

# Genotype-by-diet effects on co-variation in Lp-PLA<sub>2</sub> activity and LDL-cholesterol concentration in baboons fed an atherogenic diet

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**Abstract** Both lipoprotein-associated phospholipase A<sub>2</sub> (Lp-PLA<sub>2</sub>) activity, a biomarker of inflammation, and concentration of its primary associated lipoprotein, LDL, are correlated with adverse coronary outcomes. We previously reported a quantitative trait locus (QTL) corresponding to HSA2p24.3–p23.2 with pleiotropic effects on Lp-PLA<sub>2</sub> activity and LDL-cholesterol (LDL-C) concentration in baboons fed a basal diet. Here, our goal was to locate pleiotropic QTLs influencing both traits in the same baboons fed a high-cholesterol, high-fat (HCHF) diet, and to assess whether shared genetic effects on these traits differ between diets. We assayed Lp-PLA<sub>2</sub> activity and LDL-C concentration in 683 baboons fed the HCHF diet. We used a bivariate maximum likelihood-based variance components approach in whole-genome linkage screens to locate a QTL [logarithm of odds (LOD) = 3.13, genome-wide *P* = 0.019] corresponding to HSA19q12–q13.2 with pleiotropic effects on Lp-PLA<sub>2</sub> activity and LDL-C levels in the HCHF diet. We additionally found significant evidence of genetic variance in response to diet for Lp-PLA<sub>2</sub> activity (*P* = 0.0017) and for LDL-C concentration (*P* = 0.00001), revealing a contribution of genotype-by-diet interaction to covariation in these two traits. We conclude that the pleiotropic QTLs detected at 2p24.3–p23.2 and 19q12–q13.2 on the basal and HCHF diets, respectively, exert diet-specific effects on covariation in Lp-PLA<sub>2</sub> activity and LDL-C concentration.—Vinson, A., M. C. Mahaney, V. P. Diego, L. A. Cox, J. Rogers, J. L. VandeBerg, and D. L. Rainwater. **Genotype-by-diet effects on co-variation in Lp-PLA<sub>2</sub> activity and LDL-cholesterol concentration in baboons fed an atherogenic diet.** *J. Lipid Res.* 2008. 49: 1295–1302.

**Supplementary key words** lipoprotein-associated phospholipase A<sub>2</sub> • genotype-by-diet interaction • atherosclerosis • pleiotropy • baboon

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Both lipoprotein-associated phospholipase A<sub>2</sub> (Lp-PLA<sub>2</sub>) activity, a biomarker of inflammation, and LDL-cholesterol (LDL-C) concentration are associated with early onset atherosclerosis, endothelial dysfunction, and future cardiac events (1, 2). Additionally, Lp-PLA<sub>2</sub> activity and LDL-C concentration are positively correlated (3, 4), an observation consistent with the knowledge that the major portion of Lp-PLA<sub>2</sub> in the circulation is bound to LDL. The positive relationship between Lp-PLA<sub>2</sub> activity and LDL-C concentration and their association with cardiovascular disease probably reflects the enzymatic action of Lp-PLA<sub>2</sub> on LDL particles, which produces end products with pro-inflammatory activity that may account for many of the atherogenic properties of oxidized LDL (5, 6).

Although studies addressing the effect of genes on Lp-PLA<sub>2</sub> activity and LDL-C concentration are limited, evidence supports a genetic contribution to covariation (i.e., pleiotropy) in both traits. Polymorphism in *PLA2G7* (coding for Lp-PLA<sub>2</sub>) has substantial effects on variation in LDL-C concentration in healthy controls, and on Lp-PLA<sub>2</sub> activity in healthy controls and patients with cardiovascular disease (CVD) (7, 8). Additionally, we recently reported a genetic correlation between Lp-PLA<sub>2</sub> activity and LDL-C concentration in baboons fed a basal diet, and presented evidence for a quantitative trait locus (QTL) on chromosome 2p that influences covariation in these traits (9).

Because Lp-PLA<sub>2</sub> activity is both genetically correlated and physically associated with LDL, and both traits predict risk of CVD, knowledge of genes with pleiotropic effects on the response of these traits to an atherogenic [i.e., high-cholesterol, high-fat (HCHF)] diet would be of considerable value. However, to our knowledge, no studies have searched for genes regulating the response of Lp-PLA<sub>2</sub> activity to dietary challenge. In contrast to Lp-PLA<sub>2</sub>, substantial research has addressed the location of genes that influence the response of LDL-C concentration to

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diets enriched in cholesterol and fat, in both animal models and humans (10, as reviewed in Refs. 11–13). To date, the majority of evidence for genes that influence the response of LDL-C levels to dietary fat and cholesterol supports the involvement of polymorphisms in *LDLR*, which codes for the LDL receptor, or in *APOE* and the *APOA1-APOCIII-APOAIV* gene cluster, which code for lipid transport proteins. Despite these important advances, we still have not identified many genes that regulate the response of LDL and associated Lp-PLA<sub>2</sub> activity to an HCHF diet.

