Determinants of variation in serum paraoxonase enzyme activity in baboons

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Abstract **Paraoxonase (PON), an HDL-associated enzyme, is one of many circulating antioxidants thought to play a vital protective role. To better understand the determinants of quantitative variation in serum PON activity, we assayed PON in samples from 611 pedigreed baboons fed three diets. PON was measured enzymatically; the main determinant of variation was genetic and consisted of at least three components: two loci detected by linkage analyses and a residual polygenic component. Multipoint linkage analyses gave peak log of the odds (LOD) scores on the baboon homolog of human chromosome 7q21-22 (near *PON1*, the structural gene) of 9.1 on the low-cholesterol, high-fat diet and 4.1 on the high-cholesterol, high-fat diet (genome-wide *P* values were 1×10^{-8} and 0.0018, respectively). Surprisingly, a second locus on the baboon homolog of human chromosome 12q13 gave a LOD score of 2.9 on the high-cholesterol, high-fat diet (genome-wide *P* value was 0.032). We identified several significant covariates, including age, sex, diet, and apolipoprotein A-I concentrations. We estimate that 53% of total trait variation in baboons is explained by genes and 17% by covariates, thus accounting for ~70% of total variation in baboon PON.** **Although the generation of free radicals is influenced primarily by environmental factors, our findings suggest strong genetic regulation of one component in the antioxidant defense system that plays a major role in susceptibility to atherosclerosis.**—Rainwater, D. L., M. C. Mahaney, X. L. Wang, J. Rogers, L. A. Cox, and J. L. VandeBerg. **Determinants of variation in serum paraoxonase enzyme activity in baboons.** ***J. Lipid Res.*** **2005.** 46: **1450–1456.**

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Aerobic organisms, including humans, derive most of their metabolic energy from the reduction of oxygen and, consequently, are susceptible to the damaging effects of small amounts of O_2^-, OH, H_2O_2, and their unstable intermediates, which can react with nearly all biological macromolecules. Protection against oxidative damage has developed as a complex and poorly understood assembly of biomolecules and enzyme activities. However, protection is incomplete and variable among individuals. Oxidative damage has been invoked as a major causal factor in a number of degenerative disorders, including atherosclerosis (1, 2), diabetes (3, 4), neurological damage (5), and carcinogenesis (6) as well as in the process of aging (7).

Recently, our group has begun to characterize the components of the antioxidant system that play a role in the process of atherosclerosis. A large number of proteins that have antioxidant activity are known to be associated with lipoproteins and are hypothesized to play key roles in protection from lipid oxidation and its consequences (8). Among these, paraoxonase (PON; aryldialkylphosphatase, EC 3.1.8.1) is a well-known enzyme associated specifically with HDL particles. Although, the actual physiological role of PON is controversial, several studies have demonstrated that it is capable of preventing and reversing LDL oxidation in vitro. It is speculated that the presence of PON on HDLs accounts in large part for the cardioprotective benefit of having high levels of HDL (9).

Variation in the gene encoding PON (*PON1*) has been well studied in several human populations. Most consistently, the Q192R polymorphism is associated with major differences in enzyme activity, with the 192R form having higher and the 192Q form having lower PON activity. Other mutations in human *PON1*, such as M55L and C−108T, also are associated with variation in enzyme activity levels (10).

Baboons are a well studied model of human atherosclerosis and, in particular, of the genetic and dietary effects on risk factors for cardiovascular disease (11). In the present study, we have investigated the determinants of variation in serum PON activity in pedigreed baboons fed diets differing in levels of fat and cholesterol. We deter-

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mined that PON activity levels are strongly heritable, and we obtained evidence from a genome screen for at least two distinct genes that influence baboon PON activity.

