Genetic heterogeneity of plasma lipoproteins in the mouse: control of low density lipoprotein particle sizes by genetic factors[1]

Sheng Jiao,[2] Thomas G. Cole, Robert T. Kitchens, Barbara Pfleger, and Gustav Schonfeld

Department of Internal Medicine, Division of Atherosclerosis and Lipid Research, Washington University School of Medicine, St. Louis, MO 63110

Abstract In order to assess the genetic control of sizes and concentrations of mouse plasma low density (LDL) and high density lipoproteins (HDL), we used gel permeation fast protein liquid chromatography (FPLC) and nondenaturing gradient polyacrylamide gel electrophoresis to measure the particle sizes of LDL and HDL. Using chromatography we also quantified LDL-cholesterol and HDL-cholesterol concentrations in plasma and used them as indexes of plasma concentrations of the respective particles among 10 inbred strains (AKR/J, BALB/cByJ, C3H/HeJ, C57BL/6J, C57BL/6ByJ, C57L/J, DBA/1LacJ, 129/J, NZB/BINJ, SWR/J) and three sets of recombinant inbred (RI) strains (AKXL/TyJ, BXH/TyJ, CXB/ByJ) of mice. HDL had a dichotomous distribution among the 10 inbred strains. One group had large HDL particle sizes and high HDL-cholesterol concentrations. Another group had smaller HDL particles and lower HDL-cholesterol concentrations, and HDL sizes and HDL-cholesterol concentrations were significantly correlated. In the RI strains, HDL sizes and HDL-cholesterol concentrations clearly segregated with one or another of the progenitor strains, and RI strain distributions showed a strong linkage to the apolipoprotein (apo) A-II gene (*Apoa-2*). In contrast, LDL-cholesterol concentrations and particle sizes on FPLC did not show dichotomous distributions among the 10 inbred strains. In RI strains, the configuration of the LDL FPLC profiles and LDL-cholesterol concentrations did resemble one or another of the progenitors in the majority of cases, but LDLs of several RI strains resembled neither progenitor strain in profile configuration, and LDL-cholesterol concentrations were both greater and smaller than those of progenitor strains. However, LDL particle diameters (as judged by peaks of LDL-cholesterol profiles) did segregate with progenitors in 29/33 (88%) of RI strains suggesting that a major gene may affect LDL size. In attempting to identify a major LDL-size determining gene, we compared apoB gene restriction fragment length polymorphisms (RFLPs) to the distributions of peak LDL sizes in RI strains. Concordance rates of peak LDL sizes to apoB gene polymorphisms were 18/22 (82%) for the *Eco*RV RFLP, 5/7 (71%) for *Hind*III RFLP, and 23/29 (79%) for both (range of P values 0.90–0.95). Thus we could not unequivocally implicate the apoB gene in determining the size of LDL particles. In summary, the genetic control of LDL sizes is more complicated than is the case for HDL; however, the differences in LDL size among these strains of mice may be controlled by a major, as yet unidentified, gene. —**Jiao, S., T. G. Cole, R. T. Kitchens, B. Pfleger, and G. Schonfeld.** Genetic heterogeneity of plasma lipoproteins in the mouse: control of low density lipoprotein particle sizes by genetic factors. *J. Lipid Res.* 1990. **31:** 467–477.

Supplementary key words high density lipoprotein • cholesterol • restriction fragment length polymorphism • apoB gene

Many clinical studies have revealed that plasma low density lipoprotein (LDL) concentrations are positively correlated with the incidence of coronary heart disease while HDL concentrations are negatively correlated (1). Alterations of LDL particle sizes and compositions also are associated with atherogenesis (2, 3). Metabolic status, therapeutic interventions, and genetic factors each can alter LDL sizes (4–6). But the gene products involved in the control of LDL sizes and concentrations have not been identified, although activities of LDL-receptors (7), lipoprotein lipase (LPL) (8), hepatic triglyceride lipase (H-TGL) (9, 10), and serum cholesteryl ester transfer protein (11, 12) each seem to exert significant effects under certain conditions. Another candidate gene for regulating LDL-sizes, concentrations, or compositions is the gene specifying the transcription of apolipoprotein (apo) B-100. As the major constituent protein of

Abbreviations: VLDL, very low density lipoprotein(s); LDL, low density lipoprotein(s); HDL, high density lipoprotein(s); FPLC, fast protein liquid chromatography; apoB, apolipoprotein B; BSA, bovine serum albumin; RI strain, recombinant inbred strain; RFLP, restriction fragment length polymorphism; AKR, AKR/J; BALB/c, BALB/cByJ; C57BL, C57BL/6J; C57BLBY, C57BL/6ByJ; C57L, C57L/J; C3H, C3H/HeJ; DBA, DBA/1LacJ; NZB, NZB/BINJ; SWR, SWR/J; AKXL, AKXL/TyJ; BXH, BXH/TyJ; CXB, CXB/ByJ; Kb, kilobase(s); SDS, sodium dodecyl sulfate; LPL, lipoprotein lipase; H-TGL, hepatic triglyceride lipase.

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[2]Present address: Sheng Jiao (Noboru Sho), M. D., Ph. D., The Second Department of Internal Medicine, Osaka University Medical School, 1-1-50 Fukushima, Fukushima-ku, Osaka 553, Japan.

LDL, apoB plays pivotal roles in the assembly and secretion of triglyceride-rich lipoproteins and in the recognition of apoB-containing lipoproteins by LDL receptors during the catabolism of LDL. The structures of the apoB mRNA and gene are known (13–16) and several restriction fragment length polymorphisms (RFLPs) have been detected (17–21). In humans, some RFLPs correlate with serum cholesterol and triglyceride concentrations (22–25) and the occurrence of myocardial infarction (18). However, there are no reports regarding linkages between RFLPs of the apoB gene and LDL particle sizes, concentrations, or compositions. Investigation of these linkages in humans is difficult, because it is hard to control factors of life style such as diets and hormones that can affect the various qualities of LDL particles.