The goal of the current study was to locate QTLs influencing covariation in Lp-PLA<sub>2</sub> activity and LDL-C concentration in an HCHF diet, and to determine whether genetic effects on these traits differ between basal and HCHF diets (genotype-by-diet interaction) in pedigreed baboons, a valuable model for studying the genetics of risk factors for human atherosclerosis.

## MATERIALS AND METHODS

### Animals and dietary protocol

We obtained data for this study from a sample of 683 pedigreed baboons (*Papio hamadryas*) comprising 442 females and 241 males maintained outdoors in social groups at the Southwest National Primate Research Center (SNPRC), located at the Southwest Foundation for Biomedical Research (SFBR) in San Antonio, Texas. The age of baboons in this sample ranged from 2–29 years, corresponding approximately to a human developmental age range of 6–87 years. Animal care personnel and staff veterinarians provided routine and emergency health care to all animals in accordance with the Guide for the Care and Use of Laboratory Animals. The SFBR facility is certified by the Association for Assessment and Accreditation of Laboratory Animal Care International, and all procedures were approved by the Institutional Animal Care and Use Committee.

Baboons sampled in this study were maintained on a commercial monkey diet (basal diet, SWF Primate Diet; Harlan Teklad, Madison, WI), composed of 0.02 mg/g cholesterol and 7% fat from plant oils. The same baboons were then fed an HCHF diet for 7 weeks, composed of 6.37 mg/g cholesterol and 41% fat from lard [a detailed description of both basal and HCHF diets is given in (14)]. Animals had ad libitum access to either basal or HCHF diets, and blood samples were drawn from all baboons before and after the 7-week dietary challenge period.

For the purposes of this study, these baboons were organized into 11 distinct pedigrees, yielding a diverse array of relative pair classes: 650 parent–offspring; 577 sibling; 66 grandparent–grandchild; 81 avuncular; 6,270 half-sibling; 1,668 half-avuncular; 3 first cousin; 40 half-first cousin; 5 half-first cousin, once removed; 21 half-sibling and first cousin; 520 half-sibling and half-first cousin; 7 half-sibling and half-avuncular; and 31 double half-avuncular. These 11 baboon pedigrees may be viewed at the SNPRC website: <http://www.snprc.org/baboon/map/BPPpedigrees/bppeds.htm>.

### Phenotyping

Lp-PLA<sub>2</sub> activity and LDL-C concentration were assayed in serum samples, obtained as part of an ongoing study of the effects of diet and genotype on variation in atherosclerosis risk factors. Blood samples were taken from the femoral vein of overnight-fasted baboons sedated with ketamine. Serum was separated from whole blood by low-speed centrifugation and

stored in individual, single-use aliquots at  $-80^{\circ}\text{C}$ , protected from oxidation and desiccation (15).

Serum Lp-PLA<sub>2</sub> enzyme activity was measured at  $30^{\circ}\text{C}$  using a kit provided by Cayman Chemical Co. (Ann Arbor, MI). Hydrolysis of the substrate, 2-thio platelet-activating factor, produced a free thiol that was quantified using 5,5'-dithio-bis-(2-nitrobenzoic acid). The reaction was monitored at 405 nm using a BioTek (Winooski, VT) ELx808 microplate reader running in kinetic data acquisition mode. Rates were calculated from at least 15 min of readings in the linear phase and converted to nmol/min/ml plasma using an extinction coefficient value of 13.6/mM-cm. The between-assay coefficient of variation, based on a control sample run on each plate, was 4.3%.

Cholesterol concentrations were measured enzymatically (16) with a reagent supplied by Boehringer Mannheim Diagnostics (Indianapolis, IN) and using a Ciba-Corning (Norwood, MA) Express Plus clinical chemistry analyzer. Cholesterol carried by HDL was estimated following precipitation of apolipoprotein B (apoB)-containing lipoproteins with heparin-Mn<sup>2+</sup> as described (17), and LDL-C was calculated as the difference between total and HDL-C [in baboons, the major proportion of apoB-associated cholesterol is found on particles in the LDL size/density range (18)]. Between-assay coefficients of variation for these determinations were 2.2% and 4.6% for total and HDL-C, respectively.

### Baboon genotyping and whole-genome linkage map

Statistical genetic analyses of these two traits took advantage of a baboon whole-genome linkage map based on genotype data at nearly 300 microsatellite marker loci (mean intermarker interval = 8.9 cM) from 984 pedigreed baboons in these same 11 extended pedigrees. The physical locations in the human genome for nearly all marker loci in the baboon map are known, thus facilitating the identification of orthologous chromosomal regions in the two species. Construction of the current baboon linkage map is described in detail elsewhere (19), and additional information can be found at the SNPRC website: <http://www.snprc.org/baboon/genome/index.html>.

