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Genetic Variation in PLTP Modulates Lipoprotein Profiles in Hyperalphalipoproteinemia

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Abstract

We previously demonstrated the role of a Phospholipid Transfer Protein (PLTP) gene variation (rs2294213) in determining levels of HDL-C in hypoalphalipoproteinemia (HypoA). We have now explored the role of PLTP in hyperalphalipoproteinemia (HyperA). The human PLTP gene was screened for sequence anomalies by DNA melting in 107 subjects with HyperA. The association with plasma lipoprotein levels was evaluated. We detected 7 sequence variations: 1 previously reported variation (rs2294213), and 5 novel mutations including 1 missense mutation (L106F). PLTP activity was unchanged in the p.L106F mutation. The frequency of the rs2294213 minor allele was markedly increased in the HyperA group (7.0%) in comparison with a control group (4.3%) and the HypoA group (2.2%). Moreover, rs2294213 was strongly associated with HDL-C levels. Linear regression models predict that possession of the rs2294213 minor allele increases HDL-C independent of triglycerides. These findings extend the association of rs2294213 with HDL-C levels into the extremes of the HDL distribution.

Keywords

dyslipidemia; genetic polymorphism; atherosclerosis; cardiovascular diseases

Introduction

We define hyperalphalipoproteinemia (HyperA [OMIM #143470]) as a level of HDL-C greater than the population-based 80th percentile. In addition, it is characterized by altered HDL particle composition, metabolism, and function, including impaired antioxidant activity.(1,2)

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Rare causes of Familial HyperA include genetic deficiencies of plasma cholesteryl ester transfer protein (CETP) or hepatic lipase (HL).(3) With the exception of HL(4), the contributions of common functional polymorphisms to HyperA remain largely unknown. However, the fact that the heritability of HDL-C levels is estimated at approximately 50%(5), suggests that such polymorphisms exist.

Increasing evidence indicates an important role for phospholipid transfer protein (PLTP) in determining the plasma level of HDL-C.(6) The involvement of PLTP in lipoprotein metabolism is multifold and complex. This includes the transfer of phospholipids between plasma lipoproteins, specifically, phospholipids from triglyceride-carrying lipoproteins into HDL, and the remodeling of HDL particles in both size and composition.(7,8) In reverse cholesterol transport, ATP-binding cassette A1 (ABCA1) promotes the efflux of phospholipids and unesterified cholesterol from cell membranes in the peripheral tissues to pre- β -HDL. (9)

Pre- β -HDL is described as either discoidal in particle shape with 2–3 molecules of apo A–I (plus phospholipids and unesterified cholesterol) or as a monomolecular lipid-free/poor apo A–I.(10) Limited reports from human studies suggest that PLTP activity may be positively correlated to HDL; moreover, may also be independently and positively related to coronary artery disease.(11) PLTP overexpression in PLTP transgenic mice increases the influx of phospholipids and cholesterol into HDL, causing an increase in pre- β -HDL and decreased HDL levels.(11) In our previous study (12), we established the influence of a common PLTP gene variant (rs2294213, c.69+26G>C) in a case-control study of hypoalphalipoproteinemia (HypoA). The role of common gene polymorphisms in quantitative variation in HDL-C levels, including both HypoA and HyperA(13), suggests that examination of PLTP variations in HyperA may provide novel insight into its role in HDL metabolism. Herein, we examine the role of PLTP gene variation in HyperA as a means to demonstrate the contribution of this gene locus to quantitative variation in HDL-C.

In a case-control study of HyperA, we screened for sequence anomalies in the PLTP gene. We also tested for genetic association of discovered variations with HypoA and biochemical measurements (i.e., lipoprotein compartments). Genetic lesions leading to coding sequence missense changes were examined for potential differences in biochemical properties. We report a number of new sequence anomalies within the PLTP gene and provide further evidence that this gene is involved in determining HDL-C levels. Our findings showed that the **rs2294213** minor allele is associated with elevated levels of HDL-C.

Methods

Study Design

The design was a retrospective case-control study employing a non-Hispanic Caucasian sample of subjects from the University of California, San Francisco (UCSF) Genomic Resource in Arteriosclerosis (GRA).(14) The GRA is a repository of DNA samples from more than 12,000 patients, along with their blood samples and clinical data. Subjects with HyperA (n=107) were identified from the GRA as individuals with HDL-C levels greater than 80 mg/dL. Healthy controls (n=365) had normal lipoprotein profiles.(15) All subjects gave informed consent in a protocol approved by the UCSF Committee on Human Research. Clinical and demographic data were available for all subjects and baseline lipoprotein measurements were obtained when patients had not taken lipid-lowering medications for at least one month.