Recently, inbred strains of mice have been used for analyzing genetic effects on lipoprotein metabolism and atherosclerosis, and there is heterogeneity in the sizes, plasma concentrations, and lipid compositions of mouse lipoproteins among the various strains (26). Some strains of mice, e.g., C57BL/6J and C57L/J, also develop atherosclerotic lesions after cholesterol and fat feeding (27, 28). In our previous studies (26) and those of Lusis et al. (29), HDL sizes and HDL-cholesterol concentrations in the plasmas of 11 strains of inbred mice showed dichotomous distributions, i.e., several strains of mice had high HDL-cholesterol and large HDL particles, while other strains had low HDL-cholesterol and small HDL particles. By contrast, LDL did not show dichotomous distributions either of LDL sizes or LDL-cholesterol concentrations among the inbred strains (26).

In the present study, we investigated whether LDL sizes are determined by a major gene in RI strains of mice. Using the candidate gene approach, we evaluated the role of the apoB gene. The mouse apoB gene is located on chromosome 12, (chromosome 2 in humans) and two RFLPs of mouse apoB have been detected by means of restriction nuclease digestion with *Eco*RV and *Hind*III (30). We sought linkages between LDL sizes, compositions, and concentrations on the one hand, and RFLPs of the mouse apoB gene on the other. Similar linkages were examined for HDL and for apoA-I and apoA-II genes.

METHODS

Animals

Mice were obtained from Jackson Laboratory, Bar Harbor, ME. Inbred strains used were AKR/J (AKR), BALB/cByJ (BALB/c), C57BL/6J (C57BL), C57BL/6ByJ (C57BLBY), C57L/J (C57L), C3H/HeJ (C3H), DBA/1LacJ (DBA), 129/J, NZB/BINJ (NZB), and SWR/J (SWR). Recombinant inbred strains, AKXL/TyJ (AKXL) derived from parental inbred strains AKR and C57L, BXH/TyJ (BXH) from strains C57BL and C3H, and CXB/ByJ (CXB) from strains BALB/c and C57BLBY, were used for gene linkage analysis. All mice were female and 8–10 weeks old. They were housed in a room maintained at 24°C with 12-h light–dark cycles. Mice were given Purina mouse chow 5015 and water ad libitum. After an 18-h fast, blood was collected from the inferior vena cava under light anesthesia with diethyl ether into tubes containing sodium EDTA (1.5 mg/ml). Plasma was obtained by centrifugation at 3,000 rpm for 20 min at 4°C. As plasma obtained from a single mouse was not enough for lipoprotein analysis, plasma pools were made from three mice.

LDL sizing by gel permeation chromatography

For measurement of lipoprotein concentration and particle diameter, gel permeation fast protein liquid chromatography (FPLC) was performed as described previously (31). Briefly, plasma (450 μl) was centrifuged at 100,000 *g*-min in a microfuge and clear supernatant was loaded into a 400-μl Teflon sample loop for application to two Superose-6 columns (~50 ml of vol, Pharmacia, Sweden). Elution was at 0.5 ml/min with 1 mM EDTA, 154 mM NaCl, 0.02% NaN_3, pH 8.2. After the first 12 ml had eluted, 50 500-μl fractions were collected. Cholesterol and triglyceride concentrations in the fractions were measured by commercially available enzymatic kits (Wako Chemicals USA, Inc., Dallas, TX) on a Technicon RA-500 AutoAnalyzer.

Nondenaturing gradient gel electrophoresis of HDL

As differences between HDL particle sizes were not detected by FPLC, HDL particle sizes were analyzed by nondenaturing gradient gel electrophoresis. Mouse plasma (90 μl) was adjusted to density 1.21 g/ml by the addition of KBr solution and overlaid with a 1.21 g/ml KBr solution. Ultracentrifugation was carried out in a Beckman 42.2 Ti rotor at 42,000 rpm for 25 h at 4°C and 8-μl aliquots of the top fractions were taken for electrophoresis. To the 8 μl of sample was added 2 μl of sample buffer (40% sucrose and 0.01% bromphenol blue). The solution was applied to 4–30% polyacrylamide gradient gels (32) (PAA 4/30, Pharmacia, Sweden). Electrophoresis was carried out in a Pharmacia electorphoresis apparatus (GE-2/4 LS) at 125 V constant voltage at 10°C for 24 h in a buffer containing 90 mM Tris, pH 8.35, 80 mM boric acid, 3 mM EDTA, and 3 mM NaN_3. Thyroglobulin and bovine serum albumin (BSA) were added to the sample solution as internal standards. In addition, four reference proteins were run simultaneously for calibrating particle sizes: thyroglobulin (17 nm in diameter), apoferritin (12.2 nm), lactate dehydrogenase (8.2 nm), and BSA (7.1 nm). The slab gels were fixed in 10% sulfosalicylic acid for 1 h and stained in 0.04% Coomassie Brilliant Blue G-250 in 3.5% perchloric acid for

1.5 h, and then destained in 5% acetic acid. During fixing, staining, and destaining procedures, gels were agitated gently on a horizontal rotator. Stained gels were scanned with a model LKB 2202 Ultroscan Laser Densitometer (LKB, Sweden) at a wavelength of 632.8 nm. For particle size calibration, we measured R_f values of marker proteins relative to the migration distance of BSA and obtained a quadratic calibration curve of R_f value versus Stokes' diameter (log), and computed particle sizes.

