Localization of multiple pleiotropic genes for lipoprotein metabolism in baboons[s]

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Abstract We employed a novel approach to identify the key loci that harbor genes influencing lipoprotein metabolism in approximately 2,000 pedigreed baboons fed various diets differing in levels of fat and cholesterol. In this study, 126 overlapping traits related to both LDL and HDL metabolism were normalized and subjected to genome-wide linkage screening. As was expected, the traits were highly, but not completely, correlated. We exploited the information in these correlated traits by focusing on those genomic regions harboring quantitative trait loci (QTL) for multiple traits, reasoning that the more influential genes would impact a larger number of traits.[jlr] This study identified five major QTL clusters (each with at least two significant logarithm of the odds scores >4.7), two of which had not been previously reported in baboons. One of these mapped to the baboon ortholog of human chromosome 1p32-p34 and influenced concentrations of LDL-cholesterol on Basal and high-fat, low-cholesterol diets. The other novel QTL cluster mapped to the baboon ortholog of human chromosome 12q13.13-q14.1 and influenced LDL size properties on high-fat, low-cholesterol and high-fat, high-cholesterol, but not Basal, diets. Confirming the value of this approach, three of the QTL clusters replicated published linkage findings for the same or similar traits.—Rainwater, D. L., L. A. Cox, J. Rogers, J. L. VandeBerg, and M. C. Mahaney. **Localization of multiple pleiotropic genes for lipoprotein metabolism in baboons.** *J. Lipid Res.* 2009. **50:** 1420–1428.

Supplementary key words linkage analysis • quantitative trait locus • genetic • primate

Atherosclerosis is a complex disease with etiologies attributable to a variety of environmental, metabolic, and genetic factors. Perhaps the strongest predictor of atherosclerosis is circulating levels of lipoprotein lipids. However, lipoproteins are themselves quite heterogeneous in terms of composition, both lipid and protein, and in terms of regulatory metabolic pathways. It is not surprising, therefore, that a large number of different factors have been identified in various studies to exert significant influence on lipoprotein phenotypes and associated risk of atherosclerosis.

Our group has studied genetic factors that influence lipoprotein phenotypes in pedigreed baboons as a closely related nonhuman primate model of human lipoprotein metabolism. While it is unlikely that the same functional polymorphisms will be found in both humans and baboons, our overall hypothesis has been that genes important to baboon lipoprotein metabolism will also be important to human lipoprotein metabolism.

Of course, a gene can be identified as having great impact in baboons but appear to be unimportant in parallel human studies due to little or no functional variation. Even in this case, however, identification of the gene may help explain rare lipoprotein disorders in humans or may be useful in developing therapeutic drug regimens.

In the course of our studies, we have measured lipoprotein phenotypes in approximately 2,000 pedigreed and genotyped baboons while they were feeding on various diets differing in levels of fat and cholesterol. In this study, we pooled these data to conduct linkage analyses on a large number of interrelated lipoprotein phenotypes. As a first-step attempt at prioritization, we focus here on pleiotropic loci harboring genes that influence clusters of intercorrelated traits and that therefore are likely to play a fundamental role in lipoprotein metabolism.

Abbreviations: apo, apolipoprotein; HDL-C, HDL-cholesterol; HFHC, diet high in fat and cholesterol; HFLC, diet high in fat but low in cholesterol; IBD, identity-by-descent; LOD, logarithm of the odds; PON, paraoxonase; QTL, quantitative trait loci; TG, triglyceride.

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[s] The online version of this article (available at http://www.jlr.org) contains supplementary data in the form of a table.

<p style="text-align:center">METHODS</p>

Pedigreed baboons

The baboons from which data were obtained in this study were organized into a single, unbroken six-generation pedigree configuration with 2,426 individuals. This pedigree, with approximately 384 founders, has resulted from managed breeding of olive baboons (*Papio hamadryas anubis*), yellow baboons (*P. h. cynocephalus*), and their hybrid progeny over the past three decades. Statistical power to detect, localize, and characterize genetic effects in a pedigree derives largely from the information contained in the relative pair classes contained therein. In this complex pedigree, full sibships ranged in size from 2 (n = 372) to 12 (n = 10), with the median = 5, and there were 50 additional classes of relative pairs in this extended pedigree. Examples of these pair classes included parent-offspring (n = 350), half sibling (n = 6855), half avuncular (n = 6414), and double half first cousin (n = 754) pairs.

Diet protocol

Nearly 2,000 of these pedigreed baboons were sampled while feeding on one or more diets differing in level of fat and cholesterol (i.e., Basal diet low in fat and cholesterol, HFHC diet high in fat and cholesterol, and HFLC diet high in fat but low in cholesterol); the diets and the diet experiment have been described previously (1). Animals were fasted overnight, immobilized with ketamine, and bled from the femoral artery. Serum was frozen at −80°C in single-use aliquots, protected from desiccation and oxidation (2).

Animal care and maintenance were performed by the veterinary resources staff at Southwest Foundation for Biomedical Research, a facility certified by the Association for Assessment and Accreditation of Laboratory Animal Care International. All procedures were approved by the Institutional Animal Care and Use Committee and conformed to the Public Health Service Policy on Humane Care and Use of Laboratory Animals.

Measurement of lipid and lipoprotein traits

Methods for measuring lipid and lipoprotein traits have been reported previously; below are given brief descriptions and references for the methods employed in measuring traits used in this study. Cholesterol and triglyceride (TG) concentrations were determined enzymatically using commercial reagents in a clinical chemistry analyzer (3). HDL-cholesterol (HDL-C) was measured in the supernatant after heparin-Mn^{+2} precipitation (4), and nonHDL-C was calculated as the difference between total and HDL-C. Concentrations of apolipoprotein AI (apoA1), apoB, and apoE were determined using an immunoturbidometric approach with commercial reagents in a clinical chemistry analyzer (5, 6). Lp(a) concentrations were measured using a sandwich-style ELISA (7).

