Evidence of QTLs on chromosomes 1q42 and 8q24 for LDL-cholesterol and apoB levels in the HERITAGE Family Study[S]

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Abstract Genome-wide multipoint linkage analyses were performed to identify chromosomal regions harboring genes influencing LDL-cholesterol, total apolipoprotein B (apoB), and LDL-apoB levels using 654 markers. They were assessed in a sedentary state (baseline) and after a 20 week endurance training program. Strong evidence for two quantitative trait loci (QTLs) for baseline levels was found. There is linkage evidence in black families on chromosomes 1q41-q44 [at marker D1S2860, 238 centimorgan (cM), with a maximum log of the odds (LOD) score of 3.7 for LDL-apoB] and in white families on chromosome 8q24 (at marker D8S1774, 142 cM, with LOD scores of 3.6, 3.3, and 2.5 for baseline LDL-cholesterol, LDL-apoB, and apoB, respectively). There were no strong signals for the lipoprotein training responses (as computed as the difference in posttraining minus baseline levels).[JLR] In conclusion, QTLs for baseline apoB and LDL-cholesterol levels on chromosomes 1q41-q44 (in blacks) and 8q24 (in whites) were found. As there are no known strong candidate genes in these regions for lipids, follow-up studies to determine the source of those signals are needed.—Feitosa, M. F., I. B. Borecki, T. Rankinen, T. Rice, J-P. Després, Y. C. Chagnon, J. Gagnon, A. S. Leon, J. S. Skinner, C. Bouchard, M. A. Province, and D. C. Rao. **Evidence of QTLs on chromosomes 1q42 and 8q24 for LDL-cholesterol and apoB levels in the HERITAGE Family Study. *J. Lipid Res.* 2005.** 46: **281–286.**

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There is overwhelming evidence that the risk of coronary heart disease (CHD) varies as a function of increasing plasma cholesterol levels. The major cholesterol-carrying lipoprotein in the blood is the low density lipoprotein particle. Apolipoprotein B (apoB) is the primary surface component of LDL particles and is associated with an increased risk of CHD, as it provides a better indication of the concentration of atherogenic lipoproteins than does LDL-cholesterol. ApoB-100 is combined with lipid in the liver and converted to LDL-apoB, which is the major cholesterol-carrying lipoprotein (1). In some patients with familial hypercholesterolemia, marked increases of LDL-cholesterol result from delayed catabolism of LDL-apoB-100 associated with LDL receptor defects (2, 3). Several lines of evidence support a role for multiple genes as well as gene-environment interactions on CHD and associated risk factors, such as lipid levels, diabetes, obesity, and blood pressure (4). An important but often overlooked risk factor for CHD is physical inactivity (5). Physical activity is a behavior that has important metabolic and physiological benefits. Interestingly, although various studies have suggested several candidate genes and quantitative trait loci (QTLs) for lipid levels, they did not take into account physical activity and fitness levels.

The HERITAGE (HEalth, RIsk factors, exercise Training, And GEnetics) Family Study provides a unique opportunity to investigate these issues, because sedentary fami-

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[S] The online version of this article (available at http://www.jlr.org) contains three additional tables and two additional figures.

lies were recruited and tested, and then endurance exercise trained for 20 weeks and tested again. The percentages of variance attributable to additive genetic factors (heritability) were higher at baseline in blacks for LDL-cholesterol (80%, SEM = 0.09), LDL-apoB (80%, SEM = 0.09), and apoB (78%, SEM = 0.09) than in whites (53%, SEM = 0.07; 63%, SEM = 0.07; and 62%, SEM = 0.07, respectively). The heritability estimates are within the wide range of estimates reported in other studies (23–80%), which presumably included both physically sedentary and active families (6). Although the exercise training program in the HERITAGE Family Study had no effect on altering mean levels of LDL-cholesterol (7, 8), there was a great deal of individual variation in response to the training program. The heritability of LDL-cholesterol training response was higher in white (31%, SEM = 0.08) than in black (14%, SEM = 0.15) families. For the apoB training response, the heritability was higher in blacks (48%, SEM = 0.16) than in whites (18%, SEM = 0.08). The pattern was similar for LDL-apoB (48%, SEM = 0.17, in blacks vs. 21%, SEM = 0.08, in whites). These heritabilities for the responses are approximately half those for the respective baseline traits. The purpose of the present study was to determine whether there are QTLs influencing the variation in plasma levels of LDL-cholesterol, total apoB, and LDL-apoB at baseline and whether there are genomic regions linked to changes in response to endurance exercise training.

