Lipoproteins

Quantitative Trait Loci Influencing Blood and Liver Cholesterol Concentration in Rats

Anita C.M. Bonné, Maria G. den Bieman, Gert F. Gillissen, Ægidius Lankhorst, Christopher J. Kenyon, Bert F.M. van Zutphen, Hein A. van Lith

Objective—The LEW/OlaHsd and BC/CpbU rat inbred strains differ markedly in blood and hepatic cholesterol levels before and after a cholesterol-rich diet. To define loci controlling these traits and related phenotypes, an F₂ population derived from these strains was genetically analyzed.

Methods and Results—For each of the 192 F₂ animals, phenotypes were determined, and genomic DNA was screened for polymorphic microsatellite markers. Significant quantitative trait loci (QTLs) were detected for basal serum cholesterol level on chromosome 1 (D1Rat335-D1Rat27: total population, lod score 9.6; females, lod score 10.3) and chromosome 7 (D7Rat69: males, lod score 4.1), for postdietary serum cholesterol level on chromosome 2 (D2Rat69: total population, lod score 4.4) and chromosome 16 (D16Rat6-D16Rat44: total population, lod score 3.3), for postdietary serum phospholipid level on chromosome 11 (D11Rat10: total population, lod score 4.1; females, lod score 3.6), and for postdietary serum aldosterone level on chromosome 1 (D1Rat14: females, lod score 3.7) and chromosome 18 (D18Rat55-D18Rat8: females, lod score 2.9). In addition, QTLs with borderline significance were found on chromosomes 3, 5 to 11, 15, and 18.

Conclusions—QTLs involved in blood and/or hepatic cholesterol concentrations (or related phenotypes) in the rat were identified. This contributes to the value of the rat as an animal model in studies researching the role of cholesterol in the pathogenesis of atherosclerosis and other cholesterol-related diseases. (Arterioscler Thromb Vasc Biol. 2002;22:2072-2079.)

Key Words: cholesterol ■ phospholipids ■ quantitative trait loci ■ steroid hormones ■ rats

Atherosclerosis is a complex disorder in which genetic and environmental factors play a role. A high serum cholesterol concentration is one of the risk factors in the development of this disease. Circulating cholesterol levels do not exclusively reflect dietary habits; epidemiological studies have revealed consistently higher than average serum cholesterol levels only in particular individuals after a high dietary cholesterol intake.¹ Individual differences in serum cholesterol concentration also exist after a diet with low-fat and/or low-cholesterol content. Similar variability in serum cholesterol levels can also be observed in laboratory animals such as mice,² rabbits,³ and rats⁴ in response to control diets and to high-fat and high-cholesterol diets.⁵-⁸ Differences observed between inbred strains of these species indicate that the basal serum cholesterol concentration and the rate of increase in serum cholesterol levels after a cholesterol-rich diet are under genetic control.

To genetically analyze these phenotypes in laboratory rats, we performed a total genome scan of an F₂ population derived from the BC/CpbU and LEW/OlaHsd rat inbred strains. Cholesterol (basal and postdietary serum level and postdietary liver concentration) and cholesterol-related traits (postdietary serum phospholipid, aldosterone, and corticosterone levels) were measured in the F₂ animals. The cholesterol-related traits were included into the present study because in the F₂ population these parameters were significantly associated with postdietary serum cholesterol levels. The results of this quantitative trait loci (QTL) analysis and possible candidate genes located in the vicinity of some QTLs will be discussed.

Methods

The Methods section can be accessed online (http://www.atvb.ahajournals.org).

Results

Parental Strains

For parental strains, please see Table 1. At the beginning of the test period, all rats were of the same age, but the body weight of the LEW rats, compared with the BC rats, was higher. Males and females of the BC strain had equal body weights. In contrast, initial body weight of the LEW males

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2072
was higher than that of the LEW females. As would be expected, body weight increased, in an identical fashion, in the 2 inbred strains during the course of the experiment. At the end of the test period, the LEW rats had a higher body weight than the BC rats, and the males were significantly heavier than the females.

Pre-experimental serum cholesterol levels (baseline values) were significantly higher in the BC rats than in the LEW rats. Baseline serum cholesterol levels were similar in males and females of the BC strain. These levels were significantly higher in LEW females compared with LEW males. Because there were significant differences in initial body weight, group means of baseline serum cholesterol levels were also compared by ANOVA, with initial body weight as a covariate. The effect of the rat’s sex on baseline serum cholesterol concentration disappeared, but there tended to be a strain effect.

