

Quantitative Loci Regulating Plasma Levels of γ Glutamyl Transferase and Albumin and Their Genetic Correlations with Cardiovascular Risk Factors

TANUSHREE BOSE,^{*,†,1} V. SAROJA VORUGANTI,[‡] M. ELIZABETH TEJERO,[‡]
J. MICHAEL PROFFITT,[‡] LAURA A. COX,^{‡,§} JOHN L. VANDEBERG,^{‡,§}
MICHAEL C. MAHANEY,^{‡,§} JEFFREY ROGERS,^{‡,§} JEANNE H. FREELAND-GRAVES,^{*}
SHELLEY A. COLE,[‡] AND ANTHONY G. COMUZZIE,^{‡,§}

**Division of Nutritional Sciences, Department of Human Ecology, The University of Texas at Austin, Austin, Texas 78712; †Department of Food Science and Nutrition, California Polytechnic State University, San Luis Obispo, California 93407; ‡Department of Genetics, Southwest Foundation for Biomedical Research, San Antonio, Texas 78245-0549; and §Southwest National Primate Research Center, San Antonio, Texas 78245-0549*

γ Glutamyl transferase (GGT) and albumin (ALB) are two markers of liver function. These two proteins have been associated with non-alcoholic fatty liver disease and cardiovascular disease. The objectives of this study were to explore the genetic factors that influence variation in the plasma levels of GGT and ALB and to evaluate their genetic correlations with cardiovascular risk factors. Baboons from the Southwest National Primate Research Center at the Southwest Foundation for Biomedical Research, San Antonio, TX, were used as an animal model. The baboons were fed a standard monkey chow diet *ad libitum*. Fasting plasma concentrations of GGT, ALB, triglycerides, total cholesterol and LDL cholesterol were measured in 350 pedigreed adult baboons by standard assay procedures. A maximum likelihood-based variance decomposition approach implemented in the computer program SOLAR was used to conduct genetic analyses. The heritabilities of GGT ($h^2 = 0.55$; $P < 0.0001$) and ALB ($h^2 = 0.42$; $P < 0.01$) were significant. No statistically significant associations were found

between GGT and the cardiovascular-related phenotypes. Genetic correlations between ALB and total cholesterol, LDL cholesterol and triglycerides were significant. A QTL (LOD = 2.8) for GGT plasma levels was identified on the baboon homologue of human chromosome 22 between markers *D22S304* and *D22S280*. A QTL (LOD = 2.3) near marker *D10S1432* was detected on the baboon homologue of human chromosome 10 for ALB. These results imply that variations in the plasma levels of GGT and ALB are under significant genetic regulation and that a common genetic component influences ALB and cardiovascular risk factor phenotypes. *Exp Biol Med* 234:1519–1524, 2009

Key words: NAFLD; obesity; genome scan; atherosclerosis; oxidative stress

Introduction

The leading cause of an abnormal liver function test in overweight and obese individuals is non-alcoholic fatty liver disease (NAFLD) (1). This hepatic pathology encompasses simple steatosis, non-alcoholic steatohepatitis (NASH), fibrosis and cirrhosis (2). Many factors can lead to NAFLD. NAFLD affects approximately 14%–25% of the world's population. The prevalence is higher in the obese and in type 2 diabetics (3). Patients with NAFLD might be at a higher risk for cardiovascular disease than those without this hepatic condition (4). Epidemiological studies have shown a strong positive relationship between markers of NAFLD, such as liver enzymes and prevalence of cardiovascular disease (CVD) (5–7). The focus of this paper is on two indicators of liver function, γ glutamyl transferase (GGT) and albumin (ALB), both of which have also been

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¹ To whom correspondence should be addressed at Department of Food Science and Nutrition, California Polytechnic State University, 1 Grand Avenue, San Luis Obispo, CA, 93407. E-mail: tbose@calpoly.edu

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implicated in the pathogenesis of atherosclerosis. It is known that the variation in the plasma level of these liver-related proteins is genetically influenced. To explore these genetic factors and to evaluate the relationship of these liver markers with cardiovascular risk phenotypes, we have chosen the baboon as an animal model.

The pedigreed baboons from the colony maintained by the Southwest National Primate Research Center are an excellent model for this study. Gene and protein sequence identity are conserved between baboons and humans, and both are physiologically and developmentally similar. Baboons have proven useful for genetic studies related to atherosclerosis (8), insulin resistance (9), obesity (10) and liver gene expression (11). Although all the baboons share similar diet and housing conditions, 10% become obese spontaneously and 4% become hyperglycemic (12). Further, during weight gain, fat is deposited primarily in the abdominal area of baboons. It is known that visceral adiposity is strongly linked to several metabolic diseases, such as cardiovascular ailments, type 2 diabetes and fatty liver (13), and in obese and insulin-resistant baboons from this colony, levels of liver enzymes have been found to be altered (14).