Measurements

Genotypic and Phenotypic Studies—Genomic DNA was prepared from whole blood obtained from patients in the GRA population of UCSF.(14) Blood was drawn after a 10-hour

fast; lipoprotein quantification was carried out using standard protocols.(16–19) Standards were provided by the Centers for Disease Control (Atlanta, Georgia, USA). Baseline lipoprotein measurements were obtained when patients had received no lipid lowering medication for at least 1 month, a standard ‘wash-out’ period for such medications.(14)

Molecular Gene Scan of PLTP Gene—Denaturing High Performance Liquid Chromatography (dHPLC)(20) and Denaturing-Gradient Gel Electrophoresis (DGGE)(21) mutation detection were both employed to scan the 10 amplicons which spanned the 16 exons encoding the full length PLTP transcript (BC01984) using standard protocols. Exon 5, which is absent in one of the two known PLTP splice variants(22), was included in the analysis. Direct DNA sequencing was used further to identify the underlying DNA variations seen with wave (dHPLC) or electrophoresis (DGGE). Nomenclature guidelines for position and sequence changes described herein are available at http://archive.uwcm.ac.uk/uwcm/mg/docs/mut_nom.html.

In our previous report, (12) conditions for molecular genetic analysis of the PLTP gene (i.e., primer sequences) are provided. Sequence anomalies (i.e., mutations, polymorphisms) were screened against public sequence databases (i.e., genome.ucsc.edu, www.ensembl.org) to determine if they were novel or previously deposited as identified by Reference Sequence ID (rsID).

Site-Directed Mutagenesis—For expression of ‘wild-type’ and mutant PLTP (wild-type PLTP in pSVL plasmid was a gift from Christian Ehnholm, National Public Health Institute, Helsinki, Finland), a pSVL construct was used. Site-directed mutagenesis was carried out using the QuickChange Site-Directed Mutagenesis Kit (Stratagene Inc., La Jolla, CA).

Cell Culture—COS-7 cells were grown in DMEM supplemented with 10% FBS, L-glutamine, streptomycin, and penicillin. On the day before transfection, the cells (1.2×10^6) were seeded on 6-cm culture dishes. They were then transiently transfected using PolyFect Reagent (Qiagen Inc., Valenica, CA) according to the manufacturer’s protocol, and grown in serum-free medium overnight. Supernatants were removed after 72h, centrifuged in order to remove detached cells, and used for PLTP transfer activity and mass analysis.

Specific PLTP activity—Using a radiometric assay,(23,24) PLTP activity was measured. In this assay, the ability of PLTP to transfer phospholipids from [C^{14}] DPPC donor vesicles to ultracentrifugally isolated HDL₃ acceptor particles are measured. PLTP mass was obtained from Western blots. Culture supernatants were concentrated using acetone precipitation, separated on SDS gels, transferred onto nitrocellulose membranes and blotted using a monoclonal anti-PLTP antibody JH59(25) (a gift from Drs. Matti Jauhiainen and Christian Ehnholm, National Public Health Institute, Helsinki, Finland). The relative PLTP mass compared to the wild-type was obtained by scanning and quantitation of the PLTP bands. Specific PLTP activity was obtained by dividing the activity by mass and is expressed relative to the wild-type PLTP (wildtype PLTP activity was 727 ± 148 SD nmol/ml/h and was set to a value of 1.0). All mutations were assayed in four replicates, and included both positive and negative controls.

Statistical Methods

Statistical analyses were conducted using the SPSS for Windows (v11.0.1, 2001) system.(26) The gene-counting method was used to determine allele and genotype frequencies directly. Tests for Hardy-Weinberg equilibrium in controls, and allelic or genotypic association in cases versus controls, were evaluated by χ^2 test. Two-group comparisons of means of transformed or normally distributed variables used the independent samples Student’s t-test (two-tailed).

Natural log transformation was found to be appropriate for the normalization of variables. Two-group comparisons of means of untransformed, non-normally distributed variables used the Wilcoxon two-sample test. The general linear model (GLM) was used to fit regression models. Power transformations of potential predictor variables were examined to maximize the explanatory power of the overall model (by maximizing the F statistic). Selected interaction effects and covariate-adjusted means of the transformed responses for levels of categorical factors were tested using procedure GLM. Interaction effects with $p < 0.10$ were retained. Data in Tables are presented as the mean, $\pm$ one standard deviation.