We used composite gradient gels to resolve LDLs and HDLs on the basis of size (8) and Sudan black B to quantify lipoprotein cholesterol size distributions. Fractional cholesterol absorbance among apoB-containing lipoproteins was estimated for VLDL1 (V1-C; 36–33 nm), VLDL2 (V2-C; 33–30 nm), LDL1 (L1-C; 30–28 nm), LDL2 (L2-C; 28–27 nm), LDL3 (L3-C; 27–26 nm), and LDL4 (L4-C; 26–24 nm). Fractional cholesterol absorbance among HDLs was estimated for HDL1A (H1A-C; 24–20 nm), HDL1B (H1B-C; 20–13 nm), HDL2 (H2-C; 13–9.9 nm), and HDL3 (H3-C; 9.9–8.2 nm). In addition, serum lipoproteins also were resolved in HDL gels (9), and apoE size distributions were quantified by immunoblotting procedures as described (10, 11); fractional apoE absorbance among lipoproteins was estimated for apoB-containing lipoproteins (VL-E; 36–25 nm), HDL1A (H1A-E; 25–21 nm), HDL1B (H1B-E; 21–13 nm), HDL2 (H2-E; 13–9.9 nm), and HDL3 (H3-E; 9.9–7.2 nm). An estimate of serum concentration for each fraction was obtained by multiplication with nonHDL or HDL-C or apoE concentrations as appropriate. For cholesterol distributions only, we estimated median diameters for apoB-containing lipoproteins (Lmed-C; 24–36 nm) and HDLs (Hmed-C; 8.2–24 nm), which were defined as the diameter where half the absorbance was on larger, and half was on smaller, particles. LDL peak particle diameter was also estimated at the same time (8).

Serum activity levels of two lipoprotein-associated enzymes, lipoprotein-associated phospholipase A$_2$ (Lp-PLA2), and para-oxonase (PON) were also measured as described elsewhere. Lp-PLA2 was assayed using 2-thio-PAF as substrate in a commercial microplate-based colorimetric assay (12). PON was assayed using paraoxon as substrate in a microplate-based colorimetric assay (13).

Genotyping and construction of the map

Quantitative trait locus (QTL) localization analyses reported in this article used an updated version of the second-generation baboon whole-genome genetic linkage map that is based largely on human microsatellite markers that also are polymorphic in baboons (14, 15). This enables extrapolation of results to the orthologous human genomic locations; in this text, therefore, map locations will refer to the human chromosomal regions. Based on genotype data from 2,044 baboons, the version of the map used for this study included 287 markers mapped at 1,000:1 odds, at an average of 7.7 cM across the 20 baboon autosomes. Links to information regarding the current whole-genome linkage map for this species (including marker characteristics and genotyping conditions) can be found at http://baboon.sfbrgenetics.org/. Genotype data from all 2,044 animals on which the map is based were also used to estimate multipoint identity-by-descent (IBD) coefficients for this study.

Statistical genetic analyses

Data for 42 traits on each of three diets were available for these analyses. Because the data spanned several different experiments with differing objectives, there was a variable number of animals with data for any one trait. Each trait was preadjusted for a standard set of covariates (i.e., age, sex, and weight) that have proven to be significant covariates in many previous studies, even if one (or more) covariate was not significant for the trait. On average, these covariates accounted for 5% of total phenotypic variance (range was 0 to 32%), so the bulk of raw trait variance was retained in the residual. The residual variables were then normalized by fitting to a Gaussian distribution and standardized such that mean and SD were 0 and 1, respectively. These transformations were done in the software package SOLAR (16), and the normalized traits were used for the analyses described below.

Simple Pearson correlation analyses were performed on the transformed adjusted traits using a commercial statistics package (Stata 9.2; College Station, TX). We estimated the genetic effect, or heritability (h^2), as the proportion of the residual variance in each trait that was attributable to the additive effects of genes (i.e., σ_G^2/σ_P^2). Genome-wide multipoint linkage screening of these traits was done using maximum-likelihood-based variance decomposition routines implemented in SOLAR. Briefly, we estimated the amount of variance attributable to a QTL (σ_Q^2) by specifying the expected genetic covariances between arbitrary relative pairs as a function of the IBD relationships at a given marker locus assumed to be tightly linked to a locus that influences the trait (16). Markov Chain Monte Carlo routines, implemented in Loki (17), were used to estimate multipoint IBD probabilities for all relative pairs in the single pedigree. We conducted likelihood ratio tests for linkage at 1-cM intervals across

all autosomes. Evidence for linkage was expressed in terms of logarithm of the odds (LOD) scores, obtained as the quotient of the likelihood ratio test statistic divided by the value $\log_e 10$ (18).

Genome-wide probability values incorporated information on marker density and on the structure of the pedigree and they were calculated as suggested by Feingold, Brown, and Siegmund (19); by this calculation, a LOD = 2.75 was associated with genome-wide significance level of 0.05, a conventional criterion for QTL significance. However, based on our consideration of 126 traits, and employing a Bonferroni approach, we defined as significant in this study a QTL with a LOD score >4.7, which is the value associated with a genome-wide significance level of 0.00040 (i.e., 0.05/126) in this pedigree. We then defined as suggestive a QTL satisfying the conventional criterion of genome-wide significance (i.e., a LOD score >2.75). The purpose of imposing this relatively stringent criterion was to focus attention on the most important and reliable QTLs.

