Genome-Wide Linkage Analysis of Lipids in the Hypertension Genetic Epidemiology Network (HyperGEN) Blood Pressure Study

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Abstract—Full genome scans were performed for quantitative lipid measurements in 622 African American and 649 white sibling pairs not taking lipid-lowering medications who were ascertained through the Hypertension Genetic Epidemiology Network (HyperGEN) of the National Heart, Lung, and Blood Institute (NHLBI) Family Blood Pressure Program. Genotypes for 391 markers spaced roughly equally throughout the genome were typed by the NHLBI Mammalian Genotyping Service. Each of the phenotypes was adjusted for covariates within sex and race and then subjected to variance components linkage analysis, which was performed separately within race by using race-specific marker allele frequencies from additional random samples. The highest lod score detected was 2.77 for logarithmically transformed triglyceride (TG) on chromosome 20 (at 28.6 cm) in the African American sibling pairs. The highest score detected in the white sibling pairs was 2.74 for high density lipoprotein cholesterol on chromosome 5 (at 48.2 cm). Although no scores >3.0 were obtained, positive scores were found in several regions that have been reported in other genome scans in the literature. For example, a score of 1.91 for TG was found on chromosome 15 (at 28.8 cm) in white sibling pairs. This score overlaps the positive findings for TG in 2 other genome scans. (Arterioscler Thromb Vasc Biol. 2001;21:1969-1976.)

Key Words: genome scan ■ lipids ■ cholesterol ■ triglyceride ■ genetic linkage

Serum lipoprotein-lipid levels are major risk factors for coronary heart disease, and genes involved in lipoprotein metabolism have been implicated in coronary heart disease. Discovery of the genetic determinants of quantitative variation in lipid levels could help to elucidate the genetics of coronary heart disease.

Studies have indicated that lipid levels are significantly heritable. Point estimates of heritability for total cholesterol, HDL cholesterol (HDL-C), and plasma triglycerides (TGs) range from 42% to 65%, 45% to 83%, and 37% to 75%, respectively, with twin studies providing higher point estimates. At least half of the normal variation in LDL cholesterol (LDL-C) concentration is due to genetic factors. Several segregation analyses have suggested major genes for lipid traits. The San Antonio Heart Study reported segregation analysis evidence of a major gene for HDL-C but concluded that this locus was not any of the following major candidate loci: apoA-I/apoC-III, apoB, hepatic lipase, lipoprotein lipase, LDL receptor, or apoE. Similarly, data from the National Heart, Lung, and Blood Institute (NHLBI) Family Heart Study (FHS) show a major gene effect for mild elevation in LDL-C that is not attributable to the LDL receptor, apoE, or the cholesterol 7a-hydroxylase genes. These studies indicate that whole genome scans may reveal new major loci underlying quantitative lipid traits.

The present report describes a genome-wide search for quantitative trait loci contributing to variations in LDL-C, HDL-C, TGs, and total cholesterol in white and African American sibling pairs ascertained for hypertension. Genes accounting for variation of lipid levels within hypertensive siblings may differ from lipid genes in other populations. Familial dyslipidemic hypertension, defined as ≥1 lipid abnormality together with hypertension, has been found in approximately half of the sibships ascertained for essential hypertension, with only 15% of hypertensive siblings concordant for normal lipid levels. In familial dyslipidemic hypertension, compared with blood pressure, lipids may deviate more from normative values. Therefore, concordance of lipid levels within hypertensive sibships may identify lipid genes more closely related to risk factors for cardiovascular disease.
Methods

Subjects

Subjects in the Hypertension Genetic Epidemiology Network (HyperGEN) network come from 5 field centers located in Framingham, Mass; Minneapolis, Minn; Salt Lake City, Utah; Forsyth County, NC; and Birmingham, Ala. Eligible subjects were selected from existing databases of the population-based NHLBI FHS,16 which ascertained subjects in all of the above-mentioned field centers except Birmingham. The Birmingham center was added to increase the African American sample to approximately half the total HyperGEN sample.