Results

Baseline Characteristics of the Study Populations

The clinical characteristics of subjects with HyperA, HypoA and healthy controls are described in Table 1. Compared to healthy controls, HyperA subjects were older, had lower BMI, and were predominantly women. HyperA subjects also had higher total cholesterol (TC), LDL-C and HDL-triglycerides (TG), and lower VLDL-TG, and VLDL-C (Table 1).

Results of PLTP molecular gene scan

Mutational analysis of the 16 exons and flanking regions of the PLTP gene in the HyperA group detected several sequence anomalies, including the single nucleotide polymorphism (SNP) in intron 1 that we reported previously (**rs2294213**). (12) One novel missense mutation was identified in exon 4 (p.L106F, c.316C>T). Four novel (c.329+86T>C; c.410-21insT; c.613+121_123delTGT; c.1218+87G>T) and one previously identified (c.613+84_89delAGTCCT)(12) mutations were identified in eight subjects with HyperA.

Relative frequencies of rs2294213 in the HypoA, control and HyperA groups are 2.2%, 4.3%, and 7.0%, respectively (Table 2). There is a significantly greater frequency of the minor G allele in the HyperA group compared to the control group ($\chi^2 = 4.436$, 1 df, $p = 0.035$; odds ratio [OR] = 2.035; 95% confidence interval [CI]: 1.039, 3.985) and the HypoA group ($\chi^2 = 11.005$, 1 df, $p = 0.001$; OR = 3.224; 95% CI: 1.561, 6.661). No significant differences were seen in the minor allele frequency between sexes in the HyperA group ($\chi^2 = 1.724$, 1 df, $p = 0.19$). The genotype distribution did not deviate significantly from Hardy-Weinberg expectations ($\chi^2 = 0.122$, 1 df, $p = 0.73$).

Differences in demographic and clinical characteristics and lipoprotein components were examined by rs2294213 genotype (GG vs. GC + CC) are provided in Table 3. Carriers of the minor allele displayed elevated VLDL-C, as well as modestly elevated levels of TG and VLDL-TG. Minor allele carriers also displayed modestly decreased HDL-C.

Functional Studies of PLTP Missense Mutations

To study defects in lipid transfer activities of mutant proteins, site-directed mutagenesis was carried out, followed by transient transfection of COS7 cells and subsequent PLTP functional assay (radioisotopic method). Protein content was determined by western blotting. This variant showed specific transfer activity comparable to wild-type protein (p.L106F, c.316C>T: $111.4\% \pm 23.2\%$, $p > 0.05$, $n = 4$). The results are mean values $\pm$ S.E. of four independent experiments and are expressed as percentages of the activity of wild-type PLTP and assessed by Student's unpaired t-test, two-tailed. The mutation was synthesized and secreted from the cells as efficiently as the wild-type PLTP.

Linear Regression Analysis of Lipoprotein Measures in Carriers of rs2294213

In the model fitted for HDL-C, PLTP rs2294213 genotype (GG homozygotes versus GC+CC minor allele carriers), participant's age (in years), plasma triglyceride level (in mg/dL), sex,

clinical category (HypoA, Control, HyperA), and BMI were significant predictors retained in the model ($F = 239.66$, $p < 0.0001$) (Table 4). Controlling for clinical category, age, sex, TG, and BMI, minor allele carriers (GC+CC) had a mean HDL-C level that was 3.55 ± 1.90 mg/dL higher than common allele homozygotes (95% CI: $-0.19, 7.93$, $p=0.063$). An interaction between clinical category and genotype was observed ($p=0.013$), with carriers of the minor allele having higher levels of HDL-C in HypoA (GG: 32.69 [95% CI: $31.06, 34.33$]; GC+CC: 35.27 [95% CI: $28.21, 42.33$]) and Controls (GG: 48.81 [95% CI: $47.24, 50.38$]; GC+CC: 59.21 [95% CI: $53.88, 64.53$]) versus lower HDL-C levels in HyperA (GG: 91.46 [95% CI: $88.84, 94.07$]; GC+CC: 89.13 [95% CI: $83.03, 95.24$]). The model explained 81.7% of the variance HDL-C levels in this sample with genotype accounting for 0.7% of the variability in HDL-C and the clinical category by genotype interaction accounting for an additional 1.8% of the variability in HDL-C.

