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# Two Loci Affect Angiotensin I–Converting Enzyme Activity in Baboons

Candace M. Kammerer, David L. Rainwater, Jennifer L. Schneider, Laura A. Cox, Michael C. Mahaney, Jeffrey Rogers, Jane F. VandeBerg

**Abstract**—Serum LDL cholesterol (LDLC) concentrations and ACE activities are risk factors for the development of cardiovascular disease (CVD). However, the relationship between ACE and CVD susceptibility, and possible mechanisms of action, is controversial. With data on 622 pedigreed baboons, we used statistical genetic methods to determine the mode of inheritance of ACE activities and its relationship to LDLC on different diets. ACE activity was moderately heritable, and quantitative trait linkage analyses detected a quantitative trait locus (QTL) for ACE activity on the baboon homolog of human chromosome 17 (near the ACE structural locus, maximum multipoint lod=7.5, genomic  $P=0.000003$ ). Bivariate analyses revealed that ACE activity was genetically correlated ( $\rho_G$ ) with LDLC response (LDLC<sub>RC</sub>) to a high-cholesterol diet ( $\rho_G=0.30\pm 0.13$ ,  $P=0.01$ ) but not to LDLC on a basal diet ( $\rho_G=0.08\pm 0.13$ ). Bivariate genetic analyses indicated that a previously detected QTL for LDLC<sub>RC</sub> had significant ( $P=0.025$ ) pleiotropic effects on ACE activity levels and accounted for the genetic correlation. Therefore, we have detected 2 putative loci that affect ACE activity in baboons, one of which also affects LDLC dietary response. The existence of at least 2 genes that affect ACE activity, one of which is diet-responsive, may help explain the lack of consistency among studies of the relationship between ACE and CVD. (*Hypertension*. 2003;41[part 2]:854-859.)

**Key Words:** angiotensin-converting enzyme ■ cholesterol ■ diet ■ genetics ■ linkage ■ baboons

Hypertension and serum concentrations of LDL cholesterol (LDLC) are well-known heritable risk factors for cardiovascular disease (CVD). Numerous studies have demonstrated the central role of the renin angiotensinogen system (RAS) on blood pressure regulation and the development of hypertension. Epidemiological studies have shown that hypertension and hypercholesterolemia often occur within the same individual. Furthermore, pharmacologic inhibition of ACE, a critical RAS enzyme that cleaves the inactive angiotensin (Ang) I to form active Ang II (a potent vasoconstrictor), has beneficial effects in patients with hypertension and ischemic heart disease. Recently, researchers have reported that the magnitude of blood pressure response to infusion with Ang II is heritable<sup>1</sup> and positively correlated with serum cholesterol concentrations in both normotensive<sup>2</sup> and hypertensive<sup>1</sup> individuals. These observations suggest that common underlying pathways link the RAS and cholesterol metabolism, and this ultimately may contribute to the coordinate development of hypertension and atherosclerosis.

Serum ACE levels are moderately heritable,<sup>3</sup> and polymorphisms in the ACE structural locus (referred to as ACE or DCPI) account for 19% to 50% of the variation in serum ACE levels.<sup>4</sup> A common *Alu* repeat insertion/deletion (*I/D*) in ACE has been associated with increased blood pressure or

risk of CVD in some,<sup>5,6</sup> but not all,<sup>7,8</sup> studies. In fact, associations between CVD phenotypes and the ACE genotypes varied across populations and geographic regions.<sup>9,10</sup> Because the *I/D* polymorphism resides in an intron, these inconsistent associations may imply that the functional polymorphism is located elsewhere within ACE or even in a nearby gene. Alternatively, these observations may be indicative of the presence of gene-by-gene or genotype-by-environment interactions.

We are studying genes and environmental factors, and their interactions, that influence blood pressure regulation<sup>11</sup> and lipoprotein metabolism<sup>12</sup> in baboons, a primate model for CVD. In the present study, we report the results of our analyses of the genetic relationship between ACE activity levels and LDLC concentrations.

