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A Genetic and Correlation Analysis of Liver Cholesterol Concentration in Rat Recombinant Inbred Strains Fed a High Cholesterol Diet

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Liver cholesterol concentration in rats fed a high cholesterol diet, is under genetic control which is supported by significant differences observed among inbred strains. For instance, the Brown Norway (BN-*Lx*/Cub) rat developed a twofold higher liver cholesterol concentration than the spontaneously hypertensive rat (SHR/Ola). In the current study, we used 30 recombinant inbred (RI) strains, derived from BN-*Lx* and SHR progenitors, to locate quantitative trait loci (QTL) that are responsible for differences in liver cholesterol concentrations between the BN-*Lx* and SHR strains. The heritability of liver cholesterol was estimated to be 0.55 and a significant association was detected between concentration of liver cholesterol and the *D10Cebrp1016s2* marker on chromosome 10 (lod score = 3.3); this putative QTL was responsible for nearly 64% of additive genetic variability and thus represents a major genetic determinant of liver cholesterol concentration. Liver cholesterol concentrations significanrly correlated with intermediate density lipoprotein (IDL) cholesterol levels. © 1998 Academic Press

Consistent inter-individual differences in serum cholesterol levels exist in humans after a high cholesterol diet (1). Inbred strains of rats (2, 3, 4), rabbits (5) and mice (6, 7) can also differ in their serum cholesterol concentration response to a high cholesterol diet. Thus, it is very likely that genetic factors play an important role in this differential responsiveness to high cholesterol diet. Some experiments with inbred strains included measurements of the liver cholesterol concentration after a high cholesterol diet and significant strain differences were reported (2, 6, 8), suggesting genetic factors also influence this phenotype. The relationship between liver cholesterol and plasma lipoprotein cholesterol concentrations is not simple: for instance, when fed a high fat high cholesterol diet, the BN strain significantly increased both its plasma cholesterol and hepatic cholesterol concentrations, whereas in the LEW strain, only the plasma cholesterol was increased. The liver cholesterol concentration remained low in the LEW strain and was similar to the average observed in hyporesponsive strains that showed little or no increase in either plasma or liver cholesterol. Thus serum cholesterol levels are likely to be controlled by certain genes that are different from those regulating the hepatic concentration of cholesterol. Another possibility might be that specific lipoprotein fractions in the serum are selectively associated with the liver cholesterol concentration. For example, in rats fed increasing amounts of dietary cholesterol, the secretion of VLDL (very low density lipoprotein) from liver increased progressively along with the liver cholesterol concentration (9). In a previous study (11), we mapped quantitative trait loci (QTL) controlling the total serum cholesterol concentration and the cholesterol concentrations of the lipoprotein subfractions. The aim of this study was to locate QTLs influencing the liver cholesterol concentration and to investigate how the hepatic concentration of cholesterol correlates with the circulating concentration of cholesterol in lipoprotein subfractions.

METHODS

Animals. The recombinant inbred (RI) strains were derived from spontaneously hypertensive rats (SHR/Ola) and Brown Norway rats (BN-*Lx*/Cub) (12). The BN-*Lx* progenitor is a congenic strain that carries a segment of chromosome 8 from the polydactylous PD/Cub strain (13). A total of 36 RI strains were originally obtained from crosses of female SHR and male BN-*Lx* rats (HXB strains, n = 26) or female BN-*Lx* rats and male SHR (BXH strains, n = 10). In the

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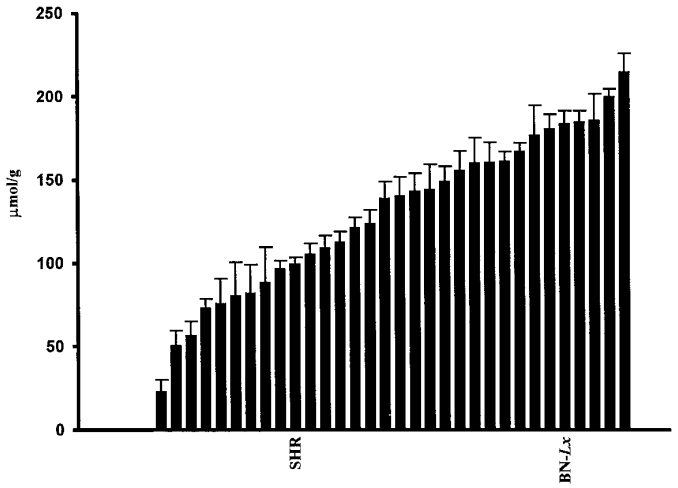

FIG. 1. The distribution of means ± SEM of the liver cholesterol concentration of recombinant inbred strain and the progenitor strains.

current study, 30 RI strains have been used (HXB strains, n = 21 and BXH strains, n = 9). In each RI strain, four to six males were tested for the liver cholesterol concentration (μmol cholesterol/g liver) and total serum cholesterol levels. The lipoprotein cholesterol levels were determined from pooled sera of each RI strain.