The high-fat high-cholesterol diet produced an increase in serum total cholesterol levels in LEW rats but not in BC rats (in fact, in BC males there was a significant decrease). This increase was more pronounced in female compared with male LEW rats. Sex and strain significantly affected the final serum cholesterol concentration. However, after correction for body weight, the sex effect was of borderline significance, but the strain effect was still highly significant. Group mean postdietary serum phospholipid levels were higher in the LEW strain than in the BC strain; this strain effect reached the level of statistical significance only in the females. After correction for body weight, there was a significant strain effect.

Although serum cholesterol levels in males of the BC strain responded with a decrease to a high-fat high-cholesterol diet, the postdietary liver cholesterol concentration was significantly higher in male BC rats compared with male LEW rats. Female rats compared with male rats of the LEW strain, but not of the BC strain, had significantly elevated hepatic cholesterol levels. In the 2-way ANOVA with final body weight as a covariate, these effects did not reach the level of statistical significance. Group mean circulating adrenal steroids levels were higher in BC rats than in LEW rats. However, after correction for body weight, this strain effect was significant only for serum aldosterone concentration.

### Genetic Mapping of Quantitative Traits

For genetic mapping of quantitative traits, please see Tables 2 and 3, Figure 1, and online Figures I to IV (available at http://www.atvb.ahajournals.org). The results of the QTL analysis using the MapQTL software are summarized in Table 2. For initial body weight, male- or female-specific

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**TABLE 1. Some Phenotypic Characteristics of BC and LEW Rats**

<table>
<thead>
<tr>
<th>Measure</th>
<th>Strain</th>
<th>ANOVA†</th>
<th>Post hoc Comparisons‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BC</td>
<td>LEW</td>
<td>BC vs LEW</td>
</tr>
<tr>
<td></td>
<td>Males</td>
<td>Females</td>
<td>S  G  S×G</td>
</tr>
<tr>
<td>Body weight, g</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 0</td>
<td>109±13</td>
<td>106±5</td>
<td>0.000 0.000 0.000</td>
</tr>
<tr>
<td>Day 28</td>
<td>223±17</td>
<td>177±4</td>
<td>0.000§ 0.000§ 0.950§</td>
</tr>
<tr>
<td>Initial vs final‡</td>
<td>0.000</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td>Serum cholesterol level, mmol/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>3.45±0.25</td>
<td>3.46±0.23</td>
<td>0.075 0.769 0.496</td>
</tr>
<tr>
<td>Postdietary</td>
<td>2.43±0.37</td>
<td>3.84±0.60</td>
<td>0.000§ 0.055§ 0.597§</td>
</tr>
<tr>
<td>Baseline vs postdietary‡</td>
<td>0.004</td>
<td>0.154</td>
<td>0.018 0.004</td>
</tr>
<tr>
<td>Serum phospholipid level, mmol/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Postdietary</td>
<td>1.51±0.19</td>
<td>1.61±0.17</td>
<td>0.018 0.100 0.476</td>
</tr>
<tr>
<td>Liver cholesterol concentration, μmol/g wet weight</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Postdietary</td>
<td>173±27</td>
<td>164±21</td>
<td>0.081 0.322 0.177</td>
</tr>
<tr>
<td>Postdietary serum adrenal steroids level, mmol/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aldosterone</td>
<td>1.38±0.44</td>
<td>1.44±0.52</td>
<td>0.011 0.464 0.890</td>
</tr>
<tr>
<td>Corticosterone</td>
<td>184±219</td>
<td>718±422</td>
<td>0.467§ 0.358§ 0.649§</td>
</tr>
</tbody>
</table>

Values are means±SD for 6 (serum parameters and body weight) or 4 (liver cholesterol concentration) animals per group. Note that a P value of 0.000 does not mean that it is zero, only that it is less than 0.0005.

†P values in two-way ANOVA with main factors strain and sex. S indicates effect of strain; G, effect of sex; S×G, interaction. Significant effects (P<0.05) are indicated in bold characters. For statistical analysis of serum and liver parameters, body weight was used as covariate.

‡P values in unpaired Student’s t test. Significant differences (P<0.0167 for serum cholesterol level and body weight; P<0.025 for the other parameters) are indicated in bold characters.

§P values in paired Student’s t test. Significant differences (P<0.0167) are indicated in bold characters.

