Cytokines, Interleukin-18, and the Genetic Determinants of Vascular Inflammation

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Inflammation and Vascular Disease
In the past decade, atherogenesis has become recognized as an active, inflammatory vascular process rather than simply the result of passive endothelial injury with subsequent lipid infiltration.1 Evidence of inflammation is found locally in the vascular wall and systemically in the circulation. Participants in the inflammatory process within atherosclerotic plaque include macrophages, T-lymphocytes, cytokines, chemokines, matrix metalloproteinases, and adhesion molecules. Systemic (circulating) markers suggesting an augmented state of vascular inflammation have been found in acute myocardial infarction, unstable angina, and chronic coronary artery disease (CAD) and in asymptomatic patients at high risk for vascular disease. These markers include the white blood cell count,2 acute-phase reactants such as high-sensitivity C-reactive protein (hs-CRP),3 serum amyloid A and fibrinogen, cytokines (eg, interleukin [IL]-6), soluble adhesion molecules, and atherosclerosis-related enzymes (eg, lipoprotein-associated phospholipase A2).3

Genetics and Atherothrombotic Disease
Unexpected atherothrombotic events are frequently observed by the clinician in individuals with few or no traditional risk factors, implicating unrecognized inherited factors.5,6 With the completion of the Human Genome Project, optimism was high that the secrets of genetic risk would soon be unveiled. The discovery of single-locus (nucleotide) polymorphisms (SNPs) promulgated the belief that the genetic underpinnings of cardiovascular disease would lie in the discovery of a limited number of these SNPs. These expectations have not yet been realized. Numerous studies have tested for associations between SNPs and clinical atherosclerosis. Candidate genes in the pathways involved in disease initiation and progression have been explored, including those related to lipid metabolism, coagulation, and inflammation. Unfortunately, many disease-association studies involving SNPs have produced contrasting results. An oft-repeated scenario is an initial report of an association between an SNP and a disease that, when independently retested, cannot be replicated; indeed, a recent review estimated that the chance of an initial positive finding being replicated is only 30%.7,8

Problems of replication can arise from a number of flaws in study design, including chance findings resulting from multiple comparisons coupled with publication bias; varying population admixture and poor phenotype description; and the complexity of the genetic structure itself—ie, the presence of linkage groups within a gene. For example, ambiguous disease associations could result when a genetic marker under investigation is nonfunctional but is linked to the functional, disease-causing genetic variant on one (or a few) linkage group(s) (“haplotype[s]”) but not on others. The study marker would appear among cases on a haplotype bearing the causative variant but could also appear among controls on other, benign haplotypes. This situation could produce a false-negative association in an inadequately powered study or lead to replication failure in samples drawn from populations of differing lineage. Thus, the use of haplotypes, rather than single SNPs alone, has been proposed to strengthen the investigation of associations with disease (phenotype), especially when the true functional SNP is uncertain.9,10 Such an approach that included the testing of haplotype associations was used in the study by Tiret et al11 in this issue of Circulation.

Cytokines, Inflammatory Cascades, IL-18, and Atherothrombosis
Plasma lipid abnormality has long been an iconic cardiovascular risk marker. Now, the interrelatedness of lipids with metabolic, oxidative, and inflammatory factors in determining risk is increasingly recognized. The hydrolysis of oxidatively modified low-density lipoprotein in the arterial intima produces proinflammatory, chemotactic factors that attract and activate inflammatory cells. The discovery and functional assessment of cytokines and cytokine networks involved in the inflammatory cascade are receiving growing emphasis. Adipose tissue is increasingly recognized as an important source of proinflammatory cytokines.
IL-18 is among the more recently recognized of these cytokines. IL-18 is a pleiotropic proinflammatory cytokine that has immunomodulatory effects on both the innate and the adaptive immune systems. It uniquely can induce T helper cell 1 or T helper 2 cell polarization as a function of the prevailing immunologic environment. Originally designated interferon-γ-inducing factor, IL-18 has received considerable recent attention. Circulating levels of IL-18 have been reported to be increased in many disease processes, which suggests that it might participate in pathophysiology. Thus, it is understandable that this cytokine would be chosen to be examined in the context of cardiovascular disease.