## Methods

### Baboon Pedigrees

Data were analyzed on a total of 760 pedigreed baboons (*Papio hamadryas*) comprising 10 pedigrees ranging in size from 42 to 107 animals. These second- and third-generation pedigrees consisted of 218 founders (33 sires and 185 dams) and their 542 offspring. ACE activities, serum lipid concentrations, and genotypic data were available on 622 of the pedigreed baboons. The 212 males and 410 females with data had a mean age of 9.7 years (SD=6.9, range; 2.2

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From the Department of Human Genetics, University of Pittsburgh (C.M.K.), Penn; Southwest Foundation for Biomedical Research (C.M.K., D.L.R., J.L.S., L.A.C., M.C.M., J.R., J.F.V.), San Antonio, Tex; and Southwest National Primate Research Center (J.R.), San Antonio, Tex

Correspondence to Candace M. Kammerer, Department of Human Genetics, Graduate School of Public Health, University of Pittsburgh, A310 Crabtree Hall, 130 De Soto St, Pittsburgh, PA 15261. E-mail ctkammerer@hgen.pitt.edu

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to 28.5) and a mean weight of 16.9 kg (SD=8.5; range, 5.2 to 45.2) at the outset of the experiment.

All animals were bred and maintained at the Southwest Foundation for Biomedical Research, a facility certified by the Association for Assessment and Accreditation of Laboratory Animal Care International. The experimental protocol was approved by the Institutional Animal Care and Use Committee.

### Dietary Protocol

Assays were performed using frozen serum samples that were obtained after the baboons had fasted overnight. As previously described,<sup>13</sup> all baboons were subjected to the same dietary challenge, and serum samples were available on each animal on each of 3 diets: (1) basal diet, a baseline monkey diet (Wayne Teklad) that was low in fat ( $\approx$ 4% of calories) and cholesterol (0.03 mg/kcal); (2) HCHF diet, a diet high in saturated fat (40% of calories from fat by the addition of lard) and cholesterol (1.7 mg/kcal); and (3) LCHF diet, a high fat-only diet (40% of calories from lard, 0.03 mg/kcal of cholesterol).

### Assay of ACE, LDLC, and Genotypes

ACE activity levels (U/L) were measured on a Ciba-Corning Express Plus Analyzer using a kit purchased from Sigma Diagnostics. The procedure is a spectrophotometric method that monitors hydrolysis of the synthetic tripeptide substrate *N*-[3-(2-furyl)acryloyl]-L-phenylalanyl-glycylglycine (FAPGG) at 340 nm. Calibrators and 2 levels of controls were purchased from Sigma Diagnostics and a pooled baboon serum sample were also run on each plate of 40 samples. Samples were diluted 1:5 with saline before analyses. The within-run and between-run coefficients of variation for this assay were 1.4% and 4.6%, respectively. ACE activity was measured on the LCHF diet only, because the maximum number of frozen samples was available on that diet.

Serum cholesterol concentrations (mmol/L) were assayed enzymatically, and LDLC was estimated after precipitation with heparin-Mn<sup>2+</sup>. LDLC was assayed on all diet samples, and LDLC<sub>RC</sub> was calculated as the difference in LDLC between the HCHF and LCHF diets (LDLC<sub>HCHF</sub>–LDLC<sub>LCHF</sub>).<sup>13</sup>

Published human primers were used to amplify 279 homologous microsatellite loci from baboon genomic DNA samples.<sup>14,15</sup> Approximately two thirds of the genotypes and 236 of these highly polymorphic markers were generated by researchers at Axys Pharmaceuticals Inc and were used to develop the baboon genomic map.<sup>14</sup> As described in detail elsewhere,<sup>14</sup>  $\approx$ 1200 human primer pairs were assayed, of which  $\approx$ 25% detected polymorphisms in baboons.

### Statistical Analyses

We used univariate quantitative genetic analysis to assess the residual heritability of ACE activity and the lipid traits while simultaneously incorporating the effects of covariates such as sex, sex-specific linear and quadratic age, and weight.<sup>13,16</sup> All parameters were estimated using maximum-likelihood methods. All covariates that had an effect ( $P \leq 0.10$ ) on ACE activity or LDLC were included in all subsequent analyses.

To determine whether any quantitative trait loci (QTLs) affected ACE activity, we performed 2-point and multipoint variance components linkage analyses, as previously described.<sup>17</sup> Briefly, we estimated the genetic variance attributable to the region around a specific genetic marker ( $\sigma_m^2$ ) by specifying the expected genetic covariances between arbitrary relatives as a function of the identity-by-descent relationships at a given marker locus assumed to be tightly linked to a locus influencing the quantitative trait. We compared the likelihood of the restricted model, in which  $\sigma_m^2=0$  (no linkage), with that of a model in which the variance due to the marker is estimated, and then we calculated the log-odds (lod score) for linkage. Because trait nonnormality can inflate lod scores, we performed 10 000 simulations to empirically obtain an adjustment factor with which to deflate the lod scores.

