On Genetics, Inflammation, and Abdominal Aortic Aneurysm : Can Single Nucleotide Polymorphisms Predict the Outcome?

A. J. Marian

The online version of this article, along with updated information and services, is located on the
World Wide Web at:

http://circ.ahajournals.org/content/103/18/2222

Editorial

On Genetics, Inflammation, and Abdominal Aortic Aneurysm
Can Single Nucleotide Polymorphisms Predict the Outcome?

A.J. Marian, MD

The late Professor Russell Ross proposed the "response to injury" hypothesis for the pathogenesis of atherosclerosis ≈25 years ago.[1–3] This shifted the paradigm from the "fibrin incrustation" and "lipid transudation" hypotheses that were formulated in the mid-19th century by von Rokitansy and Virchow, respectively. Subsequent identification of growth and mitotic factors in the vessel wall that were responsible for the proliferation and migration of smooth muscle cells,[1] along with the landmark discovery of endothelium as a biologically active inner layer of vessels by Furchgott and colleagues,[4] established the active role of blood vessels in pathological states. The findings of macrophages in the vessel wall and increased expression of proteins involved in inflammation suggested the active role of inflammation in the pathogenesis of a variety of vascular diseases, including abdominal aortic aneurysm (AAA).

See p 2260

AAA, a relatively common disease in the elderly, is a disease of the media that is characterized by degeneration of the extracellular matrix proteins. Degradation occurs as a consequence of complex interactions between genetic factors, inflammatory cytokines, matrix metalloproteinases (MMPs), tissue inhibitors of MMPs (TIMPs), and others. The ensuing phenotype is dissolution and fragmentation of collagen and elastin, leading to expansion of the vessel wall that can no longer withstand the repetitive expansile force of systolic contraction.

Extracellular matrix proteins maintain the integrity of the vessel wall. Under normal circumstances, degradation of extracellular matrix proteins is minimal because MMPs are expressed at low levels and in inactive forms.[5] The activation of MMPs requires the removal of an amino-terminal sequence by plasmin and the plasmin-generating enzymes tissue-type plasminogen activator and urokinase-type plasminogen activator, which are minimally expressed in the normal vessel wall. In addition, activated inflammatory cells, which are the main sources of collagenolytic and elastinolytic proteinases, are also absent. Furthermore, expressed TIMPs

neutralize the proteolytic activities of MMPs. Collectively, these processes maintain the integrity of the extracellular matrix proteins and the vessel wall.

In pathological states, the balance is shifted toward increased proteolytic activities of MMPs. As such, expression of MMP-9 (gelatinase B) and MMP-12 (metalloproteinase) is increased in patients with AAA.[5,6] Although the increased expression of MMPs per se does not establish causality, experimental data do suggest a causal role for the activation of MMPs in the formation of aortic aneurysms. Targeted disruption of MMP-9 (gelatinase B) results in decreased degradation of elastin fibers in response to elastase perfusion in mouse aorta and suppresses the development of aortic aneurysms.[7] Similarly, overexpression of TIMP-1 in rat aorta blocks the activity of MMPs and prevents elastin degeneration, aneurysm formation, and aneurysm rupture. Genetic studies of the monogenic disorders Marfan syndrome and Ehlers Danlos syndrome IV underscore the role the extracellular matrix proteins play in the pathogenesis of aneurysms in humans. Patients with Marfan syndrome, an autosomal-dominant disease caused by mutations in the fibrillin-1 gene, commonly develop aortic aneurysm, dissection, and valvular regurgitation.[8] Histopathological studies show loss and fragmentation of the elastic fibers.[8] Targeted deletion of the fibrillin-1 gene (*FbnI*) and reduced expression of fibrillin-1 in mice recapitulate a Marfan-like phenotype and lead to an inflammatory fibroproliferative response and the formation of aortic aneurysms.[9,10] Similarly, mutations in the type III procollagen gene (*COL3A1*) lead to aneurysm formation in patients with Ehlers Danlos syndrome IV by reducing collagen synthesis.[8] Collectively, genetic and experimental data suggest that the degradation of extracellular matrix proteins, regardless of the cause, leads to aneurysmal dilatation of the vessel.

Factors that shift the balance in favor of increased proteolytic activity in the vessel wall are largely unknown. Genetic factors are likely to be involved in susceptibility to the common forms of aneurysms, as evidenced by the familial aggregation of AAA.[11] The prevalence of AAA, which is ≈1% in siblings of subjects without AAA, increases 4-fold in siblings of patients with AAA.[11] The susceptibility genes for non-monogenic aneurysms are unknown. Increased circulating levels of interleukin (IL)-1, IL-6, tumor necrosis factor-α, and interferon-γ in patients with AAA[12] implicate genes encoding the inflammatory cytokines, which are biologically plausible candidates. Proinflammatory cytokines could not only suppress the expression of type I and III collagen fibers,[13] but also promote the degradation of elastin and

(*Circulation* 2001;103:2222-2224.)

