Inflammation is recognized for its important role in the pathophysiology of atherosclerosis (1). However, the use of genetic association studies to dissect the impact of inflammatory genes on vascular disease is challenging because of the relatively small effects expected for common variants and the difficulty of identifying surrogates that best reflect underlying disease processes. In this issue of Clinical Chemistry, Volcik and colleagues (2) suggest an original design to address these issues by looking for genetic associations with the cell-surface concentration of a pair of cognate cell-surface receptors, P-selectin and P-selectin glycoprotein ligand-1 (PSGL-1). P-selectin [encoded by the selectin P (granule membrane protein 140kDa, antigen CD62) (SELP) gene] is primarily stored in platelets and endothelial cells, but it translocates to the cell surface upon activation and binding to its counterreceptor PSGL-1 [encoded by the selectin P ligand (SELPLG) gene] located on leukocytes. Adhesion of leukocytes to endothelium, with subsequent migration into the vascular wall, similarly involves P-selectin interaction with PSGL-1. Thus, the P-selectin–PSGL-1 interaction is thought to be of central importance in thrombosis, hemostasis, and vascular inflammation. A role for the P-selectin system in atherogenesis is indicated by decreased atherosclerotic plaques among apolipoprotein E (APOE)–/– mice also deficient in either platelet or endothelial P-selectin (3). More broadly, epidemiological studies have linked increased concentrations of soluble P-selectin in plasma to a variety of acute and chronic vascular conditions.

Acquiring strong genetic evidence to corroborate these cellular phenomena in cardiovascular disease is difficult, and success has been limited. Mechanistic involvement of proteins in a disease process is no guarantee that functional genetic variations will be present at their respective gene loci. Even when such functional variations exist, evidence of their influence can be exceedingly difficult to capture because genetic effects, although they may be common, are often weak and not propagated through the multiplicity of intermediate biological steps separating a protein from a disease phenotype. Nevertheless, the T715P polymorphism of SELP, which Volcik et al. found to be associated with the concentration of P-selectin on the platelet cell-surface, is also known to be associated with concentrations of soluble P-selectin and was shown by the Coronary Artery Risk Development in Young Adults cohort to be associated with carotid intima-media thickness (4).

However, interpretation the results of these studies in a mechanistic context may be limited by the intrinsic nature of available phenotypes. Soluble P-selectin can originate from either platelets or endothelium, and might not always reflect concentration at biologically relevant sites. This limitation could equally affect genetic analysis of other biomarkers for cardiovascular disease, including, for example, concentration of plasma LDL cholesterol, for which a role in disease etiology is essentially certain, but likely are more closely related to interactions with endothelium than plasma concentration per se. More definitive insights might arise through genetic analysis of phenotypes that have more intimate connections to underlying disease etiology.

The study by Volcik et al. (2) does just that. Using flow cytometry in samples from the Atherosclerosis Risk in Communities study, the authors tested for associations between candidate functional polymorphisms in the SELP gene and expression of P-selectin on the surface of platelets as well as variants in the SELPLG gene and expression of the PSGL-1 on the surface of lymphocytes, granulocytes, and monocytes. Among 4 nonsynonymous single-nucleotide polymorphisms (SNPs) tested in SELP, the minor allele of the T715P substitution was associated with less platelet-surface expression of P-selectin among samples from white individuals. In the samples from African Americans, this trend was similar but not significant. Two of the other nonsynonymous SNPs tested, V599L and N562S, also had a significant association in the samples from whites and African Americans, respectively, but for both of these SNPs the significance level was insufficient to overcome the burden of multiple hypothesis testing. For the SELPLG gene, in the samples from African Americans the minor allele of an SNP encoding

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1 Brigham and Women’s Hospital and Harvard Medical School, Boston, MA. 
2 Nonstandard abbreviations: PSGL-1, P-selectin glycoprotein ligand-1; SNP, single-nucleotide polymorphism.
3 Genes: SELP, selectin P (granule membrane protein 140kDa, antigen CD62); SELPLG, selectin P ligand; APOE, apolipoprotein E.

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Editorials

Getting Closer to P-Selectin
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the M62I substitution had lower levels of cell-surface PSGL-1 in all 3 types of leukocytes, as did the minor allele of a second SNP in the 5’ untranslated region. In the samples from whites, the SELPLG M62I variant had much lower minor allele frequency, with an uncertain association, and the 5’ untranslated region SNP was not polymorphic. Previous studies among African Americans from this same cohort (5) had found less cardiovascular risk for the SELPLG M62I variant, consistent with the lower levels of cell-surface PSGL-1 in the present study. The authors speculate that the level of cell-surface PSGL-1 may be a mediator of cardiovascular risk. Indeed, their results show a correlation between expression level and cardiovascular risk. This association was not significant, however, and the authors invoke limiting power in the current sample of African Americans, which included only 24 and 23 cases, respectively, of coronary heart disease and ischemic stroke.

In spite of the advances reported by Volcik et al., this last hypothesis and the estimate of power remain problematic. Without knowledge of the underlying relationship between PSGL-1 concentration and incident disease, it is impossible to explain the lack of association. Certainly if expression level mediates incident disease, there would be much more power to detect this association than an association between genetic variation and disease, because only a very small proportion of the variance in surface expression is explained by the M62I polymorphism. Perhaps more relevant, however, is the focus of the study on genetic effects on expression rather than biological function, an alternative mechanism that is raised by the authors in the discussion. If the current observations are simply a byproduct of differences in biological activity, expression level may be less important for disease risk than, say, binding affinity between P-selectin variants and PSGL-1 variants.

The approach proposed by Volcik and colleagues is not limited in scope to atherosclerosis, and the ease of testing in blood samples combined with sophisticated molecular phenotyping of leukocytes suggests application to many other complex diseases with inflammatory or autoimmune etiology. Many of these diseases are known to be pathophysiological linked to specific cell-surface molecules, typified by CD40 and its ligand in systemic lupus erythematosus (6). Extended to the key cell-surface molecules for these other conditions, the study design put forward holds great promise for furthering our understanding of inflammatory diseases and their heritability.

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