MATERIALS AND METHODS

Animals and diet protocol

Data were analyzed for 611 pedigreed baboons (225 males and 386 females) that averaged 9.6 years of age (SD = 6.7) and 17.9 kg in weight (SD = 8.4). Baboons were subjected to an experimental dietary challenge protocol described previously (12). Briefly, baboons were bled while consuming a basal diet low in fat and cholesterol (basal diet sample). These same baboons were then fed each of two high-fat diets for 7 weeks before obtaining blood samples. The diets were low-cholesterol, high-fat (LCHF) and high-cholesterol, high-fat (HCHF); the two diets were separated by a 7 week washout period on the basal diet. Blood samples were taken from the femoral arteries of baboons that had been fasted overnight and immobilized with ketamine (10 mg/kg) for an average of 10 min before bleeding (range, 5–15 min). Serum was prepared by low-speed centrifugation and frozen at −80°C in small aliquots protected from oxidation and desiccation (13). Sample aliquots were stored frozen until assayed and thus were subjected to a single freeze-thaw cycle. Animals were maintained at the Southwest Foundation for Biomedical Research, a facility certified by the Association for Assessment and Accreditation of Laboratory Animal Care International, and experimental procedures were approved by the Institutional Animal Care and Use Committee.

PON activity assay

PON activity was measured at 30°C by adding serum to 100 mM Tris-HCl, pH 8.5, containing 2 mM CaCl and 3 mM paraoxon (Aldrich Chemical Co.), based on previously published protocols (14, 15). The production of *p*-nitrophenol was monitored at 405 nm using a Biotek ELx808 microplate reader running in kinetic data acquisition mode, as suggested previously (16). Rates were calculated from 15 min of readings (16 absorbance readings) in the linear phase and converted to micromoles per minute per liter of serum using an extinction coefficient value of 18.05 mM^{-1} cm^{-1}; units are given as micromoles per minute per liter of serum. Each sample was run in duplicate wells, and the average value was used. The average coefficient of variation for duplicates was 1.3% (n = 2,266 samples); the across-plate coefficient of variation, based on a control sample run on each plate, was 7.0% (n = 50 plates).

A concern with measurements of activity is the stability of the enzyme. To assess stability, we compared enzyme activity levels in two aliquots from each of eight serum samples; one aliquot was freshly thawed, and the other was left at room temperature for 30 h before assay. We found an average 3% decrease in enzyme activity (from 18.5 to 18.0 μmol/min/l) with incubation at room temperature; this decrease was not significant by ANOVA.

Baboon genotypes

Microsatellite markers were amplified from baboon genomic DNA by PCR. Amplification reactions included 2 units of TaKaRa Taq Polymerase (Shiga, Japan), 1× TaKaRa buffer, 0.33 mM deoxynucleotide triphosphate mix, 1 μM forward and reverse primers, and 50 ng of baboon genomic DNA. PCR was performed with an initial denaturing step of 5 min at 94°C, 35 cycles of 40 s denaturation at 94°C and 30 s extension at 72°C, and a final 5 min extension at 72°C. $MgCl_2$ concentrations and annealing temperatures were optimized for each reaction. Genotypes were determined by gel electrophoresis of fluorescently labeled PCR products in ABI 377 automated sequencers with Gene Scan software and analyzed using Genotyper software (Perkin-Elmer).

Data from nuclear families were used within the laboratories to identify for retyping of genotypes inconsistent with Mendelian inheritance. Genotype data were then further checked, using Markov-Chain Monte Carlo (MCMC) and descent path analysis algorithms implemented in the program SimWalk2 (17), to identify and blank two classes of genotypes: those inconsistent with Mendelian inheritance when data from the full pedigrees were considered and those that appeared to be highly unlikely (e.g., apparent double recombinants occurring within a narrow interval).

