Genetic regulation of cholesterol homeostasis: chromosomal organization of candidate genes

Carrie L. Welch,†,‡ Yu-Rong Xia,† Ishaiahu Shechter,†,‡ Robert Fares,§§ Margarete Mehrabian,† Shahab Mehdizadeh,† Craig H. Warden,∥ and Aldons J. Lusis†,§,**

Department of Pathology,† Department of Medicine,‡ Department of Microbiology and Molecular Genetics,§ and Molecular Biology Institute,*** University of California, Los Angeles, CA 90095; Department of Biochemistry,‡‡ F. Edward Hebert School of Medicine, Uniformed Services University of the Health Sciences, Bethesda, MD 20814; and Gladstone Institute,§§ San Francisco, CA 94141

Abstract As part of an effort to dissect the genetic factors involved in cholesterol homeostasis in the mouse model, we report the mapping of 12 new candidate genes using linkage analysis. The genes include: cytoplasmic HMG-CoA synthase (Hmgcs1, Chr 13), mitochondrial synthase (Hmgcs2, Chr 3), a synthase-related sequence (Hmgcs1-rs, Chr 12), mevalonate kinase (Mvk, Chr 5), farnesyl diphosphate synthase (Fdft1, Chr 3), squalene synthase (Fdft1, Chr 14), acyl-CoA:cholesterol acyltransferase (Acact, Chr 1), sterol regulatory element binding protein-1 (Srebp1, Chr 8) and -2 (Srebp2, Chr 15), apolipoprotein A-4 regulatory protein (Tfcap4p2, Chr 7), low density receptor-related protein-related sequence (Lrp-n, Chr 10), and Lrp-associated protein (Lrpap1, Chr 5). In addition, the map positions for several lipoprotein receptor genes were refined. These genes include: low density lipoprotein receptor (Ldlr, Chr 9), very low density lipoprotein receptor (Vldlr, Chr 19), and glycoprotein 330 (Gp330, Chr 2). Some of these candidate genes are located within previously defined chromosomal regions (quantitative trait loci, QTLs) contributing to plasma lipoprotein levels, and Acact maps near a mouse mutation, ald, resulting in depletion of cholesteryl esters in the adrenals. The combined use of QTL and candidate gene mapping provides a powerful means of dissecting complex traits such as cholesterol homeostasis.-Welch, C. L., Y.-R. Xia, I. Shechter, R. Fares, M. Mehrabian, S. Mehdizadeh, C. H. Warden, and A. J. Lusis. Genetic regulation of cholesterol homeostasis: chromosomal organization of candidate genes. J. Lipid Res. 1996. 37: 1406–1421.

Supplementary key words cholesterol metabolism • complex trait • quantitative trait locus • conserved synteny • disease gene

Cholesterol homeostasis is critical for normal cell function, and abnormalities of cholesterol metabolism can contribute to pathologic processes including atherosclerosis. The regulation of cholesterol homeostasis is complex, involving lipoprotein transport proteins, lipoprotein receptors, cholesterol biosynthetic and degradative enzymes, and transcriptional regulatory proteins. Rare genetic variations resulting in deficiencies or structural alterations of several of these proteins have been shown to predispose individuals to dyslipidemias and premature atherosclerosis (1). However, dissection of the genetic factors involved in common variations in plasma cholesterol levels has been hampered by environmental influences, genetic heterogeneity, and polygenic inheritance.

Animal models have become increasingly useful tools for the study of complex traits. The use of animal models in genetic studies reduces the interference of environmental differences and the problem of genetic heterogeneity. In addition, the ability to perform planned genetic crosses provides a definitive means of mapping chromosomal regions (including quantitative trait loci, or QTLs) contributing to complex traits. The availability of a dense expression map for the mouse simplifies the process of identifying the individual genes underlying QTLs (which could contain several hundred genes) by providing candidates that can be directly tested. The identification of a gene contributing to a trait by coincidental mapping of the trait and the candidate gene to the same genetic locus, has, in fact, been the most productive means of identifying disease genes in mice and is likely to become the case for humans as well. Some recent examples of the usefulness of this approach include the identification of Apoa2 as a determinant of atherogenesis in the mouse (2), Pla2g as a modifier of polymp number in colon cancer (3), and Cpe as a determinant of preproinsulinemia in obesity (4).

Abbreviations: HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; Chr, chromosome; RI, recombinant inbred; SDP, strain distribution pattern; LDL, low density lipoprotein; LRP, low density lipoprotein-related protein; QTL, quantitative trait locus; RFLV, restriction fragment length variant.

†To whom correspondence should be addressed.
‡Present address: Department of Pediatrics, University of California, Davis, CA 95616.
Our laboratory and others have begun to identify QTLs contributing to various traits related to lipid metabolism in mice, including plasma lipid levels (2, 5, 6), obesity (6-8), and cholesterol gallstone formation (9). Some of these loci fall within regions of candidate genes, others do not. While numerous candidate genes for lipid metabolism have been mapped in the mouse, including genes encoding apolipoproteins, lipoprotein receptors, and plasma lipid metabolizing enzymes, few of the genes involved in sterol biosynthesis and metabolism have been mapped. In fact, the localizations of \textit{Hmgcr} (10), encoding HMG-CoA reductase, and a family of genes encoding presumably cryptic forms of farnesyl diphosphate synthase (11, 12) are the only reported mappings of sterol biosynthetic genes in mice.

As a framework for localizing candidate genes for cholesterol homeostasis and other atherosclerosis-related traits, we have constructed a complete genetic linkage map in an interspecific mouse cross. Portions of this linkage map have been previously reported (12). We now describe the map, in entirety, and report the mapping of twelve new candidate genes for cholesterol homeostasis. We have also refined the map positions of several lipoprotein receptor genes. Finally, we have compiled a composite map of candidate genes and QTLs for sterol and lipid metabolism and discuss the current state of this "fat map."

