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Genetics

A Novel Quantitative Trait Locus on Chromosome 1 with Pleiotropic Effects on HDL-Cholesterol and LDL Particle Size in Hypertensive Sibships

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Background: High-density lipoprotein (HDL)-cholesterol, triglycerides, and LDL particle size are correlated lipid traits. Abnormal levels of these traits are frequent in hypertensive individuals and contribute to increased risk of coronary heart disease (CHD). We performed univariate and bivariate linkage analyses to identify genomic regions that influence levels of these traits and exert pleiotropic effects on the traits in hypertensive sibships.

Methods: Subjects included 691 non-Hispanic white individuals (mean age 63.1±8.5 years, 57% women, 78% hypertensive) ascertained through sibships with two or more individuals diagnosed with hypertension before age 60 years. The LDL particle size was measured by polyacrylamide gel electrophoresis and triglycerides were log-transformed to reduce skewness. Genotypes were measured at 366 microsatellite marker loci distributed across the 22 autosomes. Univariate and bivariate linkage analyses were performed using a variance components approach.

Results: Significant ($P < .001$) genetic correlations were confirmed for all pairwise combinations of the traits. Univariate linkage analyses demonstrated evidence of linkage (defined as multipoint LOD scores ≥1.3) for HDL-cholesterol on chromosomes 1p, 3p, 9q, and 18q; for log triglycerides on chromosome 10q; and for LDL particle size on chromosomes 2p and 8p. Pairwise bivariate linkage analyses of the three traits revealed a region with pleiotropic effects on HDL-cholesterol and LDL particle size on chromosome 1p (LOD score 4.48).

Conclusions: These findings indicate the presence of a quantitative trait locus on chromosome 1 that has pleiotropic effects on HDL-cholesterol and LDL particle size and may therefore influence CHD susceptibility in hypertensive sibships. Am J Hypertens 2005;18:1084–1090

Key Words: Bivariate, genetic linkage, HDL-cholesterol, triglycerides, LDL particle size.

Altered lipid metabolism in hypertensive individuals may be related to insulin resistance and manifest as decreased HDL-cholesterol, increased triglycerides, and small LDL particle size.[1,2] These lipid abnormalities are atherogenic and increase the risk for coronary heart disease (CHD). Given that at least 65 million adults in US have hypertension,[3] and that more than half of hypertensive individuals have dyslipidemia,[4] the syndrome of concomitant hypertension and dyslipidemia is an important contributor toward the burden of CHD.

Multiple genes, environmental factors, and their interactions play a role in the etiology of "complex" disorders such as concomitant hypertension and dyslipidemia.[5] A step toward elucidating the genetic architecture of a complex disorder is to study the genetic basis of quantitative "intermediate" traits of the disorder. The interindividual variation of a quantitative trait enhances the statistical power to detect genomic regions that influence the trait compared to a dichotomous characterization of "normal" and "abnormal."[6] The three quantitative lipid traits, HDL-cholesterol, triglycerides, and LDL particle size, are known to be heritable as well as correlated. Little is known about genomic regions that influence interindividual variation in these traits in hypertensive sibships. In addition,

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despite the well-recognized correlation between these traits, bivariate linkage analyses to identify loci with pleiotropic effects on these lipid traits have yet to be reported. Edwards et al[7] previously found that shared genetic effects (pleiotropy) account for a significant proportion of the phenotypic correlations between HDL-cholesterol, triglycerides, and LDL particle size. Using multivariate quantitative genetic analyses in hypertensive sibships, we similarly found that pleiotropy contributes to the additive genetic variation in these traits.[8] Therefore in addition to univariate linkage analyses, we performed bivariate linkage analyses of HDL-cholesterol, triglycerides, and LDL particle size in hypertensive sibships to identify genetic regions that influence the individual traits as well as regions that may exert pleiotropic effects on pairwise combinations of traits. Such studies have the potential to yield new insights into the genetic basis of the interindividual variation of these traits as well as susceptibility to CHD.[9]

