Phospholipid Transfer Protein and Atherosclerosis
Genetic Studies Take Aim at a Moving Target

Ronald M. Krauss, MD

Phospholipid transfer protein (PLTP) is a member of the lipid transfer/lipoplysaccharide-binding protein family that first attracted attention by virtue of its functions in intravascular lipoprotein metabolism. These include its key role in mediating transfer of phospholipids from very-low-density lipoprotein to high-density lipoprotein (HDL) in conjunction with very-low-density lipoprotein lipolysis and its capacity to remodel HDL by promoting the production of larger HDL through fusion of 2 smaller particles with generation of lipid-poor apolipoprotein (apo) AI.1 The latter has properties consistent with pre-β1 HDL, a species that can contribute to cellular cholesterol efflux2 and plays a role in reverse cholesterol transport. Together with evidence that PLTP levels are correlated with plasma concentrations of HDL cholesterol and apoAI,3 these properties of PLTP led to the suggestion that it may have an atheroprotective role; however, an increasing body of evidence from animal and human studies has pointed to its proatherogenic effects. In apoB-transgenic and apoE-deficient mice, PLTP deficiency resulted in markedly decreased atherosclerosis,4 whereas in PLTP-transgenic mice, atherosclerosis was increased5 and macrophage cholesterol efflux and reverse cholesterol transport were impaired.6 Moreover, macrophages were shown to be a source of plasma PLTP,7 and bone marrow transplantation of cells overexpressing PLTP into low-density lipoprotein-receptor–null mice resulted in proatherogenic lipid changes and increased atherosclerosis.8 On the other hand, atherosclerosis was decreased by bone marrow transplantation of macrophages expressing PLTP in double low-density lipoprotein–receptor– and PLTP-deficient mice, in conjunction with reduced plasma total cholesterol and increased HDL.9 Thus, it appears that systemic PLTP has proatherogenic effects, although in some circumstances, macrophage-derived PLTP may be atheroprotective.

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Notably, 2 forms of immunoreactive plasma PLTP have been identified with high versus low phospholipid transfer activity,10 and it has been reported that only the form with high activity is capable of forming large HDL and pre-β1 HDL with the ability to promote macrophage cholesterol efflux.11 It has been proposed that the high-activity form of PLTP is associated with apoE, and that in the process of interacting with apoAI during the transfer of surface phospholipids from triglyceride-rich lipoproteins to HDL, it loses its phospholipid transfer activity.10,12

It is likely that functional properties of PLTP other than those affecting HDL particles and reverse cholesterol transport are of importance in influencing atherosclerosis susceptibility. Notably, PLTP deficiency has been shown to reduce hepatic secretion of apoB-containing lipoproteins4 and to increase their content of vitamin E, which leads to reduced susceptibility to oxidative modification.13 In addition, reduced intestinal cholesterol absorption has been demonstrated in PLTP-deficient mice.14

In humans, higher plasma PLTP activity has been associated with coronary artery disease15 and left ventricular dysfunction16 in case-control studies and with carotid artery intimal-medial thickness in patients with diabetes mellitus,17 as well as prospectively with the occurrence of cardiovascular events in patients with coronary artery disease who are undergoing statin treatment.18 On the other hand, in nondiabetic, nonsmoking patients with peripheral vascular disease, PLTP activity was reduced compared with control subjects, despite similar levels of immunoreactive PLTP, which raises the possibility that atherosclerosis is associated with altered distribution of the high- and low-activity forms of the protein.19

Importantly, both higher PLTP levels and activity have been shown to be strongly associated with the atherogenic components of the metabolic syndrome, as well as with type 2 diabetes mellitus and inflammation.20,21 Although these relationships likely reflect concordance with factors that influence PLTP regulation, it has been shown that PLTP is relatively highly expressed in both subcutaneous and visceral adipose tissue, which leads to the possibility that a significant portion of plasma PLTP may derive from this source22 and that this may contribute to the proatherogenic effects of adiposity.23

Multiple studies have shown a variety of associations of PLTP mass and activity with various plasma lipid and lipoprotein measurements, reflecting both the complex metabolic relationships of PLTP and its high- and low-activity forms. On the basis of multivariate regression models that included body mass index and levels of lipids and lipoproteins in 39 healthy subjects, Cheung et al12 recently reported that triglyceride was the only independent correlate of PLTP mass, whereas HDL cholesterol and large HDL particles were most strongly correlated with PLTP activity. PLTP-specific activity, which primarily reflects relative amounts of the high- versus low-activity forms, was related to all components of atherogenic dyslipidemia (positively with very-low-density lipoprotein, intermediate density lipoprotein, and...
small low-density lipoprotein particles and inversely with large low-density lipoprotein and large HDL). However, after adjustment for triglyceride, only the inverse relationship with large HDL particles remained significant, such that 45% of the variance was explained by HDL particle size alone. The latter finding is consistent with the hypothesis discussed above that binding of PLTP to large HDL in the process of lipid transfer leads to loss of activity. The basis for the relationship of PLTP activity to atherogenic dyslipidemia, however, remains unexplained.