**MATERIALS AND METHODS**

**Interspecific backcross mapping**

(C57BL/6J × \textit{Mus spretus})F1 X C57BL/6J interspecific backcross (BSB) mice were generated as described (12). For the framework map, genomic DNA was extracted and mice were genotyped using previously mapped anchor clone cDNAs, randomly isolated mouse liver cDNAs, simple sequence repeat polymorphisms, and a variety of cloned cDNAs obtained from other laboratories (see Fig. 1 legend for references). For candidate gene mapping, genomic DNA from parental and F1 mice was digested with a panel of restriction enzymes (BamHI, BglII, EcoRI, HindIII, MspI, PstI, PvuII, SstI, TaqI, and XbaI) and analyzed by Southern hybridization to determine restriction fragment length variants (RFLVs). Distinct RFLVs were then selected for scoring in 67 BSB mice. The probes used for hybridization are described below. All hybridizations were performed at 65°C as described (12); wash conditions are given in Table 1. The blots were then dried and exposed to Kodak X-ray film at -70°C for 3–21 days.

Linkage maps were constructed using Map Manager v2.6.5 (13). Exact map locations were confirmed manually by the method of minimizing apparent double and multiple crossovers. All remaining double crossovers were then checked for typing errors. To minimize distortion of recombination intervals, loci exhibiting three or more double crossovers were eliminated from the framework map. The distribution patterns of candidate gene RFLVs were then compared to the distribution patterns of all other loci in the database and localized in a similar manner. Using this mapping strategy, earlier typings were repeatedly subject to evaluation as new loci were added to the map.

**Probes**

The cDNA probes used for hybridization with BSB genomic DNA are described in Table 1 and below. The following cDNAs were generous gifts: apolipoprotein A-I regulatory protein (S. K. Karathanasis, Wyeth-Ayerst, Inc.), farnesyl diphosphate synthase (P. A. Edwards, University of California, Los Angeles), low density lipoprotein receptor (H. Hobbs, University of Texas, Dallas), low density lipoprotein receptor-associated protein (J. Herz, University of Texas, Dallas), mevalonate kinase (S. Krisans, San Diego State University, and R. Gregg, Bristol Meyers Squibb), and sterol regulatory element binding protein-1 (P. A. Edwards, University of California, Los Angeles). Cytoplasmic HMG-CoA synthase and sterol regulatory element binding protein-2 cDNAs were obtained from the American Type Culture Collection (ATCC, Rockville, MD).

The mitochondrial HMG-CoA synthase cDNA probe was generated by amplification of mouse cDNA using mitochondrial-specific oligonucleotides (based on comparison of the rat mitochondrial and cytoplasmic sequences, Genbank accession #M33648 and #X52625, respectively). Briefly, mouse total cDNA was prepared using oligo(dT)12–18 primers (Gibco BRL, Gaithersburg, MD) and Superscript II reverse transcriptase (Gibco BRL) according to the manufacturer's protocol for a 20-μl reaction. One microliter of cDNA was then used as template in 10-μl PCR reactions containing 0.66 μM each primer, 1X Taq polymerase buffer, 1.5 mM MgCl₂, 0.2 mM dNTPs, and 0.5 U/μl Taq polymerase (Gibco BRL). The oligonucleotides were synthesized on an Applied Biosystems 392 synthesizer according to the manufacturer's suggested protocol and had the following sequences: 5'-ACACACGCCACCCCATAGGAT-3' (PmtsynF) and 5'-GCTTATTATCAGACTGTTGGGC-3' (PmtsynR). The amplification reaction was run on a 2% agarose gel and a single band corresponding to the expected 220 bp product was observed. The product was gel-purified and then used in Southern analysis of RFLVs as described above and in Table 1.

The cDNAs used to map the low density lipoprotein receptor-related protein (LPR)-related sequences were obtained by screening a mouse liver cDNA library using

*Welch et al. Genetic mapping of mouse genes involved in cholesterol homeostasis*
<table>
<thead>
<tr>
<th>Dimens1</th>
<th>Dimens2</th>
<th>Dimens3</th>
</tr>
</thead>
<tbody>
<tr>
<td>123</td>
<td>456</td>
<td>789</td>
</tr>
<tr>
<td>567</td>
<td>890</td>
<td>123</td>
</tr>
<tr>
<td>234</td>
<td>567</td>
<td>890</td>
</tr>
<tr>
<td>345</td>
<td>678</td>
<td>901</td>
</tr>
<tr>
<td>456</td>
<td>789</td>
<td>123</td>
</tr>
</tbody>
</table>

*Note: The table contains placeholder values for demonstration purposes.*
Fig. 1.  BSR genetic linkage map, including candidate genes for cholesterol homeostasis. Each chromosome is represented by a pair of vertical lines whose length is based on the overall genome size of 1530 centiMorgans (cM), comparable to the Chromosome Committee composite map of the mouse (16, 17). The centromeric ends are positioned at the top of each bar and distal ends at the bottom. Distances from centromeric ends are based on the most proximal locus shared with the composite map (16, 17). For each chromosome, the cumulative (cum) distance (in cM) of the most distal locus from the centromere is indicated to the left of the distal locus. For each pair of loci, the ratios of the number of recombinants to the total number of informative mice and the recombination frequencies ± standard errors (in cM) are indicated to the left of the chromosomes. For pairs of loci that cosegregate, the upper 95% confidence interval is shown in parentheses. Loci are linked with LOD scores ≥6. Candidate genes mapped in this study are indicated by bold typeface and larger font size. Gene names and references for previously published loci are given in Refs. (16, 17) with the following exceptions: Bm3.1 (61), Bm5.0 (61), Cobph2 (62), Coq3 (63), Hdhlp (64), Lgals5 (65), Pla2g2a, Pla2g2c, Pla2g5 (66), Pomp (67), Ppara, Pparg (68), Ppard (69), and Scp2 (70). Ucla markers are reported in ref. 12 or are unpublished loci. Cfr (corticotropin-releasing factor receptor), Eno2 (enolase 2), Eno3 (enolase 3), Hemox (hemoxygenase), Lgals2 (a member of the soluble lactose-binding lectin gene family) are unpublished loci and specific information can be obtained from the authors.
a partial human LRP clone. Briefly, the cDNA clone LRP5 (14) was labeled with 32P by random priming and then hybridized with a λZAP library derived from a (C57BL/6 x CBA/J)F1 female mouse (Stratawene). Phage DNA from hybridizing plaques was purified; the inserts were converted to Bluescript plasmid by in vivo excision, according to the manufacturer's protocol, and the resulting phage were used to infect E. coli strain XL1-Blue. Plasmid DNA was prepared from 50-ml cultures using Qiagen columns.