Bivariate analyses were employed to test the contrasting hypotheses of pleiotropy versus coincident linkage to explain the QTL clusters. These tests included the trait having the strongest evidence of a QTL paired with each of the other traits having a significant QTL. Implemented in SOLAR, bivariate multipoint linkage analyses identified the genomic location returning the highest joint (bivariate) LOD score within the cluster interval (20). At that point, the model partitioned the genetic variance for each trait by estimating the QTL heritability (h_Q^2), defined as the proportion of total phenotypic variance explained by the joint QTL (σ_Q^2/σ_P^2), and the residual heritability, defined as (σ_G^2/σ_P^2)$-h_Q^2$. In addition, the additive genetic correlation (ρ_Q) between traits attributable to the QTL was calculated in the model based on estimated IBD allele sharing. Hypotheses of QTL pleiotropy versus coincident linkage were tested by comparing the likelihoods of models in which ρ_Q was estimated to those of models in which it was fixed at zero; a ρ_Q significantly >0 implied pleiotropy. Complete QTL pleiotropy was tested by comparing models in which ρ_Q was estimated to models in which it was fixed at 1 (absolute value); a ρ_Q not significantly different from 1 was interpreted as evidence for complete QTL pleiotropy (21). However, in none of these tests did a pleiotropic QTL account for all the additive genetic variance.

RESULTS

Lipid and lipoprotein traits

For the purposes of multiple studies, we obtained measures of 42 lipoprotein traits for animals fed a series of three defined diets differing in levels of fat and cholesterol. The traits included various measures of lipoprotein properties, including concentrations and size distribution characteristics of several lipids and apolipoproteins. Half the traits were categorized as LDL related and half as HDL related. Each of the traits was significantly heritable at the $P < 0.05$ level, and heritabilities ranged from 6% to 71% for the 126 traits (**Table 1**).

These traits were by no means independent. Correlation analyses of the transformed traits confirmed the expected high degree of intercorrelation among the traits. For example, the average Pearson correlation coefficient (absolute value) was 0.202, and 78% of all possible pairwise correlations were significant at the $P < 0.05$ level. Thus, we anticipated developing substantial evidence for pleiotropy among these traits.

Screen for QTLs influencing lipid and lipoprotein traits

Each trait was individually preadjusted for sex, age, and weight, transformed, and then subjected to a genome-wide linkage screen. **Figure 1** plots, for LDL (upper panel) and HDL (lower panel) traits, the genomic locations of the primary QTL for each trait. For convenience, we have sorted and labeled the baboon genome using the homologous human designations. Of the 126 traits, 87 (69%; 45 LDL-related and 42 HDL-related traits) returned LOD scores >2.75 (i.e., genome-wide $P < 0.05$) and 57 of those (45%; 28 LDL-related and 29 HDL-related traits) were significant after adjustment for multiple testing (i.e., LOD >4.7; genome-wide $P < 0.00040$). The location and peak LOD score of the primary QTL for each of the 126 traits in this study are given in supplementary Table I (http://baboon. sfbrgenetics.org/Bab_SupplementalData/Rainwater2009. php?pageNum_rsDRSuppDataList=0&totalRows_ rsDRSuppDataList=126). This table also provides links to the multipoint LOD score plots for all autosomes for each of the 126 traits.

A major QTL cluster was defined as two or more significant QTLs whose 1-LOD support intervals enclosed the peak location of the predominant QTL. Under this definition, it would be possible for the cluster to result from the actions of a single pleiotropic gene. We hypothesized that such a pleiotropic gene, influencing multiple traits, might play a key role in lipoprotein metabolism. In this study, we identified five clusters of significant QTLs that satisfied this criterion, and **Table 2** lists the QTLs, their locations, and peak LOD scores. It is noteworthy that each of these clusters is anchored by a predominant QTL with LOD score exceeding 10.

Description of QTL clusters

Cluster 1 comprised three QTLs for concentrations of LDL and LDL subfractions on Basal and HFLC, but not HFHC, diets; two suggestive QTLs in the vicinity helped confirm this cluster. Human microsatellite markers mapping near this cluster included D1S2130, D1S515, and D1S192 (which were located at 48.2, 62.3, and 68.4 cM, respectively; all locations represent map distance in cM from the pter-most marker locus on the chromosome). Cluster 2 comprised six QTLs for intermediate-size lipoproteins, such as Lp(a), on all diets. Nearby markers included D6S404, D6S1698, and D6S503 (124.5, 127.2, and 146.9 cM). Cluster 3 comprised QTLs for paraoxonase activity levels on each of the three diets. Nearby microsatellite markers included D7S821 and D7S2204 (123.4 and 132.4 cM). Cluster 4 comprised 13 QTLs on chromosome 12; the QTLs are for LDL-related traits on the two high-fat diets, including various estimates of size distribution and concentration of apoB-containing lipoprotein cholesterol; four suggestive QTLs helped confirm this cluster. Four LOD scores >10 in this cluster were flanked by markers D12S297 and D12S75 (62.6 and 72.6 cM). Finally, Cluster 5 comprised 22 QTLs for a variety of HDL-related traits, including size distributions and concentrations of HDL cholesterol, apoE, and apoA1. Seventeen LOD scores >10 were flanked by human microsatellite markers D18S475,

TABLE 1. Residual heritability ± SEM (%) for 21 LDL-related and 21 HDL-related traits measured in baboons fed three diets