Families were recruited for HyperGEN if they contained at least 2 siblings with mild to severe hypertension. Severe hypertension was defined as systolic blood pressure (SBP) ≥160 mm Hg or diastolic blood pressure (DBP) ≥100 mm Hg or the use of ≥2 types of medications for the treatment of hypertension. Mild hypertension was defined as follows: 140 mm Hg < SBP < 160 mm Hg or 90 mm Hg < DBP < 100 mm Hg or the use of only 1 type of antihypertensive medication. All available affected siblings were recruited from each eligible sibship.

Random samples of age-matched persons from the same base populations (182 whites and 198 African Americans) were used to calculate gene frequencies. The randomly ascertained FHS probands were selected from the FHS centers. The Birmingham and North Carolina African American random samples were recruited by mailings to randomly selected addresses from a computerized database of Department of Motor Vehicle listings of area residents.

Subjects were excluded from HyperGEN if hypertension was first diagnosed after 60 years of age, if there was evidence of secondary hypertension, or if hypertension occurred only during pregnancy. Subjects with type 1 diabetes mellitus (insulin therapy before age 21 years) were also excluded. For more details of the design of HyperGEN, see Williams et al.17

Genotyping was performed for 938 white hypertensive sibling pairs in 470 families and 684 African American sibling pairs in 640 families. For this analysis, data from 317 white subjects and 195 African American subjects who reported that they were taking lipid-lowering medications were deleted, leaving 649 white sibling pairs and 622 African American sibling pairs. A brief comparison of results when levels for these medicated subjects were included is presented in Discussion.

Measurement of Lipids and Other Phenotypes

Subjects were asked to fast for 12 hours before their clinic visit. Evacuated tubes with no additives were used to collect samples for lipid study. Blood samples were spun at 3000g for 10 minutes at 4°C and then stored at −70°C until sufficient numbers of samples were accumulated for shipment to the Central Biochemistry Laboratory at the University of Wisconsin-Madison. Plasma TG levels were measured by using glycerol-blanked TG reagent on a centrifugal analyzer.18 LDL-C concentration was measured by using standard methods or by ultracentrifugation for subjects with TG levels >400 mg/dL. HDL-C was measured after precipitation of non-HDL-C with magnesium/dextran.21 Total cholesterol was measured by using a commercial cholesterol oxidase method.22

Anthropometric measurements were collected with subjects wearing scrub suits. Weight was measured by using a balance scale, and height was measured by using a vertical ruler mounted to a wall. Body mass index (BMI) was computed as weight (in kilograms) divided by height (in meters squared). Waist circumference was measured to the nearest centimeter at the level of the umbilicus, and hip circumference was measured at the level of the maximal circumference of the gluteus. All other variables were collected through interviews performed by trained interviewers.

Genotyping

Three 10-mL Vacutainer tubes with EDTA were drawn from each participant and centrifuged, and the cells were shipped to the HyperGEN central biochemistry laboratory. Genomic DNA was isolated from whole blood by use of standard procedures of the GEN sample. For 391 markers spaced roughly equally throughout the genome were typed by the NHLBI Mammalian Genotyping Service (Marshfield, Wis). Details on gel preparation, polymerase chain reaction conditions, and the genetic map are available in Weber and Broman (2001)23 and also from the Mammalian Genotyping Service (which can be accessed online at http://marshmed.org/genetics).

Statistical Methods

The TG variable was logarithmically transformed to normalize its distribution before all other analyses. Other lipid variables were approximately normally distributed. Adjustments were then made for linear and nonlinear effects of age as well as effects of BMI, waist-hip ratio, current smoking and drinking status, estrogen replacement, diabetes status, and field center. Regressions were performed within each sex and were separately by race. Residuals from these models, assumed to reflect more pure measures of the underlying lipid traits, were used in the subsequent genetic analysis. By making the covariate adjustments before the genetic analysis, we are not modeling potential genotype-by-environment interaction effects. In addition, we assume equal variance across research centers within race/sex groups.