Analysis of apoB gene restriction fragment length polymorphisms

A 3.0-Kb rat apoB cDNA insert (rb9E), corresponding to the 3' coding region of the mRNA, was kindly provided by Dr. A. J. Lusis, University of California, Los Angeles. The cDNA was labeled with ^{32}P by a random priming procedure (33) to a specific activity of 1-5 × 10^8 cpm/μg. Mouse genomic DNA was isolated from the liver by the proteinase K/sodium dodecyl sulfate (SDS) procedure (34). Liver (0.5 g) was removed immediately after exsanguination and frozen in liquid nitrogen. Frozen liver was pulverized by a mortar and pestle in liquid nitrogen and resuspended in 10 ml of 10 mM Tris-HCl, pH 7.4, 10 mM NaCl, 25 mM EDTA. After the addition of 1 ml of 10% SDS solution and 11 mg of proteinase K powder (Boehringer Mannheim, Indianapolis, IN), the mixture was incubated for 2 h at 37°C. Another 11 mg of proteinase K was added and the incubation was continued for an additional 2 h. After addition of 1 ml of 150 mM NaCl, DNA was extracted twice with phenol-chloroform-isoamyl alcohol 10:10:1 (v/v/v), followed by ethanol precipitation. DNA was dissolved in 10 ml of 0.1 × SSC (15 mM NaCl, 1.5 mM sodium citrate, pH 7.0). After digestion with 700 μg of deoxyribonuclease-free ribonuclease A (Boehringer Mannheim) for 30 min at 37°C, phenol-chloroform extraction, and ethanol precipitation, DNA was finally dissolved in 1 × TE buffer (10 mM Tris, pH 7.4, 0.1 mM EDTA). Ten-μg aliquots of genomic DNA were digested by 5 U/μg of restriction endonucleases, *Eco*RI, *Eco*RV, *Hind*III, *Xba*I, *Pvu*I, *Nco*I, *Cla*I, and *Sau*I (Boehringer Mannheim) for 16 h and applied to 0.7% agarose gel electrophoresis. Electrophoresis was carried out in 1 × TBE buffer (50 mM Tris-HCl, 50 mM boric acid, 1 mM EDTA) at a constant voltage of 30 V for 14 h. *Hind*III-digested lambda DNA was used as a molecular weight standard. DNA was transferred to nitrocellulose filters (GeneScreen, New England Nuclear, Boston, MA) by capillary blotting for 24 h in 20 × SSC. The filters were prehybridized in 50% deionized formamide, 0.2% polyvinyl-pyrrolidone (mol wt 40,000), 0.2% BSA (Fraction V), 0.2% Ficoll (mol wt 400,000), 50 mM Tris, pH 7.5, 1 M NaCl, 0.1% sodium pyrophosphate, 1% SDS, 10% dextran sulfate, and 125 μg/ml of denatured salmon sperm DNA at 42°C for 18 h and hybridized to ^{32}P-labeled apoB cDNA probe (1-2 × 10^6 cpm/ml) in the same buffer at 42°C for 24 h. After washing twice in 2 × SSC for 5 min at room temperature, followed by two washes (30 min each) in 2 × SSC, 1% SDS at 65°C and two washes (30 min each) in 0.1 × SSC at room temperature, the filters were exposed to Kodak X-OMAT AR X-ray film for 3-4 days at – 80°C.

Immunoblots of mouse apoB

Aliquots of whole plasma (3.3 μl) were delipidated by ethanol-diethyl ether 3:1 (v/v) and diethyl ether. Delipidated proteins were subjected to 3-6% gradient SDS polyacrylamide gel electrophoresis. Separated proteins were transferred onto a nylon membrane (Nitroplus 2000,

TABLE 1. LDL characteristics and apoB alleles in inbred strains of mice

	LDL-Lipids[a]				Polymorphism		
	CH	TG	CH/TG	Diameter	*Eco*RV	*Hind*III	*ApoB Allele*
	mg/dl			*nm*			
AKR	12.0 (1.1)	8.0 (0.3)	1.5 (0.1)	26.78	2	1	b
BALB/c	23.0 (1.2)	6.2 (0.2)	3.7 (0.1)	24.16	1	2	c
C3H	29.8 (1.2)	9.2 (0.6)	3.3 (0.2)	25.91	2	1	b
C57BL	18.8 (0.4)	5.6 (1.1)	3.6 (0.7)	27.65	1	1	a
C57BLBY	19.7	5.6	3.5	29.39	1	1	a
C57L	21.4 (0.6)	7.8 (0.1)	2.7 (0.1)	28.52	1	1	a
DBA	29.5	8.4	3.6	25.91	1	1	a
129/J	25.0 (0.9)	3.3 (0.6)	8.1 (1.8)	26.78	1	1	a
NZB	39.6 (0.8)	11.0 (0.1)	3.6 (0.1)	27.65	1	2	c
SWR	18.3 (0.2)	8.4 (0.7)	2.2 (0.2)	29.39	1	1	a

Polymorphisms of apoB gene: pattern 1 of *Eco*RV site = 10-kb + 3.9-kb, pattern 2 = 8.3-kb + 3.9-kb, pattern 1 of *Hind*III site = 11-kb + 3.8-kb, pattern 2 = 8.4-kb + 3.8-kb. Abbreviations: CH, cholesterol; TG, triglyceride.

[a]Values are expressed as mean (SEM) of three plasma pools (nine mice) except for two pools in C57BLBY and DBA.