Baboon linkage map

Genotype data were used previously to develop a first-generation genetic linkage map of the baboon genome (18). To produce the most recent version of this map, genotype data for 325 human microsatellite loci plus six novel baboon microsatellites were used in marker-to-marker linkage analyses, facilitated by the expert system program Multimap (19). Multipoint linkage analyses are highly dependent upon the validity of the linkage map. Therefore, estimates of multipoint identity-by-descent (IBD) coefficients used in the analyses described below were based on a map containing 283 of these marker loci, 275 of which were placed in unique positions at 1,000:1 odds and 8 at 100:1 odds (the latter being the standard for genetic map construction). The average heterozygosity index for the markers was 0.73 (range, 0.26–0.92), and the mean inter-marker interval in this map was 8.6 centimorgans (cM).

To allow multipoint linkage analyses to detect and localize quantitative trait loci (QTLs) for PON activity, we used the genotype data and information in the baboon whole genome linkage map to produce multipoint IBD matrices using the stochastic MCMC approach implemented in the computer package Loki (20, 21). This MCMC method yields multipoint IBD estimates that more closely approximate those obtained by exact methods that cannot be applied conveniently to large extended pedigrees, such as those in this study. Using the multipoint IBD matrices, we could perform a log of the odds (LOD) score evaluation at 1 cM intervals along each chromosome.

Baboon pedigree

The baboons were assigned to 11 extended pedigrees that included the following relative pair classes: 547 parent-offspring, 533 sibling, 34 grandparent-grandchild, 77 avuncular, 5,786 half sibling, 1,535 half avuncular, 2 first cousin, 33 half first cousin, 20 half first cousin once removed, 475 half sibling-half first cousin, 7 half sibling-half avuncular, and 30 double half avuncular.

Statistical analyses

Before analyses, PON activity levels were $\log_e$-transformed to reduce skewness and kurtosis. One animal had a PON value more than 4 SDs below the mean, and this animal was dropped from further study.

We conducted all statistical genetic analyses using a maximum likelihood-based variance decomposition approach (22) implemented in the computer program SOLAR (23). Basic univariate quantitative genetic analyses were used to simultaneously assess the additive effects of genes and the mean effects of selected covariates on PON activity data obtained from animals on each of the three diets. We estimated heritability as the proportion of the residual phenotypic variance (i.e., after accounting for the mean effects of covariates) attributable to the additive effects of genes. Using multivariate extensions to this approach (24–26), we also estimated six additional parameters: the additive genetic and random environmental correlations between PON activity levels assayed in samples from animals on the three diets. Significance in all models was assessed by likelihood ratio tests (27).

TABLE 1. PON activity levels for male and female baboons fed three diets

Diet	Males	Females
	μmol/min/l	
Basal	22.41 ± 0.47	27.64 ± 0.57
LCHF	22.90 ± 0.55	27.61 ± 0.53
HCHF	23.18 ± 0.58	28.64 ± 0.59

HCHF, high-cholesterol, high-fat; LCHF, low-cholesterol, high-fat; PON, paraoxonase. Values shown are means ± SEM.

We used the variance decomposition approach to test for evidence of QTLs that affect variation in PON activity (23). This method entails specification of the genetic covariance between arbitrary relatives as a function of the IBD relationships at a given locus. The covariance matrix for a pedigree is modeled as the sum of the additive genetic covariance attributable to the QTL, the additive genetic covariance attributable to the effects of other loci, and the variance attributable to unmeasured environmental factors. We tested the hypothesis of linkage by comparing the likelihood of a restricted model in which variance attributable to the QTL was constrained to zero (i.e., no linkage) with that of a model in which it was estimated. The LOD score of classical linkage analysis was obtained as the quotient of the difference between the two $\log_e$-likelihoods divided by $\log_e$ 10 (28).

We hypothesize that the genetic contribution to variation in many complex traits is oligogenic (i.e., resulting from the actions of two or more genes with individually measurable effects, expressed over a polygenic background). Consequently, we performed whole genome linkage screens in a sequential manner to facilitate the detection of multiple QTLs for PON activity. That is, after a first screen that detected a putative QTL, we sequentially performed a series of screens in which the previously detected QTLs were fixed in each subsequent model. This was done until a screen failed to detect a putative QTL (defined as LOD > 1.9). A more detailed description of the theory and method underlying oligogenic linkage analysis was presented previously (23).