Circulation is available at http://www.circulationaha.org

collagen by activating MMPs and inhibiting TIMPs. Increased activity of MMPs in turn could promote the activation and release of cytokines, perpetuating a vicious cycle that ultimately leads to the degradation of extracellular matrix proteins and aneurysm formation and growth.

In this issue of *Circulation*, Jones et al[14] show that plasma concentrations of IL-6 were higher in the iliac than in the brachial arteries of patients with large or inflammatory AAA, suggesting that aneurysms produce inflammatory cytokines. They also show that the −174G/C polymorphism in the *IL-6* gene was associated with the plasma concentrations of IL-6 and cardiovascular mortality. Subjects with the CC genotype had the highest plasma levels and the worst prognosis. The results of this study raise several issues. The strengths of the present study are its prospective design and the plausibility of the hypothesis. The well-established roles of IL-6 in inflammation and the activation of MMPs are consistent with the biology of aortic aneurysms. Increased plasma concentrations of IL-6 in iliac arteries, probably secreted by the cellular components in the aneurysm, is also in accord with the results of previous studies showing elevated circulating levels of inflammatory cytokines in patients with AAA.[12,15] However, IL-6 is not a specific marker of AAA, and plasma levels of it increase in response to a variety of stimuli, such as infection, malignancy, trauma, ischemia, and inflammatory disorders. There is also significant inter- and intra-individual variability. Therefore, a single measurement, although a potentially useful prognosticator in a population, is unlikely to carry a significant predictive value in a given individual.

It is possible that genetic polymorphisms that regulate the cumulative exposure to inflammatory cytokines could serve as better markers. However, polymorphisms do not exist in isolation. The human genome comprises >2 million single nucleotide polymorphisms (SNPs), and each gene has multiple SNPs that cooperatively regulate its expression. In addition, given the diversity of the regulatory factors, the impact of each SNP on gene expression is expected to be modest. The additive, synergistic, or subtractive effects of multiple SNPs, the effects of other genes, and epigenetic and environmental factors further compound this complexity. The −174G/C variants, which are located near a cAMP-binding site in the promoter region of the *IL-6* gene, in cooperation with additional polymorphisms, affect the activity of the *IL-6* promoter in in vitro assays.[16] In this aspect, the results of the study by Jones et al[14] showing the highest circulating levels of IL-6 in subjects with the CC genotype are in contrast to the results of 2 previous studies, which showed that the GG genotype had the highest promoter activity during in vitro transfection assays[16,17] and the highest plasma levels of IL-6.[17] The disparity in the results may reflect the influence of cell types and pathological states on the differential effects of the −174G/C alleles on gene expression. However, confirmation by experimentation and/or additional data sets is needed. In addition, because Jones et al[14] studied subjects with preexisting AAA, it cannot be established whether increased circulating levels of IL-6 is a cause or a consequence of AAA.

The presence of a gene-dose effect on survival, which was concordant with the observed biological effect of the

Issues to Consider When Analyzing the Results of SNP-Association Studies

Plausible biological hypothesis
Coherence with the natural history and biology
Concordant observations in several independent sets
Concordant association with secondary phenotypes
Multiple hypothesis testing
Population characteristics
Choice of a control group (in case-control studies)
Study design (prospective vs retrospective)
Size of the study population
Population admixture
Precise phenotypic characterization
Effects of confounders
Strength of association
Specificity of the effect
Biological gradient
Gene-dose effect
Temporality
Confirmation by experimentation

−174G/C variants on the plasma levels of IL-6 is a strength. However, this effect was absent for the growth of AAA. The possibility of confounders such as the concomitant presence of cardiovascular diseases other than AAA (which was previously associated with circulating levels of IL-6),[18,19] malignancy, infection, and other inflammatory disorders, should also be considered. Accordingly, the association of plasma levels of IL-6 with survival could be independent of AAA. The majority of deaths also occurred because of acute myocardial infarction and cardiovascular disease, which have been associated with increased circulating levels of Il-6.[19,20] Moreover, the strength of the observed association was weak, the sample size was small, and the number of events in a 3-year follow-up period was low. It is also noteworthy that the Hardy-Weinberg equilibrium, which is frequently used to imply the genetic balance of the study population, requires specific assumptions and does not necessarily apply to a population selected on the basis of a specific phenotype. Perhaps the most important source of spurious results in association studies is the characteristics of the study population. Thus, confirmation in multiple sets of independent populations or in a multitier study in siblings, families, and index cases is required. Finally, the results of all association studies should be considered provisional pending proof through experimentation. The Table summarizes issues to be considered when interpreting the results of a polymorphism-association study.