### Statistical genetic methods

All statistical genetic analyses were conducted using a maximum likelihood-based variance decomposition approach implemented in the computer package SOLAR (Sequential Oligogenic Linkage Analysis Routines) (20). We used this approach to partition the phenotypic variance in each trait ( $\sigma_p^2$ ) into components corresponding to additive genetic effects ( $\sigma_G^2$ ), estimated as a function of relatedness among pedigreed baboons, and environmental effects ( $\sigma_E^2$ ). We define heritability ( $h^2$ ) as the proportion of residual phenotypic variance unexplained by covariates that can be attributed to additive genetic effects ( $h^2 = \frac{\sigma_G^2}{\sigma_p^2}$ ).

Accounting for random and measured environmental contributions to the phenotypic variance can improve power to detect genetic effects. After regressing out nominally significant mean effects of age, sex, age<sup>2</sup>, age<sup>2</sup>×sex, and age<sup>2</sup>×sex, we applied an inverse Gaussian transformation to the residuals to correct for departures from multivariate normality that might inflate evidence for linkage (21). This transformation results in standardized traits with means and standard deviations approaching 0 and 1, respectively. All polygenic and linkage analyses were conducted using these normalized residual data.

*The bivariate polygenic model.* To determine the extent to which phenotypic variation in serum Lp-PLA<sub>2</sub> activity and LDL-C concentration may be affected by shared genes and shared non-

genetic factors, we conducted bivariate analyses in which both traits are considered simultaneously. In addition to the heritability for each trait, the bivariate polygenic model also estimates the additive genetic and environmental correlations between both traits. The genetic correlation ( $\rho_G$ ) is an estimate of pleiotropy between the two traits, and  $\rho_G^2$  thus estimates the portion of the additive genetic variance in each trait due to shared genetic effects. The random environmental correlation ( $\rho_E$ ) is an estimate of the shared effects of nonadditive genetic factors and unmeasured environmental variables. Using these estimates, we calculated the phenotypic correlation between the trait pair as  $\rho_P = \rho_G \sqrt{h_1^2} \sqrt{h_2^2} + \rho_E \sqrt{1 - h_1^2} \sqrt{1 - h_2^2}$  (22). We assessed the significance of  $\rho_G$  and  $\rho_E$  by means of likelihood ratio tests comparing the likelihoods of models in which the correlation was estimated to those in which it was constrained to zero (rejection of  $\rho_G = 0$  indicates pleiotropy) or to 1 (failure to reject  $|\rho_G| = 1$  indicates complete pleiotropy).

**The bivariate linkage model.** To search for pleiotropic QTLs affecting phenotypic variation in both Lp-PLA<sub>2</sub> activity and LDL-C concentration, we conducted bivariate multipoint linkage analyses using an extension to the bivariate polygenic model in which we modeled the phenotypic covariance among relatives as the sum, for each trait, of the additive genetic covariance attributable to a specified marker locus, the additive genetic covariance due to the effects of other loci, and the covariance due to unmeasured environmental factors. This extension allows estimation of the proportion of the residual variance in each of  $i$  traits that is attributable to the effects of a QTL as  $h_{Qi}^2 = \sigma_{Q_i}^2 / \sigma_{P_i}^2$ , where  $\sigma_{Q_i}^2$  and  $\sigma_{P_i}^2$ , respectively, are the QTL-specific additive genetic variance and the residual phenotypic variance for trait  $i$ . The hypothesis of linkage is supported when  $\sigma_{Q_i}^2$  is significantly greater than zero (20). In addition to the same parameters as are estimated in the bivariate polygenic model, the additive genetic correlation between the traits due to the effects of the QTL ( $\rho_Q$ ) is also estimated in the bivariate linkage model.

Our linkage analyses incorporate identity-by-descent (IBD) allele sharing estimated from genotype data at the microsatellite markers in the baboon linkage map. For marker-locus specific linkage analyses (e.g., markers within candidate genes), we estimated IBD probabilities for the pedigrees using a pair-wise maximum likelihood-based procedure (23). To facilitate our multipoint, whole-genome QTL searches we estimated multipoint probabilities of IBD among relatives throughout the baboon linkage map using Markov Chain Monte Carlo routines implemented in the computer package Loki (24). We tested linkage hypotheses at 1 cM intervals along each chromosome using likelihood ratio tests, and converted the resulting likelihood ratio statistic to the logarithm of odds (LOD) score of classic linkage analysis (25). Bivariate LOD scores are adjusted to be equivalent to univariate LOD scores in terms of degrees of freedom (26).

To control for the genome-wide false-positive rate, we calculated genome-wide  $P$  values for each LOD score using a modification of a method suggested by Feingold, Brown, and Siegmund (27) that takes into account pedigree complexity and the finite marker density of the linkage map. Accordingly, our threshold for significant evidence of linkage (corresponding to genome-wide  $\alpha = 0.05$ ) was LOD = 2.69, and that for suggestive evidence of linkage was LOD = 1.46.

