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Parallel Effects of Genetic Variation in ACE Activity in Baboons and Humans

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ABSTRACT Like humans, savannah baboons (*Papio* sp.) show heritable interindividual variation in complex physiological phenotypes. One prominent example of such variation involves production of the homeostatic regulator protein angiotensin converting enzyme (ACE), which shows heritable variation in both baboons and humans. In humans, this phenotypic variation is associated with an *Alu* insertion–deletion polymorphism in the *ACE* gene, which explains approximately half of the variation in serum ACE activity. We identified a similar *Alu* insertion–deletion polymorphism in the baboon *ACE* homologue and measured its frequency in a wild population and a captive population of baboons. We also analyzed the contribution of *ACE* genotype at this indel to variation in serum ACE activity in the captive population. When conditioned on weight, a known factor affecting ACE activity in humans, age and *ACE* genotype both accounted for variance in ACE activity; in particular, we identified a significant nonadditive interaction between age and genotype. A model incorporating this interaction effect explained 21.6% of the variation in residual serum ACE activity. Individuals homozygous for the deletion mutation exhibited significantly higher levels of ACE activity than insertion–deletion heterozygotes at younger ages (10–14 years), but showed a trend towards lower levels of ACE activity compared with heterozygotes at older ages ($\geq$15 years). These results demonstrate an interesting parallel between the genetic architecture underlying ACE variation in humans and baboons, suggesting that further attention should be paid in humans to the relationship between *ACE* genetic variation and aging. Am J Phys Anthropol 134:1–8, 2007. © 2007 Wiley-Liss, Inc.

Normal homeostatic regulation of blood pressure, salt balance, and body temperature is essential for basic health. The maintenance of fluid homeostasis therefore represents an important selective factor in the evolution of the primate lineage and may have been of particular importance in species that evolved in water-limited environments, including humans and other savannah-dwelling primates. Humans show heritable patterns of intraspecific variation in both basal measures of homeostasis and in the ability to adjust to environmental perturbations, suggesting that genetic factors contribute to these phenotypes (e.g., blood pressure: Lifton, 1996). However, identification of these factors is challenging due to the complex genetic architecture underlying these traits, including interactions between multiple genetic loci and between genetic variants and the environment.

One of the most well studied relationships between physiological phenotype and complex genetic architecture involves the gene for angiotensin converting enzyme (*ACE*). ACE is responsible for the catalytic conversion of the physiologically inactive compound angiotensinogen into the physiologically active compound angiotensin II, which helps control fluid and sodium retention, blood volume fluctuations, and vasoconstriction (Dostal et al., 1994). High concentrations of serum ACE are a risk factor for the development of certain types of cardiovascular disease (CD), including chronic hypertension and myocardial infarction (Ehlers and Riordan, 1989). Indeed, ACE inhibitors are often used to treat patients who show evidence of, or are considered at high risk of, developing CD. However, levels of serum ACE vary up to twofold even among healthy people, such that some individuals tend to have constitutively high levels of activity (Cambien et al., 1988; Rigat et al., 1990; Alhenc-Gelas et al., 1991).

In humans, serum ACE levels have a strong heritable component (Cambien et al., 1988; Cooper et al., 2000). About 50% of variation in serum ACE can be explained by association with a single *Alu* element insertion/deletion polymorphism within intron 16 of the gene (Rigat et al., 1990; Tiret et al., 1992). Individuals homozygous for the *ACE* deletion (D) allele, which lacks the polymorphic *Alu* element, exhibit higher levels of circulating ACE than those homozygous for the alternative insertion (I) allele, in which the *Alu* insertion is present. *ACE* ID heterozygotes express an intermediate phenotype (Rigat et al., 1990). In agreement with the polymorphism's physiological importance, the D allele has been associated with a higher risk of cardiovascular disease (Cam-

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bien et al., 1992; Samani et al., 1996; Seyoum et al., 2000; but see Winkelmann et al., 1996; Keavney et al., 2000), while the low-risk I allele has been associated with elite athletic performance and improved response to physical training, especially in endurance-related tasks (Montgomery et al., 1997; Gayagay et al., 1998; Moran et al., 2006). Curiously, despite the association between the D allele and cardiovascular pathologies, this allele has also been linked to centenarian-level longevity (Schachter et al., 1994; Rahmutula et al., 2002). Such an association suggests that although D allele homozygotes may be at a disadvantage in comparison to I allele homozygotes earlier in life, in later life the D allele may confer longevity- or survival-related benefits. This idea is supported by the findings of Gardemann et al. (1998), who described an elevated risk of coronary artery disease (CAD) for carriers of the D allele, but only in patients less than 61.7 years of age (the mean age in their study population). Above 61.7, they identified no relationship between CAD and *ACE* genotype (Gardemann et al., 1998). Combined with known evidence of epistatic and gene by environment interactions involving *ACE* genotype (Ye et al., 2003; Moran et al., 2005), these results suggest that the ACE phenotype and related downstream phenotypes are products of a complex set of underlying genetic interactions.