Group	Trait[a]		Basal		HFLC		HFHC	
			n	h²	n	h²	n	h²
LDL	Frac	V1-C	1,707	10.2 ± 3.6	1,298	27.6 ± 5.1	1,721	25.3 ± 4.4
LDL	Frac	V2-C	1,707	37.3 ± 4.3	1,298	45.7 ± 4.6	1,721	44.5 ± 4.6
LDL	Frac	L1-C	1,707	31.4 ± 4.9	1,298	30.1 ± 5.1	1,721	42.5 ± 5.2
LDL	Frac	L2-C	1,707	15.8 ± 4.1	1,298	23.6 ± 5.8	1,721	28.0 ± 5.0
LDL	Frac	L3-C	1,707	24.9 ± 4.6	1,298	38.6 ± 5.8	1,721	44.4 ± 5.1
LDL	Frac	L4-C	1,707	21.1 ± 4.0	1,298	34.0 ± 5.5	1,721	36.1 ± 4.6
LDL	Frac	VL-E	690	16.0 ± 6.6	690	26.7 ± 7.2	689	42.3 ± 7.7
LDL	Diam	Lmed-C	1,648	30.1 ± 4.6	1,258	39.0 ± 5.6	1,658	48.6 ± 5.1
LDL	Diam	LDLPPD	1,648	25.0 ± 4.3	1,258	29.4 ± 5.3	1,658	45.3 ± 5.1
LDL	Conc	NonHDL-C	1,937	53.2 ± 3.8	1,299	61.1 ± 4.6	1,956	58.3 ± 3.9
LDL	Conc	TG	1,085	22.9 ± 5.3	1,212	31.4 ± 5.5	1,126	29.0 ± 6.3
LDL	Conc	Lp(a)	681	70.5 ± 6.0	689	66.1 ± 6.2	686	71.2 ± 5.7
LDL	Conc	ApoB	1,229	38.8 ± 5.3	1,269	52.1 ± 5.2	1,256	46.4 ± 5.2
LDL	Conc	V1-C	1,648	21.4 ± 3.9	1,258	40.8 ± 5.1	1,658	41.0 ± 4.5
LDL	Conc	V2-C	1,648	38.8 ± 4.1	1,258	49.5 ± 4.4	1,658	53.2 ± 4.7
LDL	Conc	L1-C	1,648	38.5 ± 4.5	1,258	44.9 ± 4.9	1,658	58.0 ± 4.6
LDL	Conc	L2-C	1,648	37.3 ± 4.6	1,258	51.6 ± 5.6	1,658	30.4 ± 5.5
LDL	Conc	L3-C	1,648	25.5 ± 4.5	1,258	28.7 ± 5.7	1,658	35.0 ± 5.4
LDL	Conc	L4-C	1,648	23.7 ± 4.6	1,258	36.7 ± 6.2	1,658	27.8 ± 4.9
LDL	Conc	VL-E	590	12.0 ± 4.7	660	23.5 ± 6.3	628	47.3 ± 8.0
LDL	Act	Lp-PLA2	713	69.3 ± 7.2	706	55.7 ± 8.4	716	66.8 ± 6.8
HDL	Frac	H1A-C	1,707	24.6 ± 4.0	1,298	21.4 ± 4.6	1,721	22.6 ± 4.2
HDL	Frac	H1B-C	1,707	29.4 ± 4.3	1,298	35.4 ± 5.1	1,721	35.4 ± 4.6
HDL	Frac	H2-C	1,707	17.8 ± 4.1	1,298	33.6 ± 5.2	1,721	28.8 ± 4.0
HDL	Frac	H3-C	1,707	37.3 ± 4.2	1,298	32.6 ± 5.2	1,721	35.0 ± 4.4
HDL	Frac	H1A-E	690	23.5 ± 6.1	690	31.2 ± 7.2	689	37.1 ± 7.3
HDL	Frac	H1B-E	690	12.6 ± 7.1	690	10.9 ± 6.7	689	25.9 ± 7.5
HDL	Frac	H2-E	690	20.0 ± 5.2	690	32.8 ± 6.8	689	43.4 ± 7.3
HDL	Frac	H3-E	690	9.7 ± 5.8	690	5.8 ± 3.7	689	19.1 ± 6.2
HDL	Diam	Hmed-C	1,648	38.5 ± 4.6	1,258	40.4 ± 5.8	1,658	36.0 ± 4.7
HDL	Conc	HDLC	1,937	55.2 ± 4.0	1,299	61.8 ± 5.0	1,956	49.1 ± 4.2
HDL	Conc	ApoA1	1,234	45.1 ± 4.9	1,270	44.7 ± 4.7	1,257	39.0 ± 4.9
HDL	Conc	ApoE	1,197	34.7 ± 5.5	1,270	36.6 ± 5.4	1,239	37.5 ± 5.7
HDL	Conc	H1A-C	1,648	25.4 ± 3.9	1,258	31.7 ± 4.9	1,658	24.3 ± 4.3
HDL	Conc	H1B-C	1,648	40.7 ± 4.8	1,258	39.0 ± 5.2	1,658	41.8 ± 4.9
HDL	Conc	H2-C	1,648	43.9 ± 4.4	1,258	40.1 ± 5.3	1,658	34.5 ± 4.6
HDL	Conc	H3-C	1,648	32.2 ± 4.3	1,258	17.3 ± 4.0	1,658	30.2 ± 4.4
HDL	Conc	H1A-E	590	35.5 ± 7.8	660	38.6 ± 7.7	628	45.9 ± 8.1
HDL	Conc	H1B-E	590	26.8 ± 7.6	660	32.9 ± 8.0	628	33.8 ± 7.3
HDL	Conc	H2-E	590	13.0 ± 5.7	660	15.1 ± 6.0	628	11.1 ± 6.3
HDL	Conc	H3-E	590	19.1 ± 7.6	660	9.9 ± 5.3	628	14.4 ± 7.3
HDL	Act	PON	1,178	54.2 ± 5.4	1,155	67.7 ± 5.1	1,185	59.1 ± 5.4

LDLPPD, LDL peak particle diameter; Lp-PLA2, lipoprotein-associated phospholipase A$_2$.
[a] Trait names are defined in Methods; preceding the name is the type of measure: Frac, fraction; Diam, diameter; Conc, concentration; Act, enzyme activity.

D18S72, and D18S1156 (41.8, 51.9, and 61.5 cM). The baboon microsatellite marker D18Spha1 (57.4 cM) is located within exon 9 of the structural locus for endothelial lipase (*LIPG*) (22).