Discussion

Elevated HDL-C is a significant negative risk factor for coronary artery disease.(27–29) In the current study, the PLTP gene was chosen as a potential determinant of HDL-C levels. The effect of PLTP on HDL-C levels is attributed to its facilitated transfer of phospholipids from triglyceride-rich lipoproteins (TRL) to HDL, and the generation of pre- β -HDL in reverse cholesterol transport.

It is clear from the current findings that the rs2294213 variant of the PLTP gene is associated with the lipoprotein profile with an increased level of HDL-cholesterol in healthy subjects. Interrogation of the International HapMap database (<http://www.hapmap.org>) yields only one SNP (rs2294212) in strong linkage disequilibrium (LD, $r^2 = 1.0$) with rs2294213, located 128bp downstream (c.69+154G>C) in intron 1. Previous examinations of the human PLTP promoter have failed to include these two SNPs.(30–34) Analysis of the two alleles represented by rs2294213 using AliBaba2.1 (<http://www.gene-regulation.com/pub/programs/alibaba2>) showed the creation of the overlapping putative Simian-virus-40-protein-1 (Sp1) and Yin Yang Protein 1 (YY1) binding sites in minor allele carriers (c.69+26C). Promoter analysis of both the LDL-receptor(35) and the HDL-receptor(36) demonstrated that co-stimulation of the Sp1/YY1 sites modulates gene expression. A recent survey of SNPs and haplotypes in the PLTP gene provided evidence of an association between another intron 1 polymorphism (rs553359) and obesity-related traits.(37) Unfortunately, rs553359 (and rs2294212) was not included in the amplicons designed to analyze exon 1 and exons 2 and 3. Moreover, rs553359 was not in LD ($r^2 < 0.8$) with rs2294213. Functional studies are required to formally demonstrate the functional impact of rs2294213.

PLTP's role in phospholipid transfer from TRL, phospholipid and cholesterol acquisition from peripheral cells, and participation in hepatic synthesis of VLDL, indicates that PLTP is a central effector of HDL metabolism. In the present study, we explored further the impact of rs2294213, mapping to intron 1 of PLTP, that we previously demonstrated as being associated with anti-atherogenic changes in lipoprotein profiles.

Previously, we reported decreased PLTP activity in one (p.R235W) of four (p.E72G, p.S119A, p.S124Y, and p.R235W) missense mutations in the HypoA group using an *in vitro* activity assay.(12) Whereas the L106F missense mutation was only identified in one HyperA subject, it has the potential to further our understanding of PLTP structure and function. Predictions regarding the potential impact of this missense mutation on PLTP structure can be made within the context of the structural model proposed by Desrumaux and colleagues.(38) This mutant is predicted to be “benign” when analyzed online by PolyPhen (at <http://genetics.bwh.harvard.edu/cgi-bin/pph/polyphen.cgi>). The L106F mutation does not induce any gross structural abnormalities affecting secretion of the mutant protein. Though it

appears to be benign, it is unclear if the L106F mutation results in more subtle functional differences that the current in vitro assay is not sufficiently sensitive to discern. Though the PLTP activity assay is used to assay the impact of these missense mutations, additional analysis of these sequence variations with respect to PLTP-mediated remodeling of HDL(7), ABCA1-mediated efflux of cholesterol from peripheral tissues to HDL(9), and its participation in hepatic VLDL synthesis(39–41) is warranted.

In our previous report, we detailed potential study limitations that are also relevant to the current report.(12) These include the fact that the participants recruited into the GRA were enrolled primarily from tertiary lipid clinics, which are most commonly referred for elevated LDL-C and could explain their elevated LDL-C levels in this study. Lastly, though measurement of sequence anomalies by DGGE and dHPLC has detection sensitivity approaching 100%(42), some sequence anomalies may have gone undetected.

This is the first study to screen the entire PLTP gene for sequence anomalies in individuals with HyperA. Our findings show that the rs2294213 minor allele was enriched in individuals with HyperA. The inverse relationship between HDL-C and risk of coronary heart disease is firmly established. This and our previous report(12) are the first in-depth sequence analyses of the PLTP gene in humans and identify a novel polymorphism associated with altered lipoprotein profiles. The strikingly progressive increase in representation of this minor allele with ascending HDL-C levels presents strong inference that PLTP is an important determinant of HDL metabolism and makes PLTP a potential target for pharmacological intervention.