Here, we extend investigation of ACE-related genotype–phenotype relationships to an important nonhuman primate model of physiology and human health, the savannah baboon (*Papio* sp.). Savannah baboons are restricted to Africa, but like humans they have adapted to a very wide range of environments, from near desert to temperate montane grassland to moist evergreen forest (Jolly, 1993; Kingdon, 1997). Also like many ancient humans, most populations of baboons live in water-limited environments that experience strong seasonal changes in rainfall or temperature (Dunbar, 1990; Kingdon, 1997). Thus, like humans, baboons are subjected to ecological variability that is directly relevant to the evolution of homeostatic regulation of body fluids. Baboons are therefore a highly informative model for investigating the functional effects of genetic variation in a comparative evolutionary context.

Current knowledge about the genetic architecture underlying variation in baboon serum ACE activity suggests gene-specific similarities to humans. In particular, interindividual variation in circulating ACE has been demonstrated to have a heritable component in baboons attributable in part to a locus on baboon chromosome 17 near the baboon homologue of the human *ACE* gene (Kammerer et al., 2003). This evidence suggests that a segregating variant in the baboon *ACE* gene may associate with variation in baboon ACE activity in a manner similar to that observed in humans. Here, we report a novel baboon-specific *ACE Alu* polymorphism segregating within both captive and wild savannah baboon populations. We test the hypothesis that this polymorphism is associated with *ACE*-related physiological differences by asking whether genotype at this locus contributes to variation in serum ACE activity in the captive population.

MATERIALS AND METHODS

Subjects

This study utilized genetic samples from two populations of savannah baboons and serum protein samples from one of these populations. The first set of samples was obtained from a captive breeding colony of Guinea baboons (*Papio papio*) in residence at the Brookfield Zoo in Brookfield, Illinois (Lacy and Foster, 1988; Bruford and Altmann, 1993 for the history of this population). DNA was extracted from whole blood from 65 individuals in the Brookfield colony for molecular genetic analysis, representing close to exhaustive sampling of this population. At a later date, fresh serum samples were obtained from the subset of these 65 individuals still in residence at the colony ($n = 32$, ages 10–22 years).

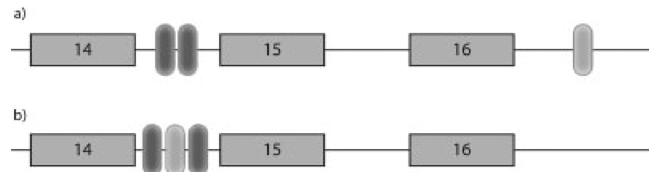

Fig. 1. Schematic of exons 14–16 (solid boxes) and intervening introns 14–16 (lines) of (**a**) human *ACE* (*Alu* indel in intron 16) and (**b**) baboon *ACE* (*Alu* indel in intron 14). Dark ovals represent fixed *Alu* polymorphisms near the baboon polymorphic *Alu* indel. Light ovals represent polymorphic *Alu* indels.

The second set of samples was obtained from a wild population of yellow baboons (*Papio cynocephalus*) from the Amboseli basin, Kenya. This population has been the subject of continuous study since 1971, with extensive demographic and behavioral data collected on a near-daily basis since that time (Altmann and Alberts, 2003; Alberts et al., 2006). DNA samples extracted from whole blood from 108 Amboseli individuals were used for molecular genetic analysis of the *ACE* locus; however, because fresh serum samples were unavailable for the Amboseli baboons, no Amboseli animals were used in serum angiotensin converting enzyme (ACE) assays. In contrast to the Brookfield sample set, the genotyped individuals from Amboseli represent a much smaller subset of the overall Amboseli study population. Nonetheless, our sampling and genotyping strategy was not subject to any known bias, and our dataset should represent a random sample from the population with respect to *ACE* genotype.