A secondary analysis was performed with adjustment for antihypertensive medication status in addition to the other listed covariates. Effects of antihypertensive medications were observed only for TGs (P = 0.04 for African American males, P = 0.002 for African American females, and P = 0.004 for white males; all other analyses were nonsignificant); this adjustment was found to have minimal effect on the lod score outcomes for TGs. Results of this secondary analysis are presented briefly in the discussion of TG lod score results. HDL-C and TGs were closely associated (P < 0.0001 for all race/sex categories); therefore, 2 analyses of HDL-C were performed: one with adjustment for TG level and the other without adjustment for TG level.

Genome scans were performed by using variance components linkage analysis as implemented in GeneHunter.24 The linkage analysis was carried out separately within race by using race-specific marker allele frequencies derived from the random samples.

Results

Table 1 shows sample characteristics of the subjects who were genotyped for the genome scan for each lipid trait and for all covariates. HDL-C, LDL-C, and total cholesterol were approximately normally distributed within age/race groups. TG was logarithmically transformed to create a more normal distribution. When data from subjects on lipid-lowering medications were added, no significant changes were observed in the means or standard deviations of the lipids or covariates. After adjustment was made for the covariates, the lipid traits did not show significant deviations from normal distributions.

Table 2 presents the significance values of the effects of each covariate used for adjustment based on type III sums of squares. The models were fit separately by race and within sex, and variables were retained to generate a residual score even if the significance did not meet the 0.05 criterion. When significant effects were observed, they caused changes as follows: Increases in age, BMI, and waist-hip ratio were associated with decreases in HDL-C and increases in LDL-C, TG, and total cholesterol levels. Current alcohol use was associated with decreases in HDL-C. Current smokers had lower HDL-C and higher TG levels, and, in African American men, lower LDL-C and lower total cholesterol levels. Estrogen use was associated with lower LDL-C, higher HDL-C, and higher TG levels. A diagnosis of diabetes was associated with higher TG and lower LDL-C levels and, in white females, lower HDL-C and lower total cholesterol levels. Differences in significance patterns across racial groups occurred most often for smoking, a phenotype found to be much more common in the African American sample.
Effects of the research center involved were included to account for possible phenotypic variation by region. In some cases (see Table 2), significant differences occurred across centers. Among African American females, LDL-C and total cholesterol levels were significantly lower for Forsyth County subjects compared with Birmingham subjects (least squares means were 111 versus 118 for LDL-C and 192 versus 200 for total cholesterol). Among white subjects, the least squares means for Framingham for logarithmically transformed TG levels (4.94 for males and 4.92 for females) were lower than those for the other sites, whereas Minneapolis TG means were the highest (5.12 for males and 5.12 for females). Salt Lake City white subjects were lowest for total cholesterol (190 for males and 203 for females); Minneapolis was highest (200 for males and 212 for females). Among white females, Framingham HDL-C least squares means were highest (42.8 for males and 56.9 for females); for males, North Carolina was lowest (40.8), and for females, Salt Lake City was lowest (51.8).

Table 3 presents the maximum lod scores and location of that score from the multipoint variance components analyses for all peaks >1.0. Detailed results of this scan can be found online at http://www.biostat.wustl.edu/data_ip/hypergen. The 5 scores >2.0 are highlighted in Table 3; graphs of the results from these chromosomes appear in the Figure. In the African American sample, the peak on chromosome 20 for TG spans ~30 cM, with scores >1.0 from 5.6 to 35.7 cM. The African American peak for total cholesterol on chromosome 2 is narrower, with scores >1.0 from the p-terminus to 28.1 cM. Finally, the peak for HDL-C on chromosome 1 spans ~16 cM, with scores >1.0 from 187.8 to 204.1 cM. When data from the subjects on lipid-lowering medications were included, each of these highest peaks diminished in magnitude (data not shown). With the medicated subjects, no peak >2.0 was observed in the white data. In the African American data including medicated subjects, only 1 peak >2.0 was observed on chromosome 21 (lod of 2.24 at 51.3 cM for LDL-C). This peak was originally 1.72 in the primary analysis without medicated subjects (see Table 3).