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References

1. Nofer JR, Walter M, Assmann G. Current understanding of the role of high-density lipoproteins in atherosclerosis and senescence. *Expert Rev Cardiovasc Ther* 2005;3:1071–1086. [PubMed: 16292998]
2. Qasim A, Rader DJ. Human genetics of variation in high-density lipoprotein cholesterol. *Curr Atheroscler Rep* 2006;8:198–205. [PubMed: 16767843]
3. Tall, AR.; Breslow, JL.; Rubin, EM. The Metabolic and Molecular Bases of Inherited Disease. Chapter 121: Genetic Disorders Affecting Plasma High-Density Lipoproteins. New York: McGraw-Hill; 2001.
4. Iijima H, Emi M, Wada M, Daimon M, Toriyama S, Koyano S, Sato H, Hopkins PN, Hunt SC, Kubota I, et al. Association of an intronic haplotype of the LIPC gene with hyperalphalipoproteinemia in two independent populations. *J Hum Genet* 2008;53:193–200. [PubMed: 18160998]
5. Austin MA, King MC, Bawol RD, Hulley SB, Friedman GD. Risk factors for coronary heart disease in adult female twins. Genetic heritability and shared environmental influences. *Am J Epidemiol* 1987;125:308–318. [PubMed: 3812436]
6. Brewer HB Jr, Remaley AT, Neufeld EB, Basso F, Joyce C. Regulation of plasma high-density lipoprotein levels by the ABCA1 transporter and the emerging role of high-density lipoprotein in the treatment of cardiovascular disease. *Arterioscler Thromb Vasc Biol* 2004;24:1755–1760. [PubMed: 15319263]
7. Settasatian N, Duong M, Curtiss LK, Ehnholm C, Jauhiainen M, Huuskonen J, Rye KA. The mechanism of the remodeling of high density lipoproteins by phospholipid transfer protein. *J Biol Chem* 2001;276:26898–26905. [PubMed: 11325961]
8. Jiang XC, Bruce C, Mar J, Lin M, Ji Y, Francone OL, Tall AR. Targeted mutation of plasma phospholipid transfer protein gene markedly reduces high-density lipoprotein levels. *J Clin Invest* 1999;103:907–914. [PubMed: 10079112]