§ANOVA after logarithmic transformation of the data.
QTLs were mapped on chromosomes 5 and 7 (please see online Figure IA), respectively. QTLs for males plus females were found for initial body weight on chromosomes 15 (please see online Figure IB) and 18 and for final body weight (females, males plus females) on chromosome 17. Kovács et al. and Klöting et al. also found QTLs influencing body weight on rat chromosomes 5 and 18.

The genome-wide scan revealed regions with significant linkage of the cholesterol and related phenotypes to loci on chromosomes 1, 2, 7, 11, 16, and 18 (Figures 1, online Figures II to IV, and Table 2). QTLs with borderline significance were found on rat chromosomes 3, 5 to 11, 15, and 18 (Table 2). Table 2 also illustrates the existence of sex-specific and sex-independent QTLs.

Table 3 shows the phenotypes of each of the 3 genotypes (LL, LB, and BB) segregating at selected loci on chromosomes 1, 2, 7, 11, 16, and 18 (Figures 1, online Figures II to IV, and Table 2). QTLs with borderline significance were found on rat chromosomes 3, 5 to 11, 15, and 18 (Table 2). Table 2 also illustrates the existence of sex-specific and sex-independent QTLs.

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TABLE 3. Cosegregation Analysis Results in F2 Progeny of LEW and BC Rats

<table>
<thead>
<tr>
<th>Marker</th>
<th>Population</th>
<th>Phenotypic Trait</th>
<th>P va $^2$ Test</th>
<th>Genotype*</th>
<th>lod Score†</th>
<th>ANOVA‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>LL</td>
<td>LB</td>
<td>BB</td>
</tr>
<tr>
<td>Chromosome 1</td>
<td>D1Rat14</td>
<td>♀ ♂</td>
<td>Postdietary serum aldosterone</td>
<td>0.458</td>
<td>0.88±0.55 (29)</td>
<td>0.61±0.30 (43)</td>
</tr>
<tr>
<td></td>
<td>D1Rat335</td>
<td>♂ ♀ ♀</td>
<td>Baseline serum cholesterol</td>
<td>0.219</td>
<td>2.88±0.42 (53)</td>
<td>3.15±0.40 (84)</td>
</tr>
<tr>
<td></td>
<td>D1Rat27</td>
<td>♀ ♂</td>
<td>Baseline serum cholesterol</td>
<td>0.034</td>
<td>2.79±0.39 (32)</td>
<td>3.19±0.42 (37)</td>
</tr>
<tr>
<td></td>
<td>D11Rat10</td>
<td>♀ ♂</td>
<td>Baseline serum cholesterol</td>
<td>0.769</td>
<td>2.89±0.39 (50)</td>
<td>3.16±0.43 (90)</td>
</tr>
<tr>
<td>Chromosome 2</td>
<td>D2Rat69</td>
<td>♂ ♀ ♀</td>
<td>Postdietary serum cholesterol</td>
<td>0.185</td>
<td>5.44±0.27 (41)</td>
<td>4.72±1.86 (91)</td>
</tr>
<tr>
<td>Chromosome 7</td>
<td>D7Rat94</td>
<td>♀ ♂</td>
<td>Initial body weight</td>
<td>0.189</td>
<td>129±12 (21)</td>
<td>132±11 (44)</td>
</tr>
<tr>
<td></td>
<td>D7Rat12</td>
<td>♀ ♂</td>
<td>Initial body weight</td>
<td>0.583</td>
<td>130±13 (22)</td>
<td>134±13 (48)</td>
</tr>
<tr>
<td></td>
<td>D7Rat69</td>
<td>♂ ♂</td>
<td>Baseline serum cholesterol</td>
<td>0.549</td>
<td>2.89±0.35 (26)</td>
<td>3.23±0.36 (40)</td>
</tr>
<tr>
<td>Chromosome 11</td>
<td>D11Rat10</td>
<td>♂ ♂ ♂</td>
<td>Postdietary serum phospholipids</td>
<td>0.064</td>
<td>1.72±0.40 (39)</td>
<td>1.48±0.33 (91)</td>
</tr>
<tr>
<td></td>
<td>D11Rat10</td>
<td>♂ ♂ ♂</td>
<td>Postdietary serum phospholipids</td>
<td>0.773</td>
<td>1.71±0.47 (27)</td>
<td>1.37±0.35 (50)</td>
</tr>
<tr>
<td>Chromosome 15</td>
<td>D15Rat5</td>
<td>♂ ♂ ♂</td>
<td>Initial body weight</td>
<td>0.809</td>
<td>145±25 (44)</td>
<td>152±25 (99)</td>
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<tr>
<td></td>
<td>D15Rat16</td>
<td>♂ ♂ ♂</td>
<td>Initial body weight</td>
<td>0.930</td>
<td>146±25 (46)</td>
<td>152±26 (92)</td>
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<tr>
<td>Chromosome 16</td>
<td>D16Rat44</td>
<td>♂ ♂ ♂</td>
<td>Postdietary serum cholesterol</td>
<td>0.067</td>
<td>5.20±2.00 (61)</td>
<td>4.54±1.82 (84)</td>
</tr>
<tr>
<td></td>
<td>D16Rat16</td>
<td>♂ ♂ ♂</td>
<td>Postdietary serum cholesterol</td>
<td>0.118</td>
<td>5.19±2.01 (60)</td>
<td>4.54±1.80 (85)</td>
</tr>
<tr>
<td>Chromosome 18</td>
<td>D18Rat55</td>
<td>♀ ♂</td>
<td>Postdietary serum aldosterone</td>
<td>0.243</td>
<td>0.90±0.60 (18)</td>
<td>0.62±0.34 (56)</td>
</tr>
<tr>
<td></td>
<td>D18Rat18</td>
<td>♀ ♂</td>
<td>Postdietary serum aldosterone</td>
<td>0.212</td>
<td>0.88±0.49 (17)</td>
<td>0.63±0.41 (48)</td>
</tr>
</tbody>
</table>