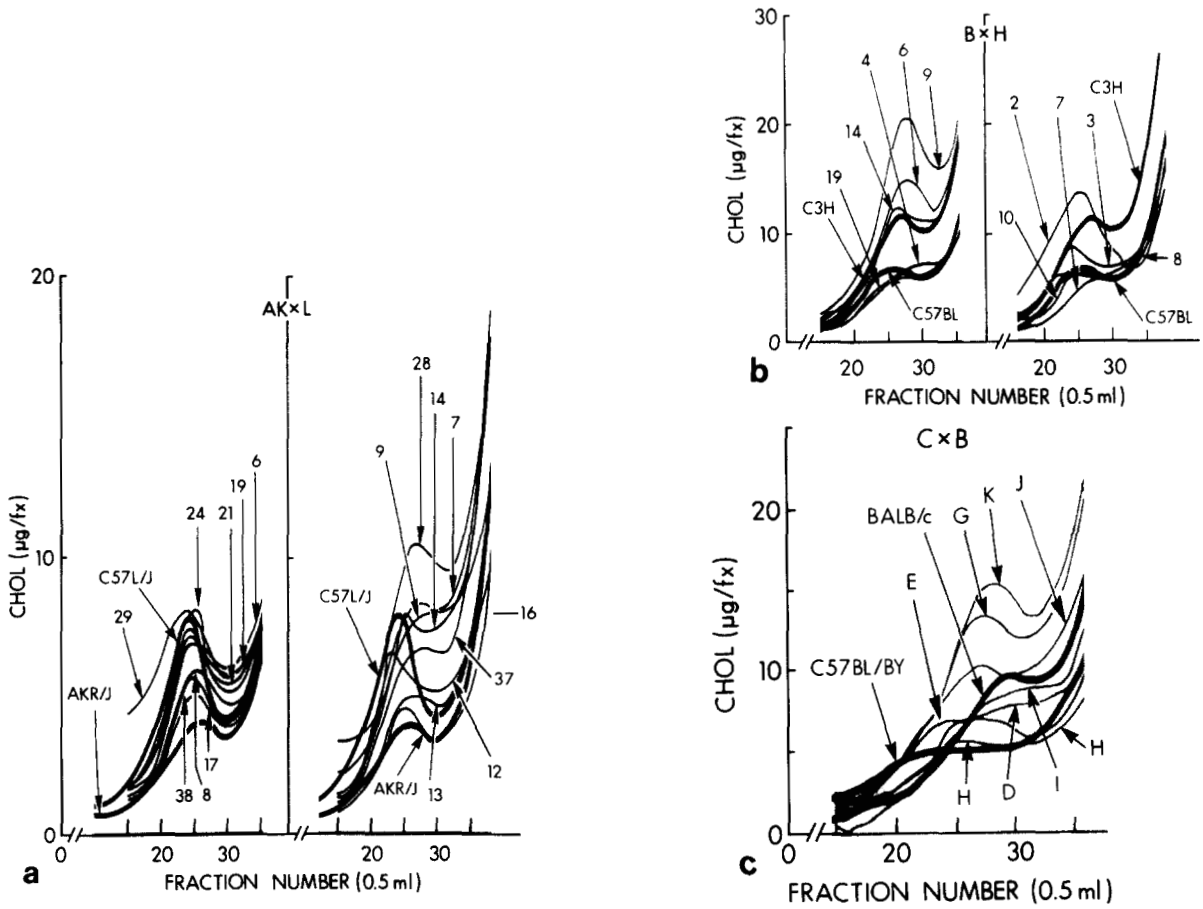


Fig. 1a–c. Gel permeation chromatography of mouse lipoproteins of AKXL/TyJ (AKXL, Fig. 1a), BXH/TyJ (BXH, Fig. 1b), and CXB/ByJ (CXB, Fig. 1c) recombinant inbred and progenitor strains. Aliquots (450 µl) of plasma pool obtained from three mice were applied to two Superose 6 columns linked in series and cholesterol concentrations in the eluted fractions were measured. Whereas VLDL and HDL peaks eluted in almost identical fractions (fraction 8 and fractions 40 or 41) for all mouse strains, LDL was eluted in different fractions for various mouse strains. Elution profiles of progenitors are presented as heavy lines. The numbers and letters refer to an individual recombinant inbred mouse.

0.45 µm pore size, Micron Separation, Inc. Westboro, MA) by electroblotting at 27 V for 18 h (35). Nylon membranes were blocked by 5% Blotto (nonfat dry milk), incubated with 1:100 diluted rabbit anti-mouse LDL serum, and then incubated with 1.38×10^6 cpm/ml ^{125}I-goat anti-rabbit IgG. Autoradiography was carried out for 1 h at −80°C.

Statistical analysis

Lipoprotein concentrations were expressed as means ± one standard error of the mean (SEM). Probability of linkage between RFLPs and lipoprotein particle sizes were obtained by a Bayesian statistical analysis described by Silver and Buckler (36).

RESULTS

Size distribution of mouse LDL

As reported previously (26), LDL-cholesterol concentrations and LDL sizes in the 10 inbred strains ranged from 12.0 mg/dl to 39.6 mg/dl and from 24.16 nm to 29.39 nm, respectively (**Table 1**). We investigated the inheritance of LDL-cholesterol profiles, LDL-cholesterol concentrations, and LDL sizes (all obtained by FPLC) using progenitor and RI strains. FPLC profiles of progenitors were performed in triplicate pools, using pooled plasma from three mice for each replicate pool. Elution profiles of the three replicate pools of each of the six progenitor strains were nearly superimposable. Thus, the LDL profiles and size estimates were highly reproducible. Only one FPLC profile was obtained per RI strain, due to limited availability and cost of the animals.

Some RI stains showed profiles resembling those of one or another of the progenitors, but the LDL profiles of many strains did not segregate to progenitors (**Fig. 1 a–c**). Similarly, there was a wide range of LDL concentrations that did not segregate to progenitors (**Fig. 2**). Since LDL compositions and LDL sizes under certain conditions can be correlated with each other (37), we also tested the inheritance of the LDL-cholesterol/LDL-triglyceride ratio, but only in the AKXL RI set, because