To suppress cross-hybridization to mouse repetitive DNA sequences, mouse COT-1 DNA (Gibco BRL) was added to hybridizations with sterol regulatory element binding protein-1 and -2 cDNAs and the mitochondrial synthase probe, according to the manufacturer's protocol. The NdeI, SmaI farnesyl diphosphate synthase promoter fragment (nt. -736 to -47) was digested with TaqI prior to hybridization to remove an AT-rich repetitive sequence. The digestion products were run on a 1% agarose gel and the 482 bp fragment (nt. -529 to -47) was purified and used as a probe.

RI strain mapping

NX129, BXD, AKXL, AKXD, and CXB RI strain DNAs were obtained from Jackson Laboratory (Bar Harbor, ME). Southern analyses of RFLVs were performed as described above. PvuI-digested NX129 DNA was hybridized with a 1.2 kb LDL receptor cDNA and washed with 1 x SSC at 50°C for 20 min (as described for BSB mapping in Table 1). BXD, AKXL, AKXD, and CXB DNA was digested with MspI, PouII, or HindIII, as indicated in Table 2, and hybridized with a previously described rat HMG-CoA synthase cDNA probe (15). The filters were then washed in 0.2 x SSC at 60°C for 20 min. The strain distribution patterns (SDPs) of the candidate gene RFLVs were compared to the SDPs in the JAX and RWE RI data files provided with Map Manager v2.6.5 (13) and the map positions were determined by minimizing the number of double and multiple crossovers.

Nomenclature and MGD accession numbers

The gene symbols utilized are consistent with the nomenclature for the corresponding human genes. Data have been deposited into the Mouse Genome Database (16) maintained by the Genome Informatics Group at the Jackson Laboratory and have the following accession numbers: MGD–CREX-638 (Chr 12), MGD–CREX-639 (Chr 13), MGD–CREX-640 (Chr 14), MGD–CREX-641 (Chr 15), MGD–CREX-642 (Chr 16), MGD–CREX-643 (Chr 17), MGD–CREX-644 (Chr 18), MGD–CREX-645 (Chr 19), MGD–CREX-646 (Chr X).

RESULTS

Framework map

A complete mouse linkage map was constructed using a set of 67 BSB mice and 322 genetic markers that span the entire mouse genome (Fig. 1). The map was anchored using a combination of 38 cDNA clones and 11 1 simple sequence repeat markers mapped in other crosses (information about specific loci is available online from the Mammalian Genome Database, ref. 16, or in the 1994 Mammalian Genome Supplement, ref. 17). The rate of anomalous typings (double recombinations that cannot be accounted for by scoring errors) was low (2 x 10^-3). The resulting map has an average resolution of 4.6 cM and an overall genetic length of 1530 cM, comparable to the length of the 1994 Chromosome Committee composite map (1538 cM) (16, 17) and in agreement with other estimates of the mouse genome size (1350 cM to 1600 cM) (18-21).

Candidate gene mapping

Using our BSB framework map and maps derived from several sets of RI strains, we have localized 12 new candidate genes for cholesterol homeostasis and refined the mapping of 3 previously reported genes. Chromosomal assignments were determined by linkage analysis of RFLVs, and genes were ordered by minimizing the number of recombinations with framework markers. Gene orders are supported by LOD scores >3.6 (most locus orders are supported by LOD scores >8; the exceptions occur in regions of lower marker density). No significant linkages were observed between the candidate genes and markers on other chromosomes. The candidate genes are widely dispersed throughout the mouse genome; gene orders, recombination distances and standard errors are given in Fig. 1.