Multivariate quantitative genetic methods<sup>13,16</sup> were used to calculate genetic ( $\rho_G$ ) and environmental ( $\rho_E$ ) correlations between ACE activity and the LDLC phenotypes and to estimate the magnitude of pleiotropic effects of underlying genes on both traits. A large genetic correlation between traits implies that the same gene or genes influence variation in both traits. In addition, to determine whether the previously detected major gene/QTL for LDLC<sub>RC</sub> concentration<sup>13,15</sup> had an effect on ACE activity, we performed a 1-locus, bivariate segregation analysis.<sup>13,18</sup> In brief, this model (unrestricted) considers the effect of the QTL for LDLC<sub>RC</sub> on both LDLC<sub>RC</sub> concentrations and ACE activities simultaneously. We compared the likelihood of a model in which genotypic means associated with the 3 LDLC<sub>RC</sub> genotypes were estimated for both traits to a model in which genotypic means were estimated for LDLC<sub>RC</sub> levels and a single mean estimated for ACE activity (restricted). Major gene/QTL pleiotropy is indicated if the likelihood of the restricted model is less than the likelihood of the unrestricted model. Using this framework, we also tested for the presence of residual genetic and environmental correlations, by comparing models in which  $\rho_G$  and  $\rho_E$  are estimated or fixed at 0.<sup>13,18</sup>

## Results

### Heritability and Linkage Analyses

Mean ACE activity for all animals was  $182.0 \pm 55.4$  U/L and ranged between 35 to 375 U/L. Univariate quantitative genetic analyses revealed that ACE activity levels were moderately heritable,  $h^2=0.47 \pm 0.09$ . In addition, females had significantly lower activities than did males (regression coefficient for sex [ $\beta_{sex}$ ],  $-22.0 \pm 3.7$ ), and activity decreased with increasing age ( $\beta_{age}$ ,  $-5.0 \pm 1.1$ ) but increased with increasing age<sup>2</sup> ( $\beta_{age^2}$ ,  $0.09 \pm 0.04$ ) ( $P < 0.05$  for all covariates). However, there were no significant effects of weight or age by sex interactions on ACE activity in these baboons. These results are similar to studies of ACE levels in humans, which also report that females have lower mean ACE levels, ACE levels decrease with increasing age, and correlation between parents and offspring is moderate.<sup>3</sup>

Because ACE activities were moderately heritable, we next performed variance components linkage analyses to determine whether any QTLs affect ACE activity.

Using 2-point linkage analyses (Table 1), we obtained highly significant evidence (lod=7.49, genomic  $P=0.000003$ ) that a QTL for ACE activity was located on baboon chromosome 16q, which is homologous to human chromosome 17q.<sup>14</sup> Multipoint analysis of baboon chromosome 16 revealed that the peak multipoint lod (=7.5) was obtained at the q-end of the chromosome, near locus D17S934 (Figure). In humans, the structural locus for the ACE enzyme (*ACE*) is located near the end of 17q at 61.2 Mb (<http://genome.ucsc.edu>). Using data on a small set ( $n=350$ ) of pedigreed baboons, we have mapped a polymorphism in the *ACE* locus in baboons to the end of baboon 16q within 4 centimorgans of D17S934 (lods for placement=3.3; C.M. Kammerer, unpublished data, 2002). These results indicate that the QTL for ACE activity in baboons may be the *ACE* structural locus.

### Correlation Between ACE Activities and LDLC

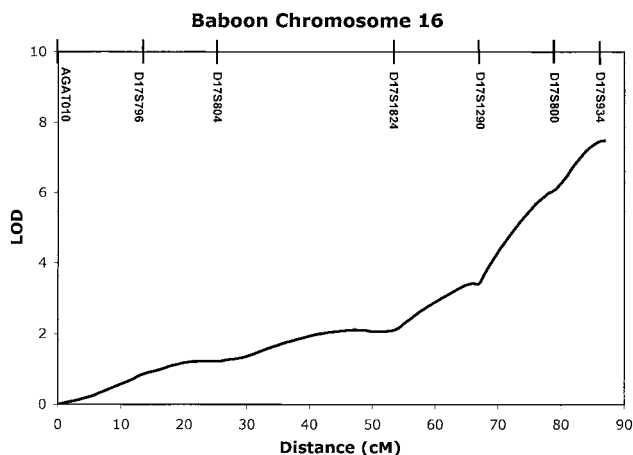
We next investigated whether there was any genetic or environmental correlation between ACE activities and another risk factor for CVD, LDLC concentrations, using quantitative genetic methods. As previously reported,<sup>13</sup> LDLC in these baboons is highly heritable on all diets (Table

