Sodium-Lithium Countertransport Activity Is Linked to Chromosome 5 in Baboons

Candace M. Kammerer, Laura A. Cox, Michael C. Mahaney, Jeffrey Rogers, Robert E. Shade

Abstract—The genes involved in the regulation of cellular sodium transport characteristics, which are correlated with some forms of essential hypertension, have not yet been identified. We are studying the genes and environmental factors that affect red blood cell sodium-lithium countertransport (SLC) activity and intracellular sodium (ICNa) concentration in 634 baboons that comprise 11 pedigrees of 2 and 3 generations each. To detect and locate possible quantitative trait loci (QTLs) that affect SLC activity and ICNa concentration, we performed a genome screen by using a maximum likelihood–based variance-components linkage analysis program (SOLAR). SLC and ICNa phenotypes as well as genotypes on 281 microsatellite loci were available for all pedigreed animals. Both SLC and ICNa traits were highly heritable (residual heritability 0.593 ± 0.083 [P < 0.0001] and 0.739 ± 0.082 [P < 0.0001], respectively). We obtained evidence that a possible QTL for SLC activity is located on the baboon homologue of human chromosome 4 between D4S2456 and D4S2365 with a maximum multipoint lod score of 9.3 (P < 10⁻⁴⁰) near D4S1645. This QTL accounts for approximately two thirds of the total additive genetic variation in SLC activity in baboons. Although ICNa concentration was highly heritable, we found no evidence for linkage to a QTL with use of this methodology. Thus, we have evidence that a gene located on the baboon homologue of human chromosome 4 (baboon chromosome 5) affects cell sodium transport in baboons. (Hypertension. 2001;37[part 2]:398-402.)

Key Words: blood pressure ■ sodium-lithium countertransport ■ linkage ■ chromosome 5

Abstract

In the present study, we report evidence that SLC activity and ICNa concentration are both highly heritable in baboons, as has been shown in humans. We also report evidence of linkage between a quantitative trait locus (QTL) affecting SLC activity and markers on the baboon homologue of human chromosome 4 (baboon chromosome 5).

Methods

Animal Model

SLC activity and ICNa concentration were measured on 634 noninbred baboons (Papio hamadryas) comprising 11 pedigrees ranging in size from 16 to 99 animals. These 2- and 3-generation pedigrees consisted of 202 founders (29 sires and 173 dams) that were not selected for blood pressure and their 432 offspring. The total 204 males and 430 females had a mean age of 9.4 years and a mean weight of 17.5 kg. All experimental procedures were approved by the Southwest Foundation Institutional Animal Care and Use Committee.

RBC Sodium Transport Phenotypes

Venous blood (20 to 30 mL) was collected into heparinized Vacutainer tubes and processed within 1 hour. The blood samples were drawn from the femoral vein after baboons were immobilized with ketamine (10 mg/kg). RBCs were separated from plasma anduffy coat by centrifugation for 10 minutes at 1000g. A 5 mL aliquot of packed cells was removed and suspended in 20 mL of a

Abstract

...the pathogenesis of some forms of essential hypertension. Numerous studies have reported a relationship between hypertension and increased SLC activity and intracellular sodium (ICNa) concentration in several ethnic groups, including whites and Asians. Ever since the original report by Canessa et al,1 numerous studies have reported a relationship between hypertension and increased SLC activity and intracellular sodium (ICNa) concentration in several ethnic groups, including whites and Asians.2,3 Approximately 50% to 80% of the population variation in SLC activity is attributable to additive genetic effects, and several studies have reported evidence that part of this variation is attributable to segregation at a single locus.4–6 Furthermore, increased SLC activity can be detected in children and adolescents.7,8 These studies suggest that sodium transport characteristics could be useful predictors of the risk of developing hypertension. Identification of the genes responsible for these phenotypes will allow better prediction of risk.

We are studying genetic and environmental factors that affect cellular sodium transport measures in baboons, a primate model used in hypertension research.9–12 In the...
LiCl loading solution for SLC measurements. The remaining RBCs were washed 3 more times, and a final 50% suspension in the washing solution was used to measure ICNa concentration.

ICNa Concentration

The 50% suspension of RBCs was diluted 1:51 with a metal-free nonionic detergent (0.02% Cationix, Scientific Products) to lyse the cells. The sodium concentration was measured by atomic absorption spectrophotometry (Perkin-Elmer). ICNa concentration was calculated as follows: (sodium concentration of 1:51 dilution×51)/hematocrit of suspension.