To control for the overall false-positive rate given the finite marker locus density in the baboon genome linkage map, we estimated the genome-wide *P* value by the method of Feingold, Brown, and Siegmund (29). In this population, a LOD of 2.696 corresponded to a genome-wide *P* value of 0.05, which is considered significant. Using the same approach, the threshold for "suggestive" evidence of linkage, as proposed by Lander and Kruglyak (30), was a LOD of 1.461.

Kurtosis can inflate LOD scores, but in no case did kurtosis exceed ±0.31 in the starting data or ±0.20 for the residual data in the null models for linkage analysis. In addition, we estimated a robust LOD score that was based on 10,000 simulations of the same trait and pedigree data with an unlinked marker (31). The simulations, implemented in SOLAR, yielded a correction factor that could be used to adjust the LOD score. However, for the three PON traits, we estimated adjustment factors >1, indicating that no adjustment was necessary.

RESULTS

Measurement of PON activity

We assayed PON activity in serum samples from 611 pedigreed baboons that had been subjected to a dietary challenge protocol; the average value was 26.1 μmol/min/l serum. **Table 1** gives average PON activity levels for male and female baboons fed each of three diets (basal, LCHF, and HCHF) and illustrates the >20% higher levels observed in females compared with males. We investigated the question of whether diet influenced enzyme activity by use of paired *t*-tests. These tests indicated no significant effect of increasing levels of dietary fat (i.e., basal vs. LCHF diet values; $P = 0.61$) but a small (3%) increase when levels of dietary cholesterol were increased (i.e., LCHF vs. HCHF diet values; $P = 0.007$).

Quantitative genetic analyses of PON activity on three diets

Genetic models, implemented in SOLAR, included covariates for age and age squared, sex, and age-by-sex, which together accounted for 9–15% of total trait variance (**Table 2**). PON activity was significantly heritable on each of the diets [heritability (h^2) for basal = 0.515; h^2 for LCHF = 0.705; h^2 for HCHF = 0.569]. Thus, between 51% and 71% of the residual variance was attributable to the additive effects of genes.

Multivariate genetic analyses of dietary effects

Multivariate models were implemented in SOLAR, and we first investigated whether the same gene(s) influenced PON activity on each of the diets. The genetic correlations were 0.981 ± 0.041 for basal/LCHF, 0.995 ± 0.019 for LCHF/HCHF, and 0.995 ± 0.021 for basal/HCHF. In no case was the genetic correlation significantly different from

TABLE 2. Mean, SD, heritability, covariate effects (β), and percentage of total variance explained by the covariates obtained from genetic analyses of activity measured in samples from baboons fed three diets

Parameter	Basal	LCHF	HCHF
Number	611	611	611
Mean	3.02 ± 0.04	3.03 ± 0.0.04	3.02 ± 0.04
SD	0.36 ± 0.01	0.37 ± 0.01	0.37 ± 0.01
Heritability (%)	51.5 ± 8.9[a]	70.5 ± 9.3[a]	56.9 ± 8.8[a]
β age	−0.012 ± 0.004[b]	−0.011 ± 0.004[b]	−0.023 ± 0.004[c]
β sex	0.18 ± 0.03[c]	0.19 ± 0.03[c]	0.23 ± 0.03[c]
β age by sex	0.023 ± 0.005[c]	0.023 ± 0.005[c]	0.035 ± 0.005[a]
β age squared	0.0005 ± 0.0003[b]	0.00002 ± 0.0003	0.0003 ± 0.0003[b]
Total variance for covariates (%)	11.6	9.1	15.1

PON activity values were $\log_e$-transformed before analysis.
[a] $P < 10^{-10}$.
[b] $P < 0.05$.
[c] $P < 10^{-4}$.