**Co-incident linkage versus pleiotropy at the QTL.** In a bivariate linkage analysis, QTLs may be found that appear to influence phenotypic variation in both traits. To distinguish the event where two traits may each be independently influenced by closely linked

genes ("co-incident linkage") from QTL pleiotropy, we conducted a likelihood ratio test of the hypothesis  $\rho_Q = 0$ . In accordance with Almasy, Dyer, and Blangero (28), failure to reject this hypothesis supports the coincident linkage of two QTLs over pleiotropic effects at the same locus.

**Detecting genotype-by-diet interaction.** Genotype-by-diet interaction occurs when there is a significant additive genetic component to the variance in response to dietary environment (29). The additive genetic variance in a phenotypic response to diet is a function of both the trait's additive genetic variance in the two diets and the additive genetic correlation between measures of that trait in the two diets. Accordingly, to assess the contribution of genotype-by-diet interaction to Lp-PLA<sub>2</sub> activity and LDL-C concentration, we tested the null hypotheses of equal additive genetic variance (i.e.,  $\sigma_{G1}^2 = \sigma_{G2}^2$ ) and complete additive genetic correlation (i.e.,  $\rho_G = 1$ ) for each trait between the two diets (30). The rejection of either null hypothesis is indicative of genotype-by-diet interaction.

To test the null hypothesis of equal additive genetic variance between the basal and the HCHF diets for each trait, we employed a Wald test, using estimates of the additive genetic variances computed directly as the product of the heritability and the phenotypic variance (i.e., the square of the phenotypic standard deviation), and using standard formulae to calculate exact sampling variances and covariances of parameter estimates for the additive genetic variances (31–33). To test the null hypothesis of complete additive genetic correlation between the two diets for each trait, we performed tests of significant deviation from  $\rho_G = 1$  between the basal and the HCHF diets using likelihood ratio tests wherein the likelihood of the null hypothesis constraining the additive genetic correlation to 1 (i.e., complete association) is compared with the likelihood of the estimated additive genetic correlation.

## RESULTS

### Sample sizes and distribution of Lp-PLA<sub>2</sub> activity and LDL-C concentration measured in basal and HCHF diets

Sample sizes and summary statistics describing the distribution of raw data for Lp-PLA<sub>2</sub> activity and LDL-C concentration measured in both the basal and HCHF diets are provided in **Table 1**. All summary statistics for Lp-PLA<sub>2</sub> activity and LDL-C concentration increased from the basal to the HCHF diet. Mean Lp-PLA<sub>2</sub> activity increased ~26%, whereas mean LDL-C concentration increased 101% from

TABLE 1. Sample sizes and distributions of Lp-PLA<sub>2</sub> activity (nmol/min/ml) and LDL cholesterol concentration (mmol/L) within and between basal and HCHF diets

	Basal Diet		HCHF Diet	
	Lp-PLA <sub>2</sub>	LDL-C	Lp-PLA <sub>2</sub>	LDL-C
N	657	679	660	683
Mean	6.09	1.07	7.66	2.15
Median	5.88	1.03	7.19	2.02
Variance	2.03	0.19	5.75	0.99
Variance/Mean	0.33	0.18	0.75	0.46
Min value	2.82	0.00	2.84	0.27
Max value	11.67	3.13	21.29	6.31

HCHF, high-cholesterol, high-fat; LDL-C, LDL-cholesterol; Lp-PLA<sub>2</sub>, lipoprotein-associated phospholipase A<sub>2</sub>.



the basal to the HCHF diet. As a proportion of the mean, the variance increased  $\sim 127\%$  and  $\sim 156\%$  from the basal to the HCHF diet, for Lp-PLA<sub>2</sub> activity and LDL-C concentration, respectively. These initial findings suggested a possible contribution of genotype-by-diet interaction to both traits, hypotheses that were addressed formally in analyses described below.

### Bivariate analyses of Lp-PLA<sub>2</sub> activity and LDL-C concentration

Because both Lp-PLA<sub>2</sub> activity and LDL-C concentration showed evidence of pleiotropy in baboons on a basal diet, we conducted bivariate analyses to characterize shared genetic effects in baboons on an HCHF diet. The results of the bivariate polygenic analysis for these traits are summarized in **Table 2**. Heritability estimates indicate that substantial additive genetic effects contribute to the residual phenotypic variance in both Lp-PLA<sub>2</sub> activity and LDL-C levels. The estimated additive genetic correlation between Lp-PLA<sub>2</sub> activity and LDL-C concentration indicates that both traits are influenced by substantial shared additive genetic effects, and that almost half of the additive genetic variance in each of the two traits is due to the effects of the same gene or genes. The genetic effects common to both traits account for approximately 30% and 29% of the residual phenotypic variance in Lp-PLA<sub>2</sub> activity and LDL-C concentration, respectively. The magnitude of these shared genetic effects accounted for much of the residual phenotypic variance shared between Lp-PLA<sub>2</sub> activity and LDL-C concentration measured in a high-fat diet (i.e.,  $\rho_P^2 = 0.43$ ). However, genetic effects on phenotypic variation that are not shared by both traits also exist, because the hypothesis of complete pleiotropy between these two traits,  $\rho_G = 1$ , was also rejected.

