Inflammation is a key component of atherosclerosis. Abundant preclinical data support the hypothesis that atherosclerosis is a chronic inflammatory disorder. Indeed, clinical trial data now provide evidence that inflammation, as reflected in serum markers such as C-reactive protein and interleukin-6, is a strong risk factor for the development and progression of atherosclerosis. The role of genetic factors in determining a predisposition or susceptibility to inflammation that exacerbates atherosclerosis is not fully known.

In-stent restenosis occurs after the deployment of an intravascular stent within an atherosclerotic lesion. The fibroproliferative response to this vascular “injury” typically develops within the first 9 months postprocedure. The response to injury follows a continuum in human arteries; some degree of cell proliferation occurs in all patients and can be thought of as a wound-healing process. In some individuals, however, the wound healing becomes excessive, leading to exuberant vascular smooth muscle cell growth and extracellular matrix synthesis, and encroachment on the arterial lumen, and resulting in a recurrence of clinical symptoms. Molecular and genetic studies suggest that cell cycle proteins, growth factors, and inflammatory cytokines regulate this process. Drug-eluting stents have dramatically reduced the prevalence of in-stent restenosis because of the local treatment of the fibroproliferation with 2 drugs, sirolimus and paclitaxel, which have antiproliferative and antiinflammatory properties. What is not known, however, is whether there is a genetic susceptibility that determines a patient’s response to stent deployment and development of in-stent restenosis.

In this issue of Circulation, Monraats et al investigate the genetics of in-stent restenosis in a case-control association study, using a candidate gene approach. The authors hypothesize that genetic variation in inflammatory genes is important in individual differences in the vascular wound-healing process, and hence they selected specific genes and polymorphisms hypothesized to be causal. They determined genotypes of 48 single nucleotide polymorphisms (SNPs) in 34 candidate genes in 3029 patients enrolled prospectively at the time of coronary intervention and studied until either target-vessel revascularization (TVR) occurred or 9 months of follow-up were complete. In this cohort, 74.4% of patients received a bare metallic stent as part of their treatment. None received drug-eluting stents. The primary end point of TVR occurred in 9.8% of patients. Using established statistical tests designed to test the association of one marker at a time, 4 SNPs were identified within the genes for ADRA2B, CD14, CSF2, and CCL11.

In testing many variables for association with disease, multiple testing is a concern because of the increased possibility of false positives. In this study, no multiple testing corrections were applied. Instead, the authors conducted a permutation test on their data set, in which the TVR and no-TVR outcome was shuffled 1000 times among the patients and the association tests were recalculated each time. In this test, any 4 SNPs were identified to be significant in 12% of permutations. To further explore their findings, the authors examined genotypes among patients with postpercutaneous transluminal coronary angioplasty restenosis versus in-stent restenosis. No differences were identified. Haplotype analysis, in which the SNPs assayed were examined for patterns between TVR and no-TVR patients, provided no additional insight. Interactions between SNPs and the association with TVR were considered, and in this analysis, a SNP in the TCF7 gene was found to interact with the CSF2 SNP previously identified, with modest significance and no multiple testing corrections. Patients were triaged into 3 risk categories: low, medium, and high, with respective TVR rates of 5.0%, 9.2%, and 12.9%. The high-risk quartile of 749 patients contained 435 patients with a specific pattern of genotypes in the TCF7, CSF2, CD14, and ADRA2B genes. The CCL11 gene polymorphism was not indicated to be part of this pattern, despite having been identified as associated with TVR.

During the past several years, we have seen a dramatic evolution in our knowledge of the genome as well as the tools with which to conduct genetic studies. We now know that there are millions of SNPs across the genome, and high-throughput genotyping assays that use SNPs as high-density genetic markers are now available, assaying ≥100 000 SNPs at a time to identify disease loci. The study by Monraats et al was initiated in 1999, a time when genetic studies using SNP markers were just coming to the forefront of genetics research. The human genome draft sequence and associated SNP databases were not well developed at that time. Traditional genetic research methods that had been developed to identify disease-causing genes in rare Mendelian disorders relied on microsatellite or other markers to identify diseases in which the effect of a single variant gene was strong. These methods identified a disease locus and further study of the
locus was needed to identify specific mutations using techniques such as positional cloning. Today, it is possible to conduct genome-wide association studies using SNPs as markers across the genome. Exciting new opportunities are on the horizon to identify disease-causing or risk-conferring alleles in complex diseases, in which disease susceptibility is hypothesized to be caused by multiple common variants, each contributing subtly to the disease.

How should we interpret the study by Monraats et al? The authors clearly outline the limitations of their study and acknowledge several important issues. First, the results of statistical testing are of marginal significance, with no multiple testing corrections applied to either the initial per-SNP analysis or the haplotypes analyses. The results of their permutation testing confirm an experiment-wide error rate of 12%, which indicates that their findings are quite possibly the result of chance and the testing considerations outlined. The functional significance of the gene variants reported are not investigated, although the literature does provide some evidence that the genes, not necessarily the SNPs investigated, could plausibly be functionally important in restenosis. Although interesting, the results cannot clearly be linked to the biology of restenosis. Only 48 markers were screened in this study. This analysis could have missed many significant SNPs because of the candidate polymorphism approach taken. Given these considerations, this study can be viewed as a screening analysis, and the results should be viewed as preliminary.

As genomic methodologies continue to evolve at an ever-rapid pace, we can look forward to a new generation of genetic studies aimed at investigating complex genetic diseases. The most common cardiovascular diseases are truly complex, with significant environmental contributions as well as likely multigenic pathogenesis. This is certainly true of vascular injury responses, in which we continue to see treatment failures even after the advent and widespread use of drug-eluting stents. Using the most cutting-edge technologies available, we now have the potential to build on early associative findings such as those reported by Monraats et al and to conduct more definitive investigations into the genetic basis of complex diseases. Importantly, for our patients, these tools are powerful means by which we will better understand the molecular basis of the most common diseases observed in the clinic and develop improved risk stratification tools and treatment modalities for cardiovascular diseases such as atherosclerosis and restenosis.

References

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