It is significant to determine the genetic factors that influence variation in circulating levels of GGT and ALB because, to understand the pathogenesis of a disease, all of the components contributing to the pathological state have to be identified. Each of the mechanisms involved in the development of a disease will not be fully understood if a component is ignored. Therefore, findings of this study will assist in future hypotheses aimed at identifying the relative contribution of various components to the pathogenesis of liver dysfunction. Additionally, if common set of genes are found to regulate variation in levels of liver proteins and cardiovascular disease risk factors, these experimental results can be used to develop future studies that will identify and explore those genes. Therefore, the purposes of this study are to identify chromosomal regions containing genes that affect the variation in the plasma levels of GGT and albumin and to determine whether genes contributing to variation in these hepatic proteins also influence variation in known cardiovascular disease risk factors as well.

Methods

Animals. The study population consisted of 350 non-inbred (254 females, 96 males) pedigreed baboons (*Papio hamadrayas*) from the colony maintained at the Southwest National Primate Research Center located at the Southwest Foundation for Biomedical Research (SFBR) in San Antonio, TX, USA (12). These animals consist primarily of olive baboons but also include yellow baboons and olive-yellow hybrids. The baboons are gang housed and fed a standard monkey chow diet *ad libitum* (Harlan Teklad 15% monkey diet, 8715, Indianapolis, IN).

Sampling and Analyses. Blood samples were

drawn after an overnight fast (12 hours) from animals sedated with ketamine. Heparin tubes were used to collect samples for analysis of GGT, ALB, triglycerides, total cholesterol and LDL cholesterol. The samples were subjected to centrifugation at 2000 g for 10 minutes, and the resultant plasma was stored in aliquots at -80°C for future analysis. The animals were weighed on a calibrated electronic scale (GSE, Chicago, IL). The Institutional Animal Care and Use Committee of the SFBR approved all procedures.

Triglycerides, total cholesterol, LDL cholesterol, GGT and ALB were analyzed by standard laboratory techniques using the Alfa Wasserman ACE clinical chemistry instrument (West Caldwell, NJ). Samples whose replicates deviated $>5\%$ were reanalyzed.

Genotyping. The animals in this study had previously been genotyped at more than 400 highly polymorphic microsatellite marker loci for the construction of a whole genome linkage map with an average marker density of 10 cM (11, 15). We made use of these maps and identity-by-descent coefficients estimated from the genotype data in our analyses.

Statistical Genetic Methods. The maximum likelihood variance decomposition methods implemented in SOLAR (16) were used to perform the statistical genetic analyses reported in this paper. We used this method to partition the phenotypic variance of the quantitative traits studied into additive genetic and non-genetic (environmental) components. From this decomposition, we estimated the proportion of the variance due to the additive effects of genes (i.e., the heritability $[h^2]$).

We further decomposed the additive genetic variance for each trait into a component for individual loci and a residual (polygenic) component, and performed multipoint whole genome linkage screens to test for quantitative loci (QTLs). Using the baboon marker map information and the multipoint IBD matrices estimated from the genotype data, we tested the hypothesis of linkage at 1 cM intervals across the baboon genome. Essentially, these tests consisted of comparing the likelihood of a restricted model for the trait in which the variance due to a QTL is constrained to zero (i.e., no linkage, null hypothesis) to an unrestricted model in which the QTL-specific variance is freely estimated. Twice the difference of the log likelihoods was asymptotically distributed as $1/2:1/2$ mixture of chi-square variable, with one degree of freedom and a point mass at zero (17). The difference between the two $\log_{10}$ likelihoods yields an LOD score, which measures the support for the hypothesis of linkage over that of “no linkage at a given chromosomal location. Our threshold for significant evidence of linkage was $\text{LOD} = 2.69$ and for suggestive evidence of linkage was $\text{LOD} = 1.46$. We obtained these genome-wide significance thresholds using a modification of an approach suggested by Feingold *et al.* (18) to control for the overall false-positive rate in our whole genome linkage screens of a single phenotype. Our approach takes into account the finite

Table 1. Relative Pairs Within the Baboon Population

Relationship	Number
Parent-offspring	44
Siblings	152
Avuncular	32
Half-siblings	2119
Half avuncular	331
Half 1st cousins	33
Half 1st cousins, 1 rem	15
Half siblings and 1st cousins	3
Half siblings and half 1st cousins	30
Half siblings and half avuncular	5

marker density in the linkage map utilized in the multipoint QTL screens and the mean recombination rate for these pedigreed baboons.