The relationship of IL-18 to atherosclerotic disease is still unfolding. To date, the most definitive information has come from mouse models. Independent studies have shown that exogenously administered IL-18 accelerates the rate of atherosclerotic lesion development and increases plaque size and inflammatory cell content. Conversely, the IL-18 binding protein (IL-18BP), a natural antagonist of IL-18, decreases inflammatory cell infiltrate and generates a stable plaque phenotype.

Previous observational clinical studies have added some limited evidence to these animal studies favoring an etiologic role for IL-18. One study identified an association between plasma IL-18 and acute coronary syndromes and reported an inverse relationship between IL-18 concentrations and left ventricular ejection fraction. Another study identified IL-18 mRNA transcript within atherosclerotic lesions and reported an association between the (semiquantitative) level of transcript and plaque stability as determined by the presence or absence of symptoms. The largest prior observational study is represented by the initial report on the present study cohort.

**The Study in This Issue**

**Study Background and Aim**

On the basis of preclinical studies suggesting a pivotal role of IL-18 in the cytokine cascade of atherosclerosis, the AtheroGene investigators previously examined the predictive value of circulating IL-18 levels among a prospectively recruited cohort of 1299 patients with documented CAD. Baseline IL-18 concentrations were found to be higher among the 95 patients who suffered a fatal cardiovascular event (median, 68 versus 59 pg/mL; \( P < 0.0001 \)) over an average follow-up of 4 years. Risk persisted after adjustment for potential confounders, including ejection fraction and the established inflammatory markers hs-CRP, IL-6, and fibrinogen, with a hazard risk ratio of 3.3 for the highest versus lowest IL-18 quartile (\( P = 0.01 \)). IL-18 was predictive for both stable and unstable angina patients. However, this association study left unclear whether IL-18 plays a causative role or whether elevated levels simply reflect an ongoing inflammatory process. Determining to what extent IL-18 levels are regulated at the genetic level represents a logical approach to gaining additional insight into the question of causality. Hence, in the study by Tiret et al., the AtheroGene investigators aimed to determine and comprehensively investigate all common sequence variations in all 4 genes comprising the IL-18 system—IL-18, IL-18 receptor (IL-R1), IL-18 receptor accessory protein (IL-18 RAP), and IL-18BP—and to relate these genetic variations (individually and collectively) to circulating IL-18 levels and to cardiovascular events, updated since their previous report.

**Study Highlights**

The cohort in the study by Tiret et al. comprised 1288 patients whose follow-up was extended to a median of 6 years. All had baseline IL-18 levels and DNA sampled. This ambitious retrospective project determined all common genetic variations of the 4 genes of the IL-18 system; 22 polymorphisms (21 were SNPs) were found and tested independently or in linkage groups (haplotypes) as predictors of IL-18 concentrations, the intermediate phenotype, and clinical events (cardiovascular death, \( n = 142 \), the clinical end point. In addition, the predictive ability of IL-18 concentrations was reassessed during the extended follow-up.

The first notable observation was that IL-18 concentrations were no longer predictive of cardiovascular deaths occurring after 4 years. A prominent genetic result was the absence of predictive ability of most SNPs tested alone, in haplotype groups, or in combinations among genes for either IL-18 levels or outcomes. In contrast, one specific SNP and one haplotype in the IL-18 gene were informative for both. The A +183G polymorphism and the GCAGT haplotype, which carried this SNP, predicted a 9% decrease in serum IL-18 concentration (\( P < 0.001 \)). Covariate adjustment caused minimal modification of the association. Despite the statistical significance of the association, the impact was small, with all haplotypes together explaining only 1.8% of the variability of IL-18 concentrations, and the A +183G polymorphism alone, carried by the GCAGT haplotype, only 1.1%.