Cholesterol biosynthetic enzymes

HMG-CoA synthase catalyzes the first reaction in the cholesterol biosynthetic pathway, condensation of acetyl-CoA with acetoacetyl-CoA to form HMG-CoA and...
<table>
<thead>
<tr>
<th>cDNA, Species</th>
<th>Clone Name</th>
<th>Reference</th>
<th>Fragment Used as Probe</th>
<th>RE</th>
<th>Wash Conditions</th>
<th>Restriction Fragment Size (kb)</th>
<th>Locus Mapped</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acyl-CoA:cholesterol acyltransferase, human</td>
<td>pHuACAT1.6</td>
<td>56</td>
<td>1.6 kb EcoRI</td>
<td>HindIII</td>
<td>0.5 x SSC, 60°C</td>
<td>3.2, 6.0, 6.9, 25, 40</td>
<td>Acat</td>
</tr>
<tr>
<td>Apolipoprotein A-I regulatory protein, human</td>
<td>Arp-1 CoZfrag</td>
<td>51</td>
<td>1.74 kb PstI</td>
<td>0.5 x SSC, 60°C</td>
<td>1.9, 3.2, 3.9</td>
<td>1.9, 3.2, 3.9, 8.8</td>
<td>Tflsop2</td>
</tr>
<tr>
<td>Farnesyl diphosphate synthase, rat</td>
<td>pFPPS-0.69</td>
<td>28</td>
<td>0.48 kb TaqI</td>
<td>1 x SSC, 50°C</td>
<td>3.3</td>
<td>7.2</td>
<td>Fdps</td>
</tr>
<tr>
<td>HMG-CoA synthase, cytoplasmic, hamster</td>
<td>p53K-312</td>
<td>ATCC</td>
<td>1.2 kb PstI, HindIII</td>
<td>0.5 x SSC, 60°C</td>
<td>2.0, 2.7, 3.8</td>
<td>2.1</td>
<td>Hmgcs1</td>
</tr>
<tr>
<td>HMG-CoA synthase, mitochondrial, mouse</td>
<td>none</td>
<td>see Methods</td>
<td>220 bp PCR product</td>
<td>EcoRI</td>
<td>0.5 x SSC, 65°C</td>
<td>5.7</td>
<td>6.3</td>
</tr>
<tr>
<td>LDL receptor, human</td>
<td>pLDLR-R4</td>
<td>57</td>
<td>1.2 kb HincII</td>
<td>PstI</td>
<td>1 x SSC, 50°C</td>
<td>1.3, 1.9, 2.1, 3.6</td>
<td>1.3, 1.4, 1.9, 2.0</td>
</tr>
<tr>
<td>LRP-related sequence, mouse</td>
<td>pLRP1</td>
<td>see Methods</td>
<td>0.8 kb EcoRI</td>
<td>EcoRI</td>
<td>1 x SSC, 50°C</td>
<td>10.3, 13.0</td>
<td>1.4, 13.0</td>
</tr>
<tr>
<td>LRP-related sequence, mouse</td>
<td>pLRP2</td>
<td>see Methods</td>
<td>1.4 kb EcoRI</td>
<td>BglII</td>
<td>0.1 x SSC, 65°C</td>
<td>1.0, 2.0</td>
<td>1.0, 3.0</td>
</tr>
<tr>
<td>LRP-related sequence, mouse</td>
<td>pLRP2</td>
<td>see Methods</td>
<td>1.4 kb EcoRI</td>
<td>MspI</td>
<td>1 x SSC, 50°C</td>
<td>0.4, 4.3, 4.7, 8.0, 9.6</td>
<td>0.4, 4.3, 4.7, 8.0, 9.6</td>
</tr>
<tr>
<td>LRP-associated protein, rat</td>
<td>RapII 145</td>
<td>58</td>
<td>3.3 kb EcoRI</td>
<td>HindIII</td>
<td>0.1 x SSC, 60°C</td>
<td>2.8, 8.8</td>
<td>2.5, 8.8</td>
</tr>
<tr>
<td>Mevalonate kinase, rat</td>
<td>SGB-1171</td>
<td>59</td>
<td>1.73 kb EcoRI</td>
<td>TaqI</td>
<td>0.5 x SSC, 50°C</td>
<td>1.8, 2.7, 4.9, 5.2</td>
<td>1.8, 2.2, 2.7, 4.5, 5.2</td>
</tr>
<tr>
<td>Squalene synthase, rat</td>
<td>pRSS1327</td>
<td>60</td>
<td>1.3 kb EcoRI, HindIII</td>
<td>1 x SSC, 50°C</td>
<td>2.8, 3.2, 3.5, 6.7</td>
<td>2.8, 3.2, 6.7, 8.0, 9.5</td>
<td>Fdft1</td>
</tr>
<tr>
<td>Sterol regulatory element binding protein-1, human</td>
<td>pCMV-CSA unpublished</td>
<td>1.5 kb SalI, EcoRI</td>
<td>PstI</td>
<td>0.5 x SSC, 60°C</td>
<td>1.8, 2.7, 3.5, 6.1, 8.9</td>
<td>1.8, 2.7, 3.2, 6.1, 8.9</td>
<td>Srebfl</td>
</tr>
<tr>
<td>Sterol regulatory element binding protein-2, human</td>
<td>pSREBP-2</td>
<td>ATCC</td>
<td>1.65 kb SalI, EcoRI</td>
<td>HindIII</td>
<td>0.5 x SSC, 65°C</td>
<td>2.8, 15.0</td>
<td>2.8, 6.0</td>
</tr>
</tbody>
</table>

The restriction fragment length variants used to score the segregation of M. spretus alleles in the backcross mice are underlined.

CoA in the cytoplasm. In liver, the condensation reaction also occurs in mitochondria, as one of the early steps of ketogenesis. The cytoplasmic and mitochondrial proteins are encoded by distinct genes; we have mapped both loci using probes specific for either the cytoplasmic (Hmgcs1) or mitochondrial (Hmgcs2) locus (Table 1). Hybridization with the Hmgcs1 probe resulted in the detection of 2.0, 2.7, and 3.8 kb fragments in PstI-digested C57BL/6 (BL/6) DNA, a 2.1 kb fragment in Mus spretus (spretus) DNA, and 2.0, 2.1, 2.7, and 3.8 kb fragments in F1 DNA. The presence or absence of the 2.1 kb fragment was followed in the backcross mice and the segregation pattern exhibited linkage to Chr 13 markers. The locus was fine-mapped to the distal end of chr 13, 15 cM from the HMG-CoA reductase gene (Hmgcr) (Fig. 1). The additional bands detected in BL/6 DNA may represent linked pseudogenes present in BL/6 but not spretus mice; other species-specific pseudogenes have been reported, including Mif (22), Odc (23), and Hmg (24) pseudogenes. The Hmgcs2 probe hybridized to a single EcoRI fragment in each parental strain DNA, 5.7 kb in BL/6 and 6.3 kb in spretus, and both...

Welch et al. Genetic mapping of mouse genes involved in cholesterol homeostasis
fragments in F1 DNA. The segregation pattern of these alleles was linked to markers on Chr 3. Conserved linkages between these mouse chromosomes and human Chr 12 and 13, which contain the human homologues of Hmgcs1 and Hmgcs2, respectively, support the presence of a related locus on Chr 12. We have designated the Chr 12 locus Hmgcs1-rs (cytoplasmic HMG-CoA synthase-related sequence).

The genes encoding three other key enzymes in the cholesterol biosynthetic pathway were mapped. Mevalonate kinase acts just distal to HMG-CoA reductase and is one of only two enzymes in the pathway in which a defect has been reported to result in a clinical disease (26). After digestion of genomic DNA with TaqI and hybridization with a mevalonate kinase cDNA probe, the BSB mice were typed for the presence or absence of a 4.5 kb spretus-specific fragment. The gene encoding mevalonate kinase, Mvk, was mapped to mouse Chr 5.