METHODS

Data and study design

The participants in the HERITAGE Family Study were measured in a sedentary state and after 20 weeks of endurance exercise training. At recruitment, subjects were required to be in good health but sedentary for at least the previous 6 months. A detailed description of the study design, exclusion criteria, exercise training protocol, and measurements is available elsewhere (9). In summary, each subject was exercise trained under supervision on a cycle ergometer three times per week for 20 weeks using a standardized training protocol. The intensity and duration of the training program were adjusted every 2 weeks, beginning at a heart rate (HR) corresponding to 55% of baseline maximal oxygen uptake (VO_{2max}) for 30 min per session and increasing gradually to a training HR that was associated with 75% of the subject's VO_{2max} for 50 min during the last 6 weeks. The power output of the cycle ergometer was adjusted automatically to maintain the desired HR of the subject at all times during all training sessions. Data from the 501 white subjects in 99 families and 277 black subjects in 101 families were studied. Race was determined by self-report. The number of sib-pairs in the marker data is given in the legend to **Table 1**. This study was approved by the institutional review boards of the participating institutions, with written informed consent obtained from each subject.

Measures

Blood samples were collected from an antecubital vein into Vacutainer tubes containing EDTA in the morning after a 12 h fast with participants in a semirecumbent position. Blood was drawn twice at baseline at least 24 h apart and at 24 and 72 h after the last training session. Samples were obtained in the early follicular phase for women. ApoB in the plasma and infranatant fraction was measured by the rocket-immunoelectrophoretic method of Laurell (10), whereas the ultracentrifuged bottom fraction was used to measure LDL-apoB levels. LDL-cholesterol was measured in the infranatant by the heparin-manganese chloride method (11). The two baseline values were averaged, as were the two posttraining values. The training response was computed as the difference between posttraining and baseline values. Posttraining values were corrected for plasma volume changes attributable to exercise (8). Extensive quality control procedures ensured the validity and reproducibility of the lipid-lipoprotein measurements (12). LDL-cholesterol, total apoB, and LDL-apoB were characterized by high-reliability intraclass correlation coefficients (ICCs) for repeated samples and split samples (ICC > 0.99) and day-to-day variation (ICC > 0.92).

TABLE 1. Descriptive statistics for baseline unadjusted phenotypes and covariates

	Whites			Blacks		
Covariate/Phenotype	N	Mean ± SD	Range	N	Mean ± SD	Range
Baseline						
Age (years)	529	35.4 ± 14.5	17.0–65.2	326	32.9 ± 11.6	17.0–65.9
BMI (kg/m^2)	522	25.9 ± 5.0	17.0–47.5	321	28.0 ± 6.2	17.4–50.9
Insulin (pmol/l)	492	64.8 ± 46.2	1.0–378.6	259	81.5 ± 68.2	1.0–519.6
Total apoB (mmol/l)	520	0.86 ± 0.24	0.21–1.56	314	0.80 ± 0.22	0.38–1.66
LDL-apoB (mmol/l)	520	0.78 ± 0.21	0.19–1.38	314	0.74 ± 0.21	0.34–1.43
LDL-cholesterol (mmol/l)	520	2.99 ± 0.81	0.73–6.04	315	2.85 ± 0.77	1.25–5.18
Training response						
BMI (kg/m^2)	481	−0.1 ± 0.8	−3.5–2.9	258	−0.2 ± 1.0	−5.2–2.9
Insulin[a] (pmol/l)	440	−5.6 ± 29.3	−145.1–138.4	185	−7.6 ± 63.5	−396.8–363.1
Total apoB (mmol/l)	468	0.00 ± 0.11	−0.39–0.32	222	0.01 ± 0.10	−0.34–0.36
LDL-apoB (mmol/l)	468	0.00 ± 0.10	−0.41–0.31	222	0.01 ± 0.10	−0.31–0.33
LDL-cholesterol (mmol/l)	468	−0.02 ± 0.37	−1.29–1.41	222	−0.01 ± 0.34	−0.79–1.49
Covariate	N	%		N	%	
Hormone use	529	30		326	50	