Derivation of the genetic linkage map of the RI strains. The strain distribution patterns (SDPs) were analyzed for a total of 560 biochemical, morphologic, immunogenetic, and molecular genetic markers. Typing methods and SDPs for these markers have previously been described (14). Amplified fragment length polymorphisms (AFLPs) provided additional molecular markers (15).

Experimental protocol. The animals were housed under natural lighting conditions. Food and tap water were available ad libitum during the whole experimental period. After weaning till the age of 7 weeks the animals were fed a commercial pelleted diet (RMH-B, Hope Farms BV, Woerden, The Netherlands). After this experimental period, the rats received the commercial diet supplemented with 5% (w/w) olive oil (Reddy, Van de Moortele NV, Oudenbosch, The Netherlands) and 2% (w/w) cholesterol (USP, Solvay/Pharmaceuticals BV, Weesp, The Netherlands) for four weeks. The experimental diet was provided in pelleted form and was stored in the freezer until use. At the end of the experimental period the animals were anesthetized with ether, exsanguinated via the inferior vena cava and the livers were removed and weighted. Three pieces of 1 g of liver were frozen immediately from each animal.

Biochemical analysis. The total serum and lipoprotein cholesterol concentrations were determined as previously described (11). The lipoprotein cholesterol concentrations were determined from the pooled sera of each recombinant inbred strains. Lipoprotein density classes were based on the pattern observed in humans: VLDL ($d < 1.006$), IDL ($1.006 < d < 1.019$), LDL/HDL1 ($1.019 < d < 1.063$), HDL2 ($1.063 < d < 1.125$), and HDL3 ($1.125 < d < 1.210$). The analysis of cholesterol levels was performed using enzymatic test kits (Boehringer-Mannheim, GmBH, Mannheim, Germany). Liver cholesterol concentration was analysed according to the method of Abell et al.(1952) (16). For each animal, three pieces of liver were analysed, each in triplicate. These pieces were homogenized in distilled water, cholesterol was extracted and analysed as referenced above.

QTL analysis. Values are expressed as means ± SEM (Fig. 1). Heritability of liver cholesterol was estimated according to the method of Plomin and McClearn (17) from the variance in mean values between and within the RI strains. The additive genetic variance was estimated to be 50% of the total variance between the mean liver cholesterol concentration of the RI strains; the environmental variance was estimated to be the average variance in mean liver cholesterol concentration within the RI strains. Narrow heritability was calculated by dividing the additive genetic variance by the sum of the additive genetic variance and the environmental variance.

Map Manager QT (version 18b) (18) was used to test for single locus associations by regression analysis and the significance of each potential association was measured using the likelihood ratio statistic (LRS) of Haley and Knott (19). The interval regression method of Map Manager QT was used to test for QTLs within marker intervals. The significance threshold for the genome wide scan was empirically determined by the Map Manager QT permutation test using the informative markers and 1000 permuted data sets as recommended by Doerge and Churchill (20). Significant linkage was defined in accordance with the guidelines of Lander and Kruglyak (21) as statistical evidence occurring by chance in the genome scan with a probability of 5% or less. Based on these criteria and the results of the permutation test, a LRS = 14.9 (corresponding to a lod score of 3.2) was established as the threshold for significant linkage in the RI strain data set. One-half of the fraction of variance attributable to each QTL in the RI strains was used to estimate the QTL effect to correct for the doubling effect of inbreeding on additive genetic variance (22, 23). The fraction of genetic variance contributed by each QTL was determined by dividing the estimated QTL effect by the heritability.

RESULTS

The SHR *vs.* BN-*Lx* progenitor strains exhibited significant differences in the liver cholesterol concentration both on control diet (7.5 ± 0.1 vs. 9.0 ± 0,5 μmol/g, $p < 0.001$) and on cholesterol rich diet (99.5 ± 3.9 vs. 183.6 ± 7.6 μmol/g, $p < 0.001$). The BN-*Lx* developed much greater liver cholesterol concentrations than the SHR after a high cholesterol diet which was in agreement with findings in previous experiments (2, 8). Since both strains shared a common environment it is likely that the genetic factors contribute to the differences in liver cholesterol concentration. The distribution of the mean liver cholesterol concentrations in the RI strains was continuous suggesting polygenic inheritance of the trait. The narrow heritability for liver cholesterol concentration was estimated to be 0.55. Given the finding of a substantial genetic component to liver cholesterol concentration in the RI strain model, we scanned for QTLs influencing this phenotype using the Map Manager QT program. The *D10Cebrp1016s2* marker on chromosome 10 showed the strongest association with the liver concentration of cholesterol (lod score = 3.3) (Fig. 2). The mean liver cholesterol concentration of the RI strains that inherited the BN-*Lx* allele for the *D10Cebrp1016s2* marker, 156.3 ± 9.4 μmol/g, was greater than the mean liver cholesterol concentration of the RI strains that inherited the SHR allele, 86.8 ± 13.3 μmol/g.