9. Oram JF, Wolfbauer G, Vaughan AM, Tang C, Albers JJ. Phospholipid transfer protein interacts with and stabilizes ATP-binding cassette transporter A1 and enhances cholesterol efflux from cells. *J Biol Chem* 2003;278:52379–52385. [PubMed: 14559902]
10. Rye KA, Barter PJ. Formation and metabolism of prebeta-migrating, lipid-poor apolipoprotein A-I. *Arterioscler Thromb Vasc Biol* 2004;24:421–428. [PubMed: 14592845]
11. Schlitt A, Bickel C, Thumma P, Blankenberg S, Rupprecht HJ, Meyer J, Jiang XC. High plasma phospholipid transfer protein levels as a risk factor for coronary artery disease. *Arterioscler Thromb Vasc Biol* 2003;23:1857–1862. [PubMed: 12947020]
12. Aouizerat BE, Engler MB, Natanzon Y, Kulkarni M, Song J, Eng C, Huuskonen J, Rivera C, Poon A, Bensley M, et al. Genetic variation of PLTP modulates lipoprotein profiles in hypoalphalipoproteinemia. *J Lipid Res* 2006;47:787–793. [PubMed: 16388083]
13. Klos KL, Kullo IJ. Genetic determinants of HDL: monogenic disorders and contributions to variation. *Curr Opin Cardiol* 2007;22:344–351. [PubMed: 17556888]
14. Pullinger CR, Hennessy LK, Chatterton JE, Liu W, Love JA, Mendel CM, Frost PH, Malloy MJ, Schumaker VN, Kane JP. Familial ligand-defective apolipoprotein B. Identification of a new mutation that decreases LDL receptor binding affinity. *J Clin Invest* 1995;95:1225–1234. [PubMed: 7883971]
15. Summary of the second report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel II). *Jama* 1993;269:3015–3023. [PubMed: 8501844]
16. Rush KLL, Turrell J. Automated simultaneous cholesterol and triglyceride determinations on the Autoanalyser II instrument: Advances in automated analysis. *Techincon International Congress* 1970;1:503.
17. Warnick GR, Benderson J, Albers JJ. Dextran sulfate-Mg²⁺ precipitation procedure for quantitation of high-density-lipoprotein cholesterol. *Clin Chem* 1982;28:1379–1388. [PubMed: 7074948]
18. Kane JP, Malloy MJ, Ports TA, Phillips NR, Diehl JC, Havel RJ. Regression of coronary atherosclerosis during treatment of familial hypercholesterolemia with combined drug regimens. *Jama* 1990;264:3007–3012. [PubMed: 2243428]
19. Havel RJ, Eder HA, Bragdon JH. The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. *J Clin Invest* 1955;34:1345–1353. [PubMed: 13252080]
20. O'Donovan MC, Oefner PJ, Roberts SC, Austin J, Hoogendoorn B, Guy C, Speight G, Upadhyaya M, Sommer SS, McGuffin P. Blind analysis of denaturing high-performance liquid chromatography as a tool for mutation detection. *Genomics* 1998;52:44–49. [PubMed: 9740670]
21. Fischer SG, Lerman LS. DNA fragments differing by single base-pair substitutions are separated in denaturing gradient gels: correspondence with melting theory. *Proc Natl Acad Sci U S A* 1983;80:1579–1583. [PubMed: 6220406]
22. Schneider M, Verges B, Klein A, Miller ER, Deckert V, Desrumaux C, Masson D, Gambert P, Brun JM, Fruchart-Najib J, et al. Alterations in plasma vitamin E distribution in type 2 diabetic patients with elevated plasma phospholipid transfer protein activity. *Diabetes* 2004;53:2633–2639. [PubMed: 15448094]
23. Jauhiainen M, Metso J, Pahlman R, Blomqvist S, van Tol A, Ehnholm C. Human plasma phospholipid transfer protein causes high density lipoprotein conversion. *J Biol Chem* 1993;268:4032–4036. [PubMed: 8440695]
24. Damen J, Regts J, Scherphof G. Transfer of [¹⁴C]phosphatidylcholine between liposomes and human plasma high density lipoprotein. Partial purification of a transfer-stimulating plasma factor using a rapid transfer assay. *Biochim Biophys Acta* 1982;712:444–452. [PubMed: 7126618]
25. Huuskonen J, Jauhiainen M, Ehnholm C, Olkkonen VM. Biosynthesis and secretion of human plasma phospholipid transfer protein. *J Lipid Res* 1998;39:2021–2030. [PubMed: 9788248]
26. SPSS, I. SPSS. SPSS for Windows. Release 11.0.1 standard version (15 Nov 2001). Chicago, IL: 2001.
27. Gordon T, Castelli WP, Hjortland MC, Kannel WB, Dawber TR. High density lipoprotein as a protective factor against coronary heart disease. The Framingham Study. *Am J Med* 1977;62:707–714. [PubMed: 193398]