TABLE 3. LOD score, genome-wide *P* values, and nearest microsatellite marker for putative quantitative trait loci obtained in sequential passes using oligogenic linkage analyses of PON activity in baboons fed three diets

	First Screen			Second Screen			Third Screen		
Diet	LOD	*P*	Marker	LOD	*P*	Marker	LOD	*P*	Marker
Basal	2.02	0.26	D7S821	1.79	0.55	D12S375			
LCHF	9.06	1×10^{-8}	D7S821	1.95	0.30	D12S375			
HCHF	4.05	0.0018	D7S821	2.88	0.032	D12S75	2.26	0.15	D7S559[a]

LOD, log of the odds. PON activity values were $\log_e$-transformed before analysis.
[a] Marker D7S559 maps ~79 centimorgan distant from marker D7S821 (see Fig. 1A).

unity, suggesting that all, or nearly all, of the same genes influenced enzyme activity on each of the diets. We estimated genetic variance for serum PON activity in a trivariate model that considered data from the three diets simultaneously. The genetic variation in PON activity on the LCHF diet ($\sigma_G^2 = 9.61$) was 27% greater than that estimated for the HCHF diet ($\sigma_G^2 = 7.56$; $P = 0.055$) but 44% greater than that for the basal diet ($\sigma_G^2 = 6.66$; $P = 0.039$); the differences in genetic variance between the basal and HCHF diets were not significant. These latter results are consistent with a genotype-by-diet interaction in which the magnitude of the genetic effect on serum PON activity is influenced by the level of dietary fat and cholesterol.

Linkage analyses

We performed a genome screen using multipoint variance components methods to search for QTLs that affect PON activity on the various diets. **Table 3** gives the noteworthy multipoint LOD scores for putative PON QTLs. On each of the diets, the strongest evidence was found for a QTL occurring on the baboon homolog of human chromosome 7 (multipoint LODs were 2.02, 9.06, and 4.05 on the basal, LCHF, and HCHF diets, respectively; genome-wide *P* values were 1×10^{-8} for LCHF and 0.0018 for HCHF). The microsatellite marker nearest these peaks was D7S821, a marker that maps to human 7q21-22. In the second screen, the strongest evidence was found for a QTL occurring on the baboon homolog of human chromosome 12 (multipoint LODs were 1.79, 1.95, and 2.88 for the basal, LCHF, and HCHF diets, respectively; genome-wide *P* value was 0.032 for HCHF). The microsatellite markers nearest these signals were D12S375 and D12S75, which map to human 12q13. One other signal (LOD = 2.26) was detected on the baboon homolog of human chromosome 7, but more than 70 cM distant from the first signal. However, this putative QTL was detected only on the HCHF diet. **Figure 1A** shows a plot of multipoint LOD scores for PON activity on the three diets together with the locations of the 19 microsatellite markers mapped on the baboon chromosome representing a fusion of genomic material found on human chromosomes 7 and 21. Figure 1B shows a similar plot of LOD scores and markers for the baboon homolog of human chromosome 12; the plots were taken from the second screen in which the dominant QTL near D7S821 was fixed in the model. Two-point LOD scores, estimated for the individual markers, showed patterns similar to those presented from the multipoint analyses (data not shown).

Effects of HDL metabolism

We tested three indicators of HDL metabolism for effects on PON activity by including them as covariates in the models presented in Table 2. **Table 4** gives the estimates of HDL effects on PON activity and the associated *P* values. By far, the strongest covariate was concentration of the dominant protein component of HDL, apolipoprotein A-I (apoA-I), which explained 3.2–6.4% of the variance. It should be noted