SLC Activities

The maximal velocity of the SLC was determined by measuring the external sodium-stimulated lithium efflux from lithium-loaded RBCs as previously reported.13 Briefly, RBCs were loaded by incubating 150 mmol/L LiCl for 3 hours at 37°C in a shaking water bath. Aliquots were then added to solution A (150 mmol/L NaCl containing 10 mmol/L ouabain, 10 mmol/L glucose, and 10 mmol/L Tris-MOPS, pH 7.4 at 37°C) or to solution B (150 mmol/L choline chloride containing 10 mmol/L ouabain, 10 mmol/L glucose, and 10 mmol/L Tris-MOPS, pH 7.4 at 37°C) and incubated at 37°C in a shaking water bath. Samples were removed at 0, 30, 50, 70, and 90 minutes and analyzed for lithium concentration by atomic absorption spectroscopy. The RBC efflux rate for incubated RBCs in sodium-free and sodium-containing media was calculated by linear regression analysis of sample time versus lithium concentration. Each sample assay was considered acceptable if the linear regression r² was ≥0.9. The SLC is the difference between the rate of appearance of lithium in solutions A and B expressed as micromoles lithium per liter RBCs per hour. Preliminary studies similar to procedures described by Smith et al14 were conducted to establish the individual parameters for loading baboon RBCs with lithium and for obtaining a linear efflux of lithium for RBCs incubated in sodium-free and sodium-containing media. These studies indicated that baboon RBC intracellular lithium concentration was 4 to 7 mmol/L RBCs after incubating for 3 hours in 150 mmol/L LiCl. Furthermore, the variation in SLC observed by incubating RBCs for 2 versus 3 hours was smaller than the variation observed between individual baboons. Therefore, we chose to standardize the method by using a 3-hour incubation period for lithium loading of RBCs.

Quality control involved submitting the fresh RBC samples as blind duplicates for analysis. Values for each group of analyses were considered acceptable when technical error was <1.5%. The intra-assay variation across the entire study for SLC activity and ICNa concentration was 8.4% and 9.4%, respectively.

Genotypes

Published human primers were used to amplify 279 homologous microsatellite loci from 963 baboon genomic DNA samples. Approximately two thirds of the genotypes were generated by researchers at Axys Pharmaceuticals, Inc, as part of another project. These highly polymorphic microsatellite markers had an average heterozygosity of 0.71 and an average spacing of 7.2 cM.15 Genotypes of the baboons were determined through gel electrophoresis of the fluorescently labeled polymerase chain reaction products in ABI 373 or ABI 377 automated sequencers with Perkin-Elmer Gene Scan software and analysis with Perkin-Elmer Genotyper software.

Statistical Analyses

We used univariate quantitative genetic analysis to assess the residual heritability of SLC activity and ICNa concentration while simultaneously incorporating the effects of covariates such as sex, sex-specific linear and quadratic age, and weight.16 Residual heritability is the proportion of variance due to additive genetic effects, after removing the variation attributable to covariates; thus, it is a measure of the strength of the genetic signal compared with unexplained noise. All parameters were estimated by using maximum likelihood methods. Significance of the residual heritability and the covariate effects was assessed by comparing the likelihood of a submodel (in which the specific parameter to be tested was fixed at 0) with that of model in which all parameters were estimated, with use of the likelihood ratio test (as described in detail elsewhere). This statistic is asymptotically distributed as χ² with 1 df.

We performed a genome scan by using a variance-components method18,19 that was extended for use on full pedigrees.20 We used the program SOLAR20 to estimate the genetic variance attributable to the region around a specific genetic marker (σ²g). This approach is based on specifying the expected genetic covariances between arbitrary relatives as a function of the identity-by-descent (IBD) relationships at a given marker locus assumed to be tightly linked to a locus influencing the quantitative trait. We tested the null hypothesis that the additive genetic variance due to a QTL influencing the sodium transport characteristics equals 0 (no linkage) by comparing the likelihood of this restricted model with that of a model in which the variance due to the marker is estimated. The difference between the 2 log₁₀ likelihoods produces a lod score for linkage. SOLAR has also been expanded to allow for multipoint interval analysis by extending the technique of Fulker et al.21 The significance of the lod scores was assessed by generating the empirical distribution of nominal lod scores. We simulated an unlinked marker locus with 5 equifrequent alleles (heterozygosity 0.80), assigned genotypes to each founder, dropped genotypes down through the actual pedigrees structure based on mendelian expectations, and then performed linkage analyses on each of the simulated markers and the actual phenotypes with the use of SOLAR. We performed 10 000 simulations of unlinked markers. Using regression analyses, we then derived a lod-score adjustment factor, which represented the slope of the empirical lod scores compared with the expected lod scores, and all multipoint lod scores were then adjusted by this value.22 On the basis of these simulations, a lod score equal to P=0.001 between a QTL and a genomic marker was considered to be evidence of linkage.

Results

Mean SLC activity over all animals was 0.242±0.099 mmol lithium/L RBCs per hour and ranged between 0.001 and 0.602 mmol lithium/L RBCs per hour, whereas ICNa concentrations ranged between 0.00 and 14.20 mmol/L RBCs (mean 6.85±2.09 mmol/L RBCs). These SLC and ICNa values are similar to those reported in several human studies.24 SLC and ICNa values that were ±4 SD from the mean were removed before the statistical genetic analyses (a maximum of 3 values for each trait).