## Discussion

Although the positive scores in the present report do not meet significance thresholds for a genome scan (a debated threshold most often placed between 3.05 and 3.616), the results still provide valuable information. Some positive regions for lipids overlap the positive regions for other phenotypes under study in HyperGEN. In these cases, using the lipid measurements as stratifying variables for linkage analysis of the other phenotypes may help to clarify equivocal linkage results. Some overlap of positive results occurred across lipid traits within the present study. Results for total cholesterol closely mirrored results of LDL-C within sex. Results for HDL-C adjusted and unadjusted for TG were also closely associated. Chromosome 15 showed some overlap for TG and HDL-C for white subjects. Chromosomes 4 and 18 showed overlap between TG and HDL-C for African American subjects. Yet beyond the scope of the present study, bivariate analyses in these regions may prove fruitful. More important, the overlap of positive HyperGEN lipid results with other published...
scans (listed in Table 4) may help to identify regions worthy of further study, even in the absence of strictly significant results within individual studies.

One of the most interesting positive HyperGEN regions was found in the white sample for TG on chromosome 15 at position 28.8 (lod 1.91). This peak overlaps peaks from 2 other genome scans (Table 4). A significant lod score on chromosome 15q between 20 and 30 cM was reported in a genome-wide scan of a TG study of Mexican Americans. In addition, a genome scan of TG in the FHS sample reported a peak on chromosome 15p (at 36 cM; D.K. Arnett, unpublished data, 2000). This FHS study also reported a significant linkage for TG on chromosome 4p (at 35 cM). This peak did not appear in the HyperGEN white sibling pair data. There was a modest peak (lod 1.10) for TG in African Americans on chromosome 4 at 13.0 cm, 22 cM from the FHS peak. The HyperGEN peak for TG on chromosome 20 at position 28.6 (lod 2.77) in African Americans coincides with a Framingham Heart Study TG peak on 20 at 35 to 40 cM. Another positive Framingham TG peak on chromosome 11 at 125 cM again overlaps a HyperGEN African American peak on chromosome 11 (lod 1.25). A TG peak was observed in HyperGEN whites on chromosome 7 (lod 1.44 at 143.4 cM). This chromosome has produced positive scores in the Framingham study (at 155 cM) and in the San Antonio study (at 94 and 186 cM). A study of Pima Indians reported a peak on chromosome 2 for TG at 45 to 50 cM; the HyperGEN white data showed a small peak near this region (lod 0.86 at 58.8 cM). Other HyperGEN peaks for TG (see Table 3) do not overlap any other reported TG-positive scores.

When subjects on lipid-lowering medications were added to the sample, the magnitude of most of the TG peaks reported above decreased. In addition, 4 peaks appeared in white subjects that were >1.0 in the primary analysis. These peaks were on chromosome 2 at 51.8 cM (lod 1.69), chromosome 12 at 130.4 cM (lod 1.14), chromosome 18 at 51.6 cM (lod 1.49), and chromosome 21 at 47.9 cM (lod 1.50). None of these peaks occurred in regions of interest from other scans. No other peaks emerged in the African American sample, although peaks on chromosomes 1, 4, and 6 increased slightly.

A modest effect on TG levels due to antihypertensive medications was also observed in white men and in African American men and women. No effect of these medications was found for other lipids. Lod scores for TG with and without a correction for this effect differed by ~0.2 lod units in African Americans and ~0.1 lod unit in whites.

For HDL-C, the HyperGEN white sample gave a lod score on chromosome 5 of 2.74 for HDL-C adjusted for TG (at 48.2 cM) and 1.49 for HDL-C unadjusted for TG (at 45.4 cM). These peaks correspond to findings from the FHS sample for HDL-C, which produced a significant lod score on chromosome 5 (at 39.9 cM; see Table 4). A small HyperGEN HDL-C peak for white subjects on chromosome 15 (lod 1.08 at 50.2 cM) overlaps a significant peak in the San Antonio scan of the Mexican American sample. Another reported peak on chromosome 5 at 186 cM coincides

