Two novel quantitative trait loci on mouse chromosomes 6 and 4 independently and synergistically regulate plasma apoB levels

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Abstract **An elevated plasma apolipoprotein B (apoB) level is a strong predictor of atherosclerosis and coronary heart disease. Epidemiologic and family linkage studies have suggested a genetic basis for the wide variations of plasma apoB levels in the general population. Using a human apoB transgenic (HuBTg) mouse model, we have previously shown that hepatic apoB-100 secretion is a major determinant of the high and low plasma human apoB levels in HuBTg mice of the C57BL/6 (B6) and 129/Sv (129) strains, respectively. In the present article, we present the identification of two novel quantitative trait loci (QTL) as major regulators of plasma human apoB levels in the F_2 and N_2 (backcrossed) offspring (n = 572) derived from crosses between the B6 and 129 mouse strains. These loci were designated *ApoB regulator genes* (*Abrg*), because the gene products are likely to be involved in the regulation of plasma apoB levels either directly or indirectly. The first locus, designated *Abrg1*, was mapped to chromosome 6 in 8-week-old male and female mice with a combined logarithm of odds ratio (LOD) score of 14 at the D6Mit55 marker (~45.9 cM). *Abrg1* contributed approximately 35% of the genetic variance. The second locus, designated *Abrg2*, was mapped to chromosome 4 with an LOD score of 8.6 in 8-week-old male mice but an LOD score of only 2.0 in 8-week-old female mice at the D4Mit27 marker (~35 cM). *Abrg2* contributed approximately 26% of the genetic variance. Epistasis between *Abrg1* and *Abrg2* was detected and accounted for approximately 12% of the genetic variance. The combination of these two QTL has major effects (>70%) on the regulation of plasma human apoB levels in the tested population. In summary, we have identified two novel loci that have a major role in the regulation of plasma apoB levels and are likely to regulate the secretory pathway of apoB. The human orthologs for the *Abrg* loci are strong candidates for human disorders characterized by altered plasma apoB levels, such as FCHL and familial hypobetalipoproteinemia.**—Ko, C., T-L. Lee, P. W. Lau, J. Li, B. T. Davis, E. Voyiaziakis, D. B. Allison, S. C. Chua, Jr., and L-S. Huang. **Two novel quantitative trait loci on mouse chromosomes 6 and 4 independently and synergistically regulate plasma apoB levels.** *J. Lipid Res.* **2001.** 42: **844–855.**

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An elevated plasma apolipoprotein B (apoB) level is a strong predictor of atherosclerosis and coronary heart disease (1). ApoB is a key structural component of triglyceride-rich lipoproteins (2). ApoB exists in two forms, B-100 and B-48. Both are products of the same gene encoding a 14-kb mRNA and share the same amino-terminal 2,152 amino acids. ApoB-48 is derived by the introduction of a stop codon at residue 2153 of the B-100 message via an RNA-editing mechanism (3). In humans, apoB-100 and apoB-48 are required for the secretion of VLDL from the liver and chylomicrons from the intestine, respectively. ApoB-100 is also the ligand for the low density lipoprotein (LDL) receptor, which mediates cellular uptake of LDL particles, hydrolyzed products of very low density lipoproteins (VLDL). Plasma apoB levels, therefore, represent the balance between the secretion and clearance rates of apoB-containing lipoproteins. Mutations in the genes encoding either apoB or the LDL receptor result in altered plasma apoB and LDL cholesterol levels in patients with monogenic disorders such as familial hypobetalipoproteinemia (FHBL) (low plasma apoB), familial defective hyperapolipoproteinemia (high plasma apoB), and FH (high plasma apoB). These mutations, however, do not account for the wide variation of plasma apoB in the general population, nor do they explain other common lipoprotein disorders. Studies have shown that known mutations

Abbreviations: *Abrg*, apolipoprotein B regulator gene; FHBL, familial hypobetalipoproteinemia; FCHL, familial hypobetalipoproteinemia; LOD, logarithm of odds ratio.

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in the apoB gene account for only a small number of subjects with low plasma apoB levels (4). Kinetic studies have shown that decreased production of apoB-containing lipoproteins is responsible for the reduced plasma apoB levels in some subjects with non-apoB-linked hypobetalipoproteinemia (5). On the opposite end of the spectrum, only a few loci have been linked to either the elevation of plasma triglyceride and/or apoB levels in subjects with familial combined hyperlipidemia (FCHL), a disorder characterized by overproduction of apoB (6). This disorder is present in 1–2% of the general population and in about 10–20% of patients with coronary artery disease (CAD). The genetic basis of FCHL is heterogeneous, and mutations in the genes regulating hepatic apoB production represent plausible causes for the disorder.

Epidemiological and family linkage studies have suggested a genetic basis for the wide variations of plasma apoB levels in the general population (7–11). These studies also suggest that plasma apoB levels are controlled by major genes other than the apoB gene. Mouse genetics provides a powerful approach to identify quantitative trait loci (QTL) involved in complex diseases or traits. Using a human apoB transgenic (HuBTg) mouse model, we have identified three mouse strains suitable for genetic analysis of factors regulating hepatic apoB secretion and hence plasma apoB levels (12). By crossing various inbred mouse strains with a congenic HuBTg mouse strain of the C57BL/6 (B6) background, we have generated HuBTg mouse strains with varying plasma human apoB levels (60–100 mg/dl), mimicking the variation of plasma apoB levels in human populations. Three mouse strains, 129/Sv, BALB/c, and C3H/HeJ, possess polymorphic alleles that, when crossed with the B6 HuBTg strain, lower plasma human apoB levels and apoB-100 secretion by 30–40% in their F_1 offspring. Our studies also suggested that differential hepatic apoB-100 secretion rates are likely to be due to post-transcriptional regulation because these mouse strains have similar hepatic human apoB mRNA levels and apoB RNA-editing activities (12). ApoB RNA-editing activity is active in the rodent liver (3), and differences in editing activity could alter the ratio of B-100 versus B-48 mRNA species and hence the synthesis and secretion of apoB-100-containing lipoproteins.

In the present article, we carried out genetic analyses in an attempt to identify factors that regulate plasma apoB levels via their effects on hepatic apoB-100 secretion rates. We chose the 129/Sv strain, a strain with low plasma apoB and hepatic apoB-100 secretion rates relative to the B6 strain, for genetic crosses with the congenic B6 HuBTg strain. From three independent crosses that generated more than 500 animals, we identified two QTL on chromosomes 6 and 4 that independently and synergistically regulate plasma apoB levels.

MATERIALS AND METHODS

Mouse crosses

A congenic HuBTg mouse strain of the C57BL/6J (B6) background was generated as described previously (12). The B6 HuBTg mouse strain has been maintained by continuously backcrossing with wild-type B6 mice purchased from the Jackson Laboratory (Bar Harbor, ME). To maintain hemizygosity of the human apoB transgene in all the animals used in this study, crosses were carried out between nontransgenic mice and hemizygous HuBTg mice. F_1 HuBTg mice (129 × B6) were generated by crossing male B6 HuBTg mice with female 129/Sv (129) mice. HuBTg offspring were identified by polymerase chain reaction (PCR), using primers specific for the human apoB gene (13). The inbred 129 strain used has been maintained in our laboratory as described (12). For genetic analysis, F_2 HuBTg mice (n = 173) were generated by intercrosses between transgenic and nontransgenic F_1 mice. In addition, two types of backcrossed offspring were also generated for mapping analysis. By convention, offspring derived from backcrosses are designated $N_{(I+1)}$, where I denotes the number of backcrossed generations. The first backcross was carried out by crossing F_1 HuBTg mice with the B6 parental strain. Offspring (n = 196) derived from this cross are designated N_2/B6. The second backcross was carried out by crossing F_1 HuBTg mice with the 129 parental strain. Offspring (n = 203) derived from this cross are designated N_2/129. To generate congenic 129 HuBTg mice for phenotype analysis, 129 × B6 F_1 HuBTg mice were continuously backcrossed to the 129 strain. Conventionally, N_{10} animals (derived after nine backcross generations) are considered congenic. We currently have N_6 animals (derived after five backcross generations), which were phenotyped to compare plasma apoB levels among strains.