Of these five major QTL clusters, two, clusters 1 and 4, had not been reported previously in baboons. **Figure 2** graphs the multipoint LOD score plots for the three traits comprising Cluster 1 on chromosome 1 (panel A) and for the 13 traits comprising Cluster 4 on chromosome 12 (panel B). Below, we further characterize the effects of genes that might be responsible for clusters 1 and 4.

Further characterization of Cluster 1 QTLs on the baboon ortholog of human chromosome 1

The gene(s) responsible for Cluster 1 obviously influences LDL metabolism, but in this case the specific effects are on cholesterol concentrations and in diets with low cholesterol levels. The trait with the highest LOD score peak

for this cluster was NonHDL-C on Basal diet (LOD = 10.76). To test the hypotheses of QTL pleiotropy versus coincident linkage, we conducted bivariate linkage analyses that paired this trait with each of the other traits with significant QTLs (**Table 3**). First, it should be noted that on average the QTL explained less than half the additive genetic variance for these traits. For the pair nonHDL-C on Basal and HFLC diets, the QTL correlation estimated to be 1, whereas for nonHDL-C and concentration of LDL1-C on Basal diet the correlation was high (0.79) but significantly different from 1. Still, the QTL correlations are all significantly greater than zero, suggesting that a single pleiotropic gene is largely responsible for this cluster.

To help narrow the region in which to search for candidate genes, we identified the 1-LOD support interval for nonHDL-C on Basal diet to be 53–65 cM on the baboon map. Selection of this region was confirmed by the observation that the peak LOD scores for the other two
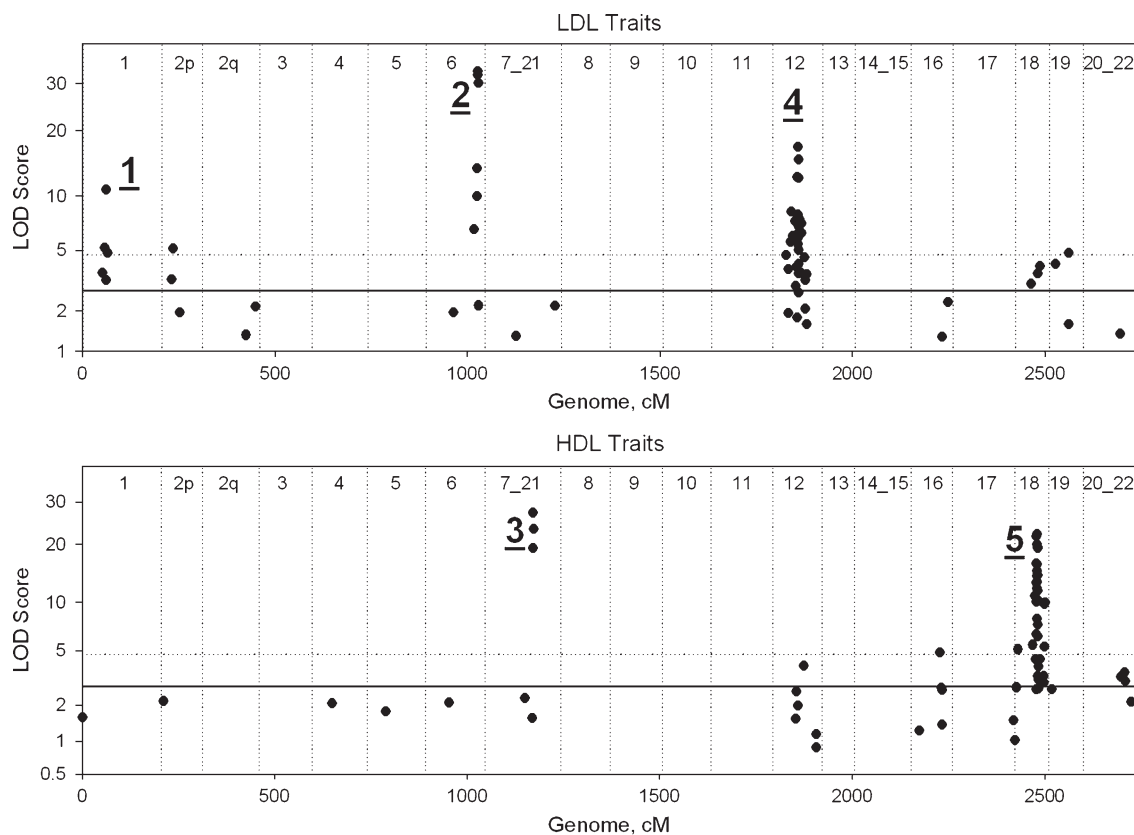
Fig. 1. Chromsomal locations of peak LOD score for the primary QTL for each of 63 LDL-related (upper panel) and 63 HDL-related (lower panel) traits. Baboon chromosomal designations are indicated as the corresponding human chromosome orthologs. The horizontal lines indicate LOD scores defined as suggestive (2.75; solid) and significant (4.7; dotted). The five major clusters are indicated by the underlined numerals.

traits with significant LODs also fell within this interval. Under the reasonable assumption of cross-species conservation in orthologous syntenic regions known to exhibit conserved microsatellite marker order (15), we interpolated QTL locations on the human physical map based on genetic distances between the QTL and flanking markers. By this process, this QTL support interval maps to 1p32-p34, a region that is 50.2–67.6 mb from pter [physical locations were taken from National Center for Biotechnology Information build 36.1 of March 2006, by use of the University of California, Santa Cruz Human Genome Browser (23)].