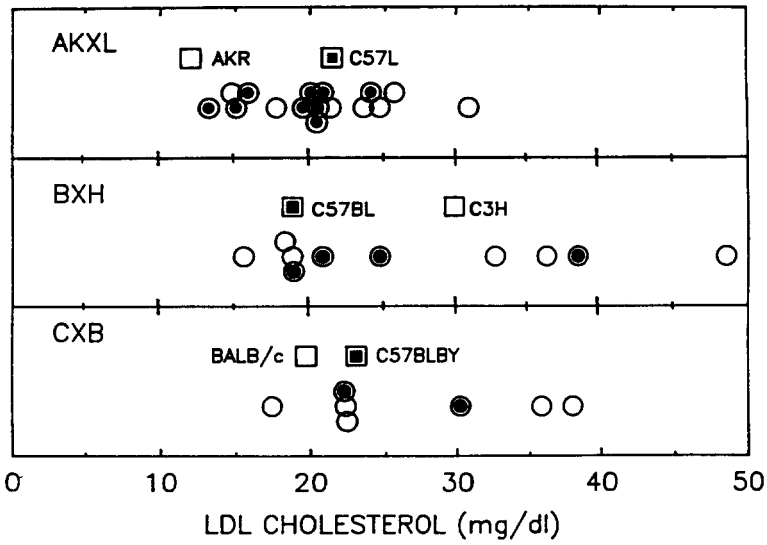


Fig. 2. Distribution of LDL-cholesterol concentrations among RI (circles) and progenitor strains (boxes). Data are from Figs. 1a-c. Identification of apoB gene polymorphism is represented as open or filled symbols.

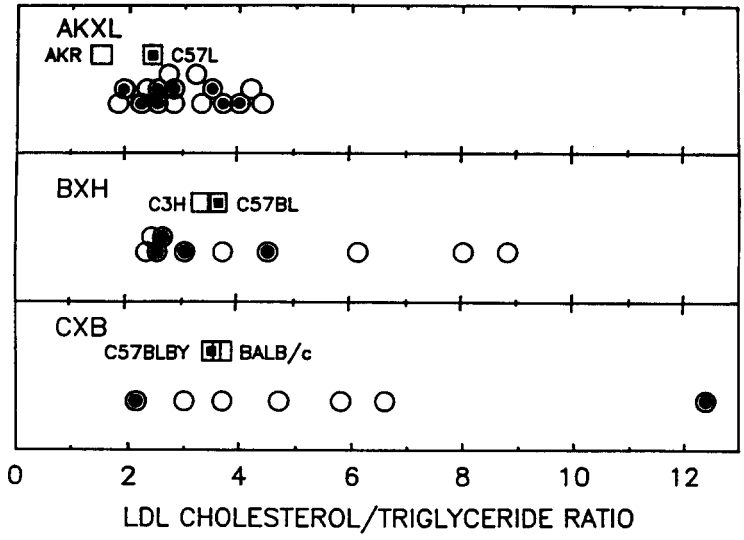
LDL ratios were different only in the progenitor strains of this RI set (Table 1). A wide range of ratio values were found, but there was no segregation to progenitors (**Fig. 3**). However, a more coherent picture emerged when we focused only on the diameters of LDL particles as represented by the peak LDL tubes of the FPLC profiles (**Fig. 4**). LDL sizes of RI strains AKXL-7, -9, -14, -24, -28, and -37 segregated to the AKR progenitor strain and RI strains AKXL-6, -12, -16, -19, -21, -29, and -38 segregated to the C57L progenitor. Strains AKXL-8, -13, and -17 showed intermediate values. RI strains BXH-4, -6, -7, -9, -14, and -19 segregated to the C3H progenitors and RI strains BXH-2, -3, and -8 segregated to the C57BL progenitors while strain BXH-10 had an intermediate size. RI strains CXB-G, -H, -I, -J, and -K segregated to the BALB/c progenitor and strains CXB-D and -E segregated to the C57BLBY progenitor. Thus 29/33 (88%) RI strains had LDL sizes resembling one or the other of the progenitor strains.

Size distribution of mouse HDL and relationships with LDL

HDL sizes of RI strains clearly segregated with the progenitor strains (**Fig. 5 and Fig. 6**). The segregation data in the BXH and CXB RI strains confirmed the previous report by Lusis et al. (29) in which HDL size was determined to be controlled by a single allele located close to the apoA-II gene (*Apoa-2*) on mouse chromosome 1, but not to the apoA-I gene (*Apoa-1*) on chromosome 9.

The concordance of segergation between LDL sizes and HDL sizes was investigated. The concordance was 17/28 (60%) in all sets of RI strains, indicating no signi-

Fig. 3. Distribution of LDL-cholesterol/LDL-triglyceride ratios among recombinant inbred (circles) and progenitor strains (boxes). Identification of apoB gene polymorphisms is represented as open or filled symbols.



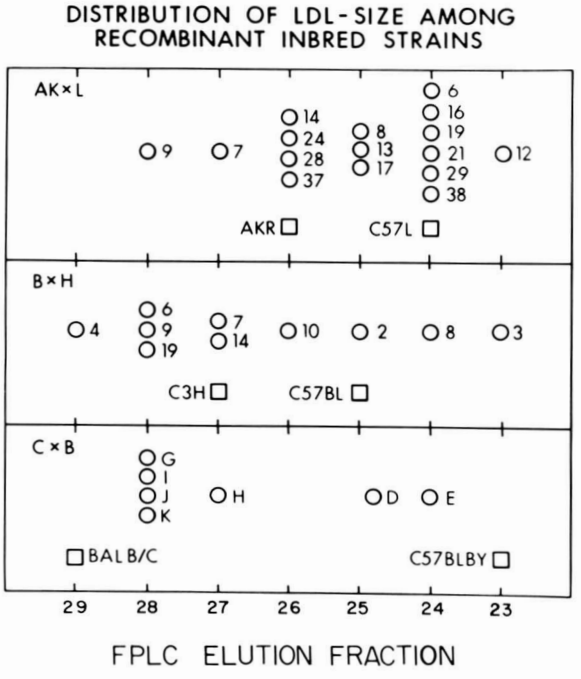


Fig. 4. LDL size distribution of AKXL, BXH, and CXB recombinant inbred strains and the progenitor strains. Data are from Figs. 1a–c. LDL sizes were taken as peaks of LDL cholesterol.

ficant linkage between LDL and HDL size polymorphisms. The cut points differentiating small from large LDL and small from large HDL are based on data in Figs. 4 and 6. For LDL and HDL in AKXL, cut points were fraction 25 and 9.4 nm, respectively. For BXH and CXB, the respective values were fraction 26 and 10 nm. In addition, the correlation between LDL diameter and HDL diameter was calculated by linear regression analysis. There was a weak, but significant, negative correlation between LDL sizes and HDL sizes in the three sets of RI strains ($r = -0.431$, $P = 0.0132$, $n = 32$). Differences in VLDL size were not detectable by the FPLC system, therefore, comparisons between VLDL size and LDL and HDL sizes were impossible.