**TABLE 1. Highest 2-Point Lod Scores for ACE Activity for Each Baboon (PHA) Chromosome and the Human (HSA) Homolog**

PHA Chromosome	HSA Homolog	Lod	Locus
1	1	0.45	D1S550
13	2p	0.75	D2S144
12	2q	0.28	D2S115
2	3	0.98	D3S1229
5	4	1.71	D4S1636
6	5	0.04	D5S1466
4	6	0.54	D6S1718
3	7/21	0.43	D7S559
8	8	0.78	D8S208
15	9	0.04	D9S156
9	10	0.33	D10S192
14	11	0.22	D11S1329
11	12	0.08	D12S375
17	13	0.41	D13S318
7	14/15	0.10	D14S261
20	16	0.32	D16S423
16	17	7.49*	D17S934
18	18	0.00	—
19	19	0.56	D19S180
10	20/22	0.44	D22S304

\*Lod&gt;2.

2). There were significant positive genetic correlations between ACE activity and  $LDLC_{HCHF}$  and  $LDLC_{RC}$  ( $\rho_G=0.26\pm 0.12$ ,  $P=0.02$ , and  $\rho_G=0.30\pm 0.13$ ,  $P=0.01$ , respectively), but not between ACE activities and  $LDLC_{basal}$  or  $LDLC_{LCHF}$ . The environmental correlations between ACE activity and LDLC were not significant on any diet. These results indicate that ACE activity is genetically correlated with LDLC response to dietary cholesterol, but not LDLC on the basal diet or response to dietary fat. In other words, gene(s) that affect variation in LDLC dietary cholesterol response have pleiotropic effects on ACE activity.



Multipoint variance components linkage analyses of ACE activities on baboon chromosome 16, which is homologous to human chromosome 17.

### Pleiotropic Effects of $LDLC_{RC}$ Major Gene on ACE Activities

Given the significant genetic correlation between ACE activity and LDLC response to dietary cholesterol, and our previous results that a major QTL affects  $LDLC_{RC}$ ,<sup>13,15</sup> we investigated whether the major gene for  $LDLC_{RC}$  had pleiotropic effects on ACE activity (Table 3). We evaluated the pleiotropic effects of the  $LDLC_{RC}$  QTL on ACE activity by calculating the natural log (ln) likelihood of an unrestricted model (model 1) in which the pleiotropic effects of the  $LDLC_{RC}$  QTL, as well as residual additive genetic pleiotropic effects ( $\rho_G$ ) on ACE activities, were estimated. Thus, in this unrestricted model, we estimated the allelic frequency ( $f[R]$ ) and genotypic means ( $\mu_{RR}$ ,  $\mu_{Rr}$ ,  $\mu_{rr}$ ) corresponding to the  $LDLC_{RC}$  major gene on both  $LDLC_{RC}$  and ACE activity. We also estimated the residual heritabilities ( $h^2$ ) for each trait, as well as the residual genetic ( $\rho_G$ ) and environmental ( $\rho_E$ ) correlations. The ln-likelihood of this model was then compared with a series of restricted models (models 2 through 6) by using the ln-likelihood ratio test.

Our results (Table 3) indicate that the  $LDLC_{RC}$  QTL has a significant pleiotropic effect on ACE activity levels. The model in which a single mean was estimated for ACE activity (model 2) was significantly worse than the one in which the mean ACE activity corresponding to each of the  $LDLC_{RC}$  QTL genotypes were estimated (compare models 1 and 2). However, after including pleiotropic effects of the  $LDLC_{RC}$  QTL on ACE activity, allowing for a residual genetic correlation was not significantly better than fixing the correlation at 0 (compare models 1 and 3). This result implies that the  $LDLC_{RC}$  QTL accounts for most, if not all, of the genetic correlation between ACE activities and  $LDLC_{RC}$ . As additional support for this interpretation, we found that allowing for QTL pleiotropy was significantly better than no pleiotropy for 2 models in which residual genetic correlations were not estimated (compare models 3 and 4;  $\chi^2=8.97$ ,  $P=0.01$ ). Finally, as expected from our previously reported results, models in which the effects of the  $LDLC_{RC}$  QTL are not estimated for either trait are strongly rejected. Because the QTL for ACE activities maps to baboon chromosome 16, and the LDLC QTL maps to baboon chromosome 4, we have evidence that at least 2 loci affect ACE activities.