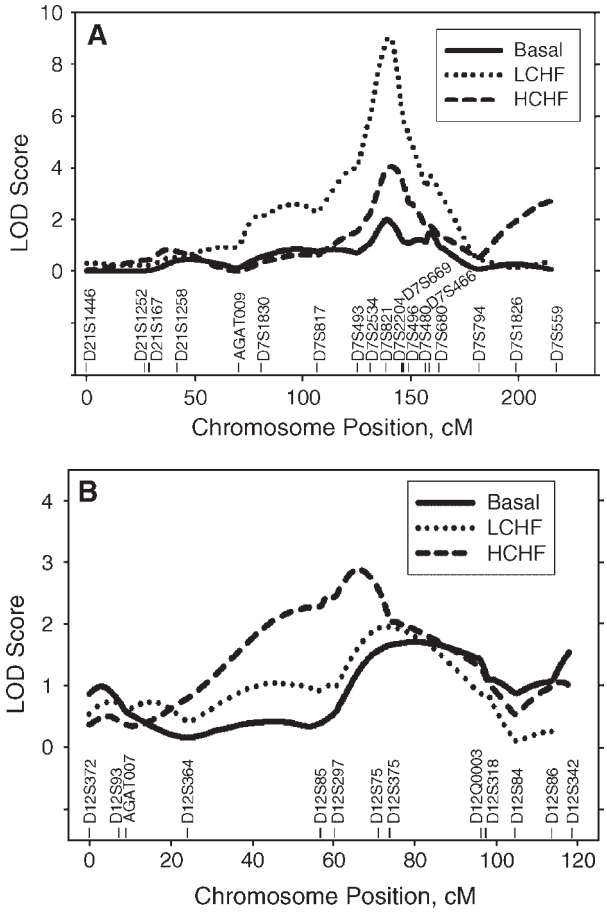

Fig. 1. Multipoint log of the odds (LOD) scores for baboon paraoxonase (PON) activity assayed on three diets [basal, solid line; low-cholesterol, high-fat (LCHF), dotted line; high-cholesterol, high-fat (HCHF), dashed line]. Scores greater than LOD = 2.8 are considered significant (i.e., genome-wide $P < 0.05$). Mapped locations of human microsatellite markers are indicated at the bottom of each panel. A: Baboon chromosome 3 (homologous to human chromosomes 7 and 21). B: Baboon chromosome 11 (homologous to human chromosome 12). cM, centimorgan.

TABLE 4. Effect (percentage of total variance) and *P* values of three measures of HDL metabolism on PON activity: concentrations of apoA-I and HDL-cholesterol and HDL median diameter

	ApoA-I			HDL-Cholesterol			HDL Median Diameter		
Diet	Number	Effect	*P*	Number	Effect	*P*	Number	Effect	*P*
Basal	482	3.2	4×10^{-8}	610	3.2	6×10^{-8}	607	1.7	0.0038
LCHF	482	6.4	2×10^{-8}	611	3.7	4×10^{-8}	611	0.6	0.10
HCHF	482	6.0	3×10^{-8}	600	2.7	8×10^{-7}	611	0.1	0.12

apoA-I , apolipoprotein A-I.

that apoA-I concentration data were not available for >100 animals in the study. HDL-cholesterol concentrations explained less variance than did apoA-I (2.7–3.7%), but all effects were significant. However, HDL median diameter, a measure of HDL particle size distributions, explained very little of the variance and was significant for only one diet. These data suggest that HDL metabolism exerts a significant effect on PON variation and that apoA-I concentration was the stronger predictor of the effect.

Components of PON variation

To approximate the relative contributions of the various determinants of PON that were detected in this study, we ran models in which we estimated the effects of the covariates (age, sex, age-by-sex, age squared), QTLs on the baboon homologs of human chromosomes 7 and 12, and a residual additive genetic component. ApoA-I levels were not included in these models because >100 animals did not have this measure; thus, the effect of apoA-I was taken from the data given in Table 4. **Table 5** gives a breakdown of the proportion of total trait variation explained by the various components we studied on each of the diets and also calculates an average value across diets. Overall, we were able to explain ~70% of PON activity variance.