Farnesyl diphosphate synthase and squalene synthase both function at a branchpoint in the biosynthetic pathway and the expression of the genes encoding these enzymes is highly regulated. The product of farnesyl diphosphate synthase, farnesyl diphosphate, is a substrate for both protein prenylation and sterol synthesis. Previously, ten loci hybridizing to the farnesyl diphosphate synthase cDNA had been mapped to mouse Chr 3, 4, 6, 10, 12, 13, 17, and X (11, 12), designated

### Table 2. Segregation of Hmgcs restriction fragment length variants in RI strains

<table>
<thead>
<tr>
<th>RI Strain Set</th>
<th>RE</th>
<th>Progenitor Strain</th>
<th>Restriction Fragment Size (kb)</th>
<th>Strain Distribution Patterns</th>
<th>Chr Linkage</th>
</tr>
</thead>
<tbody>
<tr>
<td>BXD</td>
<td>MspI</td>
<td>C57BL/6J</td>
<td>1.0, 1.2, 1.9, 3.5</td>
<td>2, 15, 14, 15, 18, 10, 21, 25, 27, 28, 29, 30</td>
<td>13*</td>
</tr>
<tr>
<td>BXD</td>
<td>MspI</td>
<td>DBA/2J</td>
<td>0.75, 1.2, 1.8, 1.9, 2.5</td>
<td>1, 5, 6, 8, 9, 11, 12, 16, 20, 22, 23, 24, 31, 32</td>
<td>12*</td>
</tr>
<tr>
<td>BXD</td>
<td>MspI</td>
<td>C57BL/6J</td>
<td>1.0, 1.2, 1.9, 3.5</td>
<td>2, 6, 11, 12, 14, 16, 21</td>
<td></td>
</tr>
<tr>
<td>BXD</td>
<td>MspI</td>
<td>DBA/2J</td>
<td>0.75, 1.2, 1.8, 1.9, 2.5</td>
<td>1, 5, 8, 9, 13, 15, 18, 19, 20, 22, 23, 24, 25, 27, 28, 29, 30, 31, 32</td>
<td>12*</td>
</tr>
<tr>
<td>AKXD</td>
<td>PvuII</td>
<td>AKR/J</td>
<td>0.82, 0.94, 2.6, 4.9</td>
<td>1.6, 7, 8, 9, 11, 12, 13, 14, 16, 21, 23, 24, 27, 28</td>
<td>15*</td>
</tr>
<tr>
<td>AKXD</td>
<td>PvuII</td>
<td>DBA/2J</td>
<td>0.82, 2.6, 5.3</td>
<td>2, 3, 10, 15, 17, 18, 20, 22, 26</td>
<td></td>
</tr>
<tr>
<td>AKXL</td>
<td>HindIII</td>
<td>AKR/J</td>
<td>15.0</td>
<td>5, 6, 9, 12, 14, 16, 17, 25, 29, 38</td>
<td>12*</td>
</tr>
<tr>
<td>AKXL</td>
<td>HindIII</td>
<td>C57L/J</td>
<td>3.1, 15.0</td>
<td>7, 8, 13, 19, 21, 24, 28, 37</td>
<td></td>
</tr>
<tr>
<td>CXB</td>
<td>HindIII</td>
<td>C57BL/6By</td>
<td>3.1, 15.0</td>
<td>J</td>
<td></td>
</tr>
<tr>
<td>CXB</td>
<td>HindIII</td>
<td>BALB/cBy</td>
<td>15.0</td>
<td>D, E, G, H, I, K</td>
<td></td>
</tr>
</tbody>
</table>

The variants used to score the segregation patterns are underlined.

*Locus is linked to chromosomal markers at the 99.99% confidence level (LOD score > 5.0).

*Locus is linked to chromosomal markers at the 95% confidence level (LOD score = 2.0).
### TABLE 3. Mouse-human synteny for candidate genes

<table>
<thead>
<tr>
<th>Mu Chr</th>
<th>Mu Gene Symbol</th>
<th>Mu Gene Name</th>
<th>Hu Chr</th>
<th>Hu Gene Symbol</th>
<th>Gene Syntenic Relationship</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Acat</td>
<td>acyl-CoA:cholesterol acyltransferase</td>
<td>1q25</td>
<td>ACACT</td>
<td>conserved linkage</td>
</tr>
<tr>
<td>2</td>
<td>Gp330</td>
<td>glycoprotein 330</td>
<td>2q24-q31</td>
<td>LRP2</td>
<td>conserved linkage</td>
</tr>
<tr>
<td>3</td>
<td>Fdps (formerly Fdpsl)</td>
<td>farnesyl diphosphate synthase</td>
<td>1q24-q31</td>
<td>FDPSSL1</td>
<td>conserved linkage</td>
</tr>
<tr>
<td>3</td>
<td>Hmgas2</td>
<td>HMG-CoA synthase, mitochondrial</td>
<td>1p13-p12</td>
<td>HMGCS2</td>
<td>conserved linkage</td>
</tr>
<tr>
<td>5</td>
<td>Msk</td>
<td>mevalonate kinase</td>
<td>12</td>
<td>MVK</td>
<td>conserved linkage; near breakpoint</td>
</tr>
<tr>
<td>5</td>
<td>Lrspap1</td>
<td>LRP-associated protein 1</td>
<td>4p16.3</td>
<td>LRPAP1</td>
<td>conserved linkage; near breakpoint</td>
</tr>
<tr>
<td>7</td>
<td>Tcfoup2</td>
<td>apolipoprotein A-I regulatory protein 1</td>
<td>15</td>
<td>TFCOUP2</td>
<td>conserved linkage</td>
</tr>
<tr>
<td>8</td>
<td>Srebfl</td>
<td>sterol regulatory element binding factor-1</td>
<td>17p11.2</td>
<td>SREBF1</td>
<td>novel segment of homology</td>
</tr>
<tr>
<td>9</td>
<td>Ldlr</td>
<td>low density lipoprotein receptor</td>
<td>19q13.2</td>
<td>LDLR</td>
<td>conserved linkage</td>
</tr>
<tr>
<td>10</td>
<td>Lrsp2</td>
<td>LRP-related sequence</td>
<td>12q13</td>
<td>LRP?</td>
<td>conserved linkage</td>
</tr>
<tr>
<td>12</td>
<td>Hmgas1-a</td>
<td>HMG-CoA synthase-related sequence</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>13</td>
<td>Hmgas1</td>
<td>HMG-CoA synthase, cytoplasmic</td>
<td>5p14-p13</td>
<td>HMGCS1</td>
<td>conserved linkage</td>
</tr>
<tr>
<td>14</td>
<td>Fdftl</td>
<td>farnesyl diphosphate farnesyl-transferase 1/squalene synthase</td>
<td>8p22-23.1</td>
<td>FDFT1</td>
<td>novel segment of homology</td>
</tr>
<tr>
<td>15</td>
<td>Srebfl2</td>
<td>sterol regulatory element binding factor-2</td>
<td>22q15</td>
<td>SREBF2</td>
<td>conserved linkage; near breakpoint</td>
</tr>
<tr>
<td>19</td>
<td>Vldlr</td>
<td>very low density lipoprotein receptor</td>
<td>9p24</td>
<td>VLDLR</td>
<td>conserved linkage</td>
</tr>
</tbody>
</table>

aData compiled from the Human Genome Data Base (55) and the Mouse Genome Database (16).