An extension of the univariate model was used for bivariate genetic analyses. The bivariate phenotype is a result of the animal's phenotypic values, population means, the additive genetic estimates and environmental effects. This model was used to calculate the genetic and environmental variance-covariance matrices in addition to genetic and environmental correlations. Both univariate and bivariate genetic analyses were conducted using the SOLAR computer program (16). Age, sex, age squared and their interactions were included as covariates for the analyses. All the traits were inverse normalized for the analyses. According to this step, observations are ranked and replaced by expected value for that rank from a standard normal distribution. Software program PEDSYS computed the group means and ranges of male and female baboons.

Results

Information describing the number of relative pairs represented in the sample for these analyses is provided in Table 1. Descriptive statistics of the phenotypes according to sex are given in Table 2. Male baboons were younger and had higher body weights than females due to the sexual dimorphism in these animals (19). Female baboons had higher levels of plasma total cholesterol, LDL cholesterol and triglycerides. The activity of GGT was elevated in male baboons, but no difference between the sexes was observed for ALB.

Table 3 shows the heritabilities of liver function markers and cardiovascular risk factors analyzed as part of this study. All were significantly heritable, with total cholesterol exhibiting the greatest heritability. Table 4 gives the genetic and phenotypic correlations between plasma GGT, albumin and plasma lipids. No statistically significant genetic association was found between GGT and the cardiovascular-related phenotypes. However, relationship between plasma albumin and lipids was negative. Significant phenotypic correlation between GGT and ALB ($\rho = 0.25$; $P < 0.0009$) and LDL cholesterol was found in this sample.

Table 2. Descriptive Statistics of Baboons

Phenotype	Male/female ^a	Range
Number	96/254	
Age (yrs)	12.90 (0.4)/15.24 (0.3)	6.9–31
Weight (kg)	31.90 (0.6)/19.54 (0.3)	12–48
Total cholesterol (mg/dl)	91.09 (2.6)/121.14 (2.6)	54–239
LDL cholesterol (mg/dl) ^b	31.53 (1.7)/53.52 (1.8)	6–135
Triglycerides (mg/dl)	46.03 (1.8)/65.20 (2.0)	19–152
γ glutamyl transferase (U/L)	46.41 (1.5)/43.43 (0.7)	2–148
Albumin (g/dl)	4.4 (0.05)/4.3 (0.03)	2.2–6

^a All data except number of patients reported as mean (standard error of the mean).

^b LDL, low-density lipoprotein.

The linkage signal for plasma GGT is displayed in Figure 1. The strongest QTL for GGT was detected between markers *D22S304* and *D22S280* on the baboon homologue of human chromosome 22, at approximately 30 cM, with an LOD score of 2.8. The maximum LOD score detected in a genome-wide linkage scan for ALB was detected on the baboon homologue of human chromosome 10, with an LOD score of 2.3 at 66 cM near marker *D10S1432* (Figure 2).

Discussion

This study has demonstrated that the variation in the plasma levels of GGT and ALB is influenced by genetic factors. The heritability estimates of these liver function markers in baboons are similar to those found in humans. Heritability estimates of GGT were 52% in Australian men and women (20) and 62% in Danish twins (21) compared with 55% found in this study. For ALB, the additive genetic effect in adult men and women was 36%, which is lower than the value calculated for baboons in this paper (22). This is the first study to publish the QTLs for GGT and ALB in baboons, and there is no information on QTLs for these proteins yet published in humans.

Even though phenotypic correlations between ALB and cardiovascular risk factors have been reported (23–25), the evidence of shared genetic effects (i.e., pleiotropy) between them has been detected for the first time in this paper. The associations found here imply that overlapping but not an identical set of genes to regulate their circulating concentrations.

Table 3. Heritabilities of Liver Function Markers and Cardiovascular Risk Factors

Phenotype	Heritability $\pm$ SEM ^a	<i>P</i>
Total cholesterol	0.68 $\pm$ 0.10	< 0.01
LDL cholesterol ^a	0.50 $\pm$ 0.10	< 0.01
Triglycerides	0.34 $\pm$ 0.11	< 0.01
γ glutamyl transferase	0.55 $\pm$ 0.13	< 0.0001
Albumin	0.42 $\pm$ 0.15	< 0.01

^a SEM, standard error of the mean; LDL, low-density lipoprotein.