Localization and preservation of the human apoB transgene in HuBTg mice

The HuBTg mice were originally generated in the FVB/NJ (FVB) strain (13). The human apoB transgene is therefore carried on a piece of donor FVB chromosome, which was transferred onto the B6 background by backcrossing. In our initial genome scan of the F_2 mice, we detected the presence of FVB alleles on chromosome 10. We subsequently localized the transgene by scanning two sets of animals, using chromosome 10 markers. The markers used were as follows: D10Mit206 (4.4 cM), D10Mit213 (6.6 cM), D10Mit282 (7.7 cM), D10Mit283 (8.7 cM), D10Mit17 (8.7 cM), D10Mit17 (12.0 cM), D10Mit214 (15.3 cM), D10Mit3 (16.4 cM), D10Mit284 (17.5 cM), D10Mit61 (24.0 cM), and D10Mit230 (47.0 cM). Nontransgenic (n = 10) and 129 × B6 F_1 transgenic (n = 10) mice were scanned for chromosome 10 markers. HuBTg mice should have both FVB and B6 alleles, whereas nontransgenic mice should have both B6 and 129 alleles where the transgene is located. B6 congenic HuBTg mice ($>N_{10}$, n = 110) were also scanned for these markers. These data showed an absolute linkage of the human apoB transgene with D10Mit214, D10Mit3, and D10Mit284 markers, which span approximately 2.2 cM of the genome. Subsequently, all transgenic animals were screened with D10Mit3 and D10Mit284 to ensure the preservation of the human apoB transgene in hemizygous animals.

Phenotype analysis

Mice were maintained on a 12-h light/dark cycle (light cycle: 7 AM–7 PM), fed a rodent chow (PicoLab Rodent Chow, No. 5001; Purina Lab Chows, St. Louis, MO), and had free access to water. Mice were fasted for 4 h (10 AM–2 PM) and retro-orbitally bled at 8 and 12 weeks of age. For plasma human apoB levels, an antibody specific to human apoB was used in immunoturbidimetric assays as described previously (12).

A genome-wide scan of selective male F_2 animals with extreme apoB phenotypes

Twenty male F_2 animals with plasma human apoB levels lower than 60 mg/dl (n = 10, the lowest 13%) or higher than 90 mg/

TABLE 1. Microsatellite markers used in the genome scan

Chromosome	Marker	cM	Chromosome	Marker	cM
1	D1Mit123	19.7		D9Mit158	36.1
	D1Mit19	37.2		D9Mit115	54.6
	D1Mit306	57.9	10	D10Mit213	6.6
	D1Mit15	86.3		D10Mit3	16.4
	D1Mit166	99.5		D11Mit20	19.7
2	D2Mit458	31.7	11	D11Mit320	39.3
	D2Mit395	55.7		D11Mit358	52.5
	D2Mit168	66.7		D11Mit167	75.4
	D2Mit456	86.3	12	D12Mit60	13.1
3	D3Mit131	4.4		D12Mit52	27.3
	D3Mit40	29.5		D12Mit7	44.8
	D3Mit352	63.4	13	D13Mit16	7.7
4	D4Mit95	12.0		D13Mit248	19.7
	D4Mit27	35.0		D13Mit256	25.1
	D4Mit12	54.6		D13Mit151	50.3
	D4Mit204	61.2	14	D14Mit26	6.6
5	D5Mit348	5.5		D14Mit60	24.0
	D5Mit309	30.6		D14Mit237	40.4
	D5Mit95	57.9		D14Mit228	55.7
	D5Mit43	77.6	15	D15Mit177	6.6
	D5Mit102	82.0		D15Mit26	21.9
6	D6Mit77	7.7		D15Mit96	49.2
	D6Mit9	27.3	16	D16Mit130	3.3
	D6Mit55	45.9		D16Mit4	25.1
	D6Mit199	57.9		D16Mit158	40.4
	D6Mit15	66.7	17	D17Mit205	35.0
7	D7Mit114	5.5		D17Mit142	41.5
	D7Mit31	31.7	18	D18Mit202	14.2
	D7Mit137	54.6		D18Mit49	33.9
8	D8Mit4	12.0	19	D19Mit41	12.0
	D8Mit224	16.4		D19Mit91	35.0
	D8Mit104	38.3		D19Mit1	43.7
	D8Mit211	50.3	X	DXMit208	18.6
9	D9Mit250	2.2		DXMit64	31.7
	D9Mit130	21.9		DXMit38	43.7

dl (n = 10, the highest 13%) at both 8 and 12 weeks of age were selected from a total of 79 male F_2 HuBTg mice. A total of 70 microsatellite markers at approximately 30-cM spacing was used to genotype these 20 animals with extreme phenotypes. The microsatellite markers used are shown in **Table 1**. The distance of the markers from the centromere of each chromosome is based on data posted by the Whitehead Institute (http://www.genome.wi.mit.edu/). For genotyping analysis, PCR primers were purchased from Research Genetics (Huntsville, AL) or custom synthesized by Life Technologies (Rockville, MD).

Logarithm of odds (LOD) score and statistical analysis

Markers on chromosomes 6 and 4 were used to define the intervals containing QTL associated with plasma human apoB levels in F_2 and N_2 HuBTg mice. The markers used were as follows: D6Mit77, D6Mit9, D6Mit178, D6Mit64, D6Mit333, D6Mit339, D6Mit199, and D6Mit15 (chromosome 6); D4Mit99, D4Mit95, D4Mit108, D4Mit89, D4Mit275, D4Mit27, D4Mit145, D4Mit12, and D4Mit127 (chromosome 4). LOD scores between genetic markers and plasma human apoB levels were computed by Map Manager QT (version QTb28) (14). The association between plasma human apoB levels among genotypes in F_2 and N_2 animals was assessed by one-way ANOVA and Student's *t*-test, respectively. To estimate the contribution of each QTL to genetic variance, a mixed model of ANOVA was conducted with the statistical package SPSS (v 9.0; SPSS, Chicago, IL).

Riboprobe preparation and RNase protection assays

Total cellular RNA was isolated from the liver by the guanidinium thiocyanate method (15). For RNase protection assays, 20-μg samples of total cellular RNA from 12-week-old male B6 and 129 mice (n = 5 per group) were used. RNA probes for mouse Apobec-1 and Sec13r were generated by amplification of the target gene from liver RNA (male B6 mice) by RT-PCR. PCR primers used and the size of amplified products for each probe are listed below. Sec13r: sense, 5′-TGG TCG AGT GTT TAT TTG GA-3′; antisense, 5′-GAT GCA CAC CCA CTG TCC GTC-3′ (191 bp); Apobec-1: sense, 5′-GGT ACT AGT TAC ACA CGA GGC CC-3′; antisense, 5′-AAG GTC TCA CTG TGT AAT TCA-3′ (203 bp). PCR products were cloned into a PCRII vector, using a TA cloning kit obtained from InVitrogen (Carlsbad, CA). DNA sequences of each clone were verified by DNA sequencing, using an ABI 377 automatic DNA sequencer (PE Biosystems, Foster City, CA).

Antisense probes were synthesized with an in vitro transcription kit obtained from Promega (Madison, WI) and [α-^{32}P]CTP (800 Ci/mmol). A *Hin*fI fragment (100 bp) including the T3 promoter was isolated from a mouse β-actin clone (Ambion, Austin, TX) and used as a reference RNA in RNase protection assays. RNase protection assays were carried out as described previously (16). Protected RNA fragments were separated in 5% polyacrylamide-7 M urea gels. Dried gels were exposed to X-ray films for 1–2 days at −80°C. For quantitation, protected RNA fragments were cut and counted in a liquid scintillation counter.