_Fdpsl_1-10, and five homologous human Chrs 1, 7, 14, 15, and X (27). Most of these loci are thought to represent pseudogenes (28). Using a promoter fragment of the functional gene (28) as a probe in Southern hybridization, we now report the mapping of a single locus presumed to encode an active prenyltransferase. The rat promoter contains a 120 nucleotide region homologous to RN55REP, a rat repetitive sequence (Genbank accession #X13424). After removing this sequence from the probe, hybridization with TafqI-digested genomic DNA resulted in the detection of a single fragment in each parental strain. The segregation pattern of the alleles was linked to the same region of Chr 3 as _Fdpsl_1. _Fdps_ now replaces _Fdpsl_1.

Squalene synthase catalyzes the formation of squalene from farnesol diphosphate, committing the pathway to sterol synthesis. The presence or absence of cosegregating 8.0 and 9.5 kb _HindIII_-generated _spretus_ fragments was followed in the backcross mice; the segregation pattern was linked to markers on the proximal end of Chr 14. The gene symbol _Fdpsl_ (farnesyl diphosphate farnesyl-transferase 1/squalene synthase) reflects the function of the encoded enzyme.

**Cholesterol esterification**

Acyl-CoA cholesterol acyltransferase catalyzes the intracellular formation of cholesteryl esters. This enzymatic activity is accompanied by down-regulation of both endogenous synthesis of cholesterol and receptor-mediated uptake of exogenous cholesterol. Hybridization of _HindIII_-digested DNA with a human cDNA probe resulted in the detection of several variant bands between BL/6 and _spretus_ mice. The distribution pattern of cosegregating 3.9 and 30 kb _spretus_-specific fragments among the BSB mice exhibited linkage to Chr 1 markers. To avoid confusion with _Acat1_ and _Acat2_ (acyt-CoA acetyltransferases) on Chrs 9 and 17, the gene symbol _Acat_ (acyl-CoA:cholesterol acyltransferase) has been assigned.

**Nuclear regulatory proteins**

Two sterol regulatory element binding proteins, _SREBP-1_ and _SREBP-2_, have been shown to regulate transcription of the low density lipoprotein receptor, HMG-CoA synthase, and farnesyl diphosphate synthase genes (29–31). In addition, there is suggestive evidence for the participation of these binding proteins in the regulation of the squalene synthase gene (32). A 1.65 kb probe corresponding to the 3' end of the _SREBP-2_ gene was used to map _Srebfl2_. Hybridization of the probe with _HindIII_-digested DNA resulted in the detection of one unique fragment in DNA from each parental strain, 15.0 kb in BL/6 and 6.0 kb in _spretus_, a common 2.8 kb fragment, and all three fragments in the F1 DNA. The segregation pattern of the variant fragments was linked to markers on Chr 15. Hybridization of _PsvII_-digested
DNA with a full-length SREBP-1 cDNA resulted in the detection of several hybridizing fragments in DNA from both BL/6 and spretus. The presence or absence of a 3.7 kb spretus-specific fragment was used to map Srebfl to Chr 8.

Apolipoprotein A-I regulatory protein 1, is a member of the steroid hormone receptor gene family and a conditional mediator of apolipoprotein A-I gene expression (33). The presence or absence of an 8.8 kb PstI spretus-specific fragment was followed in the BSB mice. The distribution pattern was linked to markers on Chr 7. The gene symbol Tfcoup2 (transcription factor chicken ovalbumin upstream promoter 2) has been assigned to be consistent with the nomenclature for other members of the gene family.

**Lipoprotein receptors and related proteins**

The uptake of exogenous cholesterol by lipoprotein receptors serves as a signal for down-regulation of the cholesterol biosynthetic pathway and up-regulation of sterol metabolizing pathways. Frank, Taylor, and Lusis (34) previously reported the localization of the gene encoding the low density lipoprotein receptor, Ldlr, to the proximal region of Chr 9 in several sets of RI strains. An apparent error in the reported SDP for NX129 mice at the Ldlr locus was recently brought to our attention. To address this discrepancy, we retyped the RI set. The correct SDP is NX129-1,2,5 = NZB/BINJ. We also typed the BSB mice to refine the mapping of Ldlr. Thus, Ldlr was mapped to the interval (cM ± SE): Gria4–7.8 ± 3.4–Ldlr–9.8 ± 3.8–D9Mit2–4.9 ± 2.8–Otf1/1.

Several low density lipoprotein receptor-related protein (LRP) cDNA clones were isolated and used as probes in Southern hybridization. Sequence analysis indicated that the clones corresponded to nt. 3815–7830 of mouse Lrp (Genbank accession #X67469). Most of the clones mapped to a single location on Chr 10. For example, hybridization of the LRP2 probe with BglII-digested DNA resulted in the detection of 1.0 and 2.0 kb fragments in BL/6 DNA, 1.4 and 3.0 kb fragments in spretus DNA, and all three fragments in F1 DNA. The distribution pattern of the 3.0 kb fragment in the BSB mice was linked to the Chr 10 marker D10Mit24 with a LOD score of 16; the RFLV was not linked to markers on any other chromosome. Using less stringent washing conditions, two RFLVs were detected which segregated with either Chr 2 or Chr 19 markers. Hybridization of LRP2 with MspI-digested DNA, followed by a final wash at 50°C, resulted in the detection of 10.3 and 13.0 kb fragments in BL/6 DNA, 1.4 and 13.0 kb fragments in spretus DNA, and all three fragments in F1 DNA. The presence or absence of the 1.4 kb fragment in DNA from the backcross mice exhibited linkage to Chr 19 markers and coincided with the reported location of Vldlr, the very low density lipoprotein receptor locus (36). Based on known sequence homologies between GP330, VLDLR, and LRP (37), and the apparent co-localizations of our LRP-related loci with these genes, we suggest that the loci mapped to Chr 2 and Chr 19 are, in fact, Gp330 and Vldlr. Thus, the mapping intervals have been refined as follows (cM ± SE): D2Mit65–3.7 ± 2.6–PgsI–12.3 ± 4.1–Gp330–1.5 ± 1.5–Acra–1.5 ± 1.5–D2Mit35 and Fth–1.5 ± 1.5–Vldlr–0.0–D19Uca2–0.0–D19Uca3– 3.9 ± 2.7–D19Mit29.

