Genetic Susceptibility to Atherosclerosis
Insights From Mice

Daniel J. Rader, Ellen Puré

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.1 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.2 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.3 Initially, the focus on C57BL/6J mice centered on differences in plasma lipid levels, especially reduced HDL cholesterol levels, in response to the atherogenic diet4 and attempts to map gene loci linked to the atherosclerotic cardiovascular disease (ASCVD) frequency at the MHC loci between C3H/HeJ (H2k) and C57BL/6J (H2d) strains 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.1 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.

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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 al9 in this issue of Circulation Research represents an important advance in defining the cellular compartments that confer genetic susceptibility to atherosclerosis in mice. They demonstrated 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 al9 used apoE-deficient mice, a well-known and extensively studied mouse model of atherosclerosis that develops atherosclerosis on a chow diet.10 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 al9 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/SW mice and C3H/SW marrow into C57BL/6J mice (along with the appropriate same-strain controls). To circumvent the incompatibility at the MHC loci between C3H/HeJ (H2d) and C57BL/6J...
Toll-like receptor 4 and exhibit normal responses to the dose of LPS used in these experiments. The status of LPS strains derived from C57BL/6J inflammatory cells. Remarkably, when recombinant inbred assayed are primarily associated with the recruitment of LDL (MM-LDL), whereas endothelial cells from C3H/HeJ and heme oxygenase-1) in response to minimally modified C57BL/6J mice exhibited substantial induction of a limited panel of proinflammatory genes (MCP-1, M-CSF, VCAM-1, C57BL/6J mice were not protected from atherosclerosis. Therefore, 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 al 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 LDL (MM-LDL), whereas endothelial cells from C3H/HeJ mice did not. It is interesting that the first 3 of the genes assayed are primarily associated with the recruitment of inflammatory cells. Remarkably, when recombinant inbred strains derived from C57BL/6J×C3H/HeJ crosses were studied, the endothelial response to MM-LDL cosegregated with atherosclerosis. These results raise the intriguing possibility that at least part of the difference between these 2 strains in atherosclerosis susceptibility relates to genetic difference in the proinflammatory response of endothelial cells to a specific inflammatory stimulus, namely MM-LDL. Interestingly, native LDL had little effect on gene expression in endothelial cells from either strain, whereas LPS induced substantial but similar gene expression in endothelial cells from both strains.

Although these findings are provocative, a number of issues about these endothelial cell experiments should be raised. First, a comparable response to lipopolysaccharide (LPS) of endothelial cells from C57BL/6J mice and C3H/HeJ mice is somewhat surprising, given that cells isolated from the latter are known to be hyporesponsive to LPS due to a missense mutation in the gene encoding for the LPS receptor Toll-like receptor 4. This was likely because of the high dose of LPS used in these experiments. The status of LPS responsiveness may be of less concern in the in vivo experiments, because C3H/HeN mice, which express functional Toll-like receptor 4 and exhibit normal responses to LPS, are also resistant to atherosclerosis. However, additional studies to directly address the role of inflammatory responses to infection in promoting atherosclerosis will be of interest. Second, these experiments were performed under static conditions, whereas endothelial cells are normally exposed to flow conditions. Demonstration of differences between the 2 strains in endothelial gene expression under different flow conditions ex vivo or in vivo, perhaps after injection of MM-LDL, would be of interest. Third, vascular smooth muscle cells also participate in atherogenesis, and ex vivo studies of inflammatory gene expression in this cell type would complement and extend the studies in endothelial cells. Finally, the genes used for readouts are primarily associated with recruitment of inflammatory cells to vessel wall. Therefore, if C3H/HeJ mice are resistant to atherosclerosis because of a relative inability of their endothelial cells to support recruitment of inflammatory cells to the vessel wall, then it might be expected that hematopoietic cells would have no chance to demonstrate an independent genetic effect on atherosclerosis. Genetic differences in hematopoietic cells might contribute to variation in atherosclerosis once the cells are effectively recruited to the vessel wall. For example, when apoE-deficient bone marrow was transplanted into wild-type mice, those mice developed significantly more lesion, demonstrating that genetic differences in hematopoietic cells can impact atherosclerosis. Therefore, it remains likely that genetic variation within the hematopoietic compartment, under some circumstances, influences atherosclerosis. Nevertheless, the present study suggests that, at least in these 2 strains of mice, genetic differences in the response of 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 study 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.
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 al 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.

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References


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