DNA sequence analysis of Sec13r cDNA

Mouse Sec13r cDNAs (953 bp) were amplified from both B6 and 129 liver RNAs, using the Access RT-PCR system kit (Promega). PCR primers used were as follows: sense, 5′-ATG GTG TCA GTA ATG AAC ACT GTG GAC A-3′; and antisense, 5′-TGG CCC TCT GTG ATG GAG GCT GAC ACA GAA C-3′. PCR products were directly sequenced with internal sequences for the Sec13r cDNA, which were obtained from the mouse Expressed Sequence Tag (EST) database (accession numbers AA870418 and AA108956). PCR products were also cloned into a PCRII vector, using a TA cloning kit obtained from InVitrogen. DNA sequences of each clone were also sequenced with both universal primers and Sec13r-specific primers. Sequence analyses were carried out with an ABI 377 automatic DNA sequencer.

RESULTS

Genetic control of apoB in two inbred mouse strains

To identify genetic factors regulating plasma apoB levels, F_2 mice hemizygous for the human apoB transgene were generated from intercrosses between 129 × B6 F_1 HuBTg and nontransgenic F_1 littermates. A total of 159 F_2 HuBTg mice were generated and their plasma human apoB levels were measured at 8 and 12 weeks of age. In addition, plasma human apoB levels of congenic B6, 129 × B6 F_1 and congenic 129 HuBTg mice were assessed. Plasma human apoB levels of 8-week-old animals are shown in **Fig. 1**. Mean plasma apoB levels of both 8- and 12-week-old animals from each cross are shown in **Table 2**.

In 8-week-old male mice, mean plasma human apoB levels (66 ± 10 mg/dl) of the F_1 HuBTg mice were significantly lower than that of the B6 parental strain (95 ± 16 mg/dl, $P < 0.0001$). They were not significantly different from that of the 129 parental strain (72 ± 9 mg/dl, $P = 0.07$). Mean plasma human apoB levels of male F_2 HuBTg mice (72 ± 17 mg/dl) were comparable to that of the 129 parental strain, but significantly lower ($P < 0.0001$) than that of B6 HuBTg mice. Plasma apoB levels

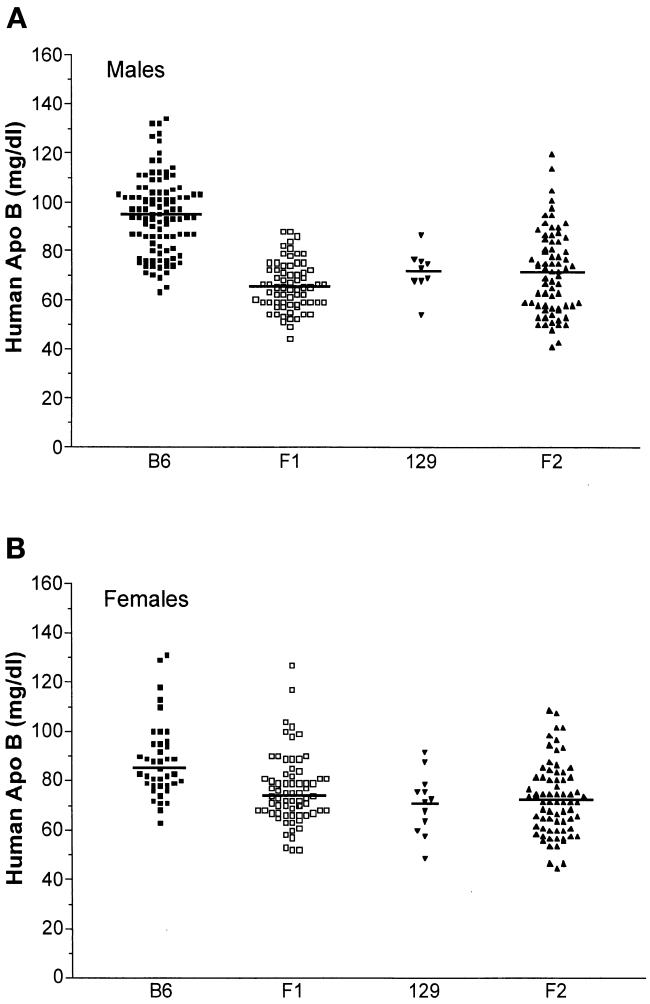

Fig. 1. Distribution of plasma human apoB levels in 8-week-old HuBTg mouse strains. A: Male HuBTg mice. B: Female HuBTg mice. Animals were bled at 8 weeks of age after a 4-h fast. B6 HuBTg mice (filled squares) were the parental strain with high plasma human apoB levels. F_1 mice (open squares) were generated from crosses between male B6 HuBTg mice and females of the wild-type 129 inbred strain. 129 HuBTg mice (filled inverted triangles) were generated by backcrossing F_1 HuBTg mice to wild-type 129 inbred mice. F_2 mice (filled triangles) were generated from crosses between F_1 transgenic and nontransgenic animals. Mean plasma apoB levels in each group of animals are shown as horizontal lines in each column and their values are shown in Table 2.

of the F_2 HuBTg mice were slightly higher ($P = 0.01$) than that of the F_1 HuBTg mice. Similar trends were observed in female mice as well as 12-week-old animals of both genders (Table 2). We also noted that mean plasma apoB levels in male mice decreased as these animals aged (8 vs. 12 weeks: B6, $P < 0.0001$; F_1, $P = 0.001$; 129, $P = 0.002$). The same phenomenon, however, was not observed in female mice. Overall, these data show that plasma human apoB levels in these animals appear to be controlled by a major gene or genes with a dominant mode of inheritance.

Two chromosomal regions associated with apoB phenotype

To favor the identification of genes with major effects on plasma human apoB levels, a selective genotyping strategy was applied to animals with extreme phenotypes. From a total of 79 male F_2 HuBTg mice, animals with plasma human apoB levels in the highest (>90 mg/dl, n = 10) and the lowest (<60 mg/dl, n = 10) 13% of the F_2 sample were subjected to a genome-wide scan, using 70 microsatellite markers spanning the mouse genome. These markers have an average spacing of 30 cM in each chromosome. The genome scan revealed possible linkages of the apoB phenotype with markers on chromosomes 6 and 4. As shown in **Table 3**, a suggestion of linkage ($\chi^2 = 8.8$, $P = 0.01$) was detected between the apoB phenotype and the D6Mit55 marker (~45.9 cM) on chromosome 6 (Table 3). The genome scan also revealed a possible linkage of plasma apoB phenotype with D4Mit204 (~61.2 cM) on chromosome 4 (Table 3). Neither marker was linked to the human apoB transgene on chromosome 10.

To establish linkage between plasma human apoB levels and the D6Mit55 and D4Mit204 markers, the remaining male F_2 and female F_2 samples were also genotyped. Link-

TABLE 2. Mean plasma apoB levels in HuBTg mice from various crosses

HuBTg Strain[a]	8-Week-Old				12-Week-Old			
	Male		Female		Male		Female	
	n	ApoB[b]	n	ApoB[b]	n	ApoB[b]	n	ApoB[b]
		mg/dl		*mg/dl*		*mg/dl*		*mg/dl*
B6	102	95 ± 16	40	88 ± 15	49	85 ± 11	10	81 ± 11
F_1	69	66 ± 10	56	76 ± 14	65	60 ± 9	61	71 ± 11
129	9	72 ± 9	12	71 ± 12	8	63 ± 6	7	72 ± 5
F_2	71	72 ± 17	72	73 ± 15	72	73 ± 15	80	73 ± 15
N_2/B6	96	82 ± 19	85	83 ± 14	101	73 ± 12	84	84 ± 14
N_2/129	106	64 ± 11	88	71 ± 9	104	61 ± 11	86	70 ± 11

[a] HuBTg mice were derived from various crosses. B6: HuBTg mice were backcrossed to the B6 strains for more than 10 generations. F_1 HuBTg mice were generated by crossing male B6 HuBTg mice with female 129 mice. 129: HuBTg mice were backcrossed to the 129 strain for five generations (N_6). F_2 mice were generated from intercrosses between F_1 HuBTg and nontransgenic littermates. F_1 HuBTg mice were backcrossed to either the B6 strain or the 129 strain to generate N_2/B6 and N_2/129 HuBTg mice, respectively. Blood samples were collected from animals at both 8 and 12 weeks of age. In a small number of animals, only one of the two time points was available for analysis.