DISCUSSION

PON activity levels were reliably measured in baboon serum samples using published approaches. The coefficient of variation for a control product run with each plate was ~7%. Baboon PON activity averaged ~26 μmol/min/l, lower than we have observed for human samples (~130 μmol/min/l; n = 1,284; data not shown) using the same assay approach.

By far, the predominant effect on PON activity was genetic, accounting for ~53% of total variance. We further partitioned the genetic variance into at least three components. One component was the locus mapping to the region of the baboon genome homologous to human chromosome 7q21-22, where the structural gene for human PON (*PON1*) is located (32). This QTL was in the significant range on the LCHF and HCHF diets but only in the suggestive range on the basal diet. The nearest microsatellite marker to the QTL was D7S821, which, in the human genome assembly (May 2004), is ~1.2 megabases from *PON1* (33). A number of studies have demonstrated polymorphisms in the human structural gene that exert significant effects on enzyme activity (10), further supporting the notion that *PON1* is responsible for this QTL. The impact of this locus on PON activity varied across the three diet samples, but on average it explained ~22% of total trait variance. Surprisingly, linkage analyses identified a second locus, on the baboon homolog of human chromosome 12, that also significantly influenced PON activity on the HCHF diet; suggestive evidence for the QTL was detected on the basal and LCHF diets. On average, this second locus explained ~11% of total trait variance. We are not aware of any other gene, whether in the vicinity of this signal on chromosome12 or otherwise, that has previously been proposed to influence PON activity, although there are several genes in the same general vicinity, such as *SOAT2*, *APOF*, and *LRP*, that might be expected to affect lipid and lipoprotein metabolism. Further studies will be required to identify this QTL.

The remaining genetic variance (19%) was not accounted for by any single locus and is designated a "residual additive" genetic effect in Table 5. Given the fact that PON is one of the many antioxidant enzymes that help maintain a delicate balance between free radicals and antioxidants, it is not surprising that other genes affecting redox pathways might also exert significant influence on PON expression.

Of the residual, nongenetic variance, we detected effects by several measured covariates that explained ~17% of total trait variance. The significant covariates that were detected in this study included age, sex, apoA-I concentrations, and diet.

In our study, females had ~20% higher PON activity levels than did males. Studies in human populations have

TABLE 5. Breakdown of the components of variation in PON activity

Component	Basal Diet	LCHF Diet	HCHF Diet	Average
Genetic	45.5	64.1	48.3	52.6
Chromosome 7	13.3	34.4	18.5	22.1
Chromosome 12	12.2	6.7	14.5	11.1
Residual additive	20.0	23.0	15.3	19.4
Covariates	14.8	15.5	21.1	17.1
Age, sex	11.6	9.1	15.1	11.9
ApoA-I	3.2	6.4	6.0	5.2
Unexplained	39.7	20.4	30.6	30.2

Data were taken from models in which a chromosome 7 and a chromosome 12 effect, plus a residual additive genetic effect, were estimated; because apoA-I concentration data were unavailable for >100 animals, the effect of apoA-I was not modeled directly but instead was taken from results given in Table 4.

also detected significant effects of sex on PON activity, with women having higher levels than men (34–36). There is disagreement among studies with regard to whether there are significant effects of age on PON activity (34–37). In none of these studies, however, was the size of the effect for sex or age estimated as we have done in the present study, which indicates that together, sex and age account for ~12% of total variance. There also was a significant positive effect of two markers of HDL metabolism, apoA-I and HDL-cholesterol, on PON activity in this study. Of these, apoA-I levels were more strongly associated than HDL-cholesterol, and they explained ~5% of total variance. The finding that an HDL measure explains a significant amount of the variance in PON is not surprising given the specific localization of PON on HDL particles. Recently, Kontush, Chantepie, and Chapman (38) reported that PON activity levels tend to increase with increasing HDL density (and decreasing particle size) and that they correlate directly with the ability of different HDL density fractions to prevent LDL oxidation. However, in our study, HDL median diameter, a measure of cholesterol distributions among size-resolved HDLs (39), was at best marginally significant, suggesting that the effect of HDL metabolism on PON activity is related to the amount of HDL rather than to its size distributions.

