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Original Article

Novel polymorphisms associated with hyperalphalipoproteinemia and apparent cardioprotection

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BACKGROUND: Hyperalphalipoproteinemia (HALP) is inversely correlated with coronary heart disease (CHD) although genetic variants associated with high serum levels of high-density lipoprotein cholesterol (HDL-C) have not been shown to be cardioprotective.

OBJECTIVE: The objective of the study was to uncover novel genetic variants associated with HALP and possibly with reduced risk of CHD.

METHODS: Exome sequencing data, HDL-C, and triglyceride levels were analyzed in 1645 subjects. They included the University of Maryland outpatients with high HDL-C (n = 12), Cardiovascular Health Study (n = 210), Jackson Heart Study (n = 402), Multi-Ethnic Study of Atherosclerosis (n = 404), Framingham Heart Study (n = 463), and Old Order Amish (n = 154).

RESULTS: Novel nonsynonymous single-nucleotide polymorphisms (nsSNPs) were identified in men and women with primary HALP (mean HDL-C, 145 ± 30 mg/dL). Using PolyPhen-2 and Combined Annotation Dependent Depletion to estimate the predictive effect of each nsSNP on the gene product, rare, deleterious polymorphisms in *UGT1A3, PLLP, PLEKHH1, ANK2, DIS3L, ACACB*, and *LRP4* were identified in 16 subjects with HALP but not in any tested subject with low HDL-C (<40 mg/dL). In addition, a single novel polymorphism, rs376849274, was found in *OSBPL1A*. The majority of these candidate genes have been implicated in fat and lipid metabolism, and none of these subjects has a history of CHD despite 75% of subjects having risk factors for CHD. Overall, the probability of finding these nsSNPs in a non-high HDL-C population ranges from 1×10^{-17} to 1×10^{-25}.

CONCLUSION: Novel functional polymorphisms in 8 candidate genes are associated with HALP in the absence of CHD. Future study is required to examine the extent to which these genes may affect HDL function and serve as potential therapeutic targets for CHD risk reduction.

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Introduction

For the past several decades, an inverse association between high-density lipoprotein cholesterol (HDL-C) and coronary heart disease (CHD) has been well recognized.[1–3] In addition to its role in reverse cholesterol transport, HDL possesses antioxidant and anti-inflammatory properties that are believed to contribute to its cardioprotective role.[4] However, while family-based and cross-sectional studies have suggested that primary hyperalphalipoproteinemia (HALP) is associated with longevity,[5,6] HDL-C-raising variants in the established gene candidates linked to HALP, cholesteryl ester transfer protein (*CETP*), hepatic lipase (*HL*), and endothelial lipase (*LIPG*), have not resulted in cardioprotection or longevity.[7–10] Moreover, a rare variant in scavenger receptor class B type 1 that raises HDL-C has been associated with elevated CHD risk,[11] and randomized human outcome trials have failed to demonstrate clinical benefit after pharmacologically mediated CETP inhibition.[12–15] Therefore, the goal of the present study was to identify novel gene candidates associated with HALP and reduced CHD risk.

Methods

We identified 72 subjects with HALP (defined as ≥80 mg/dL)[16,17] from 6 different sources with whole exome sequencing to identify novel nonsynonymous single-nucleotide polymorphisms (nsSNPs) associated with the high HDL-C phenotype. The 6 population HALP sources were the University of Maryland Preventive Cardiology clinic (Baltimore, MD; $n = 12$), the Cardiovascular Health Study (CHS; $n = 8$), Jackson Heart Study (JHS; $n = 9$), Multi-Ethnic Study of Atherosclerosis (MESA; $n = 22$), the Framingham Heart Study (FHS; $n = 15$), and the Old Order Amish population of Lancaster, PA ($n = 6$).[18–22]

HALP subjects were initially selected from the University of Maryland Preventive Cardiology clinic and derived from 2 families (Figure 1; edigree #1, pedigree #2) and 6 biologically unrelated subjects with HALP. Whole exome sequencing was performed using the Illumina Genome Analyzer platform methodology as previously described.[23] Mutation analysis included all coding, intron–exon regions and promoter regions; questionable readings were verified using the Broad Institute's Integrative Genomics Viewer. Genetic variants were confirmed within the NHLBI GO Exome Sequencing Project's exome variant server and the University of Michigan's BRAVO server. Allele frequencies were identified using the Broad Institute's Exome Aggregation Consortium browser using sequencing data from more than 60,000 subjects. Intronic or synonymous mutations were excluded from analysis. All study procedures were approved by the Institutional Review Board of the University of Maryland School of Medicine.