apoB, apolipoprotein B; BMI, body mass index. Range indicates the minimum and maximum values. Note that of 855 subjects, only 779 had genotype data, yielding a maximum of 360 white and 277 black sib-pairs at baseline with DNA data and a maximum of 286 white and 161 black sib-pairs for responses. However, all 855 subjects were used in the estimation of means, variances, and spouse and sibling resemblances in the variance components linkage analysis.

[a] Negative insulin levels for training response (postbaseline) indicate decreased levels after training.

Covariates and data adjustments

Body mass index (BMI; kg/m^2), fasting insulin level (measured by radioimmunoassay after polyethylene glycol separation) (13), and hormone use (contraceptives and hormone replacement therapy; 0 = no, 1 = yes) were included as covariates. Data adjustments were carried out separately within sex, race, and age groups (whites: <30, 30–50, ≥50 years old; blacks: <35, ≥35 years old). A stepwise multiple regression procedure was used, and only terms that were significant at the 5% level were retained. During model development, individuals with extreme scores [>4 SDs from the mean (mean ± SD)] were temporarily set aside so that they would not unduly influence the regressions. Each phenotype was adjusted for the effects of age (age, age^2, age^3), BMI, fasting insulin level, and hormonal use. The residual variances were also examined by regressing the squared residuals from the mean age regression on another similar regression model in a stepwise manner and retaining significant terms. The final phenotypes for all subjects were computed using the best regression models with the residuals standardized to a mean of 0 and a SD of 1. The responses (computed as posttraining minus baseline values) were adjusted for the similar covariates (age, response BMI, response insulin levels, and hormonal use) in addition to the respective baseline levels. The distributional properties of the adjusted data were checked for skewness, kurtosis, and extremely sparse outliers. Training response LDL-apoB values for two blacks were excluded from analysis after final adjustment because they were extreme outliers (>4 SDs from the mean and separated from the nearest interval value by at least 1 SD).

Marker data

PCR conditions and genotyping methods have been previously described (14). Automatic DNA sequences from Li-Cor (Lincoln, NE) were used to identify the PCR products, and genotypes were scored semiautomatically using SAGA software (Li-Cor). Inconsistencies attributable to non-Mendelian inheritance were identified and regenotyped (<10% were retyped). A total of 654 makers were used. Map locations were taken from the Genetic Location Database.

Linkage analysis

Genome-wide linkage scans were performed using a variance components approach as implemented in the computer program SEGPATH (15). This method is an extension of a path-analysis model of family resemblance, in which correlations among family members are modeled as a function of the allele sharing at a marker locus, taking into account residual familial correlations, representing polygenic and common environmental effects. Both sibship and parental phenotypic data were used in the estimation of the residual familial resemblance. For the linkage component, the parental marker data were used only to reduce the error in calculating the proportion of alleles shared identical-by-descent among siblings. These values were precalculated using MAPMAKER/SIBS (16) and then used as fixed data in the SEGPATH linkage analysis. The complete model includes the additive effects of a trait locus, a residual familial background modeled as a pseudopolygenic component, and a residual nonfamilial component. Estimated parameters include the heritabilities attributable to the trait locus

$$(h_g^2)$$

and the pseudopolygenic component

$$(h_r^2)$$

as well as estimates of spouse resemblance and residual sibling correlations. The linkage test is a likelihood ratio comparison between two models: a null hypothesis

$$(h_g^2 = 0)$$

and an alternative hypothesis. The difference in minus twice the log likelihoods (−2lnL) of the two hypotheses produces a likelihood ratio test that is asymptotically distributed as a 50:50 mixture of Chi-square with 1 degree of freedom and a point mass of 0. The log of the odds (LOD) score is calculated by dividing the Chi-square value by 2 × $\log_{10}$. Linkage analysis was conducted separately in blacks and whites.

A false discovery rate (FDR) (17) was used as a measure of global error for multiple testing simulations (i.e., the expected proportion of false rejections of the null hypothesis among the total number of rejections). FDR was estimated for each trait at baseline and response to training separately by white and black data using the SAS package.