Identification of a baboon-specific *ACE* polymorphism

Kammerer et al. (2003) identified a major quantitative trait locus (QTL) for variation in baboon ACE protein activity located near the baboon *ACE* homologue on chromosome 17. This result is consistent with a number of studies that have identified functionally important variation in nonhuman primates at or near sites homologous to known functional variants in humans (Trefilov et al., 2000; Newman et al., 2005; Loisel et al., 2006; Wooding et al., 2006). Although the human *ACE* indel may not itself be causally responsible for the associations observed in humans, the substantial correlation between *ACE* I/D genotype and ACE activity suggests that the functional variation is at least tightly linked to that region. Our initial attempts to identify polymorphisms in the baboon *ACE* gene therefore concentrated on baboon *ACE* intron 16, the locus homologous to the known *ACE* indel in humans (Fig. 1). Examination of baboon intron 16 revealed that no polymorphism homologous to the human indel is present in either of the two populations we sampled. This result is consistent with coalescent-based dating of the human *Alu* insertion in intron 16 to

a date after the divergence between humans and chimpanzees (Rieder et al., 1999), and is also consistent with the classification of the human *ACE Alu* insertion in the $AluY_a$ group, which is specific to hominids (Kapitonov and Jurka, 1996: dated as subfamily Sb).

We also investigated possible polymorphisms in baboon *ACE* intron 14. In humans, intron 14 of the *ACE* gene exhibits a high level of sequence variation second only to intron 16, which harbors the human I/D polymorphism. It also contains several SNP variants in absolute linkage disequilibrium with the *Alu* I/D polymorphism (Rieder et al., 1999). Amplification of the baboon intron 14 homologue resulted in the identification of an insertion–deletion polymorphism segregating in both the Brookfield and Amboseli baboon populations. Further analysis of the insertion polymorphism showed it to be of approximately the same length ($\sim$300 base pairs) as the human *ACE* polymorphism in intron 16. We therefore termed the two alleles defined by this polymorphism I_b (the insertion allele) and D_b (the deletion allele).

PCR amplification and genotyping

Individuals from the Brookfield and Amboseli populations were PCR-genotyped for the identified polymorphism using a set of primers originally designed in humans (Rieder et al., 1999) that bounds the locus of interest: ACE180 (5′-AATATGACCGGACATCCCAG-3′) and ACE181 (5′-GCTCTGTGGCCTTTCTGAAC-3′). In humans, these primers amplify a nonpolymorphic region of exon 14 and most of intron 14 (Rieder et al., 1999) (Fig. 1).

Reaction mixtures were 25 μL in total volume and contained 10.4 μL ddH_2O, 2.5 μL 10× PCR buffer (Applied Biosystems or Invitrogen), 3 μL of 25 mM $MgCl_2$ (Applied Biosystems or Invitrogen), 1.25 μL dimethyl sulfoxide (DMSO), 6.25 μL of 2 mM dNTPs (Invitrogen), 5 pM each of forward and reverse primer (Integrated DNA Technologies), and 0.5 U Taq polymerase (Applied Biosystems). DMSO was added in order to alleviate genotyping errors caused by allelic dropout (Odawara et al., 1997). Cycling conditions were as follows: 1) 80°C for 2 min; 2) 30 cycles of 94°C for 1 min; 54°C for 1 min; and 72°C for 1 min; 3) 72°C for 5 min.

PCR products were visualized on 1% agarose gels stained with ethidium bromide and compared against a 100 base pair ladder. Amplification of the baboon deletion allele (D_b) and insertion allele (I_b) yielded PCR products of $\sim$900 base pairs and 1,200 base pairs, respectively. Baboons were genotyped as D_bD_b if one band appeared at the 900 base pair mark, I_bI_b if one band appeared at the 1,200 base pair mark, and D_bI_b if one band appeared at 900 bp and a second band appeared at 1,200 bp. 37 apparent homozygotes were retyped in separate PCR reactions to check for allelic dropout. We did not retype the remaining homozygotes because among these 37 individuals, we found no incorrectly genotyped individuals.

Sequencing

Both insertion and deletion alleles were sequenced in both directions on an ABI 3700 automated sequencer using BigDye Terminator reagents (Applied Biosystems) (GenBank accession number EF101915). We used ACE180 and ACE181 as sequencing primers in addition to ACEF1 (5′-GCCTGAGCAACAGAGGGATA-3′), an internal sequencing primer that we designed that anneals directly 5′ of the polymorphic indel. The resulting sequence was visualized using the program Sequencher v. 3.1.1 (GeneCodes) and the insertion sequence was scanned for common sequence repeats using the program RepeatMasker (Smit et al., 1996–2004).

ACE serum assay

Serum samples were obtained during routine health exams for the Brookfield baboon colony. Three to five milliliters of whole blood were drawn by femoral venipuncture from anaesthetized individuals. Blood samples were stored in red-top Vacutainer tubes (BD Vacutainer) without EDTA or anticoagulants. Blood samples were allowed to clot for 2–3 h and then centrifuged for 10 min at 2,500 rpm to separate serum from blood cells. Serum was pipetted away from the blood sample and stored in 200–500 μL aliquots in thin-walled PCR tubes at −20°C until analysis.