28. Miller NE, Thelle DS, Forde OH, Mjos OD. The Tromso heart-study. High-density lipoprotein and coronary heart-disease: a prospective case-control study. *Lancet* 1977;1:965–968. [PubMed: 67464]
29. Toth PP. High-density lipoprotein as a therapeutic target: clinical evidence and treatment strategies. *Am J Cardiol* 2005;96:50K–58K. 34K–35K.
30. Laffitte BA, Joseph SB, Chen M, Castrillo A, Repa J, Wilpitz D, Mangelsdorf D, Tontonoz P. The phospholipid transfer protein gene is a liver X receptor target expressed by macrophages in atherosclerotic lesions. *Mol Cell Biol* 2003;23:2182–2191. [PubMed: 12612088]
31. Tu AY, Albers JJ. DNA sequences responsible for reduced promoter activity of human phospholipid transfer protein by fibrates. *Biochem Biophys Res Commun* 1999;264:802–807. [PubMed: 10544012]
32. Tu AY, Albers JJ. Glucose regulates the transcription of human genes relevant to HDL metabolism: responsive elements for peroxisome proliferator-activated receptor are involved in the regulation of phospholipid transfer protein. *Diabetes* 2001;50:1851–1856. [PubMed: 11473048]
33. Tu AY, Wolfbauer G, Albers JJ. Functional characterization of the promoter region of the human phospholipid transfer protein gene. *Biochem Biophys Res Commun* 1995;217:705–711. [PubMed: 8554588]
34. Urizar NL, Dowhan DH, Moore DD. The farnesoid X-activated receptor mediates bile acid activation of phospholipid transfer protein gene expression. *J Biol Chem* 2000;275:39313–39317. [PubMed: 10998425]
35. Bennett MK, Ngo TT, Athanikar JN, Rosenfeld JM, Osborne TF. Co-stimulation of promoter for low density lipoprotein receptor gene by sterol regulatory element-binding protein and Sp1 is specifically disrupted by the yin yang 1 protein. *J Biol Chem* 1999;274:13025–13032. [PubMed: 10224053]
36. Shea-Eaton W, Lopez D, McLean MP. Yin yang 1 protein negatively regulates high-density lipoprotein receptor gene transcription by disrupting binding of sterol regulatory element binding protein to the sterol regulatory element. *Endocrinology* 2001;142:49–58. [PubMed: 11145566]
37. Bosse Y, Bouchard L, Despres JP, Bouchard C, Perusse L, Vohl MC. Haplotypes in the phospholipid transfer protein gene are associated with obesity-related phenotypes: the Quebec Family Study. *Int J Obes (Lond)* 2005;29:1338–1345. [PubMed: 15953936]
38. Desrumaux C, Labeur C, Verhee A, Tavernier J, Vandekerckhove J, Rosseneu M, Peelman F. A hydrophobic cluster at the surface of the human plasma phospholipid transfer protein is critical for activity on high density lipoproteins. *J Biol Chem* 2001;276:5908–5915. [PubMed: 11083872]
39. Lie J, de Crom R, van Gent T, van Haperen R, Scheek L, Sadeghi-Niaraki F, van Tol A. Elevation of plasma phospholipid transfer protein increases the risk of atherosclerosis despite lower apolipoprotein B-containing lipoproteins. *J Lipid Res* 2004;45:805–811. [PubMed: 14993244]
40. Jiang XC, Li Z, Liu R, Yang XP, Pan M, Lagrost L, Fisher EA, Williams KJ. Phospholipid transfer protein deficiency impairs apolipoprotein-B secretion from hepatocytes by stimulating a proteolytic pathway through a relative deficiency of vitamin E and an increase in intracellular oxidants. *J Biol Chem* 2005;280:18336–18340. [PubMed: 15734742]
41. van Haperen R, van Tol A, van Gent T, Scheek L, Visser P, van der Kamp A, Grosveld F, de Crom R. Increased risk of atherosclerosis by elevated plasma levels of phospholipid transfer protein. *J Biol Chem* 2002;277:48938–48943. [PubMed: 12372822]
42. Cotton, R. Mutation detection. New York: Oxford University Press; 1997.

Table 1
Clinical characteristics of the study cohort.

Trait	HypoA (n) [†]	Control (n) [†]	HyperA (n)	Test Statistic
Age	52.7 ± 20.71 (276)	51.6 ± 20.03 (364)	58.0 ± 16.90 (107)	t = -3.386, p = 0.001
Sex (%female)	0.420 (276)	0.313 (364)	0.766 (107)	χ^2 = 9.579, p = 0.002
TG [§]				
Total	172.4 ± 76.80 (276)	124.7 ± 61.56 (364)	113.5 ± 53.21 (107)	n.s.
VLDL [*]	113.8 ± 72.70 (210)	74.5 ± 57.56 (261)	52.5 ± 37.67 (71)	t = 3.795, p < 0.001
LDL [*]	44.3 ± 18.71 (208)	34.8 ± 13.28 (259)	36.0 ± 15.99 (71)	n.s.
HDL	16.3 ± 5.80 (276)	16.6 ± 6.35 (364)	21.6 ± 9.72 (107)	t = -5.032, p < 0.001
Chol [§]				
Total	210.9 ± 72.93 (276)	193.3 ± 37.30 (364)	263.6 ± 61.25 (107)	t = -11.197, p < 0.001
VLDL [*]	27.0 ± 15.60 (221)	17.9 ± 14.52 (271)	12.0 ± 9.21 (71)	t = 4.111, p < 0.001
LDL	152.3 ± 67.64 (221)	128.4 ± 32.90 (271)	162.1 ± 62.27 (71)	n.a.
HDL	31.3 ± 6.30 (276)	48.1 ± 13.97 (364)	94.9 ± 13.67 (107)	n.a.
BMI	27.9 ± 3.212 (193)	26.0 ± 4.77 (216)	24.5 ± 5.43 (86)	z = -3.219, p = 0.001

Values are Mean ± S.D.

^{*} Natural log transformation.