Values are means±SD; number of rats is given in parentheses. Initial and final body weight are in g; baseline serum cholesterol level is in mmol/L; postdietary serum cholesterol and phospholipid level are in mmol/L; postdietary serum adrenal steroids level is in nmol/L; postdietary liver cholesterol concentration is in μmol/g wet weight. Some DNA samples failed to give a conclusive genotype, hence the number of rats typed varied slightly with each locus. Note that a $P$ value of 0.000 does not mean that it is zero, only that it is less than 0.0005.

* indicates LEW allele; B, BC allele.

† lod scores reported are at the markers indicated. In some instances the lod score between markers is higher (see Figures I through IV).

‡ One-way ANOVA with main factor genotype. For statistical analysis of serum and liver parameters, body weight was used as covariate.

§ $P$ value after logarithmic transformation of the data.

as a marker. Therefore, taken together, the segregation distortion at D1Rat335 is not a serious problem for quantitative trait linkage analysis.

Interactions
A 2-way ANOVA was used for evaluating genetic interactions (Table 4 and Figure 2) among selected loci, ie, among the loci that were flanking a segment that contains a candidate gene (see Discussion). Table 4 summarizes the significant interactions. Figure 2 illustrates the interaction between the D1Rat335 and D11Rat10 loci for baseline serum cholesterol levels. When rats were homozygous for the BC allele at the D1Rat335 locus and homozygous for the LEW allele at the D11Rat10 locus, the baseline serum cholesterol levels were highest.

Discussion
In the present study, genome-wide scanning of 192 F2 animals derived from the rat inbred strains BC and LEW for associations between marker genotypes and quantitative traits related to dietary cholesterol susceptibility resulted in the localization of quite a few QTLs with borderline significance (on rat chromosomes 3, 5 to 11, 15, and 18; Table 2) and 7 significant QTLs (on rat chromosomes 1, 2, 7, 11, 16, and 18; Tables 2 and 3, Figure 1, and online Figures I to IV). Between body weight and cholesterol or related phenotypes, there were in the F2 rats weak, but significant, associations (please see online Table I, available at http://www.atvb.ahajournals.org). This prompted us to perform a 1-way (Table 3) or 2-way (Table 4 and I) ANOVA with a covariate (body weight). Despite the use of a covariate, the genotypes at selected loci were still significantly associated with the traits (Table 3).
Furthermore, the body weight QTLs did not localize to regions where the QTLs for cholesterol and related phenotypes were found (Table 2; please see online Figure IA versus Figure 1B). Thus, it could be excluded that the QTLs for the cholesterol and related phenotypes are QTLs controlling interstrain differences in body weight. It is a common practice to study the genetic background of phenotypes with multiple environmental and genetic components by crossing 2 inbred strains that differ for the parameter under study and subsequently intercrossing or backcrossing the F1 progeny. In most studies, the progenitor strains also differ regarding body weight. We believe that in these studies, body weight should be considered, at least when there is evidence of an association between the parameters under study and body weight.