Serum ACE activity was determined using a diagnostic ACE assay kit from the American Laboratory Products Company (ALPCO). ACE activity was measured using the spectrophotometric method described by Holmquist et al. (1979), in which cleavage of the synthetic ACE substrate *N*-[3-(2-furyl)acryloyl]-L-phenylalanylglycylglycine (FAPGG) is monitored by a linear decrease over time in FAPGG absorbance at 340 nm. Twenty-five microliters of serum and 250 μL of a buffer/substrate solution were placed in a 10 mm microcuvette (Starna) and analyzed by spectrophotometer for 10 min at 37°C. The slope of the line produced by absorbance (AU) versus time was used as a measure of ACE activity and was translated into ACE units by comparison against a kit standard (1 ACE unit = amount of ACE required to cleave one micromole hippuric acid per minute per liter serum at 37°C).

We performed up to five replicate assays on $n = 30$ serum samples from Brookfield (median number of assays = 3); the three animals for whom we performed fewer than two replicate assays were excluded from subsequent analysis. Replicate assays were performed on separate days, using different serum aliquots from the same blood draw date. Aliquots were used only once each in order to minimize freeze–thaw effects on protein viability. Although spectrophotometric quantification of ACE activity by FAPGG cleavage was developed in humans, previous work has shown that this methodology is also applicable to baboons (Kammerer et al., 2003), most likely because of the high amino acid sequence conservation between the ACE proteins of humans and cercopithecine primates (comparison of the publicly available *ACE* sequence from the human and macaque genomes shows $\sim$97% sequence identity).

All individuals assayed for serum ACE were adult baboons between the ages of 10 and 22 years and in good health at the time of the blood draw. Because of sampling constraints, we were unable to conduct repeated assays of ACE activity over time within individuals; however, although ACE activity shows significant interindividual variation, evidence from humans suggests that intraindividual ACE activity is comparatively stable (Alhenc-Gelas et al., 1991).

Analysis of the genotype–phenotype relationship

We analyzed the possible contribution of *ACE* genotype to median ACE activity across individuals within

the captive Brookfield population. We focused only on D_b homozygotes ($n = 12$) and D_b/I_b heterozygotes ($n = 15$) because only three individuals within the ACE serum sample set were I_b homozygotes. Median ACE activity for each individual was chosen as the appropriate summary statistic across repeated ACE assays, because a few replicates yielded clear outliers that heavily influenced mean ACE activity values. The distribution of median ACE values for our sample did not deviate from a fitted normal distribution (Shapiro-Wilk W test: $P = 0.628$).

The literature on human ACE suggests that, although *ACE* I/D genotype accounts for approximately half of the observed variation in circulating serum ACE (Rigat et al., 1990), serum ACE concentrations and related phenotypes are the result of complex genetic interactions. In addition, our sample of baboons was highly heterogeneous with respect to sex, age, and body mass, factors that have been suggested to affect ACE activity (Gardemann et al., 1998; Harp et al., 2002; Kammerer et al., 2003; Engeli et al., 2005; Moran et al., 2006). We therefore included age, sex, and body mass in analyzing the effect of baboon *ACE* genotype on ACE activity.

We used the residuals from a regression of median ACE activity on body mass as the dependent variable in our models. In doing so, we asked whether any of the variation not explained by the known effect of mass could be explained by the independent effects of sex or age. We chose to use residuals instead of directly analyzing median ACE activity itself because of collinearity between two potentially important variables in the analysis. Baboons are a sexually dimorphic species in which males can attain close to twice the body mass of females, as exemplified by data on our captive subjects (males: mean body mass = 25.14 kg., range = 21.00—21.87 kg.; females: mean body mass = 18.48 kg., range = 14.5—21.85). Thus, body mass is strongly correlated with sex in this species ($P < 0.0001$, $R^2 = 0.670$, $n = 27$). Because the effect of body mass is difficult to disentangle from the effect of sex (i.e., all males are substantially heavier than all females), use of both as independent variables in the analysis would add little explanatory power; indeed, our method of model selection actually penalizes such additional parameterization. Consequently, we chose to examine the effects of sex (and other predictors) after controlling for mass. We chose to analyze the residuals of ACE activity on body mass rather than the residuals of ACE activity on sex for two reasons. First, body mass has been demonstrated in the literature to explain significant variation in ACE activity (Harp et al., 2002; Engeli et al., 2005); consequently, we knew that we would be controlling for a well-established and substantial source of variance that was not the central focus of our analysis. Second, because the three oldest individuals in our dataset were female, age was weakly negatively, but spuriously, correlated with body mass within our dataset ($P = 0.042$, $R^2 = 0.156$, $n = 27$ for the whole dataset, but age and body mass showed no relationship within each sex; females: $P = 0.244$, $R^2 = 0.079$, $n = 19$; males: $P = 0.732$, $R^2 = 0.021$, $n = 8$). In contrast, sex showed no significant relationship with age, because in general males and females were well-distributed across ages ($P = 0.106$, $R^2 = 0.101$, $n = 27$). However, because we could have plausibly chosen to analyze the residuals of the relationship between median ACE activity and sex instead, we also tested the robustness of our best model against an alternative that employed the residuals of ACE activity on sex.