Because the liver is the main organ involved in cholesterol metabolism, we searched for possible correlations between the liver cholesterol concentration and serum VLDL (very low density lipoprotein), IDL (inter-

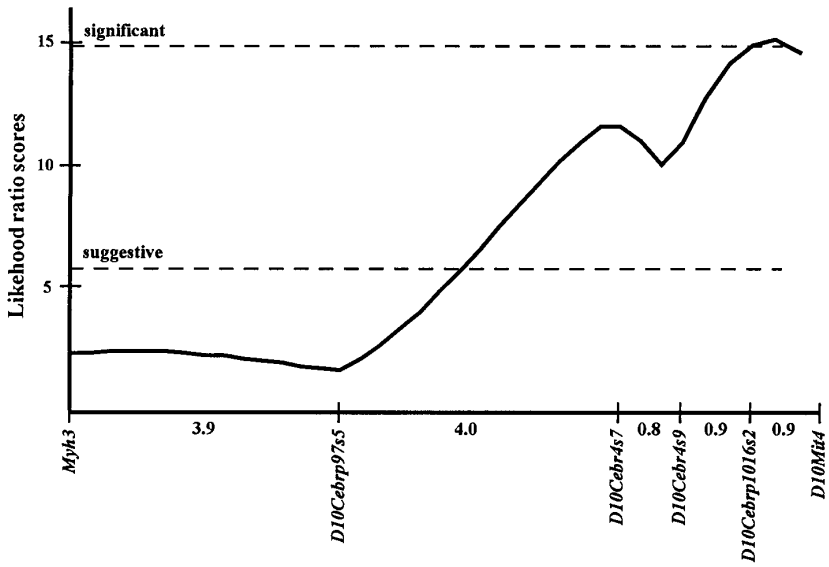

FIG. 2. Interval mapping of the QTL regulating liver cholesterol concentration. Likelihood ratio statistics from the Map Manager QT linkage analysis are plotted across the segment of chromosome 10. Estimated distances between markers in centiMorgans are determined with the Haldane map function. The horizontal line indicates the threshold for significance of the likelihood ratio statistic determined by the Map Manager QT permutation test. To convert likelihood ratio statistics to lod scores, divide by 4.6.

mediate density lipoprotein), LDL/HDL1 (low density lipoprotein/high density lipoprotein), HDL2 and HDL3 cholesterol levels in RI strains. We found a significant positive correlation of liver cholesterol concentration with IDL cholesterol levels ($r = 0.40$, $p = 0.02$).

DISCUSSION

The baseline hepatic concentration of cholesterol in the BN-*Lx* strain was significantly greater than that in the SHR strain. In addition, the BN-*Lx* strain responded to a high fat, high cholesterol diet by increasing both plasma and liver cholesterol concentrations while the SHR strain increased serum and liver cholesterol concentrations to a much lesser extent. Genetic analysis of the liver cholesterol concentration in the recombinant inbred strains indicated that this phenotype is under a polygenic control. The narrow heritability was estimated to be 0.55 which motivated us to search for responsible QTLs. Genome wide scanning for associations between marker genotypes and liver cholesterol concentrations resulted in the localization of a QTL on rat chromosome 10 near the *D10Cebrp1016s2* marker (Fig 2). Based on homologies between the segment of rat chromosome 10, where the putative QTL has been mapped, and mouse chromosome 11 and human chromosome 5q, it is possible that the ILLBP gene, which codes for the ileal lipid binding protein (24), might be a positional candidate for the regulation of hepatic concentration of cholesterol. This protein is most likely involved in the transport of bile acids through the ileocyte, participating thus in the enterohepatic circulation (25). It is well known that bile acids inhibit the activity of cholesterol 7-alpha hydroxylase at the transcriptional level (26, 27). As a result of differential activity of ileal lipid binding protein, the cholesterol 7-alpha hydroxylase could thus be more or less inhibited and the cholesterol might be available to a more or lesser extent for esterification and storage in hepatocytes. Another possibility is that SHR strain might be able to excrete more cholesterol through bile acids because of less effective transport back to circulation via ILLBP; more effective excretion of cholesterol through bile acids might explain resistence of some strains to high colesterol diets. One could speculate that in the rat molecular variation in the ILLBP gene could be responsible for the difference in liver cholesterol concentration between the two progenitor strains. Further experiments including derivation of congenic sublines are necessary to confirm and precisely map the putative QTL on rat chromosome 10.

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