We then analyzed the whole exome sequencing data from our subjects in conjunction with complete exome sequencing of HALP subjects from the CHS, JHS, MESA, FHS, and the Old Order Amish population of Lancaster, PA. As part of the NHLBI GO Exome Sequencing Project, subjects from JHS, MESA, FHS, and FHS had undergone whole exome sequencing completed using Illumina Genome Analyzer IIX or Illumina HiSeq 2000. Similarly, the Old Order Amish population of Lancaster, PA, had undergone whole-genome sequencing using Illumina HiSeq X Ten as part of the Amish Complex Disease Research Program at the University of Maryland. This available data were readily used to identify rare nsSNPs that were shared

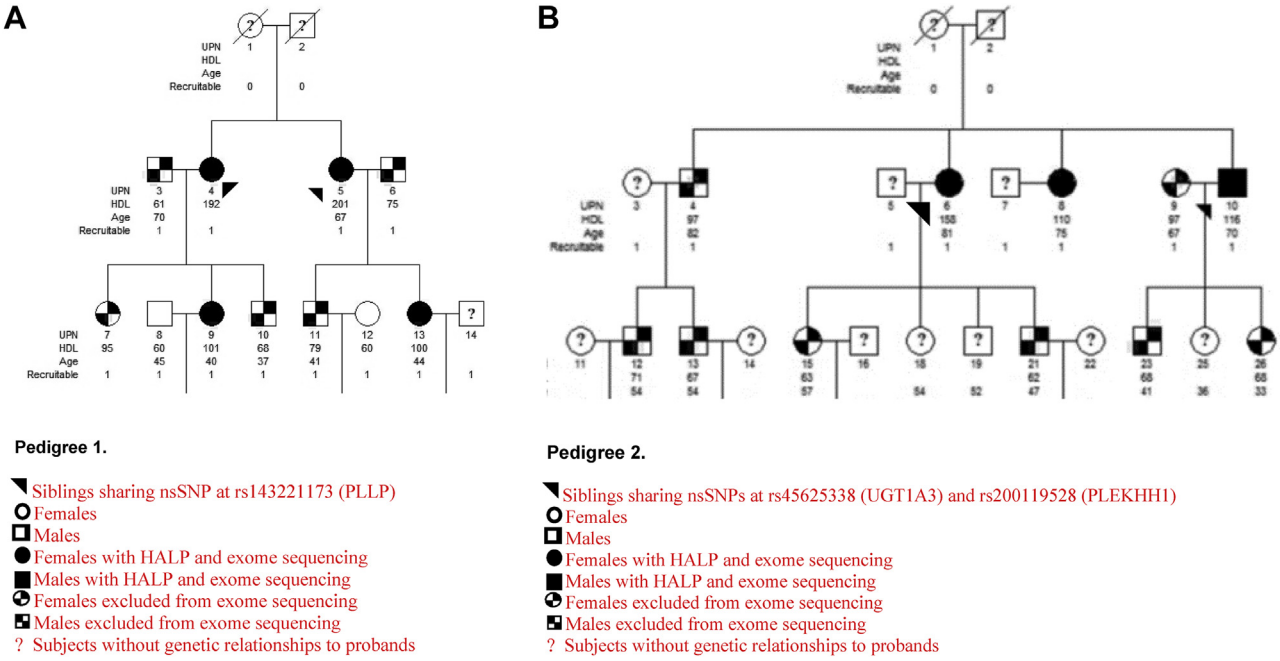

Figure 1 Two families with the HALP phenotype. HALP, hyperalphalipoproteinemia