Whole-genome bivariate multipoint linkage analysis of Lp-PLA<sub>2</sub> activity and LDL-C concentration (summarized in **Table 3**) found significant evidence for a pleiotropic QTL on the baboon (*Papio hamadryas*) equivalent of human chromosome 19 (PHA19) (plotted in **Fig. 1**). With very similar QTL-specific heritability estimates (i.e., 0.19 and 0.21), this pleiotropic QTL accounts for approximately 20% of the residual phenotypic variance in Lp-PLA<sub>2</sub> activity and LDL-C levels. The estimated additive genetic correlation between Lp-PLA<sub>2</sub> activity and LDL-C concentration at the QTL suggests that all additive genetic

TABLE 2. Bivariate polygenic analysis of variation in Lp-PLA<sub>2</sub> activity and LDL-C concentration in pedigreed baboons on HCHF diet

Parameter	Lp-PLA <sub>2</sub>	LDL-C
$h^2$	0.65 (0.07) <sup>a</sup>	0.61 (0.07) <sup>a</sup>
$\rho_G$		0.69 (0.06)
$P[\rho_G = 0]$		$1.05 \times 10^{-12}$
$P[\rho_G = 1]$		$3.86 \times 10^{-18}$
$\rho_G^2$		0.47
$\rho_E$		0.60 (0.07)
$\rho_P$		0.65

$h^2$ , heritability. Maximum likelihood parameter estimates. Parentheses enclose SEM.

<sup>a</sup>  $P < 0.0000001$ .

TABLE 3. A pleiotropic QTL on baboon chromosome 19 (PHA19) influencing variation in Lp-PLA<sub>2</sub> activity and LDL-C concentration in pedigreed baboons on HCHF diet

	Lp-PLA <sub>2</sub> , LDL-C
LOD	3.13
Genome-wide $P$ value	0.019
Baboon chromosome (PHA)	19
Location in baboon linkage map (cM from pter-most marker locus)	40
QTL-specific heritability (Lp-PLA <sub>2</sub> , LDL-C)	0.19, 0.21
$\rho_Q$	0.99
$P[\rho_Q = 0]$	0.0000462
$P[\rho_Q = 1]$	0.44
Orthologous human chromosome (HSA)	19
Marker loci used to interpolate support interval for QTL	D19S226, DM
Region in humans corresponding to QTL support interval	19q12–q13.2
Region in humans corresponding to maximum LOD in baboons	19q12–q13.12

LOD, logarithm of odds; QTL, quantitative trait locus. Summary of bivariate linkage analysis results.

effects at this QTL are shared between the two traits (i.e., the QTL exerts complete pleiotropy on both traits).

We defined a comprehensive support interval surrounding the QTL as the interval bounded by the locations on the baboon genetic map at which the LOD score was one LOD unit lower than the peak LOD score ("1-LOD drop" method) (34). We then used the markers flanking this support interval to ascertain the corresponding region of interest in the human genome, assuming a proportional relationship between genetic and physical distance between these markers. Thus, the region likely to harbor the QTL in baboons corresponds to an approximately 21 Mb interval in the human genome mapping to 19q12–q13.2 and containing 317 known RefSeq genes. A narrower, 10 Mb interval (19q12–q13.12) centered on the similarly interpolated location of the maximum LOD score harbors

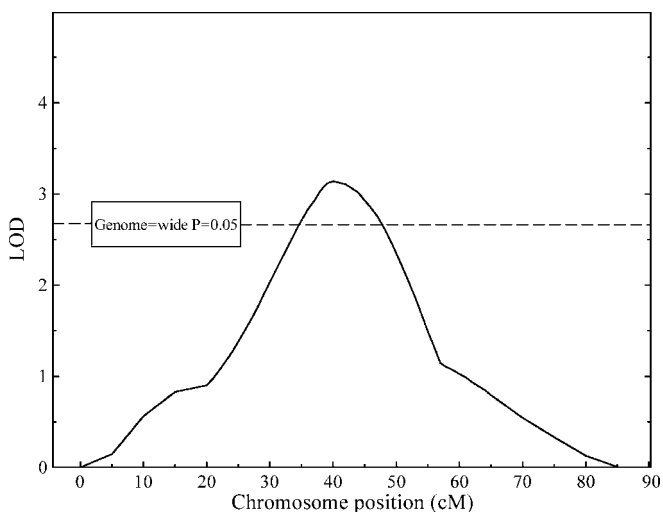


Fig. 1. Bivariate multipoint linkage results [logarithm of odds (LOD) = 3.13; genome-wide  $P = 0.019$ ] for the combined phenotype lipoprotein-associated phospholipase A<sub>2</sub> activity/LDL-cholesterol concentration on PHA19, the baboon ortholog of human chromosome 19.