We compared the ability of a series of least squares regression models incorporating all possible combinations of age, sex, genotype, and all two-variable interaction terms between these variables to explain the residual variation in serum ACE activity after regression on body mass. We chose the best model accounting for variation in residual ACE activity by identifying the model with the lowest Akaike Information Criterion (AIC_c) score, which includes a penalty for increased model parameterization and a correction for small sample size.

All statistical analyses were conducted within the statistical package JMP v. 3.2.2 (SAS).

RESULTS

Characterization of the I_b/D_b baboon polymorphism

Sequencing of the insertion and deletion alleles, and analysis using RepeatMasker identified the polymorphic baboon indel as a retrotransposable *Alu* element of the primate-specific family *AluY*. The polymorphic baboon *Alu* indel is flanked by 2 other fixed *Alu* elements, which are also found in the homologous human sequence in *ACE* intron 14 (Fig. 1). Interestingly, the human intron 16 I/D polymorphism associated with variation in human ACE serum activity is a closely related *Alu* element of the *AluY* hominid-specific subfamily, $AluY_a$ (Rigat et al., 1992).

Genotyping

The two allelic variants of the baboon *ACE* indel were found to be segregating within both the Amboseli and the Brookfield baboon populations. However, although both populations were polymorphic for the D_b/I_b *ACE* indel, we documented a large difference in allele frequencies between the two populations (Fig. 2). Specifically, in the Brookfield samples ($n = 65$) the I_b allele segregated at 36.9% and the D_b allele at 63.1%, but within Amboseli ($n = 108$) the I_b allele represented only 4.2% and the D_b allele represented 95.8% of typed alleles within our sample. Neither population deviated from Hardy-Weinberg expectations at the polymorphic site (Fig. 2).

ACE serum assay

Assays of Brookfield baboon serum samples for ACE activity ($n = 27$) resulted in a range of median ACE values from 137.47 to 359. 25 ACE units. These values fall within the range documented by Kammerer et al. (2003) for baboon ACE activity across 622 individuals (35-375 ACE U). Mean coefficient of variation across assays for the same individual was 26.2%.

Factors underlying variation in baboon ACE activity

Comparison of all least squares regression models of residual ACE activity (ACE activity unexplained by body mass) incorporating age, sex, and genotype revealed that the model with the lowest AIC_C value (the best model) included the effects of age, genotype, and an interaction of genotype with age ($R^2_{adj} = 0.216$, $P = 0.036$). The evidence ratio between this model and the second best model (genotype alone) was 3.46, suggesting that the strength of support for the best model was over three times greater than for the next best alternative model.