Table 4. Genetic (ρ_G) Correlations Between Plasma Concentration of γ Glutamyl Transferase, Albumin, and Cardiovascular Risk Factors

Phenotype	γ glutamyl transferase				Albumin			
	ρ Genetic	<i>P</i>	ρ Phenotype	<i>P</i>	ρ Genetic	<i>P</i>	ρ Phenotype	<i>P</i>
Total cholesterol	0.18	0.39	0.04	0.09	−0.60	0.01	−0.09	0.20
LDL cholesterol ^a	0.26	0.35	0.15	0.03	−0.67	0.01	−0.09	0.36
Triglycerides	−0.41	0.19	−0.03	0.13	−0.86	< 0.01	−0.09	0.44

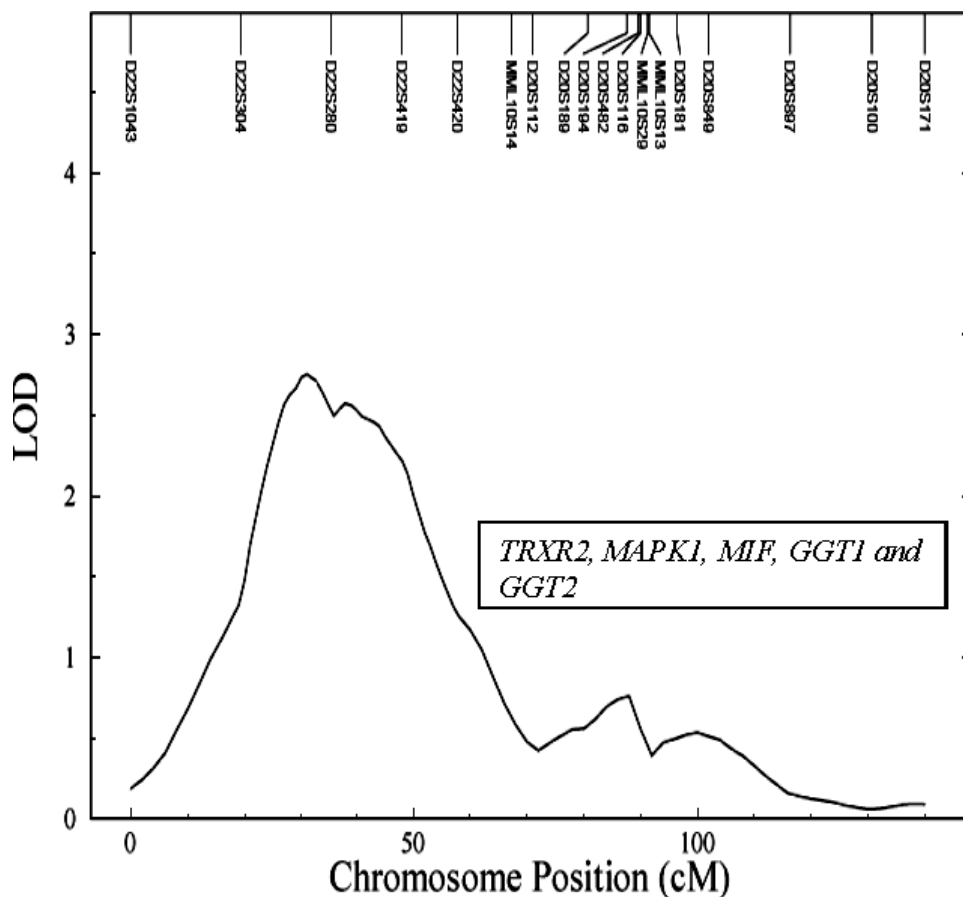
^a LDL, low-density lipoprotein.

Plasma GGT is a well-established and sensitive marker of hepatic dysfunction. The circulating concentration of GGT is elevated in CVD and NAFLD patients (26–30), implying that it might be a link between the two diseases. Yet, in this study, we did not detect any significant genetic relationship between GGT and cardiovascular risk factors. However, lack of a genetic correlation in this experimental model does not rule out the potential for a common set of genes that might operate under a different experimental design or in an atherogenic environment.

In contrast, a significant negative genetic correlation between ALB and cardiovascular risk factors was observed in this study. Traditionally, plasma ALB has been considered a good biomarker of protein malnutrition, but

recently, this protein has been associated with heart disease and was found to be an independent indicator of mortality in patients with NAFLD (23, 31). Because the liver manufactures ALB, damage to this organ reduces the blood levels of this protein (32). Lower levels of ALB raise the concentration of unbound free fatty acids that consequently increase the amount of lipid parameters in the blood (33).