Methods

Sample

Subjects included participants in the Genetic Epidemiology Network of Arteriopathy (GENOA) study, a multicenter community-based study of hypertensive sibships that aims to identify genes influencing blood pressure (BP) levels and the risk of developing hypertension.[10] In the initial phase of the GENOA study (9/1995 to 6/2001), sibships containing at least two individuals with essential hypertension diagnosed before the age of 60 years were enrolled in Rochester, MN (non-Hispanic white subjects). Between December 2000 and October 2002, 815 of the original 1583 GENOA-Rochester participants returned for a second study visit to undergo measurement of novel cardiovascular risk factors including LDL particle size and assessment of target organ damage due to hypertension. Complete genotypic and phenotypic data have been collected in 691 participants in phase II. The study was approved by the Institutional Review Board of the Mayo Clinic, and written informed consent was obtained from each participant.

Height was measured by stadiometer, weight by electronic balance, and body mass index (BMI) was calculated as kilograms per meter squared. Resting systolic and diastolic BP levels were measured in the right arm with a random-zero sphygmomanometer (Hawksley and Sons, West Sussex, UK). Three measures at least 2 min apart were taken and the average of the second and third measurements was used in the analysis. Participants were considered diabetic if they reported using insulin or oral hypoglycemic agents, or if they reported a physician diagnosis of diabetes but were not currently taking a pharmacologic agent to control their high glucose levels. Information about the use of lipid-lowering medications was obtained from a questionnaire administered to the participants.

Measurement of Lipid Variables

Blood samples were obtained by venipuncture after an overnight fast. Standard enzymatic methods were used to measure total cholesterol, HDL-cholesterol, and triglycerides.[11] The LDL particle size was measured by polyacrylamide gel electrophoresis as in Hoefner et al.[12] Plasma (25 μL) was mixed with 200 μL of loading gel (containing Sudan black-B dye and riboflavin) and added to the top of a precast 3% polyacrylamide gel tube (Quantimetrix Corporation, Redondo Beach, CA). After photopolymerization for 30 min, specimens were electrophoresed for 1 h. The dye preferentially binds to lipoprotein particles and remains with them during electrophoresis. Separation is based primarily on particle size due to the sieving action of the polyacrylamide gel. The separated lipoprotein particles were scanned with an ArtixScan 1100 (Microtek, Carson, CA). The electrophoretograms were quantitatively analyzed using the public domain NIH Image program (http://rsb.info.nih.gov/nih-image/). The program divides the electrophoretogram at designated electrophoretic mobility values and calculates the area under the curve for each mobility fraction. Particle diameter for each fraction was calculated as previously described[13] and weighted based on the percent area under the curve for each fraction in relation to the area occupied by the entire LDL band. The mean LDL particle size was then determined by averaging weighted diameters for each fraction. Two controls (mean particle diameter 264 Å and 254 Å) were analyzed with every 10 study samples. Interassay coefficients of variation were 0.77% (standard deviation, 2.0 Å), and 1.46% (standard deviation, 3.7 Å), respectively.

Genotyping

DNA from all study participants was extracted from 10 mL of EDTA-treated blood, according to standard procedures. Microsatellite markers (CHLC/Weber screening set 9.0, $n = 366$) were genotyped by standard polymerase chain reaction (PCR)-based methods by the Mammalian Genotyping Center of the Marshfield Medical Research Foundation. Marker order and genetic map distances were those provided by the Marshfield Medical Research Foundation (www.marshmed.org/genetics). Inconsistencies of the genotypes with pedigree structure were identified by the Lange and Goradia algorithm[14] as implemented in the PedCheck software.[15] Instances that could not be resolved as genotyping errors were considered as missing data.

Statistical Analyses

We first assessed whether the assumption of normality in the distribution of the lipid traits was violated.[16] Triglyceride values had excessive skew and kurtosis and were therefore natural log-transformed. HDL-cholesterol, log