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Genetic and Metabolic Influences on LDL Subclasses

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Abstract

Genetic and environmental factors influence LDL particle size and density, and expression of an atherogenic lipoprotein phenotype (ALP) characterized by predominance of small, dense LDL particles. Linkage of ALP to the LDL receptor locus has been reported previously. Quantitative sib-pair and relative-pair linkage methodologies were used to test for linkage of LDL particle size to candidate loci in 25 large pedigrees with familial coronary artery disease. Linkage to the LDL receptor gene locus was confirmed ($p=0.008$). Evidence was also obtained for linkage to the genes for apoCIII, cholesteryl ester transfer protein, and manganese superoxide dismutase. The results suggest multiple genetic determinants of LDL particle size that may involve different metabolic mechanisms giving rise to small, dense LDL and increased atherosclerosis risk.

LDL subclasses and atherogenic lipoprotein phenotype

An LDL subclass pattern characterized by a predominance of small, dense LDL particles, principally LDL3 [1], has been identified using both non-denaturing gradient gel electrophoresis [2] and analytic ultracentrifugation (Miller BD, Haskell WL, Alderman EL, Fair J, Krauss RM, unpublished data). The prevalence of this trait, which has been designated LDL subclass pattern B, is 30-35% in adult men, but is much lower in males <20 yr and in premenopausal women (5-10%)[2], and is intermediate (15-25%) in postmenopausal women [2-4]. Individuals with subclass pattern B have been shown to have higher levels of triglyceride-rich lipoproteins and remnants, reduced levels of HDL₂ [2] and a greater degree of resistance to insulin-stimulated glucose uptake than subjects with predominantly larger LDL (pattern A)[5]. Levels of plasma and LDL apoB are relatively higher in pattern B than pattern A subjects, indicative of increased numbers of LDL particles, but total LDL-cholesterol is minimally if at all higher [2], consistent with the shift toward denser, more lipid-depleted LDL particles.

The evidence that individuals with small, dense LDL have a number of interrelated metabolic features associated with increased risk of coronary artery disease led to the designation of pattern B as an atherogenic lipoprotein phenotype (ALP) [2]. Case-control studies of subjects with acute myocardial infarction [6] and angiographically documented coronary artery disease [7, 8] have shown up to a three-fold or greater [9] increase in risk associated with a predominance of small, dense LDL. However, a high degree of intercorrelation among the features of ALP have made it difficult to determine the extent to which individual components of the syndrome contribute to disease risk.

Genetic influences on LDL particle distribution

Heritability of LDL particle size, as assessed by relative concordance in monozygotic vs. dizygotic twins, has indicated that genetic factors account for 30-50% of the variation in LDL particle size in both men [10] and women [4], with the remainder due to non-genetic or environmental influences. A number of such influences have been identified, including abdominal adiposity [11], non-insulin dependent diabetes mellitus [12], use of progestin-containing oral contraceptives [13], and dietary fat and carbohydrate intake [14].

Complex segregation analyses in healthy families [15], and in families of probands with familial combined hyperlipidemia [16], have indicated that LDL subclass pattern B, as assessed by gradient gel electrophoresis, is under the influence of a major gene or genes, each with transmission consistent with autosomal dominant inheritance, and an additional polygenic or additive component. More recent studies, in which the small, dense LDL phenotype was assessed by density gradient ultracentrifugation [13] and by measurement of LDL particle size as a continuous parameter [17], have confirmed a major gene effect. The frequency of the allele(s) responsible for pattern B in these studies, which involved four different study populations, ranged from 0.1 to 0.3, consistent with the prevalence of the trait as assessed by population studies. It is of interest that in the familial combined hyperlipidemia families, the distribution of apoB levels in pattern B, but not pattern A subjects, was bimodal [18], raising the possibility of a second major gene responsible for apoB elevations that is fully expressed only in the genetic background of pattern B.

Linkage of pattern B to candidate gene loci chromosome 19p has been reported previously [19]. Using both two-point and multipoint linkage analyses, the maximal LOD (log odds ratio) score for linkage to the LDL receptor locus was 4.43 at a recombination fraction of 0.05, assuming 100% penetrance of *ALP*.