RESULTS

Table 1 shows the sample statistics for the unadjusted total apoB, LDL-cholesterol, and LDL-apoB levels, as well as several covariates, in the white and black samples. Age, BMI, and fasting insulin levels were the most consistent predictors for the baseline phenotypes, accounting for 6–40% of the phenotypic variance. For the responses to training, the baseline level was the most consistent predictor, accounting for ~15% of the phenotypic variance (see supplementary data). Significant phenotypic (within-individual) correlations were observed among adjusted total apoB, LDL-cholesterol, and LDL-apoB levels within the baseline phenotypes and within responses in both black and white families. However, correlations between baseline phenotypes and responses were largely zero to slightly negative (see supplementary data), as expected because the responses were adjusted for baseline values.

All genome-wide linkage results with LOD ≥ 2.0 are given in **Table 2** (see supplementary data). The highest LOD was 3.7 [at marker D1S2860, 238 centimorgan (cM)] for baseline LDL-apoB on chromosome 1q42 in black families. Suggestive linkage occurred for baseline total apoB at the same marker location (LOD = 2.9, at marker D1S2860, 238 cM) in black families (**Fig. 1**). A second region providing strong evidence of linkage was on 8q24 at marker D8S1774 (142 cM) in whites with LOD scores of 3.6 for baseline LDL-cholesterol and 3.3 for baseline LDL-apoB (**Fig. 2**). In the same region (8q24), there was suggestive linkage for baseline total apoB levels with a LOD of 2.5 in white families (Fig. 2). As indicated on Table 2, these LOD scores remained significant at 1q42 and 8q24 after adjusting for multiple tests using the FDR method (17). Besides these strong linkages, several other suggestive (LOD ≥ 2.0) signals were detected on chromosomes 2p25-p21, 7p21, 7p11-q11, and 12q14. Detailed genome scan results can be found in the supplementary data.

DISCUSSION

Plasma cholesterol levels are complex traits influenced by multiple genes, multiple environmental factors, and in-

TABLE 2. Genome scan results with LOD score ⩾ 2.0

Chromosome	Marker	Location[a]	LOD Score	*P*, Adjusted[b]	Phenotype	Race	Status
		centimorgan					
1q41-q44	D1S2860	238	3.7	0.01	LDL-apoB	Black	Baseline
			2.9	0.06	ApoB	Black	Baseline
	D1S2703	231	2.1	0.40	LDL-cholesterol	Black	Baseline
8q24	D8S1774	142	3.6	0.01	LDL-cholesterol	White	Baseline
			3.3	0.02	LDL-apoB	White	Baseline
			2.5	0.15	ApoB	White	Baseline
2p25-p21	D2S131	19	2.3	0.09	LDL-cholesterol	White	Baseline
7p21.3	D7S513	17	2.4	0.09	LDL-apoB	White	Baseline
7p11-q11	IGFBP1	61	2.0	0.40	ApoB	Black	Baseline
12q14.1	D12S1691	69	2.1	0.45	LDL-cholesterol	White	Training response
20q13	D20S840	59	2.2	0.45	LDL-apoB	White	Training response

LOD, log of the odds.
[a] Locations based on the Genetic Location Database.
[b] *P* values adjusted for false discovery rate (FDR) using the SAS multitest procedure (17). FDRs were estimated for all lipoprotein phenotypes in the HERITAGE Family Study, but the adjusted *P* values reported here refer to each phenotype at baseline or in response to training separately by race.

teractions among genes and environments. Therefore, one can expect multiple linkage signals of varying strengths, many of which may have only low to moderate effects. Our current findings from the HERITAGE Family Study are consistent with this expectation, and there are only two regions, 1q41-q44 and 8q24, that show strong linkage evidence (LOD ⩾ 3.0).