### Discussion

Results from epidemiologic, clinical, and genetic studies suggest that genes and their protein products that affect blood pressure regulation may also have effects on arteriosclerosis and vice versa. One of the best-studied examples of such a gene is the ACE locus, for which a common *I/D* polymorphism has been associated with serum ACE levels, as well as development of hypertension, myocardial infarction, and ischemic heart disease.<sup>10</sup> Further support for the effects of ACE on blood pressure regulation and CVD is that ACE gene expression is increased in patients with myocardial infarction, and ACE inhibitors have been successfully used to treat patients with hypertension and congestive heart failure.<sup>10</sup>

However, this possible relationship between ACE activity and development of CVD is controversial. Most recently, Keavney and colleagues<sup>8</sup> in a study of 11 000 unrelated

**TABLE 2. Heritabilities ( $h^2$ ) of All Traits and Genetic ( $\rho_G$ ) and Environmental ( $\rho_E$ ) Correlations Between ACE Activity and LDLC Concentrations**

	ACE Activity	LDLC <sub>basal</sub>	LDLC <sub>HFLC</sub>	LDLC <sub>HFHC</sub>	LDLC <sub>RC</sub>
$h^2$	0.57±0.07	0.59±0.07	0.63±0.08	0.66±0.07	0.54±0.07
$\rho_G$	—	0.08±0.13	0.11±0.15	0.26±0.12*	0.30±0.13*
$\rho_E$	—	0.08±0.11	0.08±0.12	-0.03±0.11	-0.02±0.11

\* $P \leq 0.02$ .

individuals found no relationship between the *I/D* polymorphism and CVD. Also, several studies in humans and transgenic mice have found no relationship between ACE levels and blood pressure,<sup>19</sup> which is somewhat paradoxical given that ACE inhibitors lower blood pressure. Smithies and colleagues<sup>19</sup> present results from a simulation of the RAS pathway to show that because ACE is an intermediate enzyme in the pathway, changes in expression of ACE may not result in changes in blood pressure owing to compensatory changes in Ang I. Although the simulation results correspond with the transgenic mouse studies and some studies in humans, they do not consider possible genotype-by-environment or gene-by-gene interactions that might influence the relationship between ACE polymorphisms, ACE levels, blood pressure, and CVD, and such interactions have been reported in humans.<sup>4,9</sup>

In the current study, we report that serum ACE activity in baboons is moderately heritable, as are serum ACE

levels in humans.<sup>3,4,20</sup> Furthermore, similar to the report by Zhu et al,<sup>4</sup> we detected a QTL for ACE activity (lod score=7.5) located near *ACE* on baboon chromosome 16, which is homologous to human chromosome 17.<sup>14</sup> Again, similar to some reports in humans,<sup>4,21</sup> this QTL accounted for a substantial amount (approximately two thirds) of the genetic variation in ACE activity in baboons. To determine whether there was a relationship between ACE activity and blood pressure, we also assayed ACE activity in a small group of 118 four-year-old baboons. Blood pressure in unanesthetized animals was measured as previously described<sup>22</sup> for the purposes of another study (H.C. McGill, Jr, unpublished observations, 2002). After accounting for significant sire effects, we found a significant relationship ( $P=0.02$ ) between ACE activities and mean blood pressure in these animals (C.M. Kammerer and D.L. Rainwater, unpublished observations, 2002). Although this study is very small, and the results are preliminary, one reason that