<p>| TABLE 2. Significance of F Statistics in Regression Analyses Correcting Lipids for Covariates |
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Analyses for each lipid trait were performed within sex and separately by race. Resulting F statistics and reported P values are taken from the SAS general linear models procedure (based on type III sums of squares).
with an African American HyperGEN peak for total cholesterol (lod 1.63 at 193.0 cM). Other peaks reported in the San Antonio scan are not close to HyperGEN peaks. The linkage to HDL-C, LDL-C, total cholesterol, and BMI on chromosome 13q31-32 (~75 to 80 cM) reported by Knoblauch et al. and peaks for HDL-C and total cholesterol on 3 and 19, respectively, reported in the scan of the Pima Indian subjects were not found in HyperGEN.

When lipid-medicated subjects were included, the magnitude of the HDL-C peaks for white subjects on chromosomes 1, 3, 5, 12, and 18 and the peaks for African American subjects on chromosomes 4 and 6 diminished but were still present. Several peaks emerged that were <1.0 in the primary analysis. In the data for white subjects, these peaks were on chromosome 8 (lod 1.91 at 0 cM and lod 1.58 at 36.3 cM), chromosome 10 (lod 1.04 at 75.9 cM), and chromosome 22 (lod 1.96 at 37.9 cM). In the data for African American subjects, the peaks were on chromosome 2 (lod 1.59 at 110.7 cM), chromosome 8 (lod 1.03 at 7.6 cM), chromosome 9 (lod 1.13 at 61.7 cM), chromosome 11 (lod 1.10 at 135.8 cM), chromosome 13 (lod 1.49 at 55.0 cM), chromosome 19 (lod 1.08 at 68.2 cM), and chromosome 21 (lod 1.72 at 10.1 cM). The peak on chromosome 2 is close to an HDL-C peak in the San Antonio sample. The peak on chromosome 13 is ~20 cM from an HDL-C peak in the FHS, and the peak on chromosome 11 is ~20 cM from the apoA-I/C-III/A-IV locus. Other peaks, including those for the lipid-medicated subjects, are not in regions of interest from other lipid scans.

Peaks for LDL size fractions have been described on chromosomes 3 (at 244 cM), 4 (at 126 cM), and 6 (at 162 cM); see Table 4. Although these regions were not detected by use of LDL-C in the HyperGEN data, the published scan data also showed only modest positive scores at these peaks for total LDL-C rather than the size fraction phenotypes. For total LDL-C, the highest score in the published scan was 1.3 on chromosome 12; some modest peaks occurred in the white HyperGEN data on chromosome 12, but for TG and HDL-C only. Peaks for total cholesterol mirrored those for LDL-C, although the magnitudes differed. The most interesting peak for total cholesterol was in the white data on chromosome 2 (lod 2.19 at 16.1 cM), but this is not a region that is listed as positive in other lipid scans.

When subjects on lipid-lowering medications were included, peaks for LDL-C and total cholesterol on chromosomes 2 and 13 in the white data and on chromosomes 1, 2, 5, 6, and 18 (for both phenotypes) and 21 (for total cholesterol) in the African American data decreased. Several peaks emerged that were <1.0 in the primary scan. In the white data, there were peaks on chromosome 8 (for LDL-C, lod 1.21 at 147.4 cM), chromosome 9 (for total cholesterol, lod 1.40 at 34.5 cM), chromosome 14 (for LDL-C, lod 1.20 at 31.6 cM; for total cholesterol, lod 1.27 at 31.6 cM), and chromosome 17 (for LDL-C, lod 1.23 at 31.3 cM). These peaks did not overlap those reported in published studies. In the African American data, the peak on chromosome 21 (51.3 cM) for LDL-C increased to 2.24 when lipid-medicated subjects were included. In addition, there was a peak on chromosome 22 at 0 cM (for total cholesterol, lod 1.48; for LDL-C, lod 1.07) that did not appear in the primary analysis. Neither of these peaks coincided with published results.