Strain distribution of RFLPs of the apolipoprotein B gene

Genomic DNAs obtained from 10 inbred strains were digested by restriction endonucleases (*Cla*I, *Eco*RI, *Eco*RV, *Hind*III, *Nco*I, *Pvu*I, *Sau*I, or *Xba*I). Only *Eco*RV and *Hin-d*III digestion detected polymorphisms. The mouse apoB gene showed two RFLPs at the *Eco*RV site: a 10-Kb plus 3.9 Kb pattern and a 8.3-Kb plus 3.9-Kb pattern. *Hind*III digestion also indicated two RFLPs: an 11-

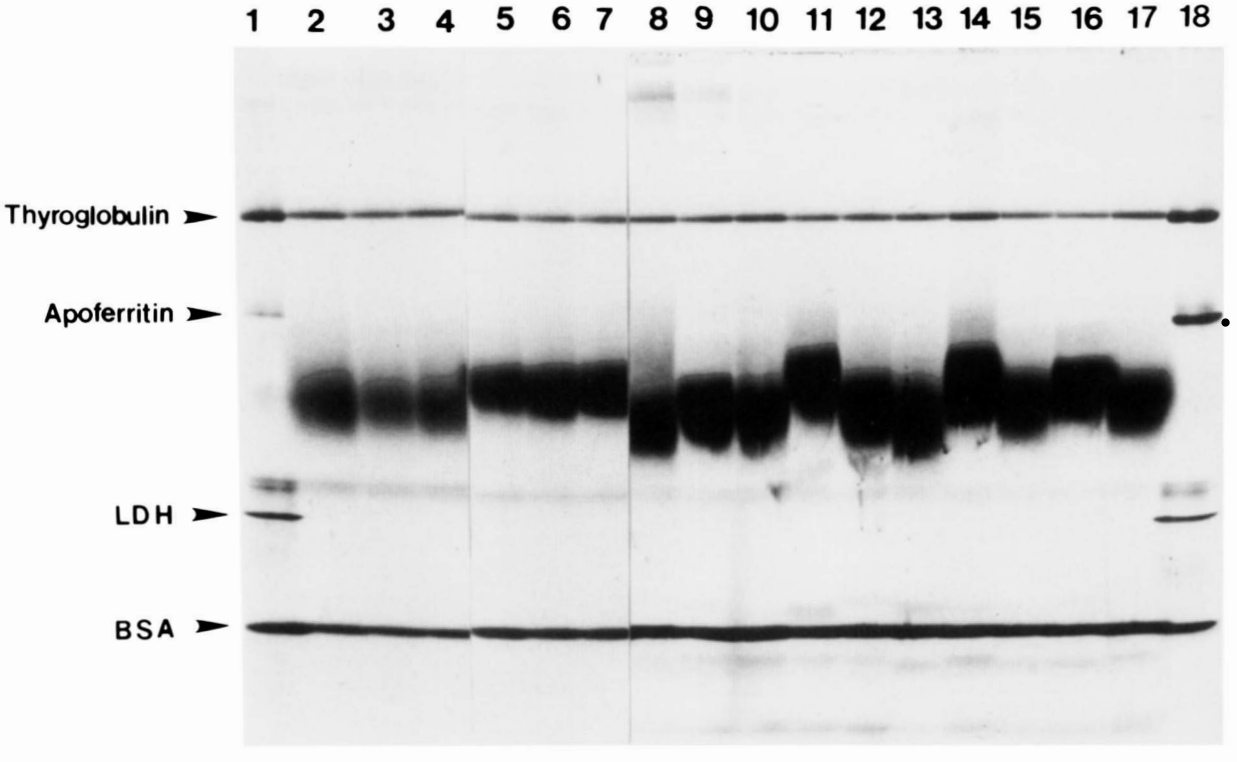


Fig. 5. Nondenaturing gradient gel electrophoresis of mouse HDL. Aliquots (8 μl) or 1.21 g/ml top fraction were applied to 4–30% polyacrylamide gradient gels. Electrophoresis was carried out at 125 V at 10°C for 24 h and gels were strained in 0.04% Coomassie Brilliant Blue G-250. Reference proteins, thyroglobulin (17 nm in diameter), apoferritin (12.2 nm), lactate dehydrogenase (LDH, 8.2 nm), and BSA (7.1 nm) were run simultaneously for calibrating particle sizes (lane 1 and 18). Thyroglobulin and BSA were added to lipoprotein samples as internal standards. Lanes 2–4, C57BL/6J; lanes 5–7, C3H/HeJ; lanes 8–17, BXH-2, 3, 4, 6, 7, 8, 9, 10, 14, and 19, respectively. Plasma pools obtained from three mice were used for each strain.

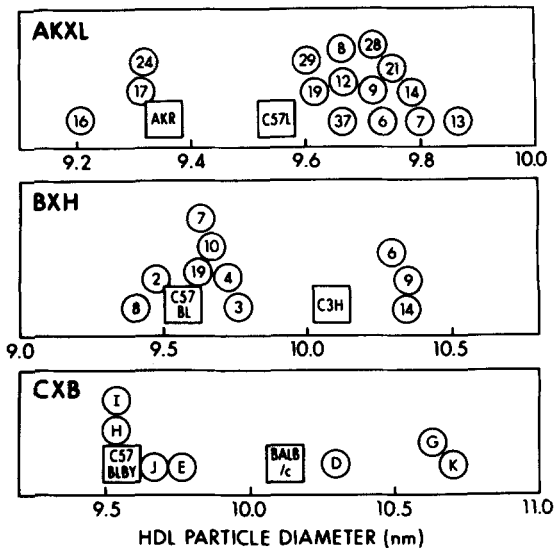


Fig. 6. HDL size distributions of AKXL, BXH, and CXB recombinant inbred (circles) and progenitor (boxes) strains.