Finally, increases in dietary cholesterol caused significant increases in PON activity in this set of animals, but we were unable to estimate the size of the effect of diet. Because increasing dietary levels of fat and cholesterol increase HDL levels in baboons (40), it is possible that the dietary category served as a surrogate indicator of HDL metabolism. Studies of diet effects in humans have identified a number of dietary components that can significantly affect PON activity, including ethanol, certain types of fat, and fruits and vegetables (41–44). Perhaps accounting for some of the reported effects of diet is a xenobiotic responsive element-like sequence within the human *PON1* promoter region that appears to mediate the effects of polyphenols on PON expression in vitro (45). In contrast to the present observations, rabbits fed an atherogenic diet showed reduced PON activity. However, the atherogenic diet also caused a 70% reduction in HDL-cholesterol levels, again suggesting that some diet effects are actually exerted through effects on HDL metabolism.

A possible concern with this report of two QTLs for PON activity is that they were not consistently significant on all diets (i.e., even though the diet means and variances were quite similar, the magnitude of the statistical support for each QTL differed noticeably). However, we can assert that the most significant signals on chromosomes 7 and 12 were replicated in analyses of data from each of the other diets by signals that were significant or suggestive at the genome-wide level (30). Although potential sources of error that could affect our level of confidence in a QTL include seemingly random biological variation and assay error, we believe that the apparent differences in linkage results were attributable, at least in part, to the effects of diet on gene expression.

Evidence for genotype-by-diet interaction is accepted if we can determine that different genes influence the trait or if the genetic variances differ according to diet (26, 40). All three possible pair-wise genetic correlations were indistinguishable from unity, suggesting that the same set of genes influences PON variation on each diet. However, our comparisons of the diet-specific variances provided evidence that the genetic contribution to the variance in PON activity on the LCHF diet was significantly greater than on the other two diets. Thus, the results of our analyses are consistent with a genotype-by-diet interaction and, therefore, a biological basis for some of the differences in genetic models observed in this study.

These results highlight the value of an animal model for characterizing gene-environment interactions that are virtually impossible to detect in free-living populations (such as humans) and yet are likely to be quite important individually.

There are, however, limitations in our ability to extrapolate results from the baboon model to the human situation. First is the fact the our observations of a QTL on the homolog of human chromosome 12 have not yet been replicated in another population or species (although we report replication in the same animals fed different diets). Such replication would increase confidence that the locus might also contribute to genetic variance in humans. Another limitation of this study is that we have assessed PON activity using only the single substrate, paraoxon. It is well documented that various isoforms of human PON can show dramatically different substrate-dependent specific activities (10, 16), so it is conceivable that our results might not be true for other substrates or that we have missed critical genetic effects by choosing to assay with a single substrate. We do not anticipate that baboons will have the same polymorphisms as exert important effects on enzyme activity in humans. Therefore, until we can identify the relevant functional polymorphisms in the baboon structural gene, we cannot determine whether our results regarding baboon PON will apply directly to the human enzyme. Given the many metabolic similarities between the two species, however, it is likely that this report of a number of factors that determine PON variation will be relevant to humans.

In summary, our study has demonstrated significant genetic regulation of an important HDL-associated antioxidant, PON. Effects of at least two genes plus several covariates (age, sex, and HDL metabolism) together account for ~70% of the total variation in PON activity. The demonstrated importance of HDL and oxidative stress to the onset and progression of atherosclerosis makes it vital to better understand the determinants of variation in the key HDL antioxidant components. Thus, characterization of the determinants of PON will not only help increase our understanding of the genetic basis of cardiovascular disease but may also create new opportunities for the development of therapeutic interventions aimed at increasing antioxidant capacity via the upregulation of PON expression.

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