142 RefSeq genes, including the lipolysis-stimulated protein receptor (*LSR/LISCH7*), three free-fatty acid receptors (*GPR40/FFAR1*, *GPR41/FFAR3*, and *GPR43/FFAR2*), and the LDL receptor-related protein 3 (*LRP3*).

### Tests of genotype-by-diet interaction for Lp-PLA<sub>2</sub> activity and LDL-C concentration

To evaluate whether genes mediate the response of Lp-PLA<sub>2</sub> activity and LDL-C concentration to an HCHF diet, we tested for the presence of additive genetic variance in response to dietary environment for both traits. For each trait, we tested the null hypotheses  $\sigma_{G(BASAL)}^2 = \sigma_{G(HCHF)}^2$  and  $\rho_{G(BASAL, HCHF)} = 1$  (results summarized in **Table 4**). The estimate of  $\rho_G$  between the basal and HCHF diet for each trait indicates that most, but not all, total additive genetic effects were shared between both diets. Equality of additive genetic variances estimated in both the basal and the HCHF diet for each trait could not be rejected; however, tests of the null hypothesis of  $\rho_G = 1$  between the basal and the HCHF diet for both Lp-PLA<sub>2</sub> activity and LDL-C concentration did reject complete sharing of the additive genetic variance between diets for each of these two traits, thus demonstrating genotype-by-diet interaction for both traits.

### Posterior analyses of effects of *LDLR* and *APOE* on variation in Lp-PLA<sub>2</sub> activity and LDL-C concentration

Although they lie outside the support interval for this QTL, because substantial research has identified their effects on variation in circulating LDL-C concentration and on subsequent CVD risk, we conducted two additional series of analyses to assess the possible contributions of the genes encoding the LDL receptor (*LDLR*) and apo-E (*APOE*) to variation in Lp-PLA<sub>2</sub> activity and LDL-C concentration. First, we performed measured genotype analyses (35) using restriction fragment-length polymorphism (RFLP) data in *LDLR* and *APOE* that were available for a subset of these same animals ( $n \approx 400$ ) (36, 37). In these analyses, we included additive and dominance effects of an *Ava*II polymorphism in *LDLR* and a *Ban*II polymorphism in *APOE* in our genetic models for each of the two traits. These analyses detected significant additive genetic effects of *LDLR* on variation in both Lp-PLA<sub>2</sub> activity ( $P = 0.0006$ ,  $N = 390$ ) and LDL-C concentration ( $P = 0.0020$ ,  $N = 405$ ). However, the estimated effect of *LDLR* on genetic variance in both traits was small, accounting for 6% or less of the additive genetic variance in each trait. Our

measured genotype analyses detected no significant evidence for an effect of *APOE* on additive genetic variance in Lp-PLA<sub>2</sub> activity or LDL-C concentration. Second, using marker-locus-specific estimates of IBD (based on genotype data at these same RFLP sites) for all animals with phenotype data, we conducted bivariate linkage analyses to assess whether either of these two genes contributed to the effects of a pleiotropic QTL. Our analyses yielded no significant evidence for linkage between either *LDLR* or *APOE* and a QTL influencing variation in the Lp-PLA<sub>2</sub>/LDL-C bivariate phenotype ( $\text{LOD} \leq 0.19$ ,  $P \geq 0.35$ ).

## DISCUSSION

The simultaneous increases observed in Lp-PLA<sub>2</sub> activity and LDL-C concentration in baboons fed an HCHF diet is consistent with reports by others of associations between both traits (3, 4, 7), and with what is known of the functional biological relationship between Lp-PLA<sub>2</sub> and the LDL particle. Diet-induced increases in LDL-C concentration have been reported previously (38, 39) and the effects of increased LDL-C concentration on risk of CVD are well known (40). However, to our knowledge, this is the first report of a parallel increase in Lp-PLA<sub>2</sub> activity, a biomarker of inflammation independently associated with increased CVD risk, in response to dietary challenge. Although details of the mechanism underlying the relationship between increased LDL-C levels and Lp-PLA<sub>2</sub> activity are poorly understood, the parallel increase in both traits observed in baboons fed an HCHF diet suggests that the same or similar diet is likely to increase measures of both biomarkers in humans, and further exacerbate risk of CVD.