Further characterization of Cluster 4 QTLs on the baboon ortholog of human chromosome 12

The gene(s) responsible for Cluster 4 obviously influences LDL metabolism and, more specifically, it appears to influence LDL size distributions on the high-fat diet. This conclusion is based on the observation that most of the significant QTLs were for fractions or diameter data, including the three highest LODs in the cluster. The trait with the highest LOD score peak was Lmed-C on HFHC diet (LOD = 17.11). To test the hypotheses of QTL pleiotropy versus coincident linkage, we conducted bivariate linkage analyses that paired this trait with each of the 12 other traits exhibiting significant LOD scores (Table 3). In this cluster, the QTL explained about a quarter of the additive genetic variance in each trait. All of the 12 QTL correlations were significantly different from zero at the $P < 0.05$ level, and all but one of the QTL correlations was indistinguishable from unity. These observations suggest that a single pleiotropic gene is responsible for the major QTL cluster observed on the baboon ortholog of human chromosome 12.

To help narrow the region in which to search for candidate genes for this QTL, we determined the 1-LOD support interval for Lmed-C on HFHC to be 61–69 cM on the baboon map. Further corroborating the selection of this interval, the LOD score peaks for all but two of the clustered traits in Table 2 fell within this same interval. Interpolations based on nearby human microsatellite markers suggested this interval corresponds approximately to cytogenetic location 12q13.13-q14.1 and physical location 48.7–61.2 mb on the map of human chromosome 12.

DISCUSSION

In this study, we conducted genome screens for 126 lipid and lipoprotein traits that included size and concentration measures of protein and lipid components of LDLs and HDLs. A key focus of the experiments underlying this study was detection of diet effects on QTLs, namely the

TABLE 2. Locations of peak LOD scores for traits comprising five major QTL clusters

Cluster	Chromosome	cM	LOD	Trait		Diet
1	1	55	5.16	Conc	NonHDL-C	HFLC
1	**1**	**60**	**10.76**	**Conc**	**NonHDL-C**	**Basal**
1	1	64	4.81	Conc	L3-C	Basal
2	6	124	6.63	Frac	V2-C	HFLC
2	6	132	13.63	Frac	V2-C	Basal
2	6	132	10.01	Conc	V2-C	Basal
2	**6**	**134**	**32.98**	**Conc**	**Lp(a)**	**HFLC**
2	6	134	32.09	Conc	Lp(a)	HFHC
2	6	135	30.09	Conc	Lp(a)	Basal
3	7_21	124	19.21	Act	PON	Basal
3	**7_21**	**124**	**27.25**	**Act**	**PON**	**HFLC**
3	7_21	126	23.37	Act	PON	HFHC
4	12	45	5.59	Frac	L3-C	HFLC
4	12	51	6.04	Conc	L2-C	HFHC
4	12	63	12.37	Diam	LDLPPD	HFHC
4	12	64	5.43	Frac	V1-C	HFHC
4	**12**	**64**	**17.11**	**Diam**	**Lmed-C**	**HFHC**
4	12	64	5.87	Conc	V1-C	HFHC
4	12	65	7.96	Frac	L1-C	HFLC
4	12	65	6.23	Diam	Lmed-C	HFLC
4	12	66	5.01	Conc	L1-C	HFLC
4	12	67	15.02	Frac	V2-C	HFHC
4	12	67	6.86	Frac	L3-C	HFHC
4	12	67	12.26	Conc	V2-C	HFHC
4	12	69	7.53	Conc	L3-C	HFHC
5	18	46	5.46	Frac	H3-C	HFLC
5	18	51	10.92	Frac	H1A-C	HFHC
5	18	54	16.11	Conc	HDLC	Basal
5	18	54	10.13	Conc	H1B-E	HFLC
5	18	54	12.88	Conc	H1A-C	HFHC
5	18	55	6.39	Frac	H1A-C	HFLC
5	18	55	21.71	Conc	HDLC	HFLC
5	18	56	19.92	Diam	Hmed-C	HFLC
5	**18**	**56**	**22.18**	**Conc**	**H1B-C**	**HFLC**
5	18	56	13.99	Diam	Hmed-C	HFHC
5	18	57	13.04	Frac	H1B-C	Basal
5	18	57	11.93	Diam	Hmed-C	Basal
5	18	57	16.03	Conc	H1B-C	Basal
5	18	57	14.85	Frac	H1B-C	HFLC
5	18	57	8.04	Conc	H1A-C	HFLC
5	18	58	19.34	Frac	H2-C	HFLC
5	18	58	19.19	Conc	ApoE	HFLC
5	18	58	6.21	Conc	H1A-E	HFLC
5	18	58	14.10	Frac	H2-C	HFHC
5	18	58	11.63	Conc	ApoE	HFHC
5	18	59	7.37	Conc	ApoE	Basal
5	18	60	10.15	Frac	H2-C	Basal

Chromosome numbering is based on that of the homologous human chromosomes. Traits indicated in bold exhibited the highest LOD score for a cluster.

effects of increasing levels of dietary fat (comparing Basal and HFLC diets) and of increasing levels of dietary cholesterol in the high-fat environment (comparing HFLC and HFHC diets). In some cases, a QTL showed no evidence of diet effects; for example, PON and Lp(a) QTLs on each diet mapped to the same genomic regions. However, more intriguing was the detection of QTLs for a trait only on certain diets or even on different chromosomes depending on diet. For example, significant QTLs for nonHDL-C were detected on chromosomes 1 (Basal and HFLC diets) and 19 (HFHC diet), suggestive of diet-by-genotype interaction. There can be many reasons why QTLs influencing a trait might differ across diets, including the possibility that our criterion for declaring significance was too strin-

gent (or not sufficiently stringent). While we cannot exclude the possibility that Type 1 and Type 2 errors were responsible for apparent diet differences, a likely interpretation is genotype-by-diet interaction, wherein the effects of different genes predominate depending on dietary environment. In fact, some of our previous baboon studies have reported evidence of diet-by-genotype interaction for HDL- and LDL-related traits (13, 24–26). However, additional explorations of novel diet-by-genotype interactions developed in this study await future analyses.