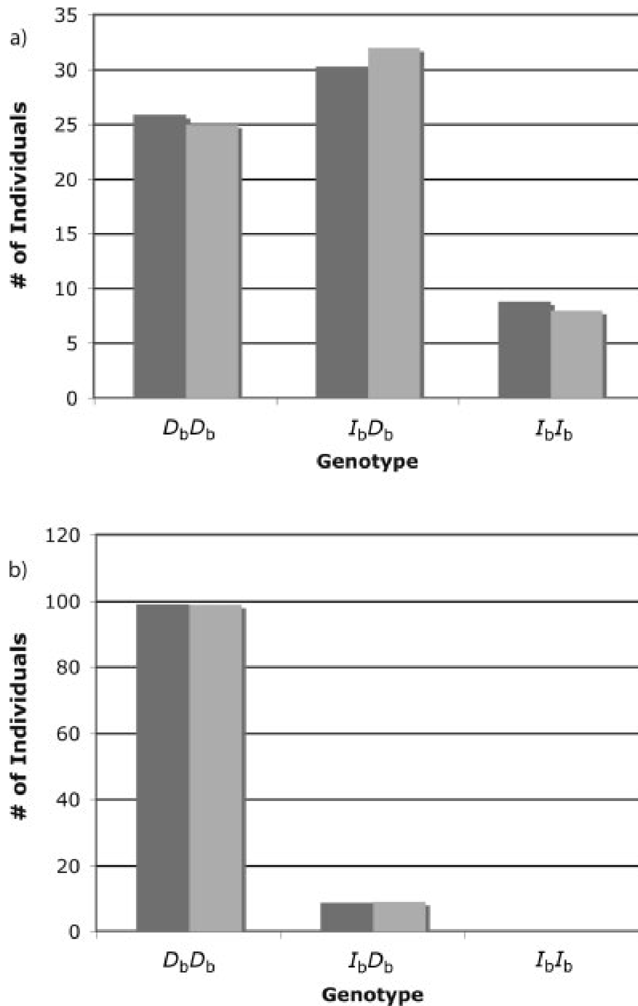

Fig. 2. Observed (dark gray) and expected (light gray) genotype frequencies in (**a**) Brookfield (n = 65) and (**b**) Amboseli (n = 108). Neither population significantly deviates from Hardy-Weinberg expectations.

An increased sample size would be likely to increase the support for this model over the nearest alternatives, because the higher parameterization of this model relative to these alternatives is penalized to a larger degree by the small sample size correction. The age + genotype + age × genotype model also significantly explained variation in residual ACE activity that was unexplained by sex (R^2_{adj} = 0.248, P = 0.022), demonstrating that these results are robust to which of the two correlated variables, sex or body mass, was conditioned out of the analysis.

The age, genotype, and age × genotype model explained over 20% of the variation in baboon ACE activity after taking into account the effect of body mass. This result was driven largely by the interaction effect between genotype and age (Table 1). Removal of the genotype by age term produced a nonsignificant overall model that reduced the explained variation in residual ACE activity by 21.6% (R^2_{adj} = 0.000, P = 0.402).

The age by genotype interaction effect

Interindividual variation in ACE activity was primarily driven by the effects of body mass and the age × genotype interaction, suggesting that in baboons, as in humans, *ACE* genotype is one of several important factors underlying variation in serum ACE activity.

TABLE 1. Parameter estimates and standard errors for terms in the best fit model for residual ACE activity: $a + g + g \times a$

Term	Estimate	Standard error	P
Intercept	−34.835	51.943	–
Genotype	−15.308	9.686	0.128
Age	2.312	3.447	0.509
Genotype × age	9.580	3.337	0.011

Overall model: R^2_{adj} = 0.216, N = 27, P = 0.036.
P-values describe the probability that the size of an individual effect is actually equal to 0. Genotype is treated as a continuous regressor variable within the model in order to estimate variance components. Only the genotype × age effect is statistically significant as a model effect.

The genotype by age interaction indicates that, although D_bD_b individuals exhibited higher levels of ACE activity at younger ages compared to D_bI_b heterozygotes, this trend disappeared at more advanced ages. This result was further supported when we examined the correlation between genotype and median ACE activity but stratified this analysis by age (Fig. 3a,b), or conversely when we regressed ACE activity on age separately for each genotype (Fig. 3c,d). We found a strong relationship between genotype and ACE activity in individuals below the median age of 15 years (P = 0.0006, R^2 = 0.709, n = 12), and a nonsignificant trend in individuals 15 and older (P = 0.113; R^2 = 0.182, n = 15). D_bD_b individuals exhibited a negative correlation between ACE activity and age (P = 0.030, R^2 = 0.392, n = 12), whereas D_bI_b baboons showed a suggestive positive correlation between these two variables (P = 0.079, R^2 = 0.218, n = 15). Consequently, in older individuals the distribution of ACE activity values is actually shifted higher in heterozygotes relative to D_bD_b homozygotes.

DISCUSSION

We report an interacting effect of age and genotype that, together with the additive effects of age and genotype, significantly explained 21.6% of the phenotypic variation in baboon serum ACE activity after controlling for the small but statistically significant effect of body mass. Although D_bD_b individuals exhibited a higher level of ACE activity as young adults than D_bI_b heterozygotes, this pattern was statistically undetectable in older adults, and in fact the trend for older adults was in the opposite direction. Modeling baboon ACE activity with this set of parameters explained 21.6% more variation in the overall ACE residuals dataset compared to the same model without the age by genotype interaction effect, and exhibited approximately three and a half times the support of the next best model. While the age at which the genotype-ACE activity relationship seems to disappear or reverse (age 14–15) is advanced for baboons (Alberts, 1995; Alberts and Altmann, 2003; Alberts et al., 2006; Beehner et al., 2006), many individuals do live past fifteen years in the wild (maximum observed lifespan in Amboseli is 27 years for females and 22 years for males: see Alberts and Altmann, 2003). Thus, the described age by genotype interaction effect is likely to be relevant in the context of natural populations.