The complex functional properties and metabolic associations of PLTP present the likelihood of multiple mechanisms affecting atherosclerosis, with the possibility that proatherogenic or antiatherogenic effects might be manifest under different physiological and pathological conditions. Identification of genetic variants that affect PLTP function or regulation affords the prospect of more directly assessing its overall impact on cardiovascular disease (CVD) in humans. Recently, Jarvik et al.\(^\text{24}\) reported that among a panel of 14 PLTP single-nucleotide polymorphisms (SNPs) tested for association with severe carotid artery disease in 442 male patients and 497 control subjects, the minor alleles of 3 SNPs (2 in intronic and 1 in the 5′ region) were associated with a protective odds ratio for carotid disease, whereas the minor allele of a fourth intronic SNP was associated with higher risk. One of the low-risk SNPs (rs6065904) was associated with reduced PLTP activity in both a subset of 87 individuals from the primary study and a replication cohort of 210 individuals, whereas no consistent effects on PLTP activity were found for the other 3 SNPs. In addition, 4 SNPs that were consistently associated with PLTP activity were not related to carotid artery disease risk. Among these was rs7679, which represented a large linkage disequilibrium block and had been reported previously to have a concordant association with both HDL cholesterol and hepatic PLTP gene expression, as well as an inverse association with plasma triglycerides.\(^\text{25}\)

Findings for an additional SNP imputed from HapMap (rs22494213) showed a relationship with PLTP activity but did not confirm earlier reports of an association with HDL cholesterol.\(^\text{26}\) Interestingly, among traits tested for relationships with PLTP activity, the strongest were for insulin or hemoglobin A1C, which together with age, sex, body mass index, and various combinations of genotypes explained about half of the total variance for this trait.\(^\text{24}\) However, after adjustment for the nongenotype covariates, there were no consistent relationships of any plasma lipids or lipoproteins, including HDL cholesterol, with those SNPs found to be associated with PLTP activity, which leaves open the question as to their pathophysiological consequences.

In this issue of *Circulation*, Vergeer et al.\(^\text{27}\) have provided compelling genetic evidence for a relationship between PLTP and CVD in humans using findings from 7 clinical studies in 3 northern European countries. On the basis of consistency of associations with PLTP activity in 2 of the study populations, 2 SNPs (rs387114 and rs6065904) were selected from a total of 6 candidates to construct a PLTP gene score that consisted of 0 to 4 of the combined alleles that were associated with reduced PLTP activity. The SNP associations were generally consistent with the findings of Jarvik et al.\(^\text{24}\) described above. Their functionality was supported by the demonstration that the PLTP gene score was strongly and linearly related to reduced hepatic PLTP transcript levels, although results for the individual SNPs were not provided. Most notably, the gene score was associated with a significantly lower odds ratio for CVD in 3 of the 5 clinical cohorts tested and in the combined data from all 5, with a 31% reduction in risk in the group with the highest score, representing approximately 5% of the total population. On the basis of the principle of Mendelian randomization, these findings, together with the results of Jarvik et al.\(^\text{24}\) point strongly to a role for altered PLTP regulation in the pathogenesis of atherosclerotic CVD. Again, however, there were no significant relationships between the PLTP variants and standard plasma lipid and lipoprotein biomarkers of CVD risk, including HDL cholesterol and apoAI.

However, Vergeer et al.\(^\text{27}\) have suggested that changes in HDL particle subpopulations may be involved in mediating the reduced CVD susceptibility associated with the combined PLTP genotypes. In 1 of their clinical cohorts (EPIC [European Prospective Investigation of Cancer]-Norfolk), the PLTP gene score was strongly and linearly related to concentrations of small HDL particles as measured by nuclear magnetic resonance spectroscopy, with a corresponding inverse relationship with large HDL particle size. They speculated on the basis of these findings that reduced PLTP activity may retard HDL remodeling, such that smaller HDL particles, perhaps with an enhanced capacity for promoting reverse cholesterol transport, accumulate in plasma. Although this is an appealing hypothesis, there are a number of considerations that raise questions as to its validity. Ideally, if the PLTP-related changes in HDL subclasses are in the CVD causal pathway, then their inclusion with the gene score in a multivariable regression model should reduce the statistical significance of the association of the score with CVD risk. As the authors point out, limited statistical and analytical power may be responsible for the lack of such a finding, but its absence nevertheless weakens the inference of functional significance of the HDL particle changes. Another concern is raised by the apparent inconsistency of their results with the earlier report, cited above, that PLTP activity is inversely related to levels of large HDL particles and HDL particle size,\(^\text{12}\) and thus, the generalizability of their findings to other populations is uncertain. Moreover, although pre-B1 HDL is very small, it constitutes only a minor proportion of the small HDL particles in the size range determined by nuclear magnetic resonance,\(^\text{2}\) and there is no compelling evidence that small HDL have enhanced atheroprotective functions compared with large HDL. On the contrary, there is evidence that smaller HDL particles can be associated with increased CVD risk, in part due to their relationship with other components of the metabolic syndrome.\(^\text{28}\) Finally, the multiplicity of potential effects of PLTP on atherogenic processes beyond those related to HDL function also needs to be strongly considered.

Overall, the studies by Jarvik et al.\(^\text{24}\) and Vergeer et al.\(^\text{27}\) reinforce the value of using both informative SNPs and measurements of gene function to establish a specific gene’s
association with CVD risk. But in the case of PLTP, the basis for the connection of gene to disease remains elusive, and multiple paths remain to be explored.

Disclosures

Dr Krauss has been a consultant to Celena Corporation.

References


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