A substantial proportion of the variation in Lp-PLA<sub>2</sub> activity and LDL-C concentration in baboons fed an HCHF diet may be attributed to the additive effects of genes, and some of these genes exert pleiotropic effects on variation in both traits. This finding is consistent with the results of our previously reported analyses from these same animals fed a basal diet containing much lower amounts of fat and cholesterol. In both studies, shared genetic effects, indicative of pleiotropy, account for substantial portions of the total additive genetic variance. However, in the present study, pleiotropic effects account for nearly twice the additive genetic variance as they did in the earlier study, i.e., approximately 47% versus 25%, respectively.

Detection of a pleiotropic QTL influencing Lp-PLA<sub>2</sub> activity and LDL-C concentration is also consistent with the results of our earlier study in these same baboons; however, several characteristics distinguish the two QTLs. It is clear that the gene(s) underlying the QTLs detected in the two different dietary environments are different, because the QTL detected in analyses of baboons fed the HCHF diet maps to a baboon chromosomal region corresponding to HSA19q12–q13.2, whereas the QTL detected in baboons fed a basal diet maps to a baboon chromosomal region corresponding to HSA2p24.3–p23.2. Fur-

TABLE 4. Shared genetic effects between basal and HCHF diets and evidence for genotype-by-diet interaction affecting Lp-PLA<sub>2</sub> activity and LDL-C concentration in pedigreed baboons

	Lp-PLA <sub>2</sub>	LDL-C
$\rho_G$ (between basal and HCHF diets)	0.94 (0.03)	0.86 (0.05)
$\rho_G^2$ (between basal and HCHF diets)	0.89	0.73
$P[\rho_G = 1]$	0.0017	0.00001
$P[\rho_G = 0]$	$2.31 \times 10^{-25}$	$4.42 \times 10^{-19}$
$P[\sigma_{G(BASAL)}^2 = \sigma_{G(HCHF)}^2]$	NS	NS

Maximum likelihood parameter estimates. Parentheses enclose SEM.

ther, the QTL detected in the HCHF diet accounts for approximately 20% of the residual phenotypic variance in both traits, and all additive genetic effects at this QTL are shared between the two traits. In contrast, the QTL detected in the basal diet (9) had a smaller effect, i.e., it accounted for approximately 15% of the residual phenotypic variance in both traits, and additive genetic effects at the QTL were only partially shared between the two traits. These findings suggest the possibility that under HCHF dietary stress, Lp-PLA<sub>2</sub> activity and LDL-C levels may be more closely regulated by a gene or genes with greater phenotypic effects than gene(s) regulating both traits in a less stressful dietary environment.

In conjunction with the results of our previous study of covariation in Lp-PLA<sub>2</sub> activity and LDL-C concentration in baboons fed a basal diet, the results of this study of the same traits in baboons fed an HCHF diet provide compelling evidence for genotype-by-diet interaction in determining covariation in these two CVD risk factors. A contribution of genotype-by-diet interaction to variation in both traits may be inferred from the observation that the phenotypic variances observed for both traits differ substantially between diets, whereas the magnitude of additive genetic effects does not change between diets. However, genotype-by-diet interaction is demonstrated conclusively by the rejection of the statistical null hypothesis (and its associated biological null hypothesis of no genotype-by-diet interaction) that the residual additive genetic correlation between the same trait measured in two dietary environments equals 1. Further, the extent of the genotype-by-diet interaction effect on each trait may be inferred by examining the proportion of the additive genetic variance that is shared between measures of the trait (i.e., the squared genetic correlation) in the basal and HCHF diets. A logical conclusion drawn from finding genotype-by-diet interaction effects on both traits is that there are diet-specific additive genetic components to the variance in both Lp-PLA<sub>2</sub> activity and LDL-C concentration. This result suggests a situation in which different genes or suites of genes contribute to variation in both traits, or, alternatively, the relative contributions of the genes involved may change as a function of exposure to increased dietary cholesterol and fat. A scenario in which the additive genetic variance in both traits is relatively stable irrespective of dietary fat and cholesterol, whereas different genes may contribute (or contribute differentially) to this variance as a function of environmental exposure, is supported further by our localization of pleiotropic QTLs for Lp-PLA<sub>2</sub> and LDL-C to two different chromosomes. We conclude from these results that the QTL that mapped to chromosome 2p24.3–p23.2 in our previous study, and the QTL that mapped to chromosome 19q12–13.2 in this study, harbor genes that exert effects specific to the basal and HCHF diets, respectively.

In contrast to the QTL implicated on HSA2p that exerted incomplete pleiotropic effects on both traits in a basal diet (9), our results suggest that the QTL on HSA19q implicated in this study may exert complete pleiotropy on both Lp-PLA<sub>2</sub> activity and LDL-C levels in an HCHF diet.