**TABLE 3. Results of One-Locus Bivariate Analyses of Pleiotropy**

Model Characteristics	Model No.					
	1	2	3	4	5	6
Model includes:						
LDLC <sub>RC</sub> QTL	Yes	Yes	Yes	Yes	No	No
QTL pleiotropy	Yes	No	Yes	No	No	No
$\rho_G$ and $\rho_E$	Yes	Yes	No	No	Yes	No
Parameter:						
Frequency (R)	0.74	0.74	0.74	0.74	—	—
Trait=LDLC <sub>RC</sub> , mmol/L						
$\mu_{RR}$	1.72	1.72	1.72	1.72	1.77	1.77
$\mu_{Rr}$	1.80	1.80	1.80	1.80	= $\mu_{RR}$	= $\mu_{RR}$
$\mu_{rr}$	2.18	2.18	2.18	2.18	= $\mu_{RR}$	= $\mu_{RR}$
SD	0.12	0.12	0.12	0.12	0.16	0.16
$h^2$	0.15	0.15	0.15	0.15	0.55	0.54
Trait=ACE activity, U/L						
$\mu_{RR}$	187.7	197.5	188.8	197.3	195.9	197.3
$\mu_{Rr}$	202.9	= $\mu_{RR}$	202.6	= $\mu_{RR}$	= $\mu_{RR}$	= $\mu_{RR}$
$\mu_{rr}$	222.0	= $\mu_{RR}$	222.0	= $\mu_{RR}$	= $\mu_{RR}$	= $\mu_{RR}$
SD	49.8	51.0	50.0	51.0	51.0	51.0
$h^2$	0.44	0.47	0.44	0.47	0.47	0.47
$\rho_G$	-0.17±0.32	0.17±0.29	[0]	[0]	0.30±0.13	[0]
$\rho_E$	0.05±0.09	0.07±0.09	[0]	[0]	-0.02±0.11	[0]
Ln-likelihood	-4370.607	-4374.306	-4370.779	-4375.265	-4418.441	-4422.844
$\chi^2$ vs model 1 (df)	—	7.40 (2)	0.34 (2)	9.36 (4)	95.7 (5)	104.5 (7)
$P$	—	0.02	NS	0.05	10 <sup>-17</sup>	10 <sup>-18</sup>

Values in brackets were fixed in the analysis.

we may have found a relationship between ACE activities and blood pressure, whereas some studies in humans have not, is that both the ACE activities and blood pressure were obtained on animals of similar age and on a controlled diet.

One risk factor that may interact with RAS to affect blood pressure and development of CVD is cholesterol. Several investigators have recently reported that in both normotensive and hypertensive individuals,<sup>1,2</sup> serum LDLC was the best predictor of blood pressure response to Ang II infusion, a method that is used to characterize essential hypertension. Because dietary cholesterol raises serum cholesterol levels to varying degrees in humans and other animal model species,<sup>12</sup> it is perhaps not surprising that investigators on the MRFIT study of 11 342 men reported a relationship between dietary cholesterol and blood pressure.<sup>23</sup> Figueroa and Vijayagopal<sup>24</sup> suggested that a mechanism by which the RAS may promote atherogenesis is via modulation of proteoglycans in smooth muscle cells. They reported that in smooth muscle cells, Ang II stimulated proteoglycan synthesis, and the synthesized proteoglycan bound LDL with high affinity. Thus, elevation of Ang II not only would increase blood pressure but also could contribute to the initial stages of atherogenesis.

In our study of baboons, we detected genotype-by-environment interaction effects on the correlation between ACE activities and LDLC. In other words, there was no relationship between ACE activities and LDLC on the basal or high fat diets, even though ACE activities were measured on the latter (LCHF) diet. Instead, we detected strong genetic correlations on the high-cholesterol diet, and we have previously reported that a major QTL located on baboon chromosome 4 affects this LDLC response in baboons.<sup>13,15</sup> Subsequent bivariate genetic analyses revealed that this previously detected QTL accounts for most of the observed correlation between ACE activities and LDLC. The presence of this genotype by environment interaction on the relationship between ACE activities and LDLC is consistent with reported associations between RAS, blood pressure, and LDL cholesterol in some studies. In addition, the presence of such genotype by diet interactions in humans could help explain the lack of associations reported in other studies in which it was not possible to control dietary factors.

### Perspectives

Evidence is accumulating that gene-by-gene and gene-by-environment interactions affect risk factors for CVD, although identification of such interactions is difficult and the mechanisms by which such interactions occur is unclear. Our study provides evidence that at least 2 genes affect ACE activity levels in baboons: one that may be the ACE structural locus, and one that exerts diet-dependent pleiotropic effects on blood pressure regulation and lipid metabolism.

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