Lrpap1, encoding the LRP-associated protein, was localized to Chr 5 using a rat cDNA probe. After digestion with HindIII, genomic DNA from BL/6 mice exhibited hybridizing bands of 2.8 and 8.8 kb, whereas DNA from spretus mice yielded bands of 2.5 and 8.8 kb. The RFLV segregated with marker D5Mit54 on proximal Chr 5, in a region of conserved homology to human Chr 4p16 which contains the human LRP-associated protein gene (Table 3).

**DISCUSSION**

We have constructed a complete mouse linkage map and have utilized the map to localize candidate genes for cholesterol homeostasis (Fig. 1). The framework map has a resolution of <5 cM and is anchored with 149 loci typed in other mouse crosses, facilitating the incorporation of our mapping data into the composite map of the mouse genome (16, 17). Using the BSB framework map, we have localized 14 candidate genes for cholesterol homeostasis by linkage analysis of RFLVs. The genes encode cholesterol biosynthetic and metabolic enzymes, nuclear regulatory factors, and lipoprotein receptors. In addition, we have mapped a novel locus related to HMG-CoA synthase in several sets of RI strains. The localization of genes in the mouse provides candidates for mapped phenotypes and clues regarding evolutionary relationships between loci and conserved interspecies synteny.

**Candidate genes for plasma lipoprotein QTLs**

Our primary objective in mapping genes involved in cholesterol homeostasis is to clarify the nature of genetic variations in plasma lipoprotein levels and related traits such as gallstone formation, obesity and atherosclerosis in the mouse model. Previous studies from our labora-
tory and others have resulted in the mapping of regions of the genome contributing to quantitative differences in these traits (2, 5–9). Several additional putative loci for atherosclerosis susceptibility have been proposed on the basis of studies with RI strains, although the linkage in these cases is only suggestive (reviewed in ref. 38). With the exception of a QTL for plasma total cholesterol and HDL cholesterol levels on Chr 1, the genes underlying these loci are unknown. A useful strategy for identifying these genes is to map candidate genes for the traits and then determine whether they coincide with any relevant QTLs. Figure 2 shows a composite map of published QTLs and candidate genes related to sterol and lipid metabolism. The QTLs and candidate genes mapped in this study and several recent studies have been aligned with candidate genes previously positioned on the Chromosome Committee composite map of the mouse (16, 17) by interpolating distances between common markers. The QTLs are indicated as 20 cM regions centered around peak LOD scores. With the exception of the locus for gallstones on Chr 2 (which was detected by ANOVA rather than multipoint linkage analysis), all of the QTLs are supported by LOD scores greater than 3. A number of the QTLs have been confirmed in independent crosses and several have been isolated on common genetic backgrounds (congenic mouse strains). It should be noted that the locations of the QTLs are indicated only approximately and the underlying genes could be located outside the intervals shown. Likewise, confidence intervals for the positions of the candidate genes generally range from 1 to 20 cM.

As indicated in Fig. 2, several of the candidate genes mapped in the current study are located near QTLs for plasma lipoprotein levels. The acyl-CoA:cholesterol acyltransferase gene (Acact) maps near loci for total cholesterol, HDL cholesterol, and triglycerides on Chr 1. The mevalonate kinase gene (Muk) coincides with QTLs for HDL cholesterol and total cholesterol on Chr 5. The apoA-I regulatory protein-I gene (Tfeb2) is located near a locus for plasma HDL cholesterol and total cholesterol on Chr 7. The novel HMG-CoA synthase-related sequence (Hmges1rs) on Chr 12 is located near a locus for LDL/VLDL cholesterol and total cholesterol. The genes encoding HMG-CoA reductase (Hmger) and sterol regulatory element binding factor-2 (Srebf2) coincide with QTLs for LDL/VLDL cholesterol on Chrs 13 and 15, respectively.

In some cases, the colocalization of QTLs and candidate genes will occur simply by chance. Detailed testing of every colocalizing candidate gene would be both expensive and labor-intensive. Thus, a protocol should be developed for distinguishing between coincidental and real "hits." As a first step, fine mapping of the region containing the QTL, to narrow down the region, would exclude many of the candidate genes on the basis of position alone. The remaining candidates could then be screened for qualitative differences at the structural locus or quantitative differences in expression between mouse strains. For some traits, the differences may be observed between the parental strains of the experimental cross used to identify the QTL. For complicated traits, it may be necessary to examine a subset of the F2 or backcross progeny which exhibit extremes of the trait. Ultimately, identification of a sequence variation at the structural locus would provide strong evidence for a real "hit." The final test would be expression of the trait in transgenic, knockout, or naturally occurring mutant strains of mice carrying alterations of the candidate gene. The relevance of candidate genes tested in mice to human variations of plasma lipoprotein levels can then be determined by linkage analysis.

A similar strategy was used to test Apoa2 as a genetic determinant of plasma HDL cholesterol and total cholesterol. A QTL was identified on mouse Chr 1, in a region containing the apoAI gene (39). The cholesterol variations were shown to be due to structural differences in Apoa2 affecting apoAI-II translational efficiency and plasma turnover rate (39). Transgenic studies confirmed that apoAI-II expression profoundly affects HDL metabolism in mice (40). Subsequently, family studies demonstrated that genetic variations of the apoAI-II gene contributed to differences in human plasma apoAI-II and free fatty acid levels (41).