None of the polymorphisms predicted deaths over 6 years. Considering only deaths occurring during the first 4 years of follow-up, a significant global association between haplotypes of IL-18 and cardiovascular death was found, which persisted after adjustment for cardiovascular risk factors. Considering individual IL-18 haplotypes, again GCAGT was singled out, and it was found to be associated with a protective effect on mortality both before and after adjustment for traditional risk factors (HR = 0.57, \( P = 0.021 \)). Furthermore, the association was markedly weakened after additional adjustment for baseline IL-18 levels. The IL-18/A +183G polymorphism, located in the 3'UTR, was postulated to affect mRNA stability, providing a molecular rationale for the observations.

**Study Strengths and Limitations**

Overall, this is an impressive research effort using current genetic techniques in a sample of moderate size and numbers of clinical events. The “system approach” to IL-18 is to be commended and emulated. Indeed, it can be expected that common polymorphisms, even when functionally active, are unlikely to individually explain a high proportion of clinical risk in a complex, multifactorial, and multigenic disease such as CAD, especially when applied in a global fashion across groups with diverse genetic and environmental backgrounds. Otherwise, these variants likely would be suppressed or
eliminated by natural selection over time.) However, the results are complex and the clinical consequences appear to be limited.

Baseline IL-18 predicted mortality initially but not during longer-term follow-up. It is unfortunate that additional sera were not available to track IL-18 during follow-up; future studies should obtain serial samples and determine the dynamics of IL-18 and the predictive value on cardiovascular risk of variations in concentration over time. Until then, the utility and applicability of serum IL-18 concentration as a clinical risk indicator must be questioned. Also, inflammatory marker levels might interact with pharmacological therapy (eg, statins, antiplatelet therapy, converting enzyme inhibitors), affecting the marker level and predictive value.\(^\text{19}\)

Similarly, differing disease states (acute versus chronic coronary syndrome) might affect marker level, magnitude, and even direction of risk association. These factors were not well accounted for in the present study.

A minor allele of IL-18 and its associated haplotype were found to predict IL-18 levels but accounted for only 1% to 2% of IL-18 variability. This haplotype also predicted outcome (ie, it was protective) and IL-18 levels primarily accounted for this predictive effect, but, again, the variant explained only a minority of events. However, if IL-18 provides independent, complementary information to that of other cytokines and inflammatory markers (eg, IL-6, CRP), then it might be useful to develop a cytokine or inflammatory marker score in which a cluster of markers, each contributing incremental risk information, is assessed together to optimize risk prediction.\(^\text{20}\)

A concern arising in observational studies such as this is the use of multiple comparisons: ie, among 4 genes, 22 SNPs, and multiple haplotypes, which raises the chance of false-positive findings. By chance alone, a few associations would be expected to achieve statistical “significance.” Hence, replication in a prospectively studied, independent cohort is needed.\(^\text{7,8}\) Also, as Tiret et al\(^\text{11}\) note, although the study was inspired by interest in interactions among different genes, the multilocus exploratory approach was unrevealing and, admittedly, the study had limited power to detect these interactions, raising concerns about false-negative findings as well.

On the positive side, the study was based on sound biological rationale and on findings of preclinical and preliminary clinical studies, and a consistency of results between the intermediate phenotype (IL-18 level) and clinical outcome (CV death) was noted: Risk was accounted for by phenotypic effect, and an appealing mechanistic interpretation was proposed.

**Study Implications**

A valuable lesson of this study is the comprehensive, systems-based genetic approach taken, which might usefully be applied to other atherosclerosis-related molecular biological systems. In this study only one promising IL-18 gene-related SNP/haplotype association was found, and its contribution to risk was modest, the mechanism speculative, and the likelihood of replication uncertain. Hence, additional studies are needed. However, such modest steps in understanding the genetic underpinnings of atherosclerosis are likely to be the rule rather than the exception, and in combination with contributions from several other systems, these small steps might well account for the greater part of the heritable basis of atherothrombosis risk.

**Conclusion**

A comprehensive approach to the assessment of a genetic risk contribution of the IL-18 system to cardiovascular disease (ie, mortality) was undertaken. Results suggested the IL-18/A +183G polymorphism and its SNP-carrying haplotype to be good candidates for further investigations. Study limitations, including those noted above, suggest that results should be considered hypothesis generating and be replicated in further studies.

**References**


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