The QTL on chromosome 1q41-q44 (230–261 cM) for baseline LDL-apoB (LOD = 3.7 at 238 cM) and total apoB (LOD = 2.9 at 238 cM) levels was detected in blacks. Prior studies showed results supportive of our findings in this region (1q44, 261 cM) for LDL-cholesterol (LOD = 1.1) and total cholesterol (LOD = 1.6), also in black Americans (18). This finding in the HERITAGE Family Study is particularly noteworthy given the small size of only 277 black sib-pairs (Table 2). In comparison, the LOD scores in this region were much lower (0.7 at 242 cM for LDL-apoB and 1.1 at 243 cM for total apoB) in the larger white

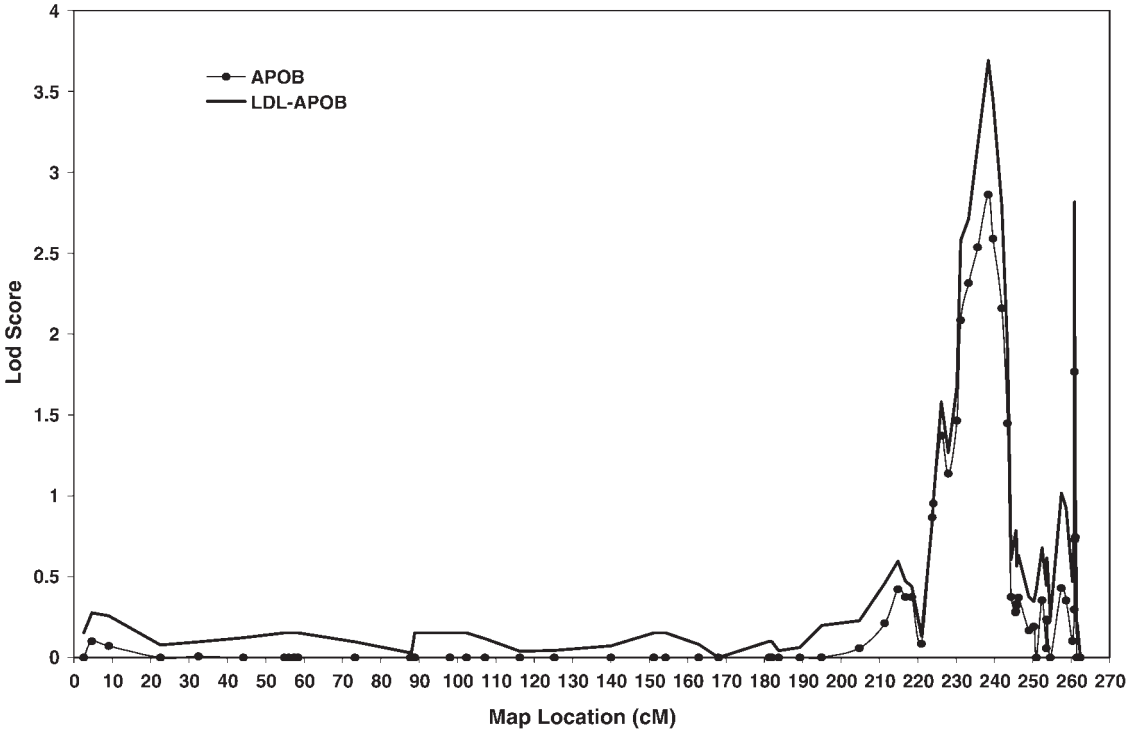

Fig. 1. Overview of the multipoint linkage results on chromosome 1 for baseline apoB and total apoB levels in black families. Log of the odds (LOD) scores are along the y-axis, and map locations [centimorgan (cM)] are on the x-axis (1 LOD intervals are 231 and 243 cM).