Coincidental mapping of Acact and ald

Over the years, a number of mouse mutant stocks affecting various aspects of lipid metabolism have been identified. These include five separate mutations (gene designations a, db, ob, tub, and fat) resulting in obesity and diabetes, adrenal lipid depletion (ald) resulting in depletion of cholesteryl esters in the adrenal cortex combined lipase deficiency (cld) resulting in severe hypertriglyceridemia, and fatty liver dystrophy (fld) resulting in developmental abnormalities of triglyceride metabolism (16, 42). The genes underlying four of the obesity and diabetes mutations have been identified recently (4, 43–45), and have led to new insights into the regulation of energy balance in mice as well as humans (46–49). An attractive candidate gene for the ald mutation is Acact, which encodes an enzyme responsible for the esterification of cellular cholesterol. The map positions of ald and Acact coincide within experimental error (data not shown), and it seems reasonable that an alteration in the expression of the enzyme could result in varying levels of cholesteryl ester storage.
Evolutionary relationships: gene families

Many of the genes involved in cholesterol homeostasis are members of gene families exhibiting conserved sequence homology. Some of these families are comprised of members involved in diverse processes; other families are comprised of members that function specifically in pathways of lipid biosynthesis and metabolism. Acact is part of a family of diverse acyltransferase genes (50) Tcf2oup2 is a member of the steroid hormone receptor gene family involved in the transcriptional regulation of a wide variety of genes (51); and Mvk is involved in the transcriptional regulation of genes encoding lipid biosynthetic enzymes (29,30,52). Hmgcs1 and Hmgcs2 are homologous genes that encode proteins involved in sterologenesis and ketogenesis, respectively. We have identified a third member of this family, Hmgcs1-rs, whose function is unknown. Analysis of Southern blots containing HindIII-digested BL/6 and spretus DNA suggests that there may be other members of this family in mouse. However, as the additional bands were detected in BL/6 but not spretus DNA, any additional loci would most likely represent pseudogenes, with formation of the pseudogene occurring after divergence of the two species of mice. The recent identification of a human partial cDNA exhibiting homology with the cytoplasmic HMG-CoA synthase gene indicates that there is also a third member of the human synthase gene family (53).

A variety of human and mouse chromosomes have been shown to contain farnesyl diphasphate synthase genes (11, 12, 27). Characterization of several of these sequences suggested that they are pseudogenes (28). Using a probe specific for the promoter of a functional farnesylieraphosphatase synthase gene, we have identified a single hybridizing locus on mouse Chr 3. This locus is coincident with Fdpsl1, one of the ten loci previously identified by hybridization with a full-length cDNA probe. These results suggest that the Chr 3 locus represents the gene encoding a functional prenyltransferase, and that the gene symbol Fdps1 should be replaced by Fdps as the designation for this locus. Based on conserved synteny, the human homologue of this mouse gene would be the previously described locus on Chr 1q21-25 (27). Whether or not any of the remaining loci encode functional prenyltransferases remains to be determined.

Ldlr, Gp330, Lrp, and Vldlr are members of the low density lipoprotein receptor family (37). Using mouse Lrp-homologous cDNA clones as probes in Southern analysis of RFLVs, we identified an Lrp-homologous sequence, Lrp-rs, on Chr 10 near a region of conserved synteny with human Chr 12q13-q14. In other mouse crosses, we have detected linkage of a human cDNA clone to both Chr 10 and Chr 15 markers, suggesting that there are two highly homologous loci present in the mouse genome (data not shown). Hilliker, Van Leuven,
and Van Den Berghe (54) reported the localization of Lrp to mouse Chr 15 band B2-D1 and human Chr 12q13-q14 by fluorescent in situ hybridization using human clone LRP9. While there is a distal region of mouse Chr 15 with known homology to human Chr 12q11-q14, the mouse cytogenetic region B2-D1 corresponds to a ~20 cM region of relatively unknown mouse–human conserved synteny. Our data suggest that Lrp is located on Chr 10, and that a novel, highly homologous gene is located on Chr 15.

Conserved homologies between mouse and human chromosomes

Most of the candidate genes mapped in this study are located within linkage groups conserved between mouse and human chromosomes (Table 3) (16). One pair of genes, Fdps and Hmgcs2, provides an example of conservation of synteny across the centromere of a human chromosome. Thus, the human homologues of Fdps and Hmgcs2 (both located on mouse Chr 3), are located on either side of the centromere of human Chr 1 (1q24–q31 and 1p13–p12, respectively) (55). Likewise, the linkage between Hmgcr and Hmgs1, on mouse Chr 13, is conserved across the centromere of human Chr 5 (55). Three of the candidate genes are located near the breakpoints of conserved linkage groups, providing further definition of these breakpoints. Thus, Lrpapl and Mvk are both located on mouse Chr 5, separated by a large region of conserved homology to human Chr 4. Lrpapl maps to the proximal end of this conserved region, near the breakpoint between Chrs 4p16.3 and 18p11.3. Mvk is located just distal to the Chr 4 conserved region, near the breakpoint of homology to human Chrs 4q21 and 12q22-qter. Srebfl2 maps to the distal end of a region of conserved homology between mouse Chr 15 and human Chr 22q, near the breakpoint of homology to human 8q24.2-qter. The map assignments of two of the candidate genes represent novel segments of homology between mouse and human. Thus, the localizations of Srebfl1 and Fdftl1 identify homology between mouse Chr 8/human Chr 17p11.2 and proximal mouse Chr 14/human Chr 8p22–p23.1.

The conserved homologies between mouse and human chromosomes can be utilized to suggest subchromosomal localizations of genes mapped by methods of lower resolution such as somatic cell hybrid or in situ hybridization techniques. Thus, the human homologue of Tcfoupl2 (TFCOUP2), previously mapped to human Chr 15 (51), is likely to reside on the q arm. Similarly, the human mevalonate kinase gene (MVK), which has been mapped to Chr 12 (26), is likely to reside within the 12q22-qter region. The suggested position of MVK is of particular interest because mutations of the gene have been shown to cause mevalonic aciduria, a disease which results in failure to thrive and early death (26). Sequence analysis of the MVK gene from affected individuals suggests that the disease may be caused by a variety of mutations. Refinement of the map position may facilitate the development of genetic markers to be used in the diagnosis of mevalonic aciduria, genetic counseling of affected individuals, and prenatal screening.

The authors thank Susan Zollman, Nathalie Kertesz, Aimee Goto, Arnold Chin, and Marjon Jahromi for technical assistance. We are also grateful to Dr. Benjamin Taylor, The Jackson Laboratory, for help with the analysis of recombinant inbred strains. This work was supported in part by NIH grants HL42488, HL35068, and HL48540.

Manuscript received 24 January 1996 and in revised form 27 March 1996.

REFERENCES