Kb plus 3.8-Kb pattern and an 8.4-Kb plus a 3.8-Kb pattern. These RFLPs defined three alleles: a, b, and c (Table 1). Analysis of the RI strains demonstrated the segregation of RFLPs to one or the other of the progenitor strains. These RFLP data were consistent with the observation of Lusis et al. (30).

TABLE 3. Inheritance of *Hind*III apoB gene polymorphism, LDL size polymorphism, and other chromosome 12 markers

CXB (BALB/c X C57BL/6ByJ) loci/RI	D	E	G	H	I	J	K
LDL size	B	B X	C	C	C	C X	C
Apob	B	C	C	C	C	B	C X
Ly-18[a]	B	C	C	C	C	B	B X
Ah[b]	B X	C	C	C	C	B	C
D12Nyu1[c]	C	C	C	C	C	B	C

The symbols C and B are used as generic symbols for alleles inherited from the progenitor strains BALB/c and C57BL/6ByJ, respectively. Abbreviations are shown in Table 2.
[a]According to Tada et al. (37).
[b]According to Poland et al. (41).
[c]According to D'Eustachio (38).

Linkage between LDL size polymorphism and apoB gene

Twenty three of 29 RI strains showed concordance between LDL size and RFLP type (**Table 2 and Table 3**).

TABLE 2. Inheritance of *Eco*RV apoB gene polymorphism, LDL size polymorphism, and other chromosome 12 markers

AKXL (AKR/J X C57L/J) loci/RI	6	7	8	9	12	13	14	16	17	19	21	24	28	29	37	38
LDL size	L	A X	?	A	L	?	A	L	?	L	L	A	A X	L X	A	L
Apob	L X	L	A X	A	L X	L	A	L X	A	L	L	A	A X	A	L X	L
Ah[a]	A	L	L	A	A	L	A	A	A	L	L	A	L X	A	L	A
Ly-18[b]	A	L	L	A	A	L	A	A	A	L	L	A	L	A	L	A
D12Nyu1[c]	A	L	L	A	A	L	A	-	A	L	L	L	L	A	L	A

BXH (C57BL/6J X C3H/HeJ) loci/RI	2	3	4	6	7	8	9	10	14	19
LDL size	B	B	H X	H	H	B	H	?	H	H
Rnr-12[d]	B	B	B	H	H	B	H	H	H	H
Apob	B	B	B	H	H	B	H	H	H X	H
D12Nyu2[c]	B	B	B	H X	H	B	H	B	H	H
Ly-18[b]	B	B	B	B	H	B	H	B	H	H

The symbols A, L, B, and H are used as generic symbols for alleles inherited from the progenitor strains AKR/J, C57L/J, C57BL/6J, and C3H/HeJ, respectively. The pairs of loci showing discordance are represented as X. Symbol "?" denotes the RI strain showing intermediate LDL size of progenitors. Symbol "-" denotes the RI strain has not been identified regarding the loci. Abbreviations: *LDL size*, a putative gene locus controlling LDL particle size; *Rnr-12*, chromosome 12 ribosomal RNA gene; *Apob*, apolipoprotein B gene; *Ah*, arylhydrocarbon hydroxylase gene; *Ly-18*, lymphocyte alloantigen 18 gene; *D12Nyu1* and *2*, chromosome 12 DNA markers.
[a]According to Poland et al. (41).
[b]According to Tada et al. (37).
[c]According to D'Eustachio (38).
[d]According to Arnheim et al. (39).

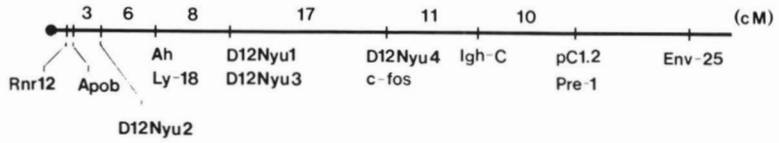


Fig. 7. Gene loci in mouse chromosome 12 (Refs. 30, 39, 42). The centromere is to the left. The numbers above the line are the distance (centimorgans, cM) between the loci.

This yields a probability of $0.90 < P < 0.95$ that LDL size is linked to the apoB gene using the Bayesian statistical approach (36). When the strain distribution of LDL size was compared with the strain distribution of the other marker loci on chromosome 12 [aryl hydrocarbon hydroxylase gene (*Ah*), lymphocyte alloantigen gene (*Ly-18*), chromosome 12 ribosomal RNA gene (*Rnr12*), and DNA markers *D12Nyu1* and *D12Nyu2*, references 20, 38–42] (Tables 2 and 3), the putative LDL size locus was assigned 7.5 ± 3.9 centimorgans (cM, mean ± standard deviation) from the apoB gene toward the centromere (**Fig. 7**), an unlikely location since the apoB gene has previously been assigned to a distance of ~4 cM from the centromere and mouse chromosomes are acrocentric.

Western blot of mouse apoB

To assess apparent molecular weight of mouse apoB, immunoblot assay was performed by 3–6% gradient SDS polyacrylamide gel electrophoresis. ApoB was detected with polyclonal rabbit anti-mouse LDL antibody (**Fig. 8**). Apparent molecular weights of apoB-100 were not different among strains.

