Uncovering cardiovascular disease genetics

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February is American Heart Month, a great time to take a closer look at cardiovascular disease (CVD). According to the World Health Organization (WHO), about 17.5 million people die each year from CVD, a figure representing 31% of all deaths worldwide. Physicians recommend regular exercise and a diet rich in fruits and vegetables to prevent development of CVD, but many healthy individuals still die from this disease. As with other complex diseases, both genetic and environmental factors influence disease risk, and some families are highly predisposed to the disease.

A recent study published in *PLOS Genetics* takes a closer look at CVD heritability in mice, using 100 strains of mice to identify genetic pathways that increase CVD risk. Bennett et al. find that CVD is an incredibly complicated disease that is more than the sum of its parts – with gene-by-gene interactions playing a major role in CVD risk.

Using Mice to Understand Cardiovascular Disease Genetics

CVD includes many disease subtypes, but most are related to a process called atherosclerosis, or "hardening of the arteries." Atherosclerosis occurs when cholesterol-rich plaques build up inside arteries. These plaques harden, narrowing the arteries, obstructing blood flow, and increasing the risk of a blood clot. Unstable plaques may also break off from the artery wall and block blood flow, potentially causing a heart attack or stroke. Atherosclerosis is an incredibly complicated disease involving interactions between the liver, immune system, heart, and

arteries. Genome-wide association studies (GWAS) have been used to identify risk loci, or regions of the genome harboring variants more common in people with CVD than without. This strategy helped researchers identify the blockbuster drug target PCSK9, but results so far explain only a small percentage of CVD heritability.

Rather than examining humans, Bennett and colleagues used 100 distinct strains of inbred mice to examine the genetic basis of atherosclerosis. Mice within each strain are essentially genetically identical, and each strain has unique characteristics and disease susceptibility due to its genetic background. One hundred strains represent a very large pool of genetic diversity, allowing the researchers to closely examine the genetics underlying atherosclerosis risk. As mice are less athero-prone than humans, these mice were subsequently crossed to C57BL6/J mice carrying the athero-promoting ApoE-Leiden transgene. These mice were further challenged with a high cholesterol diet to increase the severity of atherosclerosis. At sacrifice, the researchers quantified plaque formation in the aorta as a measure of atherosclerosis, and they harvested tissues and plasma for gene expression and metabolite analyses, respectively. They also measured traits known to be associated with atherosclerosis, such as insulin/glucose levels, lipid levels, and obesity.

The researchers noticed a surprisingly high level of variation in atherosclerotic plaque formation; some mouse strains had very large plaques, while other strains showed very small plaques. Due to the large magnitude of these differences, they hypothesized that gene-by-gene interactions play a role in determining plaque size. To test this hypothesis, they compared two measures of heritability: narrow sense and broad sense. Narrow sense heritability considers each risk locus to add risk incrementally: a + b + c… = total risk. In contrast, broad sense heritability includes environmental factors, multiplicative gene-by-gene interactions, and the effects of dominant traits. For this study, broad sense heritability of atherosclerosis is higher than narrow sense

heritability (0.63 vs 0.21), indicating that non-additive factors play a role. Given that these mice were exposed to a controlled environment, researchers conclude that gene-by-gene interactions are important in atherosclerosis.

In addition to differences among strains, sex differences in plaque area within a given strain were also evident, with male mice displaying much smaller plaques than female mice. These sex differences are especially interesting given that CVD manifests differently in men and women, with new data showing that heart attacks are underdiagnosed and undertreated in women. Genome-wide association studies found four loci in females and one locus in males associated with plaque area. Bennett and colleagues also found significant associations of monocyte and cytokine levels with plaque area, further showcasing the importance of the immune system in the development of atherosclerosis.

As mentioned above, working in mice allows one to deeply probe gene expression in regions of interest. Perhaps unsurprisingly, the researchers found that distinct gene sets in liver and aorta were associated with atherosclerosis, notably immune response genes in the liver and genes related to smooth muscle cell function in the aorta. The researchers sorted highly co-expressed genes into regulatory modules in order to understand which pathways were most affected. One key finding was the predicted involvement of the nitric oxide pathway, known to be important for blood vessel dilation and previously identified in basic and clinical studies of atherosclerosis. Interestingly, metabolite screening also implicated the nitric oxide pathway, as plasma levels of arginine and ornithine, two pathway components, were correlated with plaque size. These parallel findings indicate the power of multilevel systems analysis to further our understanding of disease mechanisms.

Research Applications and Open Questions

In sum, Bennett and colleagues studied more than 1,800 mice representing 100 strains, generating a tremendous amount of data. Their conclusions with regard to atherosclerosis are equally complicated. Atherosclerosis risk can be influenced by alterations in many distinct pathways, including lipid metabolism and various aspects of the immune response. These pathways themselves each include many genetic loci, painting a complex, varied, and multilayered picture of atherosclerosis. The researchers term this finding an "infinitesimal model" of disease, likely applicable to other common diseases, in which variation in thousands of genes across multiple biological systems impacts risk. This model may also explain why some therapies are more effective in certain ethnic groups than in others, as observed with CVD drugs such as warfarin and ACE inhibitors.

The infinitesimal nature of atherosclerosis may explain why GWAS studies have uncovered so little of CVD heritability, as GWAS often require either large effect sizes or extremely large sample sizes to uncover significant associations of risk loci. Another piece of the puzzle is the gene-by-gene interactions uncovered in this study. The work described here shows a multiplicative model of CVD risk rather than an additive one – variants together exert effects much stronger than those predicted based on the sum of their individual effects. These sorts of interactions are difficult to model using GWAS, as GWAS studies commonly use a parsimonious additive model of risk. Gene-by-environment interactions not explored in this paper could also be multiplicative in nature – this area warrants thorough investigation in the future.

Another important finding of this paper is the association of various metabolites with plaque size. Trimethylamine N-oxide (TMAO), a metabolite previously shown to be correlated with CVD risk in humans, is also correlated with female mouse plaque size in this study. TMAO is an intriguing new drug target for CVD, as it appears to connect the gut

microbiome to CVD risk, and a study inhibiting one step of TMAO formation decreased atherosclerosis in mice. More broadly, the research community is highly interested in developing metabolite-based models to assess one's risk of CVD and other chronic diseases, as standard blood panels may not accurately predict risk. Harnessing the variation found in inbred mice may help researchers to build a more complete predictive model.

Data from this paper are publicly available at the UCLA Systems Genetics Resource. Although working in inbred mice brings the benefits of high genetic variation and the power of targeted gene expression analysis, it's important to remember that results obtained in mice must be verified in and analyzed alongside those from humans. Encouragingly, a few of the loci found in this work were previously noted in human GWAS studies. Bennett et al. note that while individual genes may not be of the same importance in mice and humans, pathway importance is more likely to be conserved.

Despite the physiological differences between mice and humans, it's clear that mouse studies can help researchers uncover new information related to complex diseases like CVD. A better understanding of the genetics behind CVD may lead to new drug targets or, in the future, treatments personalized based on one's genetics. During American Heart Month this year, your doctor may tell you to eat healthy, exercise, and have your cholesterol levels checked, but in a few decades, a CVD genetic risk profile could be added to those guidelines!

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