Kovács et al.11 reported significant linkage of the marker D1Mit14 with basal serum cholesterol levels in a study of a backcross derived from SHR/Mol and BB/OK rats. Genetic analyses of OLETF/H11003 (OLETF/H11003, Fischer 344) rats revealed statistically significant linkage between D1Rat306 and basal serum cholesterol levels.12 These QTL regions are located at the telomeric part of the q arm of rat chromosome 1 and do not colocalize with the QTL region D1Rat335-D1Rat27 as found in the present study (Figure 1A). The markers D1Rat306 and D1Mit14 are located at 133 cM in the rat genome database map, whereas D1Rat27 is located at 45 cM.

Interestingly, the region around D1Rat27 (Figure 1A) contains the Apoe gene.13 ApoE plays a pivotal role in the catabolism of triglyceride-rich lipoproteins by serving as a ligand for lipoprotein receptors. The Apoe gene might be a positional candidate for this QTL. In humans, allelic variation of the Apoe gene has been associated with differences in serum total cholesterol14 and LDL cholesterol15 levels. Transfer of a segment of chromosome 1 containing the Apoe gene from the BN/Cr rat inbred strain into the genetic background of the SHR/Ola rat16 caused a significant increase in LDL/HDL1 cholesterol level. The SHR.BN-D1Wox6-D1Mgh11/
Ipcv (n=6) compared with the SHR/Ola (n=6) had increased LDL/HDL cholesterol (on average, 65% higher level; \( P = 0.001 \) by unpaired Student \( t \) test; unpublished data).

For mice, several genetic analyses have been published that revealed QTLs influencing hepatic or plasma cholesterol concentration after a high-cholesterol diet.\(^2,18,19\) However, to the best of our knowledge, this is the first report dealing with rats in which a genome-wide search successfully identified multiple chromosomal regions linked to circulating cholesterol levels after a high-fat high-cholesterol diet (Tables 2 and 3 and online Figure II). The present study also supports our previous findings of a QTL on rat chromosome 2 influencing postdietary IDL cholesterol levels.\(^20\)

Bottger et al\(^21\) localized the gene (\( Lpl \)) coding for lipoprotein lipase (LPL) to rat chromosome 16. In the radiation hybrid map, \( Lpl \) is located near the marker \( D16Rat6.\)\(^13\) In the present study, the latter marker is located in the vicinity of the QTL controlling serum cholesterol levels on rat chromosome 16. LPL plays a major role in lipoprotein metabolism,\(^22\) and in humans, LPL mutations have been associated with atherosclerosis and dyslipidemia.\(^23\) The SHR-BN-\( D16Mit5/Cub \) congenic strain is a rat strain with the genetic background of the spontaneously hypertensive rat (SHR) onto which a segment from the BN strain, containing the \( Lpl \) gene, has been transferred.\(^16\) This SHR-BN congenic strain (n=4) has a significantly increased serum cholesterol level compared with the SHR/Ola (n=6) progenitor strain after the feeding of a high-fat high-cholesterol diet (on average, 81% higher level; \( P = 0.014 \) by unpaired Student \( t \) test).\(^17\) Thus, \( Lpl \) could be the gene on rat chromosome 16 that is controlling postdietary circulating cholesterol levels.

Up until now, 2 QTLs controlling serum phospholipid levels have been described in the rat. Bottger et al\(^21\) and Kovács et al\(^24\) found a QTL for basal serum phospholipid levels on rat chromosome 4. Postdietary HDL\(_2\) phospholipids were associated with the same region of rat chromosome 4\(^24\) or with rat chromosome 20.\(^21\) In the present study, we could not confirm the aforementioned associations. However, we now found a QTL for postdietary serum phospholipid levels on rat chromosome 11 (Tables 2 and 3 and online Figure III). As to the gene involved, we can only speculate. This segment of rat chromosome 11 is homologous with mouse chromosome 16 and human chromosome 3q, where the gene for CTP:phosphocholine cytidylyltransferase (\( Pcyt1a \)) is located. This gene is involved in hepatic phospholipid metabolism.\(^25\) On the basis of homology, one might speculate that this gene is responsible for the strain difference in serum phospholipid level in rats fed high-fat high-cholesterol diets.