Weaker linkage was observed with the insulin receptor locus on chromosome 19p (LOD score 1.78 at a recombination fraction of zero). Utilizing nonparametric quantitative sib-pair and relative-pair linkage methodologies, we have tested for linkage of a gene or genes determining LDL particle size to the LDL receptor locus and other candidate gene loci in 278 members of 25 pedigrees in whom the proband and at least one other blood relative had coronary artery disease. We obtained evidence for linkage of a locus controlling LDL particle size to a three-allele marker within the LDL receptor gene locus ($p=0.008$). With relative-pairs, significant evidence for linkage ($p = 0.001-0.007$) was also observed for the apoCIII gene on chromosome 11, the gene for cholesteryl ester transfer protein on chromosome 16p, and the manganese superoxide dismutase gene locus on chromosome 6q. No linkage was observed for other candidate loci tested: apoB, apoAII, apo(a), apoE-CI-CII, lipoprotein lipase, and HDL binding protein.

While these genetic loci have been identified by polymorphic DNA markers that do not indicate the presence of causative mutations in the respective genes, it is intriguing that their protein products have connections to metabolic pathways that may be involved in the generation of the pattern B phenotype. Small, dense LDL have been shown to have reduced affinity for the LDL receptor [20], and, conceivably, altered LDL receptor function or regulation could result in further impairment of plasma clearance of these LDL or their metabolic precursors. Variation in insulin receptor function or regulation could be responsible for the insulin resistance associated with increased triglyceride, reduced HDL, and small, dense LDL [5].

It has been demonstrated that apoCIII gene haplotypes are associated with variation in plasma triglyceride levels [21], which in turn could affect levels of small, dense LDL [22]. It is also of interest that some investigators have

demonstrated association [23] and linkage [24] of polymorphisms in the AI/CIII/AIV cluster to familial combined hyperlipidemia.

In the case of CETP, there is evidence for polydispersity and increased mass of small, triglyceride-rich LDL particles in patients with homozygous CETP deficiency [25], and that in vitro incubation of LDL with CETP results in conversion to a larger and more uniform size distribution [26]. On the other hand, CETP may facilitate lipolytic conversion of larger to smaller LDL particles by promoting triglyceride transfer into the LDL core [27]. A possible mechanistic association of lipoprotein metabolism with MnSOD activity is much more speculative, but it is noteworthy that in vitro susceptibility of LDL to copper-induced oxidation increases as a function of decreasing LDL particle size [28].

Other candidate mechanisms for the small, dense LDL phenotype include those that result in overproduction and/or impaired clearance of triglyceride-rich lipoproteins. These particles could include precursors of small, dense LDL, or could promote their formation by cycles of triglyceride exchange and lipolysis as described above. There is evidence for reduced exogenous triglyceride clearance in pattern B subjects, independent of fasting triglyceride level [12], as well as recent evidence from stable isotope kinetic studies for reduced fractional catabolism of VLDL and IDL fractions in comparison with pattern A subjects (Krauss RM, La Belle M, Blanche PJ, Shames D, and Hellerstein M, unpublished observations). While pattern B subjects have been found to have reduced levels of heparin-releasable lipoprotein lipase activity [29, 30], and patients with heterozygous lipoprotein lipase deficiency have a lipoprotein phenotype that appears similar to that found in subjects with pattern B [31], the prevalence of genetic lipoprotein lipase deficiency appears to be much lower than that of pattern B, and LDL particle size was not linked to the lipoprotein lipase gene locus in the present study. However, it remains possible that other genetically influenced factors resulting in

retardation of catabolism of triglyceride-rich lipoproteins or their remnants, such as increased levels of apoCIII as suggested above, may have an etiologic or contributory role in many subjects with the small, dense LDL phenotype.

The findings described above have led to the hypothesis that several different genetic loci contribute to the expression of the small, dense LDL phenotype, that these genes cumulatively influence the prevalence of the trait in the general population, and that in any family one or more of the loci are responsible for the major gene and additive effects identified by complex segregation analyses. Moreover, the results suggest that different genetically determined metabolic mechanisms may give rise to ALP, and that these differences, as well as gene-environment and gene-gene interactions, may result in variability of metabolic and pathologic manifestations among affected individuals.