[b] Fasting plasma samples were measured for plasma human apoB levels (mg/dl) and all values shown are means ± SD. Comparisons between any two groups of animals were carried out using a Student's *t*-test. The results are described in text.

TABLE 3. χ^2 analysis of chromosome 6 and 4 markers in selected male F_2 HuBTg mice

Marker	ApoB Phenotype[a]	n	Genotype (No. of Mice)			df[b]	χ^2	*P*[b]
			B6/B6	B6/129	129/129			
D6Mit55	Low	10	0	6	4	2	8.8	0.01
	High	10	6	3	1			
D4Mit204	Low	10	0	5	5	2	5	0.08
	High	10	2	7	1			

[a] Low-apoB phenotype: F_2 HuBTg mice with plasma human apoB levels <60 mg/dl at both 8 and 12 weeks of age. High-apoB phenotype: F_2 HuBTg mice with plasma human apoB levels >90 mg/dl at both 8 and 12 weeks of age.

[b] df, Degrees of freedom. *P* values were derived from χ^2 analysis.

TABLE 4. LOD score analysis of chromosome 6 and 4 markers with plasma human apoB in animals from three independent crosses

Marker	Sex	Age	LOD Score F_2 (79)	N_2/B6 (109)	N_2/129 (110)	Total (298)
		weeks				
D6Mit55	M	8	2.6	1.4	3.2	7.2
		12	2.8	4.0	2.7	9.5
D4Mit204	M	8	2.6	0.4	2.0	5.0
		12	1.5	2.3	1.6	5.4
			(80)	(87)	(93)	(260)
D6Mit55	F	8	3.0	1.0	2.8	6.8
		12	0.9	3.6	2.1	6.6
D4Mit204	F	8	0.7	0.7	0.4	1.8
		12	0.5	0.3	0.02	0.8
			(159)	(196)	(203)	(558)
D6Mit55	M + F	8	5.6	2.4	6.0	14.0
		12	3.7	7.6	4.8	16.1
D4Mit204	M + F	8	3.3	1.1	2.4	6.8
		12	2.0	2.6	1.6	6.2

Plasma human apoB levels from both 8- and 12-week-old animals were used for linkage analyses. LOD scores were computed by the Map Manager QT program. The sum of LOD scores for each marker from all three crosses (F_2, N_2/B6, and N_2/129) are shown in the "Total" column. The number of animals is shown in parentheses. In a small number of animals, only one of the two time points for plasma apoB levels was available for analysis.

age was assessed by likelihood ratio statistics, using Map Manager QT (14). The results are shown in **Table 4**. The LOD scores for D6Mit55 were 2.6 and 3.0 for 8-week-old male and female mice, respectively. The combined LOD score was 5.6, indicating a significant linkage between D6Mit55 and plasma human apoB levels in 8-week-old F_2 mice. Linkage between D6Mit55 and plasma human apoB levels (combined LOD = 3.7) was also significant in 12-week-old male and female F_2 mice. Table 4 also shows a suggestive linkage (LOD = 2.6) of the D4Mit204 marker with plasma human apoB levels in 8-week-old male F_2 mice. There was no apparent linkage of the D4Mit204 marker to plasma human apoB levels in female F_2 mice.

Confirmation of the linkages between plasma human apoB levels and chromosomes 6 and 4 markers in two backcrosses

To independently confirm the linkages between chromosomes 6 and 4 markers with plasma human apoB levels, two additional crosses were generated. Male F_1 HuBTg mice were backcrossed to female mice of the B6 or 129 strain to generate N_2/B6 and N_2/129 mice, respectively. Plasma human apoB levels from 8-week-old N_2 mice are shown in **Fig. 2**. Mean plasma apoB levels from both 8- and 12-week-old N_2 animals are shown in Table 2. As with the F_2 cross, the distribution of plasma apoB levels from N_2 mice also suggested a dominant mode of inheritance of gene(s) involved in the regulation of plasma apoB levels.

All N_2 mice were genotyped with D6Mit55 and D4Mit204. Linkages between these two markers and plasma human apoB levels were assessed. The results are shown in Table 4. In male N_2/B6 mice, D6Mit55 was significantly linked to plasma human apoB levels in 12-week-old animals (LOD = 4.0) but not in 8-week-old animals (LOD = 1.4). This age effect was similarly observed in female N_2/B6 mice. In both male and female N_2/129 mice, significant (or highly suggestive) linkage was observed in both age groups. Table 4 also shows that gender, age, and genetic background all have an effect on the degree of linkage between D4Mit204 and plasma human apoB levels in N_2 mice. As observed for the female F_2 mice, D4Mit204 failed to show a linkage in either female N_2/B6 or female N_2/129 mice. In male mice, linkage was suggestive in 12-week-old N_2/B6 mice (LOD = 2.3) and in 8-week-old male N_2/129 mice (LOD = 2.0).

The combined data from all three crosses showed a significant linkage of plasma human apoB levels to D6Mit55 in both male (LOD = 7.2–9.5, depending on age) and female mice (LOD = 6.6–6.8). These data also showed a significant linkage to D4Mit204 in male mice (LOD = 5.0–5.4), but not in female mice (LOD = 0.8–1.8). Overall, these data confirmed that in addition to the F_2 cross, both D6Mit55 and D4Mit204 markers were linked to plasma human apoB levels in the N_2 crosses.

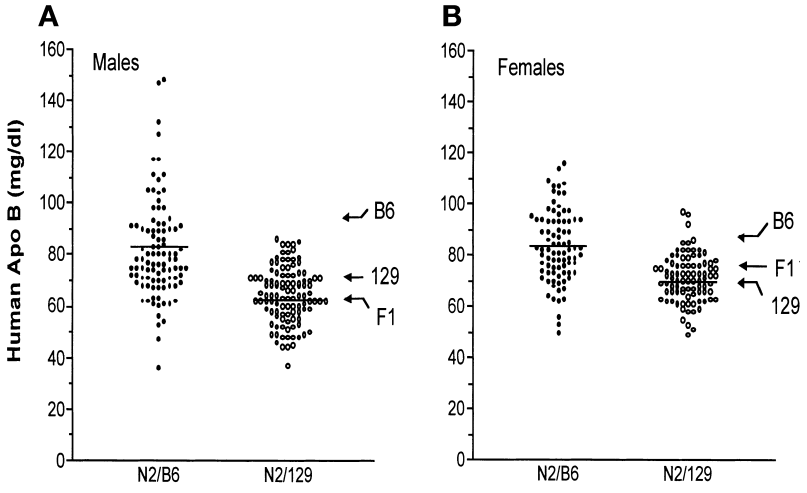

Fig. 2. Distribution of plasma human apoB levels in 8-week-old backcrossed animals. A: Male HuBTg mice. B: Female HuBTg mice. Animals were bled at 8 weeks of age after a 4-h fast. N_2/B6 mice (solid circles) were generated by crossing male F_1 HuBTg mice to female wild-type B6 mice. N_2/129 mice (open circles) were generated by crossing male F_1 HuBTg mice to female wild-type 129 mice. Mean plasma apoB levels in each group of animals are shown as vertical lines in each column and their values are shown in Table 2. The position of arrows indicates the mean plasma apoB levels for B6, 129, or F_1 animals.