### Table 4. Significant (\( P < 0.05 \)) Locus-Locus Interactions

<table>
<thead>
<tr>
<th>Locus 1 × Locus 2</th>
<th>Population</th>
<th>Phenotypic Trait</th>
<th>( P ) (interaction) ANOVA*</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1Rat335 × D11Rat10</td>
<td>( \varnothing \varnothing \times \varnothing \varnothing )</td>
<td>Baseline serum cholesterol</td>
<td>0.002</td>
</tr>
<tr>
<td>( \varnothing \varnothing \times \varnothing \varnothing )</td>
<td>Postdietary serum phospholipids</td>
<td>0.049</td>
<td></td>
</tr>
<tr>
<td>D11Rat10 × D16Rat44</td>
<td>( \varnothing \varnothing \times \varnothing \varnothing )</td>
<td>Postdietary serum corticosterone</td>
<td>0.037</td>
</tr>
<tr>
<td>D11Rat10 × D16Rat66</td>
<td>( \varnothing \varnothing \times \varnothing \varnothing )</td>
<td>Postdietary serum corticosterone</td>
<td>0.007†</td>
</tr>
</tbody>
</table>

*Two-way ANOVA with main factors locus1-genotype and locus2-genotype. For statistical analysis body weight was used as a covariate.
†\( P \) value after logarithmic transformation of the data.
For humans, a significant interaction between Apoe and Lpl variants for circulating HDL cholesterol levels has been described. In the present study, there was no interaction between the segment of rat chromosome 1 that contains the Apoe gene and the Lpl containing chromosomal segment (rat chromosome 16). However, we detected a significant association between chromosome 11 and 1 or 16 (Table 4). This association points to a gene–gene interaction of Pcy1la with Apoe and Lpl. To the best of our knowledge, such an interaction has not been described previously.

The reciprocal relationship between cholesterol metabolism and adrenal steroid hormone activity is well established. Therefore, we anticipated that QTLs affecting serum cholesterol concentration might also be associated with variations in serum corticosterone. However, no significant QTLs for corticosterone were identified, even though there were significant interactions between the markers flanking a cholesterol-QTL (D1Rat335, D16Rat6, and D16Rat44) and the D11Rat10 marker (Table 4), thus suggesting a relationship between circulating cholesterol and corticosterone levels. Interestingly, QTLs for aldosterone were revealed on regions of chromosomes 1 and 18, where QTLs for high blood pressure have previously been identified. The genes responsible for these aldosterone variations are not known but may, indirectly, relate to the regulation of electrolyte metabolism.

In the mouse, several QTLs also involved in cholesterol metabolism have been localized. The chromosomal positions of the QTLs as found in the present study have been compared with the chromosomal locations of QTLs found in the mouse. A homologous location of the QTL for basal serum cholesterol levels on rat chromosome 1 (Figure 1) has been confirmed by 2 studies in the mouse. Gu et al and Purcell-Huynh et al found a QTL on mouse chromosome 7 in the vicinity the Apoe gene. For serum total cholesterol levels, a QTL on rat chromosome 2 was found (please see online Figure II). On the homologous region of mouse chromosome 3, a QTL for serum cholesterol levels was also identified. Furthermore, on mouse chromosome 18, a QTL for phospholipids levels has been found. Mouse chromosome 18 is homologous to rat chromosome 18, where we have found a suggestive QTL for this trait (Table 2). These findings seem to indicate that these traits have been conserved in the evolutionary differentiation of these species and thus may play a major role in cholesterol metabolism.

In summary, the present study indicates that rat chromosomes 1 to 3, 5 to 11, 15, 16, and 18 each contain at least 1 QTL that is involved in blood and/or hepatic lipid concentrations (or related phenotypes). Because the QTL mapping data were obtained with a relatively small number of animals, further experiments, including the development of (double) congenic strains or knockout strains after gene cloning, are necessary to precisely map the QTLs and to confirm the role of the suggested candidate genes.

References
17. Deleted in press.


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Materials and Methods

The research project was approved by the Animal Experimentation Committee of the Utrecht Faculty of Veterinary Medicine.

Animals and housing
All animals were kept under SPF conditions with 12 hours per day light (7.00 h - 19.00 h) and controlled temperature and humidity regimen. Six males and six females of the inbred strains BC/CpbU (BC) (obtained from the Central Laboratory Animal Institute of the Utrecht University, The Netherlands) and LEW/OlaHsd (LEW) (obtained from Harlan, UK) were tested for cholesterol response, as described in the experimental protocol (see below). From each gender and strain two animals were used for reciprocal matings. The F1-hybrids were intercrossed producing an F2 progeny.