This result is consistent with a single gene or polymorphism at this QTL with fully coordinated effects on both traits, and suggests that the gene or polymorphism detected here is part of a biological pathway that includes Lp-PLA<sub>2</sub> and LDL in close proximity. One biological pathway that is consistent with the complete pleiotropy at the QTL on HSA19q is the LDL catabolic pathway, because LDL and Lp-PLA<sub>2</sub> are associated at the point of LDL clearance. Genes lying at the 10 Mb region in humans that encompasses the point of maximum linkage evidence in baboons (HSA19q12–q13.12) include at least one with potential effects on LDL catabolism. *LSR/LISCH7*, the lipolysis-stimulated lipoprotein receptor, codes for a receptor that is differentially stimulated by free fatty acids of different chain lengths and is able to bind apoB- and apoE-containing lipoproteins (41). Furthermore, LSR is hypothesized to be an alternate pathway of LDL clearance in the liver separate from the LDLR or the LDL-receptor related protein (LRP) pathways (42). Other potentially relevant genes at HSA19q12–q13.12 include three members of the G-protein-coupled receptor family, *GPR40*, *GPR41*, and *GPR43*, also known as fatty-acid receptors *FFAR1*, *FFAR3*, and *FFAR2*, respectively. *GPR40*, *GPR41*, and *GPR43* code for proteins known to be differentially activated by medium- and long-chain fatty acids (*GPR40*) (43) and short-chain fatty acids (*GPR41* and *GPR43*) (44, 45). In particular, *GPR40* is stimulated by ligands that are most prevalent in plasma, i.e., palmitate, oleate, stearate, linoleate, and linolenate, all components with proportions that differ significantly between the basal and the HCHF diet. Finally, *LRP3*, a member of the LDLR family, is also found in this region, although its function in lipoprotein metabolism, if any, is unknown (46). Interestingly, in this study, we did not detect an effect of the structural gene coding for Lp-PLA<sub>2</sub> (*PLA2G7*, located at 6p21.2–p12) on either trait in either diet. Although other studies have detected effects of this gene on Lp-PLA<sub>2</sub> activity and CVD risk (7, 8), *PLA2G7* does not appear to be the primary regulator of its own enzymatic activity in this population under either dietary regimen.

Two well-studied genes on chromosome 19, *APOE* and *LDLR*, exert significant effects on LDL-C concentration in humans, and have been shown to influence risk of CVD in multiple studies (as reviewed in Ref. 12). Additionally, recent genome-wide association analyses have detected significant association of single-nucleotide polymorphisms in these two genes with LDL-C and coronary artery disease (47, 48). However, the fact that polymorphisms in neither gene were linked to a QTL influencing the bivariate phenotype, coupled with the fact that both genes map outside the estimated support interval for our QTL, leads us to conclude that neither *LDLR* nor *APOE* are responsible for the pleiotropic QTL that we have detected on the baboon ortholog of human chromosome 19. This conclusion does not exclude these genes as contributors to covariation in these traits in baboons or, by extension, humans; indeed, our observation that polymorphism in baboon *LDLR* is significantly associated with variation in both LDL-C concentration and Lp-PLA<sub>2</sub> activity supports such a contribu-



tion. However, in the HCHF dietary environment to which these baboons have been exposed, the effect of polymorphism in *LDLR* on covariation in these two traits is relatively small, compared with that of the pleiotropic QTL, and contributes primarily to the additive genetic (polygenic) background. That we detected no similar contribution of polymorphism in *APOE* to the additive genetic background in these two traits may be attributable to the fact that baboons (and other cercopithecoid primates) appear to be monomorphic for the specific *APOE* allele associated with variation in lipoprotein metabolism and CVD risk in humans (49–51). This result may also be due to the possibility that the *APOE* polymorphisms analyzed in this study are either not functional or not in linkage disequilibrium with functional polymorphisms that may contribute to variation in LDL-C concentration and/or Lp-PLA<sub>2</sub> activity in these baboons. Nevertheless, neither our linkage nor association analyses provide any evidence that either *APOE* or *LDLR* is responsible for the effect of the pleiotropic QTL localized in this study.

Localizing QTLs with effects on Lp-PLA<sub>2</sub> activity and LDL-C concentration is an efficient first step toward identifying a set of positional candidate genes for further analysis. In this study, we have described a QTL, detected in the HCHF diet and corresponding to 19q12–q13.2, that appears to exert complete pleiotropy on covariation in Lp-PLA<sub>2</sub> activity and LDL-C concentration in a nonhuman primate model for the genetics of atherosclerosis risk factors. To our knowledge, this is the first report of a QTL affecting both Lp-PLA<sub>2</sub> activity and LDL-C concentration detected in an HCHF dietary environment. We have also demonstrated the contribution of genotype-by-diet interaction to covariation in Lp-PLA<sub>2</sub> activity and LDL-C concentration measured in both basal and HCHF dietary environments. Knowledge of the genes regulating both traits under the controlled dietary conditions possible with a nonhuman primate model with close genetic and physiological similarity to humans will ultimately improve our understanding of how Lp-PLA<sub>2</sub> activity and LDL-C concentration contribute to increased risk of human cardiovascular disease. ■

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