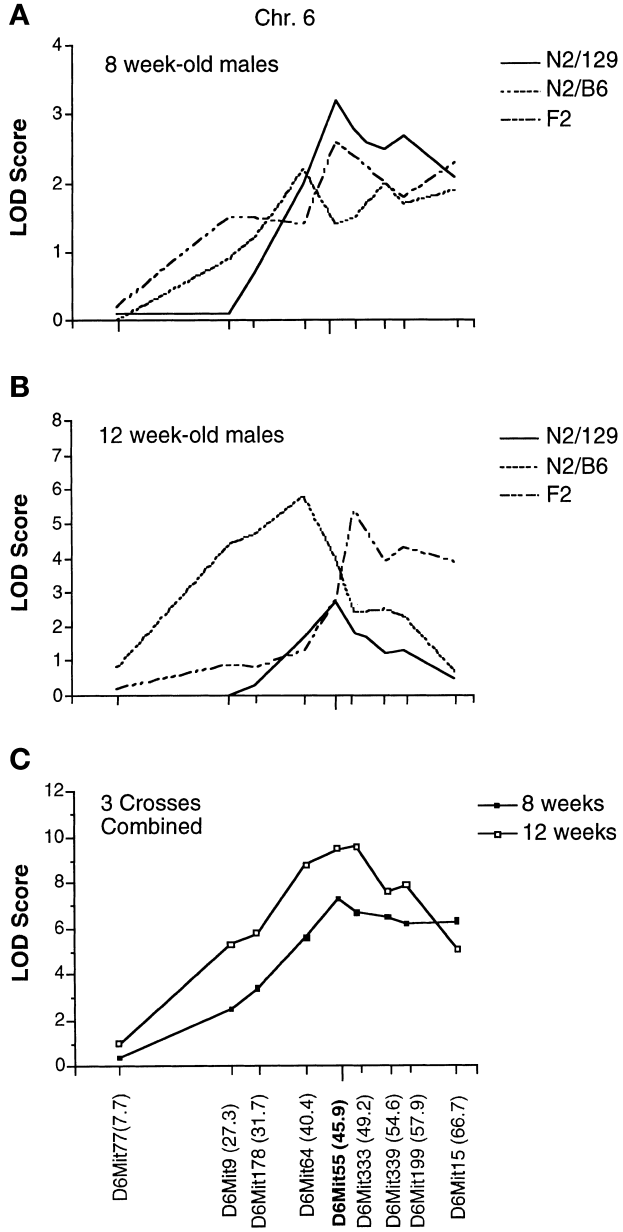

Fig. 3. LOD score analysis for the *Abrg1* locus on chromosome 6. Male HuBTg mice from F_2, N_2/B6, and N_2/129 crosses were genotyped for chromosome 6 microsatellite markers. The map distance (centimorgans) to the centromere for each marker is plotted on the x axis and is indicated in parentheses after each marker. The LOD score for each marker is shown on the y axis. A: Plasma apoB levels from 8-week-old male animals were used for LOD score analysis. B: Plasma apoB levels from 12-week-old male animals were used for LOD score analysis. C: LOD scores from all three crosses of the same age group (either 8 or 12 weeks) were combined.

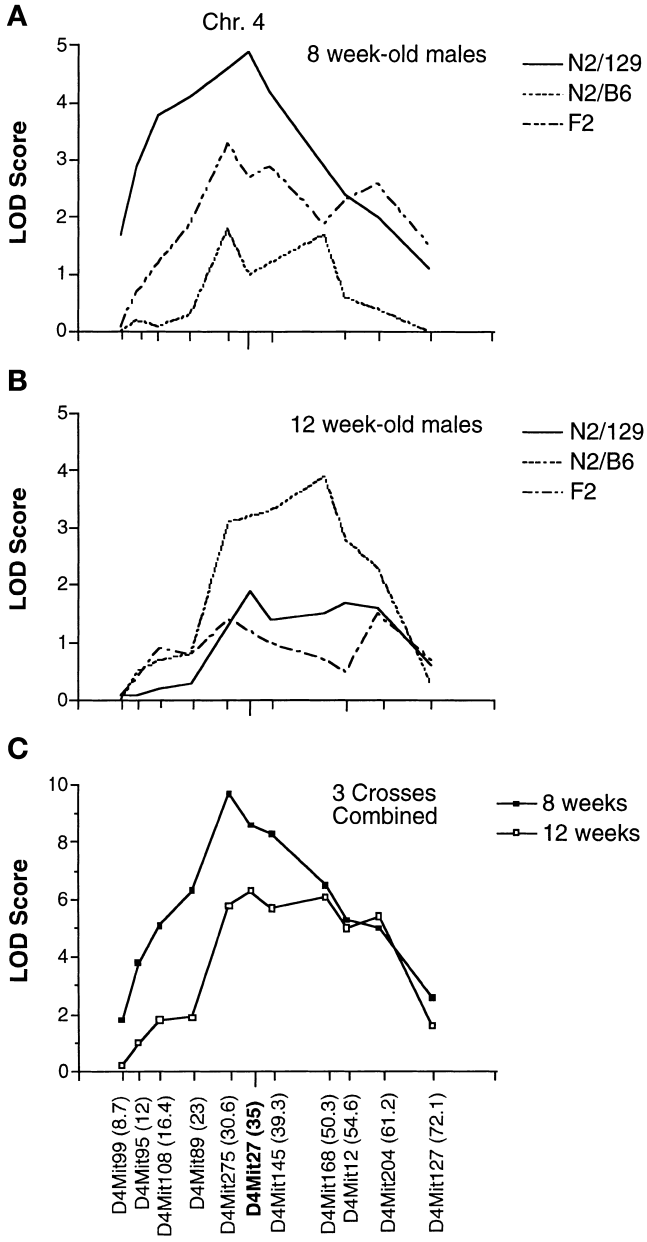

Fig. 4. LOD score analysis for the *Abrg2* locus on chromosome 4. Male HuBTg mice from F_2, N_2/B6, and N_2/129 crosses were genotyped for chromosome 4 microsatellite markers. The map distance (centimorgans) to the centromere for each marker is plotted on the x axis and is indicated in parentheses after each marker. The LOD score for each marker is shown on the y axis. A: Plasma apoB levels from 8-week-old male animals were used for LOD score analysis. B: Plasma apoB levels from 12-week-old male animals were used for LOD score analysis. C: LOD scores from all three crosses of the same age group (either 8 or 12 weeks) were combined.

Definition of the chromosome 6 and 4 QTL intervals

To define the intervals on chromosomes 6 and 4 that were associated with plasma human apoB levels, markers flanking either D6Mit55 or D4Mit204 were used to genotype male animals from F_2 and N_2 crosses. LOD scores were computed for each marker on chromosomes 6 and 4 and plotted in **Figs. 3** and **4**, respectively. As shown in Fig. 3C, the combined data from all three crosses showed that the LOD score peaked at D6Mit55. These data suggested that the QTL is likely to be near D6Mit55 and within an interval of ~28 cM (40.4–57.9 cM), spanning D6Mit64 and D6Mit199 on chromosome 6. However, when each cross was considered separately (Fig. 3A and B), a smaller interval of ~9 cM (40.4–49.2 cM) encompassing D6Mit64 and D6Mit333 could be defined.