DISCUSSION

In an earlier study we observed mouse strain-related heterogeneities in the sizes of LDL and HDL particles

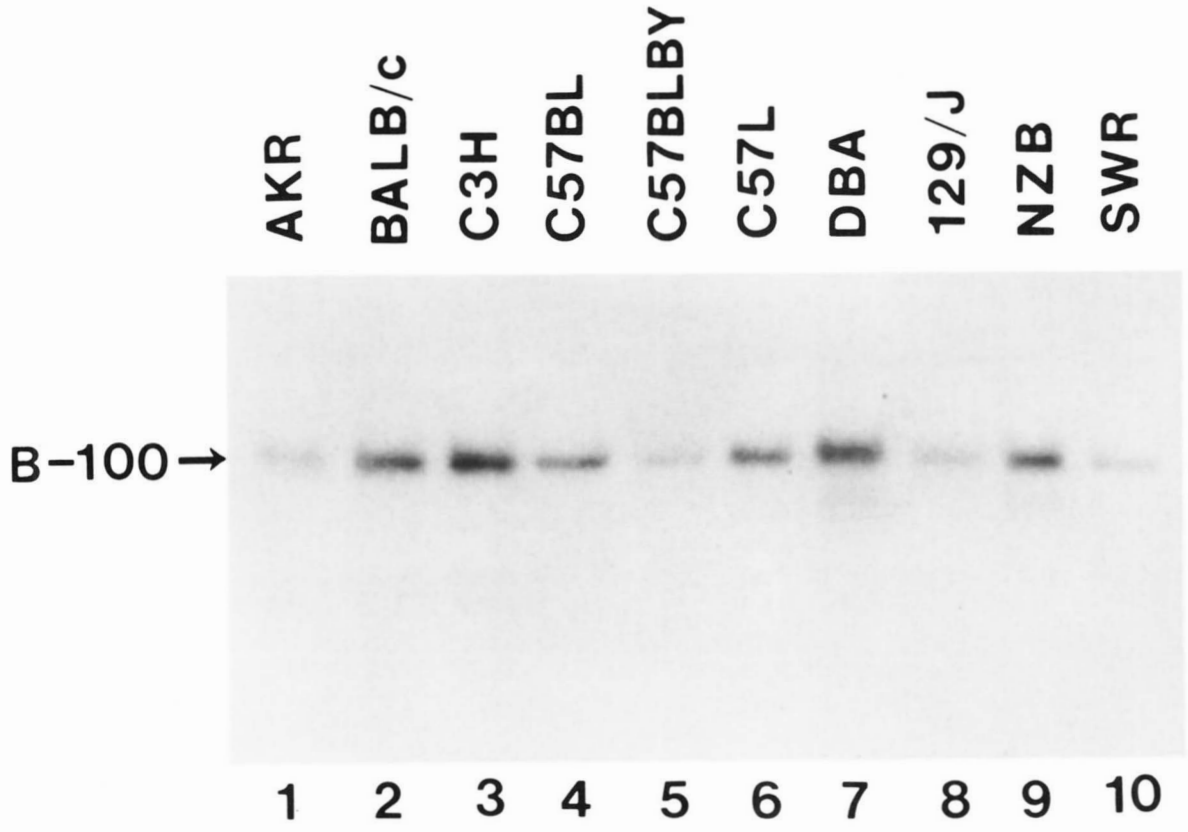


Fig. 8. Western blot of mouse apoB. Delipidated proteins obtained from 3.3 μl of plasma were subjected to 3–6% gradient SDS polyacrylamide gel electrophoresis, and transferred onto a nylon membrane by electroblotting. The nylon membrane was incubated with rabbit anti-mouse LDL serum (1:100 dilution), and incubated with ^{125}I-labeled goat anti-rabbit IgG. Autoradiography was performed for 1 h at −80°C.

(26). Our aim was to ascertain whether LDL-size was an inherited trait in mice as it appears to be in humans (7, 10), and whether a major gene could be implicated. We tested whether that major gene could be apoB, the major protein of LDL. Other qualities of LDL such as the configuration of its profile on FPLC, its composition, and concentration also were tested.

Although, as noted earlier, products of several candidate genes such as LPL, H-TGL, cholesteryl ester transfer protein, and LDL receptors could be involved in the regulation of LDL size, it appeared logical to test the role of apoB in determining LDL size for several reasons. *a*) Since apoB-100 comprises > 20% of LDL mass, alterations in its size could affect the size of the particle; this has been described in humans (35). *b*) Alterations of crucial amino acids in the lipid-binding regions could affect hepatic assembly of lipoproteins. *c*) Amino acid alterations in or near the LDL-receptor recognition domain could affect residence times of LDL in plasma; this has been described in humans (43, 44). *d*) Still other alterations could affect susceptibility to digestion by lipases. We ruled out large changes in apoB size by direct analysis on Western blot in 10 strains (Fig. 8). Ruling out the relatively more subtle alterations requires analysis of protein or gene sequence. However, it is possible to assess whether a major gene is likely to be implicated in determining LDL-size by studying the segregation of LDL-sizes among RI strains, and then it is possible to evaluate the role of the apoB gene by seeking linkages between RFLPs of apoB and LDL-size.

The findings on LDL were compared and contrasted with HDL since the role of a major gene (*Apoa-2*) in the inheritance of HDL size had already been reported (29). Indeed, our analysis of HDL sizes by nondenaturing gradient gel electrophoresis in three pairs of progenitor strains and the accompanying sets of RI strains confirmed the findings of Lusis et al. (29). By contrast, shapes of LDL elution curves, concentrations of LDL-cholesterol, and ratios of LDL-cholesterol/LDL-triglycerides of RI strains did not segregate to progenitors, suggesting that multiple genes and/or metabolic factors affected those qualities of LDL. On the other hand, LDL sizes of RI strains did segregate to one or another progenitor in 88% of the cases, implying that LDL size may be controlled by the product of a major gene and modified by the products of other genes such as LPL and H-TGL. However, when the segregation patterns of LDL sizes were compared to those of tissue LPL activities and RFLP of LPL gene reported by Kirchgessner et al. (45), no significant linkage was found (data not shown). Attempts at linking LDL-size to the apoB gene by apoB RFLP analysis yielded only marginally significant results. Thus, if a major gene does determine LDL-size, its identity remains to be determined.

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