Several findings have been reported for familial lipid syndromes (familial hypercholesterolemia and familial com-
bined hyperlipidemia (FCHL)). A region for FCHL on chromosome 1q has been identified in several independent samples. In the white HyperGEN sample, a peak occurs \(15 \text{cM}\) centromeric from this locus for HDL-C adjusted for TG (lod 1.15 at 159.9 cM), and another peak occurs \(20 \text{cM}\) telomeric for HDL-C not adjusted for TG (lod 2.13 at 198 cM). Of the other positive locations reported in a whole genome scan of FCHL, none overlaps the HyperGEN sample. Other findings reported for FCHL and familial hypercholesterolemia also do not overlap the HyperGEN peaks.

In summary, our primary analysis of the HyperGEN lipid data revealed several interesting linkage peaks. These findings are based on lipid phenotypes adjusted before analysis for covariates and, therefore, do not reflect any potentially important genotype-by-environment interaction effects. The incorporation of such effects, in addition to multilocus models and multitrait models, may provide a fruitful next step in the analysis of these data. Results including lipid-mediated subjects generally tended to decrease the magnitude of those peaks that overlapped most closely with results from other scans, and although some modest peaks emerged, they were not often in previously reported regions. Because the true lipid values of these individuals are unknown, these peaks may be more likely to be false-positive results. Although isolated positive scores should not be discounted as possible true positives, those results that show the highest degree of overlap with other studies may represent the most promising locations for follow-up studies. In particular, chromosomes 15, 20, and, perhaps, 7 for TG appear interesting. For HDL-C, chromosome 5 shows 2 potential regions of interest, and chromosome 15 may be worth additional follow-up. As data from more genome scans accumulate, such overlapping findings will direct future targeted gene searches.

### List of HyperGEN Participating Institutions and Principal Staff

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TABLE 4. Summary of Locations of Positive Results From Published Genome Scans of Lipid Traits

<table>
<thead>
<tr>
<th>Study</th>
<th>TG</th>
<th>HDL-C</th>
<th>LDL-C</th>
<th>Total Cholesterol</th>
<th>FHCHA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Almasy et al,22 1999 (San Antonio scan of unesterified HDL-C in size classes 2a, 2b, 3a, and 3b)</td>
<td>2 (140 cM), 2a</td>
<td>4 (78 cM), 3a</td>
<td>5 (186 cM), 2b</td>
<td>8 (68 cM), 2b</td>
<td>8 (150 cM), 2a</td>
</tr>
<tr>
<td>Arnett et al,24 2001 (FHS TG scan)</td>
<td>4 (35 cM)</td>
<td>15 (36 cM)</td>
<td>11 (60–70 cM)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aouizerat et al,46 1999 (FCHL scan in Dutch families)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duggirala et al,27 2000 (San Antonio TG scan)</td>
<td>15 (20–30 cM)</td>
<td>7 (94 cM)</td>
<td>7 (186 cM)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Imperatore et al,28 2000 (scan in Pima Indians)</td>
<td>2 (45–50 cM)</td>
<td>3 (182 cM)</td>
<td>13 (75–80 cM)</td>
<td>13 (75–80 cM)</td>
<td></td>
</tr>
<tr>
<td>Knoblauch et al,23 2000 (scan in families ascertained for FH)</td>
<td></td>
<td>10 (70 cM)</td>
<td>2 (186 cM)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pajukanta et al,29 1999 (scan of Finnish FCHL families)</td>
<td>10 (174 cM)</td>
<td>10 (174 cM)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peacock et al,31 2000 (scan of HDL-C in the FHS)</td>
<td>5 (40 cM)</td>
<td>13 (28 cM)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rainwater et al,26 1999 (San Antonio scan of LDL-C size fraction phenotypes)</td>
<td></td>
<td></td>
<td>3 (244 cM)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shearman et al,25 2000 (Framingham scan of TG and TG/HDL-C ratio)</td>
<td>3 (140 cM), ratio</td>
<td>7 (155 cM), TG and ratio</td>
<td>11 (125 cM), ratio</td>
<td>16 (70–75 cM), TG and ratio</td>
<td>20 (35–40 cM), TG and ratio</td>
</tr>
</tbody>
</table>

FH indicates familial hypercholesterolemia. Studies are listed alphabetically by author.

References


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