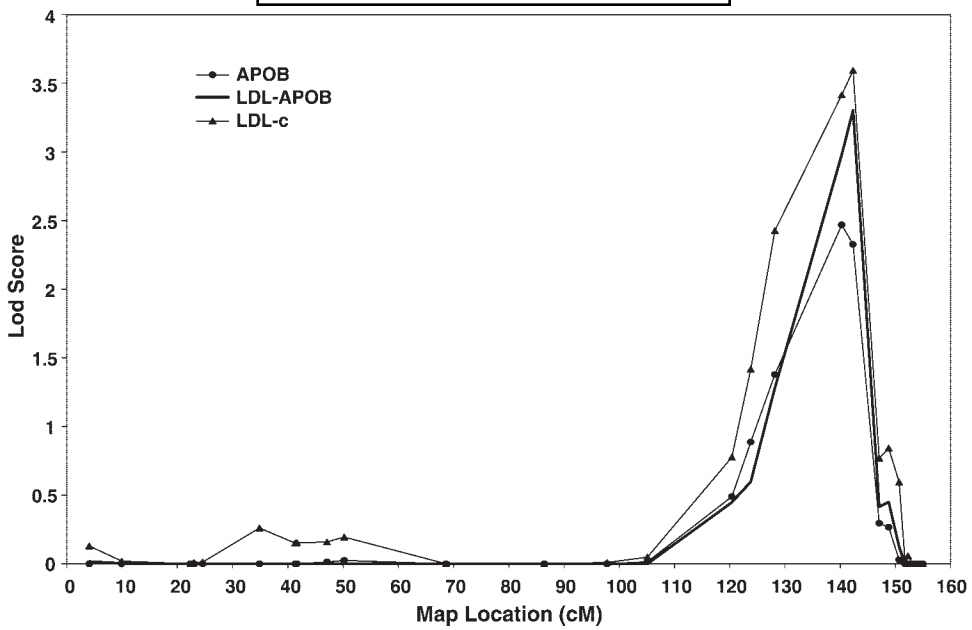

Fig. 2. Overview of the multipoint linkage results on chromosome 8 for baseline LDL-cholesterol, total apoB, and LDL-apoB levels in white families. LOD scores are along the y-axis, and map locations (cM) are on the x-axis (1 LOD intervals are 129 and 147 cM).

sample (360 sib-pairs) at baseline. Given this contrast and the fact that the only other supporting study also involved African-Americans, this novel QTL may be particularly relevant for blacks. No obvious candidate genes for lipid metabolism were found in this region. Consequently, this region warrants follow-up studies to narrow the linkage peak and discover the causative gene(s).

The second strong linkage result was on chromosome 8q24 (128–142 cM) for baseline LDL-cholesterol, LDL-apoB, and total apoB levels in whites. Previous reports of linkage on 8q23-q24 were with other lipoproteins, such as low HDL-cholesterol (LOD = 4.7, 124 cM) in Finnish families (19) and HDL-cholesterol levels (LOD = 4.9, 145 cM) in Mexican-Americans (20). However, there was no linkage for HDL-cholesterol in the HERITAGE Family Study (M. F. Feitosa, personal communication). No prominent candidate genes for lipid metabolism are known on 8q23-24.

For training response LDL-cholesterol and LDL-apoB, only suggestive signals were noted in white families on 12q14 (LOD = 2.1) and 20q13 (LOD = 2.2), respectively. This is likely a function of the lower power that arises for several reasons. First, the sample size was reduced, as shown in Table 2; the number of sib-pairs was reduced by 21% in whites and 42% in blacks for the training responses. Second, the training response was computed as a difference score between baseline in post measures, consequently entailing twice as much error or noise in the variance compared with baseline data. Third, likely as a function of the nature of difference scores, the heritabilities for the training responses are approximately halved (31% and 21% for LDL-cholesterol and LDL-apoB, respectively) compared with the baseline data (53% and 63%, respectively). However, notwithstanding these problems concerning the power of the response data, these results are interesting because they represent the only known genome scans for the unique assessment of lipid response to exercise training. Although one candidate gene is the low density lipoprotein receptor-related protein 1 (LRP1) gene that maps to 12q13 (at 58 cM), an association of lipids with LRP1 has not been described in the literature. There are no known lipid- and lipoprotein-related genes on 20q13.

In summary, this study provides three unique findings. First, the genome-wide scan provides linkage evidence in the sedentary state for variations in LDL-cholesterol (8q24 in whites) and LDL-apoB (1q41-q44 in blacks) levels. Second, these linkage signals were not replicated across blacks and whites, which may simply reflect differences in allele frequencies or could be indicative of interactions between QTLs and environmental factors. Third, relatively weaker linkage results for responses to exercise may be attributable to smaller sample sizes and smaller heritabilities (compared with baseline) that reduce the power of the linkage analysis. Consequently, the results for these training responses are inconclusive, but they do represent the only known data of their kind to date. These findings may be useful in guiding further fine-mapping and association studies.

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