In Fig. 4C, the combined data showed that the LOD

TABLE 5. Association of *Abrg1* with plasma human apoB levels

Cross	Sex	Age	ApoB: Grouped by Genotypes[a] B6/B6	B6/129	129/129	P^b
		weeks		*mg/dl*		
F_2	M	8	84 ± 12 (n = 16)	70 ± 15 (n = 36)	65 ± 22 (n = 17)	0.003
		12	88 ± 21 (n = 19)	70 ± 29 (n = 41)	58 ± 15 (n = 17)	0.002
	F	8	78 ± 14 (n = 18)	74 ± 13 (n = 38)	61 ± 9 (n = 14)	0.001
		12	77 ± 14 (n = 23)	73 ± 11 (n = 40)	67 ± 22 (n = 15)	0.1
N_2/B6	M	8	87 ± 15 (n = 40)	77 ± 20 (n = 56)	NA	0.01
		12	78 ± 11 (n = 45)	69 ± 10 (n = 58)	NA	<0.0001
	F	8	87 ± 13 (n = 38)	81 ± 14 (n = 47)	NA	0.04
		12	90 ± 11 (n = 39)	79 ± 14 (n = 45)	NA	0.0002
N_2/129	M	8	NA	68 ± 10 (n = 55)	60 ± 10 (n = 48)	0.0003
		12	NA	64 ± 11 (n = 54)	57 ± 10 (n = 48)	0.0009
	F	8	NA	74 ± 9 (n = 48)	67 ± 8 (n = 40)	0.0004
		12	NA	73 ± 10 (n = 44)	66 ± 11 (n = 41)	0.002

[a] Genotypes of animals were grouped using the D6Mit55 marker for *Abrg1* at the chromosome 6 interval. ApoB levels are shown as means ± SD. The number of animals (n) for each group is given in parentheses. NA: This cross did not yield offspring with the genotype shown in the column.

[b] *P* values were derived from one way analysis of variance (ANOVA) for F_2 cross and derived from a Student's *t*-test for N_2 crosses.

score peaked around D4Mit275 (30.6 cM) and D4Mit27 (35 cM). Because previous analyses (Table 4) failed to show linkage of D4Mit204 with plasma apoB levels in females, we genotyped female HuBTg mice from all three crosses (n = 260) with the D4Mit27 marker. The results showed a suggestive linkage between D4Mit27 and plasma human apoB levels in female mice (8 weeks old, LOD = 2.0; 12 weeks old, LOD = 2.6) (data not plotted). Overall, this second QTL likely exists near the D4Mit275 and D4Mit27 markers and within an interval of ~30 cM (30.6–61.2 cM) spanning the D4Mit275 and D4Mit204 markers. However, it did not appear to be as important, if important at all, in female mice.

The products of the genes residing in the aforementioned intervals likely regulate plasma apoB levels. We suggest the provisional designation, *ApoB regulator genes*, for these newly identified loci. The QTL at the chromosome 6 interval will be designated *ApoB regulator gene 1* (*Abrg1*), and the QTL at the chromosome 4 interval will be designated *ApoB regulator gene 2* (*Abrg2*). The LOD score for *Abrg1* is 14 in 8-week-old animals, using D6Mit55 as the marker. Using D4Mit27 as the marker, the LOD score for *Abrg2* is 8.6 in 8-week-old male mice and 2.0 for 8-week-old female mice.

Relationship of plasma human apoB levels with Abrg1 and Abrg2 loci

To assess the relationship between *Abrg* alleles and plasma human apoB levels, animals were grouped on the basis of genotypes, using D6Mit55 and D4Mit27 for *Abrg1* and *Abrg2*, respectively. The results for *Abrg1* and *Abrg2* are shown in **Tables 5** and **6**, respectively. As shown at the top of Table 5, male 8-week-old F_2 mice heterozygous for *Abrg1* had plasma human apoB levels (70 ± 15 mg/dl) be-

TABLE 6. Association of *Abrg2* with plasma human apoB levels

Cross	Sex	Age	ApoB: Grouped by Genotypes[a] B6/B6	B6/129	129/129	P^b
		weeks		*mg/dl*		
F_2	M	8	84 ± 14 (n = 17)	68 ± 17 (n = 39)	67 ± 14 (n = 12)	0.003
		12	82 ± 32 (n = 20)	70 ± 25 (n = 43)	61 ± 20 (n = 13)	0.07
	F	8	80 ± 12 (n = 14)	73 ± 13 (n = 33)	70 ± 15 (n = 20)	0.1
		12	75 ± 12 (n = 16)	76 ± 12 (n = 39)	70 ± 20 (n = 20)	0.3
N_2/B6	M	8	86 ± 17 (n = 36)	78 ± 19 (n = 60)	NA	0.03
		12	78 ± 9 (n = 39)	70 ± 12 (n = 64)	NA	0.0001
	F	8	86 ± 13 (n = 48)	80 ± 14 (n = 37)	NA	0.04
		12	88 ± 12 (n = 47)	80 ± 14 (n = 37)	NA	0.003
N_2/129	M	8	NA	69 ± 10 (n = 52)	60 ± 9 (n = 53)	<0.0001
		12	NA	64 ± 11 (n = 50)	58 ± 11 (n = 53)	0.004
	F	8	NA	72 ± 9 (n = 51)	70 ± 9 (n = 37)	0.4
		12	NA	71 ± 11 (n = 49)	69 ± 10 (n = 36)	0.3

[a] Genotypes of animals were grouped using the D4Mit27 marker for *Abrg2* at the chromosome 4 interval. ApoB levels are shown as means ± SD. The number of animals (n) for each group is given in parentheses. NA: This cross did not yield offspring with the genotype shown in the column.

[b] *P* values were derived from one-way analysis of variance (ANOVA) for F_2 cross and derived from a Student's *t*-test for N_2 crosses.

tween those of homozygotes for either the B6 (84 ± 12 mg/dl) or the 129 (65 ± 22 mg/dl) allele. Similar trends were observed in 12-week-old male and female F_2 mice as well as in N_2/B6 and N_2/129 mice of both genders. These data suggest a codominant mode of inheritance for the B6 and the 129 alleles of the *Abrg1* QTL.

The effect of *Abrg2* on plasma apoB levels varied dependent on age, genetic background, and gender (Table 6). In male N_2/B6 mice, heterozygotes had significantly lower plasma apoB levels than did homozygotes for the B6 allele. In male N_2/129 mice, heterozygotes had significantly higher plasma apoB levels compared with homozygotes for the 129 allele. These results suggest that the B6 and the 129 alleles of *Abrg2* are likely to be codominantly expressed in male N_2 mice. However, in male F_2 mice, heterozygotes for *Abrg2* had similar plasma apoB levels compared with those of homozygotes for the 129 allele. These data suggested that the dominance of these alleles varied by genetic background.

Our previous data analysis suggested a possible linkage (LOD = 2.0–2.6) of *Abrg2* to plasma human apoB levels in female mice. Table 6 confirms that there was a significant association between the two in female N_2/B6 mice. Female N_2/B6 mice heterozygous for *Abrg2* had significantly lower plasma human apoB levels than homozygotes for the B6 allele. However, female N_2/129 mice heterozygous for *Abrg2* had plasma human apoB levels similar to those of animals homozygous for the 129 allele mice. These data suggest that, in female mice, the B6 allele of the *Abrg2* QTL may be recessive relative to the 129 allele, and its effect on plasma human apoB levels was realized only in the context of the B6 background.

Epistasis between the Abrg1 and Abrg2 loci

To assess potential interactions between *Abrg1* and *Abrg2*, animals were grouped according to genotype, using D6Mit55 and D4Mit27 as markers for the *Abrg1* and *Abrg2* QTL, respectively. Results from 8-week data and 12-week data were similar. Only 8-week data are shown in **Table 7**. In male F_2 mice (Table 7, top), plasma human apoB levels were lower when animals had at least one 129 allele in both loci. For example, animals doubly heterozygous for both loci had plasma human apoB levels (67 ± 13 mg/dl) that were significantly lower than those in heterozygotes for the *Abrg1* locus (83 ± 13 mg/dl) or heterozygotes for the *Abrg2* locus (83 ± 10 mg/dl). These results demonstrated the existence of epistasis between *Abrg1* and *Abrg2* in the determination of plasma human apoB levels. Similar interactions were observed in male N_2 mice of either the B6 (Table 7, middle) or the 129 (Table 7, bottom) background. An interaction between the two loci was also observed in female mice (Table 7). However, unlike in male mice, homozygosity of the 129 allele for *Abrg2* had no additive effect on lowering plasma human apoB levels in female F_2 and N_2/129 mice. This was likely due to the apparent dominant nature of the 129 allele in female mice as described earlier (Table 6).