Although our sample size is small, previous reports of a major QTL for ACE activity near the baboon *ACE* gene give us increased confidence that we have identified a

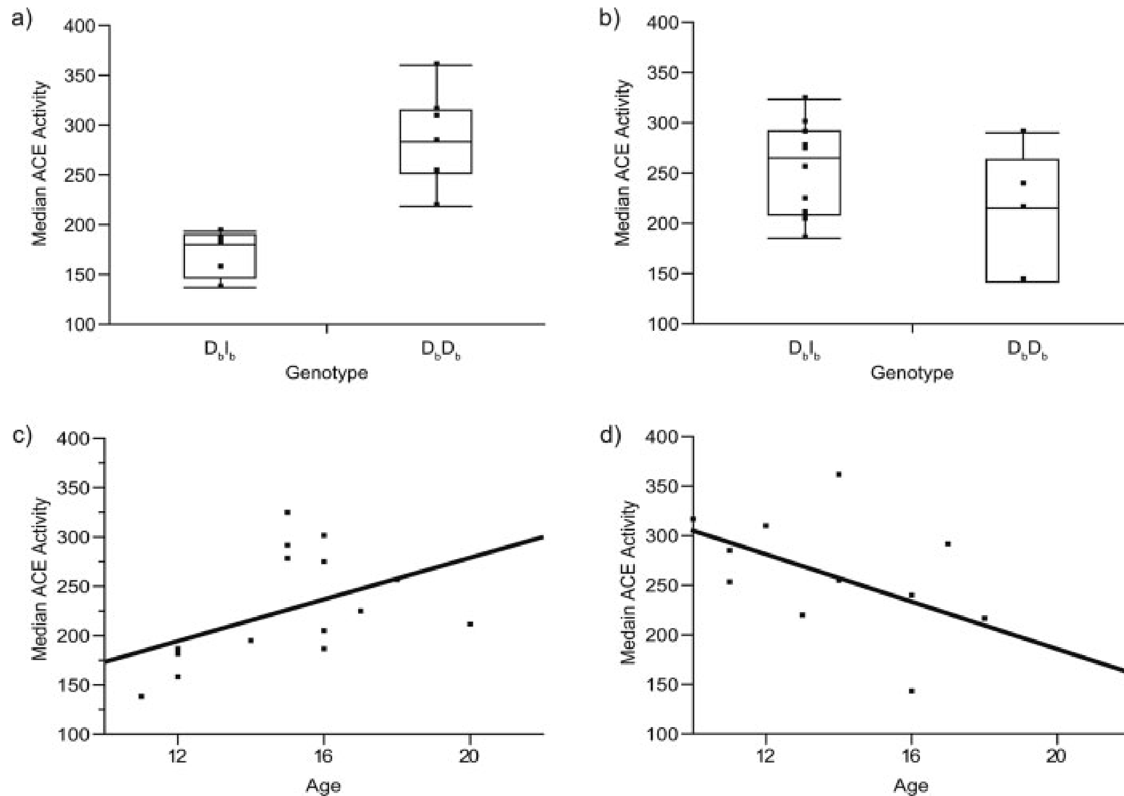

Fig. 3. Bivariate relationships between *ACE* genotype and median ACE activity for (**a**) subjects below the median age of 15 years ($P = 0.0006$, $R^2 = 0.709$, $n = 12$); (**b**) subjects at or above the median age of 15 ($P = 0.113$; $R^2 = 0.182$, $n = 15$); and univariate regressions of median ACE activity on age for (**c**) D_bI_b individuals ($P = 0.079$, $R^2 = 0.218$, $n = 15$); (**d**) D_bD_b individuals ($P = 0.030$, $R^2 = 0.392$, $n = 12$).

real effect (Kammerer et al., 2003). Unlike Kammerer et al. (2003), we identified no effect of sex independent of body mass effects. Their sex effect may have resulted from the very strong relationship between sex and body mass in baboons, which we accounted for by analyzing the residuals of the regression of ACE activity on body mass instead of ACE activity directly. Thus, our analysis of the effects of sex on ACE activity, unlike that of Kammerer et al. (2003), was independent of the effect of body mass. Kammerer et al. (2003) also identified a negative correlation between ACE activity and age. Our analysis demonstrates that the magnitude of that effect is conditional on *ACE* genotype.

