothelial cells will cast a much wider net with regard to the strain differences in endothelial response to MM-LDL. As noted above, extending these studies to primary vascular smooth muscle cells would add another dimension. It may be possible to genetically map the differences in endothelial cell response to specific loci and ultimately determine the specific genes responsible for the difference in this response. Definitive proof will come with endothelial-specific transgenic and gene knockout studies that confer susceptibility to resistant strains and resistance to susceptible strains through the genetic manipulation of the expression of a single or a limited number of genes. However, it is important to keep in mind that there are many genetic differences between B6 and C3H mice, and although these studies suggest that the endothelial response is one source of variation in atherosclerosis susceptibility, there are likely to be other genetic differences affecting genes in other cells and tissues that may influence atherosclerosis susceptibility as well.
The present study also has implications for research in humans. Although difficult, it may be possible to isolate primary microvascular endothelial cells from human subjects with different genetic atherosclerosis susceptibility to determine whether similar ex vivo differences in response to inflammatory stimuli exist. Even skin fibroblasts may be able to be used as a surrogate cell type for assessing differences among individuals, similar to their use in a variety of other genetic conditions in which the fibroblast is not the primary cell involved in the disease but is easily obtained. Furthermore, it may be of interest to prioritize inflammatory response genes in genetic association studies of ASCVD. One can imagine that once specific susceptibility genes are identified, single nucleotide polymorphisms in these genes could be used as part of an atherosclerosis genetic-susceptibility panel to predict future risk of ASCVD. Finally, these results support the concept of developing new systemic antiatherosclerotic therapies that are targeted toward cells in the vessel wall to reduce their response to inflammatory stimuli. If the mechanism for responsiveness can be determined, it may provide an upstream target for therapy without having to define all the downstream gene targets. Such therapies could be useful in individuals with genetic predisposition to vascular inflammatory responses as well as more broadly applicable for all types of atherosclerosis, just as statins are protective even in persons who have average cholesterol levels.

In summary, research into the genetic basis of atherosclerosis susceptibility is still in its infancy. Studies in mice with different genetic susceptibility of atherosclerosis will continue to provide major insights that will drive research in humans. The studies by Shi et al9 are an elegant demonstration of the power of murine studies and provide exciting new directions for investigation. The future holds the promise of using genetics to determine with much greater precision which individuals are at risk for developing premature atherosclerosis and to guide the specific development and use of novel therapies to prevent and treat ASCVD.

Acknowledgments
The authors would like to thank Dr Carolyn Cuff (The Wistar Institute) for helpful discussions.

References

Key Words: atherosclerosis • genetics • lipids • inflammation
Genetic Susceptibility to Atherosclerosis: Insights From Mice
Daniel J. Rader and Ellen Puré

Circ Res. 2000;86:1013-1015
doi: 10.1161/01.RES.86.10.1013

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/86/10/1013

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/