We also assessed these data from 8-week-old animals by using a mixed model ANOVA. These analyses showed that approximately 30% of the total variance of human apoB levels in 8-week-old F_2 mice was contributed by genetic variance. The *Abrg1* and *Abrg2* loci contributed approximately 35% and 26%, respectively, to genetic variance. The interaction between the two loci accounted for approximately 12% of genetic variance. Thus, *Abrg1*, *Abrg2*, and the epistasis between the two loci contributed to a majority (73%) of the genetic variation seen in plasma human apoB levels in crosses derived from the B6 and 129 strains.

Analysis of positional candidate genes

Possible positional candidates for *Abrg1* and *Abrg2* were considered, using the Mouse Genome Informatics database (http://www.informatics.jax.org/). On the basis of the known functions of the genes, we identified three positional candidates on chromosome 6 that could potentially regulate apoB secretion. These genes, *Sec13r*, *Pparg*, and *Apobec1*, are localized between D6Mit55 and D6Mit333. Both the human homologs *SEC13R* (3p25-24) and *PPARG* (3p25) were mapped to chromosome 3, whereas the human ortholog *APOBEC-1* was localized to chromosome 12p31 (17–19). Segments of human chromosomes 9 and 1 were found to be syntenic to the chromosome 4 interval containing *Abrg2*. However, no apparent positional candidates were detected. Detailed information on syntenic hu-

TABLE 7. Epistasis between the *Abrg1* and *Abrg2* loci

Cross	*Abrg2*	*Abrg1* (8-Week-Old Males) B6/B6	B6/129	129/129	*Abrg1* (8-Week-Old Females) B6/B6	B6/129	129/129
F_2	B6/B6	85 ± 25 (n = 4)[a]	83 ± 13 (n = 9)	85 ± 17 (n = 4)	86 ± 6 (n = 4)[b]	81 ± 10 (n = 8)	63 ± 13 (n = 2)
	B6/129	83 ± 10 (n = 7)	67 ± 13 (n = 22)[a]	60 ± 23 (n = 10)	79 ± 14 (n = 11)	72 ± 11 (n = 18)[b]	58 ± 8 (n = 4)
	129/129	83 ± 15 (n = 4)	59 ± 7 (n = 5)	59 ± 3 (n = 3)[a]	66 ± 18 (n = 3)	75 ± 17 (n = 10)	64 ± 10 (n = 7)[b]
N_2/B6	B6/B6	90 ± 18 (n = 19)	85 ± 16 (n = 17)	NA	86 ± 14 (n = 21)	86 ± 13 (n = 27)	NA
	B6/129	84 ± 12 (n = 12)	75 ± 21 (n = 39)	NA	87 ± 12 (n = 17)	74 ± 13 (n = 20)	NA
	129/129	NA	NA	NA	NA	NA	NA
N_2/129	B6/B6	NA	NA	NA	NA	NA	NA
	B6/129	NA	72 ± 8 (n = 30)	64 ± 10 (n = 21)	NA	75 ± 9 (n = 9)	68 ± 8 (n = 26)
	129/129	NA	62 ± 8 (n = 25)	58 ± 10 (n = 28)	NA	73 ± 9 (n = 20)	66 ± 8 (n = 14)

Animals were grouped by genotype, using markers D6Mit55 and D4Mit27 for the *Abrg1* and *Abrg2* loci, respectively. Plasma human apoB levels are shown as means ± SD (mg/dl). The number of animal (n) for each group is included in parentheses. Comparisons were made among three groups of animals using one-way ANOVA in the F_2 cross. Comparisons between two genotypes in the N_2 crosses were made by a Student's *t*-test. Groups of animals compared are shown with the same alphabetical letter, with *P* values as shown: [a] $P < 0.05$; [b] $P < 0.01$.

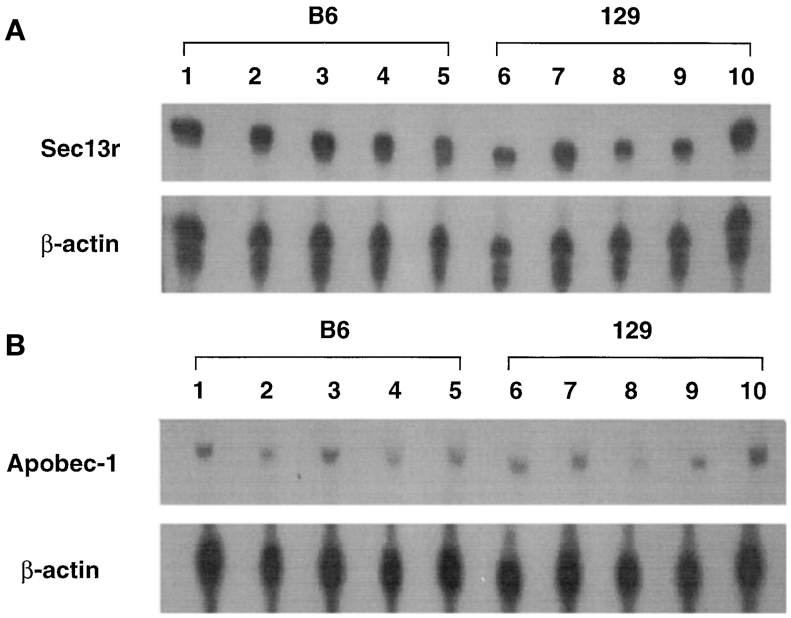

Fig. 5. Hepatic expression of positional candidate genes in B6 and 129 mice. Total liver cellular RNAs from B6 (lanes 1–5) and 129 (lanes 6–10) mice were subjected to RNase protection assays. Autoradiograms obtained from the assays are shown. Sec13r (A) and Apobec-1 (B) riboprobes were used along with a β-actin riboprobe as an internal control. Protected fragments were cut and counted. After normalization, no differences in hepatic Sec13r (100 ± 24% vs. 100 ± 11%) and Apobec-1 (B6 vs. 129 = 100 ± 22% vs. 110 ± 18%) mRNA levels were found between the two mouse strains.

man chromosome segments can be found at the National Center of Biotechnology Information (http://www.ncbi.nlm.nih.gov/Homology/).

We compared the expression levels of the *Sec13r* and *Apobec1* genes between B6 and 129 mice by RNase protection assays. **Figure 5A** shows that there was no difference in hepatic Sec13r mRNA levels between B6 and 129 mice (100 ± 24% vs. 100 ± 11%). Figure 5B shows that there was no differences in the hepatic Apobec-1 mRNA levels between B6 and 129 mice (100 ± 22% vs. 110 ± 18%). We also attempted to determine potential structural variations of the *Sec13r* gene between the two strains. Sec13r cDNAs were amplified from liver RNA samples and sequenced. These analyses, however, did not reveal any variation in the coding regions of the gene between the two strains.

DISCUSSION

Plasma apoB levels are regulated by the secretion and clearance of apoB-containing lipoproteins. We have previously demonstrated the suitability of specific HuBTg mouse strains for the identification of genetic factors involved in the regulation of plasma apoB levels. In these strains, plasma human apoB levels can be used as a surrogate marker for hepatic apoB-100 secretion (12). In this article, we present the genetic analysis of F_2 HuBTg animals and animals backcrossed to either the B6 (N_2/B6) or the 129 (N_2/129) strain. Genetic variance accounted for approximately 30–40% of the total plasma apoB variance in mature animals at 8 and 12 weeks of age. Our results revealed two novel loci, designated *ApoB regulator gene* (*Abrg*), which were associated with plasma human apoB levels in the HuBTg mice. The first locus, *Abrg1*, was mapped to chromosome 6 in both male and female mice with a combined LOD score of 14–16 (depending on age) at the D6Mit55 marker (~45.9 cM). *Abrg1* contributed to approximately 35% of genetic variance. The second locus, *Abrg2*, was mapped to chromosome 4 with an LOD score of 6.3–8.6 in male mice but an LOD score of only 2.0–2.6 in female mice at the D4Mit27 marker (~35 cM). *Abrg2* contributed to approximately 26% of genetic variance. Epistasis between *Abrg1* and *Abrg2* was detected and accounted for approximately 12% of genetic variance. We have, therefore, identified two QTL, which together have major effects (> 70%) on the regulation of plasma human apoB levels in the tested population.