The issues discussed here illustrate the complexities of identifying the genetic determinants of susceptibility to polygenic traits, which are the common causes of morbidity and mortality in humans. Perhaps it is not surprising that the results of the vast majority of the polymorphism-association studies performed during the past 20 years have not stood the test of time. The conventional linkage techniques have limited power to map genes with small or moderate effects,

and linkage-disequilibrium studies using SNPs to detect an association with a phenotype are subject to a high rate of spurious results. Recently, SNP maps of the human genome have been generated with the hope of performing whole-genome linkage-disequilibrium studies. It is estimated that 500 000 to 1 000 000 SNPs, used alone or as haplotypes, will be needed to perform genome-wide linkage-disequilibrium studies. However, there is a considerable debate regarding the usefulness of SNPs in mapping the susceptibility genes for complex disorders. Optimists anticipate that the development of comprehensive SNP maps of the human genome and highly efficient high-throughput genotyping techniques, along with the advances in bioinformatics, will afford the opportunity to search for the susceptibility genes. Genome-wide association studies in thousands of phenotypically well-characterized subjects could provide information that, in conjunction with functional genomics, proteomics, and our enhanced understanding of the molecular biology of human diseases, could lead to identification of the individual susceptibility genes for complex traits. Similar large-scale studies will afford the opportunity to identify the genetic determinants of the clinical outcome, pharmacogenetics, and response to medical and surgical interventions. Until then, we all are like the proverbial blind men attempting to describe an elephant.

References

1. Ross R, Glomset JA. Atherosclerosis and the arterial smooth muscle cell: proliferation of smooth muscle is a key event in the genesis of the lesions of atherosclerosis. *Science*. 1973;180:1332–1339.
2. Ross R, Glomset JA. The pathogenesis of atherosclerosis (first of two parts). *N Engl J Med*. 1976;295:369–377.
3. Ross R, Glomset JA. The pathogenesis of atherosclerosis (second of two parts). *N Engl J Med*. 1976;295:420–425.
4. Furchgott RF, Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature*. 1980;288:373–376.
5. Curci JA, Liao S, Huffman MD, et al. Expression and localization of macrophage elastase (matrix metalloproteinase-12) in abdominal aortic aneurysms. *J Clin Invest*. 1998;102:1900–1910.
6. Thompson RW, Holmes DR, Mertens RA, et al. Production and localization of 92-kilodalton gelatinase in abdominal aortic aneurysms: an elastolytic metalloproteinase expressed by aneurysm-infiltrating macrophages. *J Clin Invest*. 1995;96:318–326.
7. Pyo R, Lee JK, Shipley JM, et al. Targeted gene disruption of matrix metalloproteinase-9 (gelatinase B) suppresses development of experimental abdominal aortic aneurysms. *J Clin Invest*. 2000;105:1641–1649.
8. Towbin JA, Casey B, Belmont J. The molecular basis of vascular disorders. *Am J Hum Genet*. 1999;64:678–684.
9. Pereira L, Lee SY, Gayraud B, et al. Pathogenetic sequence for aneurysm revealed in mice underexpressing fibrillin-1. *Proc Natl Acad Sci U S A*. 1999;96:3819–3823.
10. Pereira L, Andrikopoulos K, Tian J, et al. Targeting of the gene encoding fibrillin-1 recapitulates the vascular aspect of Marfan syndrome. *Nat Genet*. 1997;17:218–222.
11. Baird PA, Sadovnick AD, Yee IM, et al. Sibling risks of abdominal aortic aneurysm. *Lancet*. 1995;346:601–604.
12. Juvonen J, Surcel HM, Satta J, et al. Elevated circulating levels of inflammatory cytokines in patients with abdominal aortic aneurysm. *Arterioscler Thromb Vasc Biol*. 1997;17:2843–2847.
13. Siwik DA, Chang DL, Colucci WS. Interleukin-1β and tumor necrosis factor-α decrease collagen synthesis and increase matrix metalloproteinase activity in cardiac fibroblasts in vitro. *Circ Res*. 2000;86:1259–1265.
14. Jones KG, Brull DJ, Brown LC, et al. Interleukin-6 (IL-6) and the prognosis of abdominal aortic aneurysms. *Circulation*. 2001;103:2260–2265.
15. Reilly JM, Miralles M, Wester WN, et al. Differential expression of prostaglandin E2 and interleukin-6 in occlusive and aneurysmal aortic disease. *Surgery*. 1999;126:624–627.
16. Terry CF, Loukaci V, Green FR. Cooperative influence of genetic polymorphisms on interleukin 6 transcriptional regulation. *J Biol Chem*. 2000;275:18138–18144.
17. Fishman D, Faulds G, Jeffery R, et al. The effect of novel polymorphisms in the interleukin-6 (IL-6) gene on IL-6 transcription and plasma IL-6 levels, and an association with systemic-onset juvenile chronic arthritis. *J Clin Invest*. 1998;102:1369–1376.
18. Schieffer B, Schieffer E, Hilfiker-Kleiner D, et al. Expression of angiotensin II and interleukin 6 in human coronary atherosclerotic plaques: potential implications for inflammation and plaque instability. *Circulation*. 2000;101:1372–1378.
19. Ridker PM, Rifai N, Stampfer MJ, et al. Plasma concentration of interleukin-6 and the risk of future myocardial infarction among apparently healthy men. *Circulation*. 2000;101:1767–1772.
20. Volpato S, Guralnik JM, Ferrucci L, et al. Cardiovascular disease, interleukin-6, and risk of mortality in older women: the Women's Health and Aging Study. *Circulation*. 2001;103:947–953.