The interactive effects of genotype and age on serum ACE activity in our sample are particularly suggestive in the context of results from human studies. In humans, the D allele appears to confer a seemingly paradoxical survival advantage in very long-lived individuals, despite its association with increased susceptibility to cardiovascular disease (Schachter et al., 1994; Rahmutula et al., 2002), a major source of mortality in many human populations. Because many studies conducted on humans have conducted ACE activity analyses on age-matched samples or have excluded elderly participants (Rigat et al., 1990; Winkelmann et al., 1996), it seems possible that the human *ACE Alu* polymorphism, like the baboon polymorphism, may also interact with age in explaining ACE phenotypic variation. This possibility is suggested by the results of Gardemann et al. (1998), who found that the development of coronary artery disease is related to human *ACE* I/D genotype in individuals younger than their mean population age of 61.7, but not in older individuals. If our sample had included only adult baboons in their prime (ages 10–14), we also would have identified a straightforward relationship between *ACE* genotype and serum ACE activity, but no age by genotype interaction (Fig. 3).

Recent evidence from studies in humans (Hoffjan et al., 2005), nonhuman primates (Barr et al., 2004; Newman et al., 2005), and animal model systems (Dilda and Mackay, 2002; Montooth et al., 2003) demonstrates that interaction effects between multiple genetic variants and between genetic variants and the environment are major contributors to the genetic architecture of complex traits. Consistent with these findings, we identified a significant interaction effect that influenced variation in serum ACE activity, a physiologically important complex trait. However, the genotype by age interaction we have documented is difficult to classify as either a G $\times$ E effect or an epistatic effect. We surmise that "age" is a variable capturing aspects of both environmental and genetic effects that contribute to the aging process. Thus, the age by genotype interaction we have documented may in fact represent a quantitatively accessible proxy for a more specific, age-related change in baboon physiology, such as a change in the proportion of fat mass versus muscle mass, or a change in general cardiovascular health.

Our results also suggest some intriguing similarities between the genetic architecture of ACE activity in humans and baboons. As in humans, the baboon *ACE* gene is polymorphic for an intronic *Alu* insertion–dele-

tion variant identifiable across multiple populations. In both species, genotype at the polymorphic *Alu* indel is a significant predictive factor for ACE activity. Our results join a growing body of work suggesting that variation within homologous genes across primate species may have similar intraspecific functional effects (Trefilov et al., 2000; Barr et al., 2004; Wang et al., 2004; Newman et al., 2005; Loisel et al., 2006; Wooding et al., 2006). However, it is less clear whether associations that hold across species are due to strictly homologous variation, or instead result from independent evolutionary events (Wang et al., 2004; Wooding et al., 2006). It is also unclear whether the mechanistic manifestation of independently derived functional variation will be similar. Fine mapping and functional studies that identify the human and baboon *ACE* variants specifically underlying these associations will shed light on these outstanding questions.

Interestingly, we detected a large difference in allele frequencies at the polymorphic *ACE Alu* insertion between the captive Brookfield population and the wild Amboseli population. Allele frequencies in Brookfield are similar to those described for the human *ACE Alu* variant, which range between 43 and 61% for the I allele (39–57% for the D allele) in Caucasian and Asian populations (Cambien et al., 1992; Samani et al., 1996; Gayagay et al., 1998). The small size of the Brookfield colony, its management by humans, and documented inbreeding in its history (Lacy and Foster, 1988; Bruford and Altmann, 1993) suggest that the similarity between the Brookfield dataset and human populations is probably coincidental, because allele frequencies in small, inbred populations will be heavily influenced by random genetic drift and founder effects. Alternatively, allele frequencies at the *ACE Alu* polymorphism may be different in guinea baboons than in yellow baboons, possibly due to selection on ACE activity or its downstream phenotypic effects. More detailed population genetic analyses would be required to provide support for one of these hypotheses.

CONCLUSIONS

We identified an *Alu* insertion–deletion polymorphism in the baboon *ACE* gene that shares intriguing similarities with an *Alu* polymorphism in human *ACE*, and tested whether genotype at the baboon *Alu* indel is associated with variation in the activity of serum ACE protein. In our analysis, *ACE* genotype was indeed significantly associated with ACE activity, but this relationship was contingent on age. Young and middle-aged adults that were homozygous for the deletion allele of the *Alu* polymorphism exhibited higher ACE activity than heterozygotes. This effect was undetectable, and potentially reversed, in older baboons.

These results reinforce the utility of studying the genetic architecture of complex traits in nonhuman primates as a model for homologous traits in humans. Identification of parallel polymorphisms such as the *Alu* indels in human and baboon *ACE* will be useful when using nonhuman primates as models of human health and disease. More generally, these results have implications for the management and care of captive primates, and for understanding the consequences of genetic variation in natural populations. Finally, the parallel between our results and those found in humans suggests that related species occupying similar ecological niches may also exhibit similarities in the genetic architecture underlying ecologically relevant traits. Hence, attention to the shared evolutionary and ecological histories of primates will be informative for understanding both human evolution and human health.

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