[§] Lipid measurements expressed in mg/dL. N.a., not applicable. N.s., not significant. TG, triglyceride; VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; BMI, body mass index.

[†] Clinical characteristics for the control and hypoaliproteinemia comparison groups reproduced from our previous report.(12)

Table 2
PLTP rs2294213 Allelic and Genotype Frequencies.

	HypoA	Control	HyperA
C/C	264	335	92
C/G +G/G	12	29	15
Total	276	365 [‡]	107 [‡]
G allele	2.2%	4.3%	7.0%

Clinical characteristics for the control and hypoalphalipoproteinemia comparison groups reproduced from our previous report.(12) Allelic and genotypic frequencies determined directly by gene counting.

[‡] $\chi^2 = 4.436$, 1df, $p = 0.035$. Odds Ratio (OR) = 2.035, 95% Confidence Interval (CI): 1.039, 3.985.

[†] $\chi^2 = 11.005$, 1df, $p = 0.001$. OR = 3.224, 95% CI: 1.561, 6.661.

Table 3

HyperA Group Demographic and Clinical Characteristics according to PLTP rs2294213 genotype.

		GG (n=92)	CG+CC (n=15)	Statistical Test
Age (yrs)		57.9 ± (16.92)	58.9 ± (17.34)	t = -0.219 p = 0.827
TG [§]	Total *	109.9 ± (52.54)	135.9 ± (53.6)	t = -1.736, p = 0.085
	VLDL [‡]	50.4 ± (37.53)	74.3 ± (34.82)	z = -1.861, p = 0.063.
	LDL [‡]	36.1 ± (16.28)	35.2 ± (13.62)	z = -0.062, p = 0.951
	HDL	21.0 ± (9.02)	25.6 ± (12.88)	z = -1.020, p = 0.308
Chol [§]	Total *	263.9 ± (63.19)	262.0 ± (49.48)	t = -0.027, p = 0.979
	VLDL [‡]	11.5 ± (9.34)	17.3 ± (5.72)	z = -2.071, p = 0.038
	LDL [‡]	161.5 ± (64.00)	168.3 ± (42.61)	z = -0.641, p = 0.522
	HDL	95.8 ± (13.78)	89.7 ± (12.12)	z = -1.944, p = 0.052
BMI [£]		24.1 ± (4.95)	26.9 ± (7.59)	t = -1.223, p = 0.244

* Natural log transformation.

[§] Lipid measurements expressed in mg/dL.

[‡] Measurements for this lipoprotein compartment were only available in 65 participants with the GG genotype and 6 participants with the GC or CC genotypes.

[£] Measurements for BMI were only available in 74 participants with the GG genotype and 12 participants with the GC or CC genotypes. TG, triglyceride; Chol, cholesterol; VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; BMI, body mass index.

Table 4

Regression Analysis of Lipoprotein Measures HDL-C.

Outcome	Predictor	Coefficient	Standard Error	95% CI	p-value
HDL-C (n=494)	Genotype	3.55	1.90	-0.19, 7.93	0.063
	Sex	7.67	1.05	5.61, 9.74	<0.001
	HypoA	-20.03	2.35	-24.64, -15.41	<0.001
	HyperA	36.29	2.21	31.95, 40.63	<0.001
	Constant	59.43	0.95	57.55, 61.30	<0.001
Overall model fit: F = 239.66, p < 0.0001, adjusted R ² = 0.817					

Multiple linear regression analysis of HDL-C levels. Predictors evaluated in the model included: age (years), sex (female vs. male), plasma TG (mg/dL), BMI (kg/m2), clinical category (Control vs. HyperA, HypoA), PLTP rs2294213 genotype (GG vs GC+CC), and genotype-by-clinical category interaction. An interaction between clinical category and genotype was observed (p=0.013), with carriers of the minor allele having higher levels of HDL-C in HypoA (GG: 32.69 [95% CI: 31.06, 34.33]; GC+CC: 35.27 [95% CI: 28.21, 42.33]) and Controls (GG: 48.81 [95% CI: 47.24, 50.38]; GC+CC: 59.21 [95% CI: 53.88, 64.53]) versus lower HDL-C levels in HyperA (GG: 91.46 [95% CI: 88.84, 94.07]; GC+CC: 89.13 [95% CI: 83.03, 95.24]). Covariates appearing in the model are evaluated at the following values: age = 52.93 years, TG = 147.1 mg/dL, BMI = 26.5.