This study focused on characterizing loci that harbor genes important to lipoprotein metabolism. For nearly 70% of the 126 traits, we detected a QTL that was significant by conventional criteria (i.e., genome-wide $P < 0.05$). To minimize the possibility of Type 1 error due to the multiple tests, we elected to restrict the criterion for significance to be a genome-wide P value < 0.0004, which is equivalent to a LOD score of 4.7 in our baboon pedigree. Using this criterion, we detected significant QTLs for 57 (45%) of the traits. It should be noted that this criterion is probably overly conservative, given the observed degree of intercorrelation among traits that serves to reduce the effective number of independent traits. The significant QTLs were found on the baboon orthologs of human chromosomes 1, 2, 6, 7, 12, 16, 18, and 19.

To help prioritize for further characterization, we attempted to localize pleiotropic QTLs that influenced multiple lipoprotein traits under the assumption that the genes underlying them are likely to play fundamental roles in
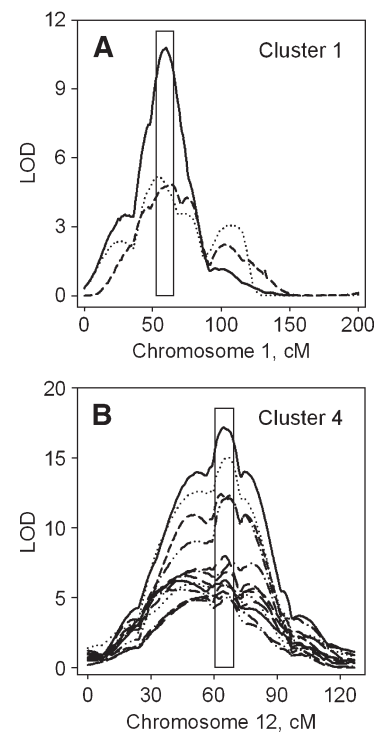

Fig. 2. Multipoint LOD score plots for the traits comprising QTL Clusters 1 (A) and 4 (B) on the baboon orthologs of human chromosomes 1 and 12, respectively. The boxes indicate the 1-LOD drop intervals for the predominant QTL.

TABLE 3. Results from bivariate linkage analyses

Trait 1 (Diet)	Trait 2 (Diet)	Joint LOD	Trait 1 h_R^2	h_Q^2	Trait 2 h_R^2	h_Q^2	ρ_Q	P ($|\rho_Q| = 1$)
Chromosome 1								
Conc NonHDL-C (Basal)	Conc NonHDL-C (HFLC)	11.2	26.8	22.8	43.1	14.5	1.000	1.000
Conc NonHDL-C (Basal)	Conc L3-C (Basal)	13.2	28.0	23.9	11.9	13.6	0.795	0.001
Chromosome 12								
Diam Lmed-C (HFHC)	Frac L3-C (HFLC)	17.8	29.2	16.9	29.3	6.5	−1.000	1.000
Diam Lmed-C (HFHC)	Conc L2-C (HFHC)	18.0	30.0	15.7	24.0	7.4	−0.472	0.008
Diam Lmed-C (HFHC)	Diam LDLPPD (HFHC)	16.2	30.2	16.5	29.0	13.9	0.983	0.100
Diam Lmed-C (HFHC)	Frac V1-C (HFHC)	15.5	29.8	16.1	18.8	5.7	0.880	0.301
Diam Lmed-C (HFHC)	Conc V1-C (HFHC)	16.2	29.4	17.9	36.1	6.4	0.932	0.362
Diam Lmed-C (HFHC)	Diam Lmed-C (HFLC)	17.5	28.5	16.3	26.8	7.6	0.999	0.993
Diam Lmed-C (HFHC)	Frac L1-C (HFLC)	19.2	29.9	15.7	18.5	9.6	1.000	1.000
Diam Lmed-C (HFHC)	Conc L1-C (HFLC)	16.6	29.9	15.4	36.8	6.7	0.991	0.918
Diam Lmed-C (HFHC)	Frac L3-C (HFHC)	19.2	33.0	14.3	33.1	8.0	−1.000	1.000
Diam Lmed-C (HFHC)	Conc V2-C (HFHC)	16.4	29.9	17.1	42.4	11.4	0.957	0.165
Diam Lmed-C (HFHC)	Frac V2-C (HFHC)	17.0	29.5	15.9	31.8	13.1	0.987	0.431
Diam Lmed-C (HFHC)	Conc L3-C (HFHC)	15.9	29.6	16.4	24.3	9.4	−0.979	0.493

The models included the indicated trait pairs from Cluster 1 on chromosome 1 and Cluster 4 on chromosome 12; the table gives joint LOD score, proportion (%) of trait variances attributable to the residual (h_R^2) and QTL (h_Q^2) genetic components, the QTL correlation (ρ_Q), and the P value for the test of complete QTL pleiotropy. ρ_Q is the QTL-specific additive genetic correlation; the P value is from the $\log_e$-likelihood ratio test of the hypothesis that $|\rho_Q| = 1$.

lipoprotein metabolism. Therefore, we defined a major QTL cluster as two or more QTLs whose 1-LOD support intervals enclosed the peak of the predominant QTL. In fact, 47 of the 57 significant QTLs in this study colocalized into one of five major QTL clusters under this definition.

Two of these major clusters (Cluster 2 and Cluster 3) mapped to chromosomal regions containing the appropriate structural locus. *LPA* is the structural locus on chromosome 6 for the Lp(a)-specific protein apo(a). Previous studies in baboons (7, 27, 28) and humans (29) have demonstrated genetic variation at this locus accounts for the majority of variation in Lp(a) levels. In this study, the locus also appears to influence lipoprotein particles in V2-C size range (30–33 nm), which is the size range that would contain baboon Lp(a) particles. *PON1* is the structural locus on chromosome 7 for the PON protein found in serum. Our previous study in baboons detected a QTL in this region (13), and numerous studies in humans (30–33) have demonstrated significant effects of *PON1* polymorphisms on PON activity.

