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## Genetics of Common Forms of Heart Disease:

### A Long and Winding Road

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Most of our current understanding of the genetic underpinnings of heart disease, including atherosclerosis, heart failure, and arrhythmias, is based on Mendelian forms of these disorders or on transgenic/knockout mouse models. But the vast majority of heart disease is complex as a result of the interactions of many genetic and environmental factors. Clearly, an understanding of the genes and interactions contributing to these common forms of disease has the potential to improve diagnosis and treatment greatly. In this issue of *Circulation Research*, Rodriguez et al<sup>1</sup> use mouse models to convincingly identify a novel gene contributing to a complex form of atherosclerosis. The gene, *Raet1e*, seems to act by influencing the immune system, independently of traditional risk factors such as plasma cholesterol levels. In addition, the work is important as an exemplar of the dissection of a complex trait in mice.

The story has its beginning  $\approx$ 2 decades ago with the development by Plump, Breslow, and colleagues<sup>2</sup> of a spontaneous mouse model for atherosclerosis, the apolipoprotein E (*ApoE*) knockout mouse, followed by the finding several years later that the effects of the *ApoE* mutation differed remarkably between different inbred strains of mice. For example, although C57BL/6J (B) mice carrying the mutation exhibited large lesions, strain FVB (F) mice were almost free of lesions.<sup>3</sup> To identify loci contributing to genetic differences in atherosclerosis susceptibility, a cross was constructed between strains B and F on an *ApoE* null background. Atherosclerotic lesion size was determined in each of the progeny, and the segregation patterns of chromosomal regions were followed using genetic markers polymorphic between the strains. These studies identified several quantitative trait loci for atherosclerosis and suggested complex inheritance with susceptibility alleles derived from both parental strains.<sup>4</sup>

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The strongest locus mapped to a proximal region of mouse chromosome 10 and disease susceptibility was conferred by the F allele. To further examine the properties of the chromosome 10 locus and to begin fine mapping the region, a congenic strain was constructed by intercrossing F and B mice and then repeatedly backcrossing the progeny to B mice and selecting at each generation for the presence of F allelic markers at the chromosome 10 locus. After multiple generations of crossing, a mouse strain composed entirely of the B background, except for the selected chromosome 10 region derived from the F strain, was produced. The final congenic strain, studied both on *ApoE* and *Ldlr* null backgrounds, exhibited the expected properties in terms of lesion development: mice carrying F alleles had greater lesion development than controls carrying B alleles. The introgressed region encompassed  $\approx 22$  cM and included  $\approx 382$  genes.<sup>5</sup>

Congenic strains facilitate fine mapping because they essentially Mendelize a complex trait by removing all genetic differences between the 2 parental strains, except for the isolated region. The strategy is an old one, dating back to the 1940s when Snell and colleagues<sup>6</sup> first identified the major histocompatibility complex (*H2*) in mice. To subdivide the region, the congenic strain was crossed to B mice, and progeny were tested for recombination within the chromosome 10 interval. Strains generated from these progeny were then tested for atherosclerosis susceptibility compared with the B parental strain. This showed that the chromosome 10 region actually contained 2 loci influencing atherosclerosis: a proximal female-specific locus designated 10a and a distal locus affecting both sexes designated 10b.<sup>7</sup>

The current study successfully completes the mapping strategy and identifies the underlying genetic polymorphism (ie, causal mutation). The 10b locus was further narrowed, using a new subcongenic strain, to a region containing 4 coding genes plus the promoter of *Raet1e*. Each gene was analyzed by sequencing and examining the expression levels in relevant tissues. The results showed a significant expression difference only for *Raet1e*, which exhibited greatly decreased aortic and liver expression in the congenic compared with the background strain. The causal role of the *Raet1e* variation was confirmed by overexpression of the B allele in the congenic background, resulting in decreased atherosclerosis. Furthermore, the nucleotide responsible for the expression difference was identified using a strategy of promoter-reporter constructs, comparing B and F sequences. A T $\rightarrow$ C variation (single nucleotide polymorphism rs50817078) in the transcription initiation region of F-derived *Raet1e* confers decreased promoter activity relative to the B-derived variant (T).<sup>1</sup>

This is an exceptional story because there are few successful examples of the identification of a gene contributing to a complex trait using a mapping strategy in mice. The main difficulty with the approach is that, similar to human populations, common diseases such as atherosclerosis tend to be complex among mouse populations. Thus, although 2 different inbred strains of mice may differ dramatically for a trait, there are often many loci involved, and the effect of each locus tends to be small. Frequently, the effects of such loci are context dependent, both in terms of genetic background and environmental sensitivity.<sup>8</sup>

The discovery of *Raet1e* as an atherosclerosis susceptibility gene using this unbiased approach opens up a new and promising area of investigation. *Raet1e* belongs to a family of genes encoding cell surface proteins that are members of the major histocompatibility class

1–related family. *Raet1e* proteins are generally upregulated by stress, resulting in activation of natural killer cells, B cells, T cells, and macrophages. Thus, it was somewhat surprising that the F strain, carrying a low expression allele, is associated with increased atherosclerosis. It is interesting to note that although atherosclerosis is clearly an inflammatory disease involving both macrophages and lymphocytes, human GWAS for atherosclerosis traits have identified few loci containing inflammatory genes.<sup>9</sup>

Given that this story has been  $\approx 2$  decades in the making and is one of the few successes, it is fair to ask whether quantitative trait loci mapping of complex traits in mouse is a viable strategy. It may seem that GWAS in human populations offer a much more powerful and relevant approach to the discovery of genes, contributing to common diseases. But it is important to remember that the identification of associated loci is one thing, and the identification of causal genes and mechanisms is quite another. Although there have been some impressive follow-ups of human GWAS hits,<sup>10</sup> these have been relatively few. The difficulty in understanding the 9p21.3 locus, an early poster child for GWAS in the area of cardiovascular disease, illustrates the difficulty.<sup>11</sup> Although the number of successes of gene discovery for complex traits in mouse is not large, there have been several notable findings, including a number in the cardiovascular field. Furthermore, as in human genetics, gene discovery studies in mice are being revolutionized by high-throughput sequencing and systems-based approaches. For example, the sequencing of the genomes of numerous inbred strains of mice has made possible a high-resolution association strategy that is analogous to human GWAS.<sup>12</sup> Thus, the laborious congenic strain approach to fine mapping can be largely avoided. At this point, it seems best to keep an open mind.

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