Cardiovascular Genetics: A News Round up

Increasing Power
Multivariate Analysis of Metabolic Networks Reveals Novel Atherosclerosis Loci

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Study Hypothesis
Many of the phenotypic risk markers that are known, or suspected, to be involved in disease are interrelated. Given that many genes have pleiotropic effects, where the genetic variant is associated with multiple outcome variables, it is likely that multiple related phenotypes have a common genetic basis. At present, majority of genome-wide association studies analyze the effect of single nucleotide polymorphisms (SNPs) on individual phenotypes in univariate analyses. Where multiple phenotypes are examined, applying a traditional Bonferroni correction for multiple testing may be overly stringent, and does not take into account the common genetic origin of the correlated phenotypes. Inouye et al hypothesized that by analyzing multiple related phenotypes together in a multivariate genetic analysis, rather than individually in a typical univariate analysis, they would have increased power to detect genetic associations with disease-related phenotypes.

How Was the Hypothesis Tested?
The discovery effort focused on 2 separate population-based cohorts from Finland with genome-wide SNP genotypes, and extensive serum metabolite profiles. Using data from 6,600 individuals, the authors first identified 11 metabolic networks from the 130 metabolites profiled. This metabolic panel included metabolites known to be related to atherosclerosis and metabolic disease, such as lipoproteins, lipids, and amino acids. The authors then looked for genetic associations with these networks, as well as with the individual metabolites, to compare the results of the multivariate analyses versus univariate analyses. To interrogate the relationship between the top loci and cis gene expression, the authors used mouse models to examine the relationship between tissue-specific gene expression and development of atherosclerosis. In addition, they examined the expression of the top genes in human atherosclerotic plaque samples.

Principal Findings
In the genome-wide association analysis of SNP associations with serum metabolites, the multivariate analyses yielded a greater number of significant associations than the univariate analyses, even after applying stringent correction for multiple testing. Although the multivariate analyses detected almost all of the associations found in the univariate analyses, the univariate analyses failed to detect a number of interesting associations which were significant in the multivariate analyses. Among the top 34 loci discovered through the multivariate analyses, 27 had previously been found to be associated at the level of genome-wide significance with individual metabolites or with metabolic phenotypes. Of the 7 novel loci, the 2 strongest signals were in SERPINA1 (best P=5.4×10^{-48}) and AQP9 (best P=2.9×10^{-32}), with both loci being associated with multiple metabolic networks. These 3 loci were selected to take forward for additional in-depth analyses. Using PolyPhen to predict the effect of the mutations, the top SNP in the SERPINA1 gene, which was nonsynonymous, was predicted to be benign, whereas the top SNP in the AQP9 gene, located in an intron, was predicted to be damaging. In 3 separate expression quantitative trait loci study samples, the top SERPINA1 SNP was associated with SERPINA1 expression in blood and liver, but not adipose. The top AQP9 SNP was associated with AQP9 expression in liver, but not adipose or blood. There was evidence of higher adipose SERPINA1 and AQP9 expression in individuals with low high-density lipoprotein compared with high high-density lipoprotein cholesterol. Expression of SERPINA1 and AQP9 in blood was found to be associated with several metabolites, some of them components of the same networks that were associated with the SERPINA1 and AQP9 SNPs in the discovery sample. Thus, these data outlined a potential relationship between gene variants, tissue-specific expression of the gene, and downstream phenotypes related to atherosclerosis. The authors extended these suggestive findings into a mouse model of disease, using a sample of F2 mice derived from the backcross of an atherosclerosis-susceptible background.
with an atherosclerosis-resistant strain, on an Apo-E knockout background. Liver gene expression as well as atherosclerotic plaque lesion area in the mice was determined after 16 weeks on a high-fat diet. Although there was no evidence of association of atherosclerotic lesions with Serpina1 expression, there was a significant association between higher Aqp9 expression and increased lesion area in the mice. In human atherosclerotic plaque samples, both SERPINA1 and AQP9 were found to be expressed at higher levels in plaque samples compared with controls, lending further support to the evidence of both genes being involved in atherosclerosis, albeit through distinct mechanisms.

**Implications**

Using a multivariate approach, the authors demonstrated that the simultaneous analysis of correlated phenotypes has greater power to detect genetic associations than analysis of each phenotype sequentially. The authors gathered evidence from a variety of sources to establish a comprehensive analysis of the relevance of their top 2 novel loci with atherosclerosis. Both SERPINA1 and AQP9 are interesting candidate genes, which may have distinct roles in the development or progression of atherosclerosis. In addition, this study serves as an example of the importance of the analytical method in discovery of novel candidates for disease. As it becomes increasingly feasible to measure large numbers of metabolites and phenotypic risk markers in human studies, taking advantage of more powerful multivariate models may be crucial to fully exploit the potential power of discovery in these studies.

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**Disclosures**

None.
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