Cluster 5 mapped to the baboon ortholog of human chromosome 18 and included 22 significant QTLs for HDL traits. The significant traits tended to be for larger HDLs that are enriched in apoE (10). This QTL cluster confirms our previous study involving many of the same traits and animals and in which we reported promoter variants in *LIPG*, the structural locus for endothelial lipase on chromosome 18, to regulate HDLC levels (22).

Finally, two of the QTL clusters, Cluster 1 and Cluster 4, identified chromosomal regions in which we have not previously localized QTLs for lipoprotein-related traits. Both these clusters had multiple significant QTLs and they reflected pleiotropic effects on various LDL traits. However, the two clusters differed markedly in the types of LDL measures that were affected. Below, we discuss the two clusters in greater detail.

Cluster 1 included three significant and two suggestive QTLs that represented measures of LDL-C and LDL subfraction concentrations on the two low cholesterol diets. The QTLs clustered in a genomic region corresponding to human chromosome 1p32-p34. Falling within the 1-LOD support interval for the largest signal are 105 genes (23, 34), including *LRP8* (encoding LDL receptor-related protein 8, apoER2, which is an apoE receptor), *SCP2* (encoding sterol carrier protein-2), and *PCSK9* (encoding proprotein convertase subtilisin/kexin type 9). Although it binds apoE, apoER2 is found only in brain and testis and so is not likely to play a role in lipoprotein metabolism of the circulation (35). SCP-2 plays important roles in intracellular lipid metabolism and signaling (36) but does not appear to play a direct role in LDL metabolism. However, *PCSK9* is a particularly strong candidate, based on an extensive list of polymorphisms that modulate LDL receptor levels, in both positive and negative directions, and thereby LDL-C levels [reviewed in (37)]. In fact, there was a strong correlation ($r = 0.54$, $P = 0.001$, n = 115) in diabetic humans between circulating levels of PCSK9 protein and LDL-C (38). *PCSK9* hepatic transcript expression in mice is substantially reduced by dietary cholesterol (39), which could help explain why we have detected significant QTLs only on low cholesterol diets in baboons.

Genome-wide single-nucleotide polymorphism association studies have been reported recently for various populations, and several have investigated effects on LDL-related measures (40–44). These studies have identified significant associations of LDL-C with polymorphisms in various genes. With respect to chromosome 1, several genes have been implicated for LDL-C [*CELSR2/PSRC1/SORT1* (40, 42, 43) and *PCSK9* (40, 43, 44)] and for TG [*ANGPTL3* (40, 43) and *GALNT2* (43)]. Of these implicated genes, the closest to our QTL cluster is *PCSK9*. Although *PCSK9* is an excellent candidate for the QTL cluster, our bivariate

analyses do not exclude entirely the possibility that several loci on chromosome 1 independently influence LDL-C concentrations. Future research will focus on identifying functional polymorphisms associated with variation in baboon LDL-C.

Cluster 4 included 13 significant and four suggestive QTLs that represented measures of LDL size and concentration properties on the two high-fat diets. Given the number of significant signals and the stringent criteria we imposed for declaring significance, there can be little doubt this cluster reflects a key locus influencing LDL metabolism. The peaks of all but two of the significant QTLs in this cluster mapped within the 9-cM 1-LOD drop interval of the predominant QTL for Lmed-C on HFHC diet. Falling within this interval on the human physical map are 212 genes (23, 34). There are several among these genes that play key roles in lipoprotein metabolism: *SOAT2* (encoding SOAT2, steroyl *O*-acyltransferase 2, which has long been known as acyl-CoA:cholesterol acyltransferase 2 or ACAT2), *APOF* (encoding apoF or lipid transfer inhibitor protein), and *LRP1* (encoding low density lipoprotein-related protein 1 or α2-macroglobulin receptor). LRP1 is ubiquitously expressed and functions in endocytosis and signal transduction (35, 45). In binding and internalizing a number of lipoprotein ligands, LRP1 plays a key role in atherosclerosis (46). ApoF is a lipoprotein constituent that is an inhibitor of cholesteryl ester transfer protein; its presence on lipoproteins modulates cholesteryl ester transfer protein-mediated remodeling between VLDL and both LDL and HDL particles (47, 48). SOAT2 expression is specifically localized to hepatocytes and enterocytes; the enzyme produces cholesteryl esters that are incorporated into apoB-containing lipoproteins secreted by these cell types (49). Studies in humans and other primates suggest that variation in SOAT2 is associated with response to dietary cholesterol and plasma lipid levels (50, 51).

Each of these genes is a plausible biological candidate for QTL Cluster 4. Given that all but one of the 12 traits tested had genetic correlations with Lmed-C on HFHC that were high and not different from one, the data suggest there is only one pleiotropic gene responsible for this cluster. Future research will be directed toward identifying that gene. We also will want to determine whether specific polymorphisms at this locus underlie variation in human LDL phenotypes. However, it may be difficult to extrapolate the present findings to free-living human populations, given that our ability to detect this gene in baboons was no doubt due, at least in part, to our ability to precisely control diet.

In summary, we have adopted a novel approach to identify highly influential QTLs for lipoprotein metabolism using data from a large baboon pedigree. Our results identified five major QTL clusters in the baboon genome. Three of the clusters confirmed previously reported signals, but two of the QTL clusters were novel and had not been reported previously in baboons. The novel clusters reflected genes influencing various aspects of LDL metabolism and they localized to genomic regions in which plausible candidate genes for lipoprotein metabolism are found in humans. Future research will focus on identifying the gene(s) responsible for each QTL cluster and, ultimately, identifying the specific polymorphisms in baboons and humans that are responsible for LDL variation.

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