Experimental protocol
After weaning, at the age of three weeks, the animals were fed a commercial, pelleted diet (RMH-B®, Hope Farms BV, Woerden, The Netherlands) till the age of seven weeks. The chemical composition of this commercial diet has previously been described. Then, at the age of seven weeks, the commercial diet was supplemented with 5.0% (w/w) olive oil (Reddy, Van de Moortele NV, Oudenbosch, The Netherlands) and 2.0% (w/w) cholesterol (USP, Solvay/Pharmaceuticals BV, Weesp, The Netherlands) during four weeks. This pelleted test-diet had been stored in the freezer until use. The animals had free access to food and water before and during the test-period. At the start and at the end of the test-period, after a 16 hour fast, blood samples were taken by orbital puncture under diethyl-ether anesthesia (between 8.00 and 10.00 h). Serum was collected after centrifugation and stored at -20°C. Following the last blood sampling, the rats were anaesthetised with diethyl-ether, exsanguinated via aorta puncture and the livers and spleens were removed. The tissues were frozen immediately at -70°C.

Chemical analyses
In a total of 192 F2-animals (92 males and 100 females) basal serum cholesterol levels, postdietary liver cholesterol concentrations, and serum phospholipid, cholesterol, aldosterone and corticosterone levels were determined. Lipids were extracted from liver homogenates according to a modification of the method of Abell et al. Liver cholesterol, serum cholesterol and serum phospholipids were measured enzymatically using the appropriate kits supplied by Boehringer Mannheim GmbH (Mannheim, Germany). Serum aldosterone and corticosterone concentrations were determined by radioimmunoassays as previously described.

Genome scan
DNA was isolated from the spleen using a standard procedure. A total of 256 microsatellite (SSLP) markers, polymorphic between the BC and LEW strain, were used for screening of the F2-progeny. These markers were randomly dispersed throughout the rat genome. Primers flanking the microsatellites were obtained from Research Genetics Inc. (MapPairs™, Huntsville, USA). When a microsatellite in BC and LEW differed less than 10 basepairs, the forward primer was 5'-end labelled with [γ-32P]ATP. Twenty nanogram genomic DNA was used for PCR according to manufacturers protocol. Products were separated in standard sequencing gels. When the microsatellites differed more than 10 bp between BC and LEW, 100 ng genomic DNA was used in the PCR reactions. These non-radioactive PCR products were separated in standard agarose gels.
**Map construction**

Segregation ratio of the genotypes of individual markers was checked by means of the Chi-squared goodness-of-fit test. None of the markers showed significant segregation distortion. The genetic map distance for the markers was computed with JoinMap™, version 3.0.6 For the establishment of linkage groups, a critical minimal LOD score of 3.0 was used. For calculation of map distances and estimating most likely gene orders, a critical LOD score of 0.05 was used. Recombination frequencies were converted to map distances in centiMorgans using the Kosambi function. Output from JoinMap was converted to figures using the graphics program MapChart7.

**Statistical analysis**

Both for the parental strains and for the F2-intercross rats, all statistical analyses of the measured phenotypes were carried out according to Petrie and Watson8 using a SPSS PC+ computer program.9 Two-side probabilities were estimated throughout.

**a. Parental strains**

The phenotypic characteristics of the BC and LEW rats were checked for normality using the Kolmogorov-Smirnov one-sample test. All results within groups were found to be normally distributed. Student's one sample t test for paired data was used to evaluate changes with time within groups. The significance of the differences between groups was calculated by a two-way analysis of variance (ANOVA) with strain and gender as main factors. Homogeneity of the variances was tested by Bartlett's test. For some phenotypes the variances had to be equalized by transformation (i.e. logarithmic) of the data.8 After transformation the variances were similar and the transformed within-group data still were normally distributed. For the serum and liver parameters the two-way ANOVA was performed with body weight as covariate, because there were significant differences in body weight between the two strains.

If the ANOVA showed significant effects the group means were further compared with the unpaired Student's t test. These tests were performed with pooled (for equal variances) or separate (for unequal variances) variance estimates. The equality of variances was tested using a F test. To take into account the greater probability of a type I error due to multiple comparisons, the level of significance for the Student's t tests was pre-set at P<0.05/[times a group is used for a comparison] instead of P<0.05, according to Bonferroni's adaptation.8 In all other cases, the probability of a type I error <0.05 was taken as the criterion of significance.

**b. F2-animals**

Within each gender, all traits were normally distributed (Kolmogorov-Smirnov one-sample test). Gender appeared to have a significant influence on the phenotypes (unpaired Student's t test, p<0.05), except on final body weight and baseline serum cholesterol level. For the genetic analysis of the gender-influenced traits in the combined male and female population, these phenotypes were first normalized in each gender. The measured levels were subtracted by the mean established for that gender and then divided by the standard deviation of that gender. Within the combined male and female population the (transformed) variables were normally distributed according to the Kolmogorov-Smirnov one-sample test.