The heritabilities of SLC activity and ICNa concentration were high (residual heritability 0.593±0.083 [P<0.0001] and 0.739±0.082 [P<0.0001], respectively), indicating that genes affected much of the variation in these 2 traits (Table). In contrast, covariates accounted for <10% of the total variation in SLC activity (6.8%) and ICNa concentration (1.1%). There were significant effects of sex-specific age and age-squared on SLC activity but not of sex or weight (Table). There were no significant covariate effects on ICNa concentration.

To search for potential QTLs affecting SLC activity and ICNa concentration, we performed genome screens with the use of multipoint variance-components linkage analysis. For SLC activity, we obtained a maximum unadjusted lod score of 6.4 on baboon chromosome 5, which is homologous to human chromosome 4 (Figure 1). There was no evidence for additional QTLs affecting SLC activity; the maximum unadjusted lod score was <1.6 for all other chromosomes. We also obtained no evidence for linkage to any QTLs affecting ICNa concentration; the maximum unadjusted lod score was <1.6 for all chromosomes (results not shown).
As described in Methods, we performed 10,000 simulations of the SLC phenotype with an unlinked polymorphic marker to assess the significance of the multipoint lod scores. We obtained 12 lod scores: 2.00 (expected = 12) and 3 lod scores >3.00 (expected = 1). Based on these simulation results, our nominal lod scores for SLC are slightly inflated, and so we used regression analyses to estimate a lod-score adjustment factor of 0.96. All adjusted multipoint lod scores were modified downward by this factor.

We also identified 2 additional highly polymorphic loci, D4S1554 and D4S3248, genotyped the 634 animals in this study for the 2 markers, and used Multimap24 and CRIMAP25 to map them onto baboon chromosome 5 (Figure 2). The logarithmic odds for placement of these 2 loci were >2.0. Using all available genotypic data for the baboon chromosome 5 map, we recalculated the multipoint IBD matrices for this chromosome and reanalyzed SLC activity for linkage by use of SOLAR (Figure 2). The adjusted maximum multipoint lod score was 9.3 ($P < 10^{-15}$) at 67 cM between markers D4S3248 and D4S2365. This QTL accounts for 0.436 of the total variance and 70% of the additive genetic variance in SLC activity. Thus, we have strong evidence that a QTL for SLC activity is located on the baboon homologue of human chromosome 4.

Discussion

Cation transport characteristics in RBCs are potentially useful as predictors of hypertension because they are correlated with hypertension in different populations, are reliably assayed by different laboratories, are highly heritable, and are measurable in children and young adults.6 In addition, there are reports of a correlation between increased SLC activity and insulin-dependent and non–insulin-dependent diabetes, hyperlipidemia, and nephropathy,3 as well as a relationship between sodium-dependent hypertension, especially nonmodulation,26,27
Although SLC activity is correlated with hypertension, current thinking is that the factors responsible for SLC activity do not have a role in causing pathology per se but reflect aspects of membrane properties that may cause or be caused by disease. Because SLC activity and ICNa concentration are both highly heritable, identification of the gene(s) that affects SLC activity or ICNa concentration would facilitate our understanding of the mechanisms by which these cation transport phenotypes may be associated with hypertension. With the exception of an association and the QTL for SLC on baboon chromosome 5 (homologous to human chromosome 4) are shown.

We have reported previously that systolic blood pressure and diastolic blood pressure are heritable in baboons. In a small study of 84 baboons, we observed that mean SLC activity was significantly higher (P<0.00001) among offspring of sires with high blood pressure versus offspring of sires with low blood pressure (0.286±0.015 versus 0.207±0.016 μmol lithium/L RBCs per hour, respectively; C.M. Kammerer and R.E. Shade, unpublished data, 2000). These results indicate that baboons should be a useful model for genetic and physiological studies of cell sodium transport characteristics and blood pressure.

Multipoint genome screens were performed in the baboons to locate possible QTLs affecting SLC activity or ICNa concentration. Although both traits were highly heritable, we found no evidence for linkage of possible QTLs for ICNa concentration by using this methodology and these covariates. In contrast, we found strong evidence for linkage (adjusted maximum lod score 9.3) between a QTL for SLC activity and microsatellite markers in the region between D4S414 and D4S413 on the baboon homologue of human chromosome 4. This result is consistent with a small sib-pair study linking SLC activity in white men to the MN blood group, which is also located on chromosome 4. As yet, we have identified no strong candidate genes for SLC activity in this chromosomal region, although we are continuing the development of additional microsatellite markers to narrow the chromosomal region of linkage. Future studies to identify and characterize this gene may elucidate fundamental processes that contribute to the development of hypertension.

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