Insulin resistance and oxidative stress are hypothesized to be the link between fatty liver abnormalities and heart disease (34). Another plausible mechanism associating these two disorders could be abnormal lipoprotein metabolism in individuals with steatotic liver (i.e., elevated plasma concentrations of triglycerides and low-density lipoprotein cholesterol (LDL) (35). The high prevalence of cardiovas-

**Figure 1.** Map depicting LOD scores (X axis) and marker distances (Y axis) for plasma gamma glutamyl transferase on chromosome 22.

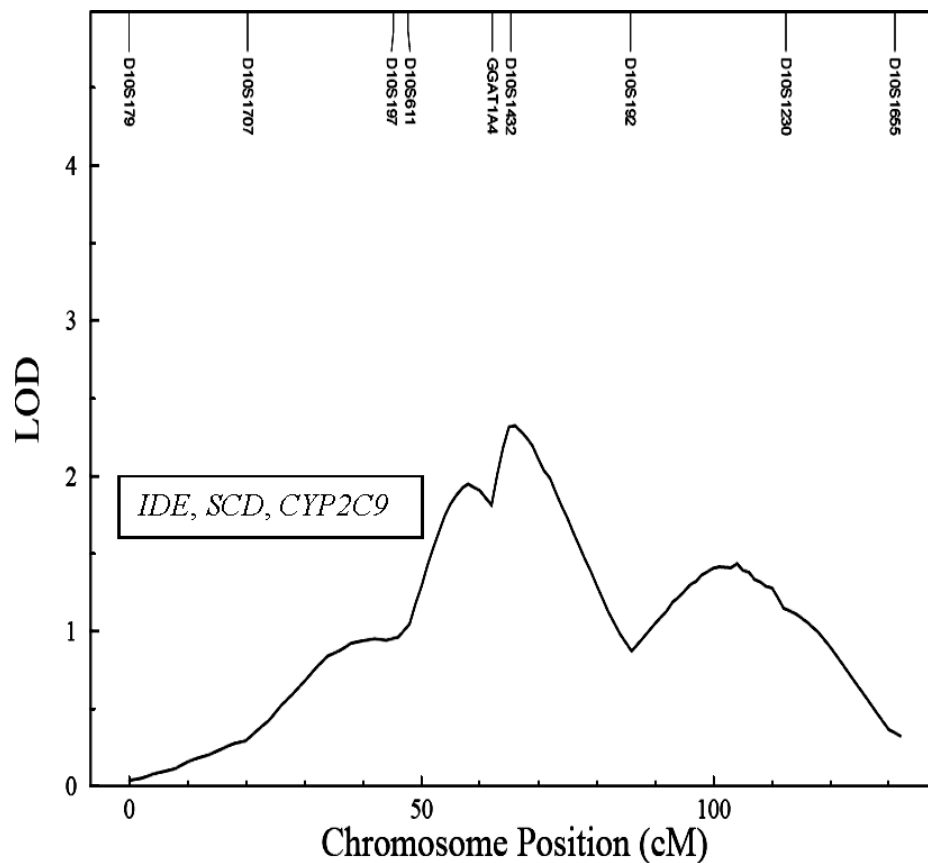


Figure 2. Map depicting LOD scores (X axis) and marker distances (Y axis) for plasma albumin on chromosome 10.

cular disease in NAFLD patients could be due to insulin resistance that results in dyslipidemia (36).

For GGT, potential positional candidate genes (based upon the homologous region in humans) that are present within the one LOD-support interval of the maximum linkage signal on chromosome 22 include selenoprotein Z (*TRXR2*) (37), mitogen-activated protein kinase 1 (*MAPK1*) (38), macrophage migration inhibitory factor (*MIF*) (39), GGT 1 (*GGT1*) and GGT 2 (*GGT2*) (40). The most promising positional candidate genes within this QTL are the two structural genes that produce the two isoforms (*GGT1* and *GGT2*) of GGT. The genes of interest within the one LOD-support interval of the ALB QTL on chromosome 10 are insulin-degrading enzyme (*IDE*) (41), stearyl-CoA desaturase (*SCD*) (42), cytochrome P450 subfamily IIC and polypeptide 9 (*CYP2C9*) (43). All of these genes are related to oxidative stress.

In conclusion, this study indicates that genetic factors significantly influence the variation in circulating levels of GGT and ALB, and a common set of genes appear to regulate plasma levels of ALB and cardiovascular-related risk factors in baboons.

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