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References

1. Krauss RM, PJ Blanche. *Curr. Opin. Lipidol.* 1992;3:377-383.
2. Austin MA, MC King, KM Vranizan, RM Krauss. *Circulation* 1990;82:495-506.
3. Campos H, E Blijlevens, JR McNamara, JM Ordovas, BM Posner, PWF Wilson, WP Castelli, EJ Schaefer. *Arterioscler Thromb* 1992;12:1410-1419.
4. Austin MA, B Newman, JV Selby, K Edwards, EJ Mayer, R M Krauss. *Arterioscler Thromb* 1993;13:687-695.
5. Reaven GM, Y-DI Chen, J Jeppesen, P Maheux, R M Krauss. *J. Clin. Invest.* 1993;92:141-146.
6. Austin MA, JL Breslow, CH Hennekens, JE Buring, WC Willett, RM Krauss. *JAMA* 1988;260:1917-1921.
7. Campos H, JJ Genest Jr., E Blijlevens, JR McNamara, JL Jenner, JM Ordovas, PWF Wilson, EJ Schaefer. *Arterioscler Thromb* 1992;12:187-195.
8. Coresh J, PO Kwiterovich Jr., HH Smith, PS Bachorik. *J Lipid Res* 1993;34:1687-1697.
9. Griffin BA, DJ Freeman, GW Tait, J Thomson, MJ Caslake, CJ Packard, J Shepherd. *Atherosclerosis* 1994;106:241-253.
10. Lamon-Fava S, D Jimenez, JC Christian, RR Fabsitz, T Reed, D Carmelli, WP Castelli, JM Ordovas, PWF Wilson, EJ Schaefer. *Atherosclerosis* 1991;91:97-106.
11. Terry RB, PD Wood, WL Haskell, ML Stefanick, R M Krauss. *J. Clin. Endocrinol. Metab.* 1989;68:191-199.
12. Feingold KR, C Grunfeld, M Pang, W Doerrler, RM Krauss. *Arterioscler Thromb* 1992;12:1496-1502.
13. de Graaf J, DW Swinkels, AFJ de Haan, PNM Demacker, AFN Stalenhoef. *Am. J. Hum. Genet.* 1992;51:1295-1310.

14. Dreon DM, HA Fernstrom, B Miller, RM Krauss. *FASEB J* 1994;8:121-126.
15. Austin MA, MC King, KM Vranizan, B Newman, RM Krauss. *Am J Hum Genet* 1988;43:838-46.
16. Austin MA, JD Brunzell, WL Fitch, RM Krauss. *Arteriosclerosis* 1990;10:520-530.
17. Bu X, RM Krauss, D Puppione, R Gray, JI Rotter. *Am. J. Hum. Genet.* 1992;51:A336.
18. Austin MA, H Horowitz, E Wijsman, RM Krauss, J Brunzell. *Atherosclerosis* 1992;92:67-77.
19. Nishina PM, JP Johnson, JK Naggert, RM Krauss. *Proc Natl Acad Sci USA* 1992;89:708-712.
20. Nigon F, P Lesnik, M Rouis, MJ Chapman. *J Lipid Res* 1991;32:1741-1753.
21. Dammerman M, LA Sandkuijl, JL Halaas, W Chung, JL Breslow. *Proc Natl Acad Sci USA* 1993;90:4562-4566.
22. Krauss RM. *Am Heart J* 1987;113:578-582.
23. Tybjaerg-Hansen A, BG Nordestgaard, LU Gerdes, O Færgeman, SE Humphries. *Atherosclerosis* 1993;100:157-169.
24. Wojciechowski AP, M Farrall, P Cullen, T Wilson, JD Bayliss, B Farren, BA Griffin, MJ Caslake, CJ Packard, J Shepherd, R Thakker, J Scott. *Nature* 1991;349:161-164.
25. Sakai N, Y Matsuzawa, K Hirano, S Yamashita, S Nozaki, Y Ueyama, M Kubo, S Tarui. *Arteriosclerosis* 1991;11:71-79.
26. Lagrost L, H Gandjini, A Athias, V Guyard-Dangremont, C Lallemand, P Gamber. *Arterioscler Thromb* 1993;13:815-825.
27. Lagrost L, P Gamber, C Lallemand. *Arterioscler. Thromb.* 1994;14:1327-1336.

28. Tribble DL, LG Holl, PD Wood, RM Krauss. Atherosclerosis 1992;93:189-199.
29. Jansen H, W Hop, A Van Tol, AVG Bruschke, JC Birkenhäger. Atherosclerosis 1994;107:45-54.
30. Campos H, DM Dreon, RM Krauss. Associations of hepatic and lipoprotein lipase activities with changes in dietary intake and low density lipoprotein subclasses. J. Lipid Res. (in press).
31. Miesenböck G, B Hölzl, B Föger, E Brandstätter, B Paulweber, F Sandhofer, JR Patsch. J Clin Invest 1993;91:448-455.