**QTL analysis**

The location of the QTLs affecting the measured (transformed) quantitative traits were determined using the interval-mapping module of the MapQTL computer package (version 4.0).10 QTL analysis was also performed by MQM-mapping10 on the MapQTL computer
program. QTL-likelihood plots were produced by using the markers that flank the LOD score peak of identified QTLs in the interval-mapping method, as cofactors for QTL mapping on the MapQTL computer program.

For each trait and chromosome the LOD score thresholds were calculated by permutation analysis\(^{11}\) in order to achieve the chromosome-wide significance levels of 5%. Based on the paper of Lander and Kruglyak\(^{12}\) an association was assumed suggestive when the LOD score was between 57.6% and 100% of the LOD score threshold for significance.

If a DNA marker and the trait of interest are segregating independently, the values of the trait will be equally distributed among the homozygote and heterozygote genotypes. All data within genotype groups were found to be normally distributed by using the Kolmogorov-Smirnov one-sample test. For each group (females and males, females or males), co-segregation of phenotypes with alleles at marker loci was evaluated by comparing the values between different genotypes via one-way ANOVA with or without body weight as co-variate. For serum and liver parameters body weight was used as co-variate, since in F\(_2\) rats there were between some of these parameters and body weight weak, but significant linear associations (Table I). Pearson’s linear correlation coefficients (r) were calculated; significance was assessed by a two-tailed test. In the ANOVA tests, homogeneity of variances was tested (Bartlett’s test). When necessary, the variances were equalized by logarithmic or logistic transformation of the data.\(^8\) After transformation the within-group data were still normally distributed.

**Interactions**

Selected two-locus pairs (i.e. the loci that were flanking a candidate gene) were tested for epistatic interactions with a two-way ANOVA with body weight as a co-variate.

**References**

9. SPSS Inc. *SPSS/PC+™ 4.0, Base Manual for the IBM PC/XTAT and PS/2V (Release 4.0).* 1990; SPSS Inc., Chicago, IL USA.

**Table I. Associations between body weight and serum or liver parameters.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pearson’s linear correlation</th>
<th>F2 rats</th>
<th>Males (n = 92)</th>
<th>Females (n = 100)</th>
<th>Males plus females (n = 192)</th>
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<tbody>
<tr>
<td>Baseline serum cholesterol&lt;sup&gt;b&lt;/sup&gt;</td>
<td>r</td>
<td></td>
<td>-0.2202</td>
<td>-0.1306</td>
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<td></td>
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<td>0.195</td>
<td><strong>0.034</strong></td>
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<td>Postdietary serum cholesterol&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>0.1421</td>
<td>0.1087</td>
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<td>0.158</td>
<td>0.133</td>
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<tr>
<td>Postdietary serum phospholipid&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>0.155</td>
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<td><strong>0.041</strong></td>
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</table>

<sup>a</sup> Association with initial (Day 0) body weight.

<sup>b</sup> Association with final (Day 28) body weight.

<sup>c</sup> Significant associations (P < 0.05) are indicated in bold characters.
Fig. I  The genetic map (left) and LOD score plot (right) for initial body weight in the (LEW x BC)F₂-intercross. The thick horizontal line represent the threshold value of the LOD score considered as significant for linkage. (A) Rat chromosome 7 (RNO7), females. (B) Rat chromosome 15 (RNO15), males plus females.
Fig. II  The genetic map (left) and LOD score plot (right) for postdietary serum cholesterol level in the (LEW x BC)F₂-intercross. The thick horizontal line represent the threshold value of the LOD score considered as significant for linkage. (A) Rat chromosome 2 (RNO2), males plus females. (B) Rat chromosome 16 (RNO16), males plus females.
Fig. III  The genetic map (left) and LOD score plot (right) for postdietary serum phospholipid level in the (LEW x BC)F2-intercross. The thick horizontal line represent the threshold value of the LOD score considered as significant for linkage. Rat chromosome 11 (RNO11); 1 = males plus females, 2 = females.
Fig. IV The genetic map (left) and LOD score plot (right) for postdietary serum aldosterone level in the (LEW x BC)F₂-intercross. The thick horizontal line represents the threshold value of the LOD score considered as significant for linkage. (A) Rat chromosome 1 (RNO1), females. (B) Rat chromosome 18 (RNO18), females.