Using a HuBTg mouse model, we have, for the first time, successfully identified two novel loci that are involved in the regulation of plasma apoB levels. These two loci were not linked to the endogenous mouse apoB gene (chromosome 12) (20) or the human apoB transgene (chromosome 10). As described in Materials and Methods, the human apoB transgene (with its native promoter) was inserted into the proximal end (15.3–17.5 cM) of chromosome 10 and was maintained in hemizygosity in tested animals by intercrossing hemizygous transgenic animals and nontransgenic animals in all crosses used in this article. We previously noted that strain differences in plasma apoB-100 levels between B6 and 129 are parallel between mouse and human apoB-100 proteins and that the plasma human apoB level is a suitable marker for the hepatic apoB-100 secretion rate in these animals (12). These data implied that the cellular machinery regulating mouse apoB secretion is also effective on the transgenic human apoB proteins.

In this article, we showed that in 8-week-old male mice, plasma human apoB levels of the congenic B6 HuBTg mouse strain (~95 mg/dl) were significantly higher than those of the congenic 129 HuBTg mouse strain (~72 mg/dl). Male F_1 HuBTg mice had slightly lower, but not statistically significant, plasma apoB levels (~66 mg/dl) compared with the 129 HuBTg mice. The two *Abrg* loci and their interaction may explain a majority of the differences in plasma apoB levels between the two mouse strains. The

B6 and 129 alleles of the *Abrg1* locus appeared to affect plasma apoB levels codominantly in both male and female mice. Whereas both the B6 and 129 alleles of the *Abrg2* locus affected plasma human apoB levels codominantly in male mice, the 129 allele appeared to be dominant in female mice. An additive effect on plasma apoB levels was also observed when animals were heterozygous for both alleles. Despite the codominant nature of the *Abrg* loci in male mice, mean plasma apoB levels in the F_1 HuBTg mice were similar to those of 129 HuBTg mice. These data suggest the involvement of other, unidentified *Abrg* loci in the regulation of plasma apoB levels in these mice. This is in agreement with the fact that approximately 30% of genetic variance of apoB was not accounted for by the *Abrg1* and *Abrg2* loci.

Using male animals from F_2 and N_2 crosses, we defined the intervals for the two *Abrg* loci. The chromosome 4 interval for *Abrg2* was defined by two markers spanning approximately 30 cM (D4Mit275–D4Mit204: 30.6–61.2 cM). *Abrg2* is likely to be located near the D4Mit27 marker (35 cM), because this marker had the highest LOD score. No apparent positional candidates for *Abrg2* were detected in or near the defined interval. The *Abrg1* locus is likely to be near or within an interval of approximately 9 cM defined by D6Mit64 (40.4 cM) and D6Mit333 (49.2 cM). The LOD score of the interval peaked at the D6Mit55 marker (45.9 cM). Three positional candidates were detected that could potentially regulate the assembly and secretion of apoB lipoproteins. These genes are *Sec13r*, *Pparg*, and *Apobec1*, which mapped between the D6Mit55 (45.9 cM) and the D6Mit333 (49.2 cM) markers. Both the human homologs *SEC13R* (3p25-24) and *PPARG* (3p25) were mapped to chromosome 3, whereas the human ortholog *APOBEC-1* was localized to chromosome 12p31 (17–19).

Mouse *Sec13r* (17) encodes a protein homologous to yeast secretory pathway protein 13 (*Sec13*). Sec13 participates in vesicular transport between the endoplasmic reticulum and the Golgi apparatus (21). Although specialized vesicles have not been directly implicated in intracellular apoB transport, they are plausible regulators of the apoB secretory pathway (22, 23). Our data showed that hepatic Sec13r mRNA levels were similar between B6 and 129 strains. No structural variations in the coding region of the gene were found between the two strains. However, the candidacy of *Sec13r* for *Abrg1* remains open because the possibility that variations in the 5′ and/or 3′ untranslated regions of the Sec13r mRNA exists, which may affect the efficiency of protein translation, resulting in variations in protein levels and/or functions. The second candidate, *Pparg*, encodes a protein, peroxisome proliferator-activated receptor γ (PPARγ), which is predominantly expressed in adipose tissue and plays an important role in adipogenesis (24). Genetic variations in human *PPARG* have been associated with body mass index, insulin sensitivity, and severe obesity (25–28). Strain differences in PPARγ may lead to differential adiposity which, in turn, may affect plasma free fatty acid levels and alter the output of apoB-containing lipoproteins from the liver. However, mice heterozygous for PPARγ deficiency have no significant differences in body weights or basal plasma free fatty acid levels compared with wild-type mice (29). Comparison studies to determine the candidacy of *Pparg* for *Abrg1* are currently underway. Finally, the third candidate, *Apobec1*, encodes an enzyme involved in the editing of apoB mRNA (30). Strain differences in RNA-editing activity could alter plasma apoB-100 levels by altering the ratio of apoB-100 and apoB-48 mRNAs. However, we found no differences in the Apobec-1 mRNA between B6 and 129 mice. In a previous study, we tested the activity of apoB RNA editing in both the B6 and 129 × B6 F_1 mouse strains (12). Similar amounts of mouse and human apoB mRNA were shown to be edited in the two strains. Taken together, these data make *Apobec1* a less likely candidate for the *Abrg1* gene.

The identification of novel loci that regulate plasma apoB levels may allow for the identification of candidate genes involved in human disease in which plasma apoB levels are altered. Family linkage studies have shown that the apoB gene is not linked to the low apoB phenotype in certain forms of FHBL (31, 32). A study has localized a novel FHBL susceptibility locus to chromosome 3p21.1-22 in a 40-member FHBL kindred (33). The secretion rates of apoB-containing lipoproteins were also shown to be reduced in these FHBL subjects (5). The chromosome segment (3p21.1-22) containing the human FHBL susceptibility locus is in close proximity to the human chromosome 3 segment (3p25-24) syntenic to the chromosome 6 interval containing the mouse *Abrg1* locus. It is therefore tempting to speculate that the mouse *Abrg1* locus is a possible mouse ortholog for the human FHBL susceptibility locus.

Mouse *Abrg* loci may also provide candidate genes for the form of FH that is not linked to the LDL receptor, apoB, or apoE genes (34–36). In addition, they are potential candidate genes for FCHL, which is characterized by the overproduction of apoB (37–41). Genome scan studies have identified several chromosome segments associated with altered plasma LDL cholesterol levels in FH (35, 36) and FCHL (42–45). Neither *Abrg1* nor *Abrg2* mapped to any of the chromosome intervals linked to the disorders mentioned above. Therefore, the human orthologs for the newly identified mouse *Abrg* loci likely represent novel loci regulating plasma human apoB levels.

We have previously shown that the main determinant for plasma apoB levels in the two mouse strains (B6 and 129) is the hepatic apoB-100 secretion rate via posttranscriptional mechanisms (12). Therefore, mouse *Abrg1* and *Abrg2* are likely involved in the regulation of the assembly and secretion of apoB-containing lipoproteins. Posttranscriptional and particularly posttranslational regulation has been amply documented in cultured cells to play a crucial role in the regulation of apoB secretion (22, 46). Our studies have shown that *Abrg1* and *Abrg2* interact with each other to regulate plasma apoB levels in vivo. The identification of *Abrg1* and *Abrg2* may reveal new pathways involved in the regulation of apoB secretion, potentially providing novel sites for pharmacological therapy (47) in subjects with elevated plasma apoB and cholesterol levels and hence with a high risk for atherosclerosis and CAD.

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ERRATA

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