Genetic control of hepatic apoB-100 secretion in human apoB transgenic mouse strains

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Abstract Plasma apolipoprotein B (apoB) levels vary widely in the general population and elevated plasma levels of apoB are associated with higher risk for atherosclerotic coronary heart disease. Determination of genetic factors regulating population variance of plasma apoB levels is complicated by the genetic heterogeneity of human populations. Using a congenic human apoB transgenic mouse strain in the C57BL/6 background (B6 HuBTg), we assessed genetic effects on the variance of plasma apoB, and on hepatic apoB-100 secretion rates. Six inbred mouse strains were crossed with the B6 HuBTg strain. Mean plasma apoB levels in the parental B6 HuBTg strain were 95 ± 14 mg/dl. F1 human apoB transgenic offspring displayed plasma human apoB levels ranging from 60 to 105 mg/dl. In three F1 strains, the BALB/B6, C3H/B6 and 129/B6 strains, markedly lower plasma apoB levels (61 ± 11, 64 ± 5, and 67 ± 8 mg/dl) were due to lower apoB-100 secretion rates. Human apoB mRNA levels in these three F1 strains were similar to those of the parental B6 strain suggesting that the mechanism for varying apoB secretion rates is most likely not transcriptional. In summary, we have identified three inbred mouse strains possessing polymorphic alleles which, when crossed with the B6 strain, lower plasma apoB levels and apoB-100 secretion in their F1 offspring. These mouse strains provide a powerful tool for genetic analysis of factors regulating apoB-100 secretion and hence plasma apoB levels.—Voyiaziakis, E., C. Ko, S. M. O'Rourke, and L-S. Huang. **Genetic control of hepatic apoB-100 secretion in human apoB transgenic mouse strains.** ***J. Lipid Res.*** **1999.** 40: **2004–2012.**

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Wide variations of plasma low density lipoprotein (LDL) cholesterol (C) levels are observed in the general population (1). The risk for atherosclerosis and coronary heart disease (CHD) increases in individuals with elevated plasma levels of LDL-C and apolipoprotein B (apoB) (2, 3). ApoB is a mandatory constituent of plasma LDL and is required for the secretion of very low density lipoproteins (VLDL), the precursor of LDL. ApoB exists in two forms, B-100 and B-48 (4). Both are products of the same gene encoding a 14-kb mRNA and share the same amino-terminal 2152 amino acids. Specific editing of human B-100 mRNA in the intestine, but not in the liver, introduces a stop codon at residue 2153 to produce B-48 (5, 6). In rodents, unlike humans, approximately 60% of B-100 mRNA is edited to B-48 mRNA in the liver (7, 8). Consequently, a large portion of apoB-containing lipoproteins derived from mouse liver are B-48-containing VLDL particles which are cleared rapidly from the circulation through the remnant pathway. This phenomenon may contribute to the low levels of LDL-C observed in rats and mice.

Mutations in the LDL receptor gene and in amino acid 3500 of apoB result in elevated plasma LDL-C levels (9, 10). However, most mutations in the apoB gene either abolish apoB synthesis or produce truncated apoB proteins, leading to absent or reduced plasma apoB levels (11). Mutations in the gene encoding the large unit of the microsomal triglyceride transfer protein (MTP) complex, an obligatory factor in VLDL assembly, result in a complete absence of plasma apoB-100 (4, 12, 13). However, these monogenic disorders account for only a small percentage of individuals with altered plasma LDL-C and apoB levels (4, 9, 11). Indeed, common apoB and LDL receptor variants are associated with minimal to absent changes in plasma levels of LDL-C and apoB (14–19). Nevertheless, studies using complex segregation analyses have demonstrated a significant genetic component in the familial aggregation of high apoB levels (14–16, 18, 20). These studies also show that >40% of the variability of total plasma apoB levels can be explained by an unknown major codominant gene(s) (14–18). Recently, a promoter polymorphism of the MTP gene has been implicated in a lower plasma LDL-C level in the general population (21). A common polymorphism in the gene encoding cholesterol 7α-hydroxylase, the rate-limiting enzyme in bile acid biosynthesis, has also been linked to population variance of plasma

Abbreviations: apoB, apolipoprotein B; CHD, coronary heart disease; MTP, microsomal triglyceride transfer protein; LDL, low density lipoprotein; VLDL, very low density lipoprotein; HuBTg, human apoB transgenic mouse; TG, triglycerides; SR, secretion rate.

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LDL-C levels, but notably only to high plasma LDL-C levels (19). To date, the variants of apoE, which affect clearance rates of chylomicron and VLDL remnant particles, are the only common DNA polymorphisms consistently shown to be associated with significant heritable variation in plasma LDL-C in several populations (22, 23). Overall, very few of the factors known to be involved in LDL metabolism have been shown to play a role in the variation in plasma LDL-C and apoB levels observed in the general population. Of these, even fewer have been shown to be directly involved in regulating apoB secretion.

In this report, we assessed the genetic control of plasma apoB levels using inbred mouse strains and a congenic mouse strain carrying a human apoB transgene. A congenic human apoB transgenic mouse strain synthesizing human apoB only in the liver (24, 25) can provide the means to determine hepatic apoB-100 secretion rates in a mouse model without being confounded by intestinal apoB production. In addition, the high levels of human apoB expression in these mice allow for both easier detection and better discrimination of plasma apoB-100 levels among mouse strains than is possible using wild-type mice. Inbred mouse strains represent reproducible sources of genetic variation in biological traits including plasma apoB levels (26, 27). We hypothesized that inbred mouse strains possess polymorphic alleles for the gene(s) regulating hepatic apoB secretion rate and hence plasma apoB levels. Once these alleles are introduced into congenic human apoB transgenic mice, they will vary hepatic human apoB secretion rates and hence plasma human apoB levels. As numerous in vitro studies have shown that the regulation of apoB secretion is predominantly post-translational (see reviews, refs. 28, 29), these mouse strains provide powerful tools to identify genetic factors involved in post-translational regulation of apoB secretion, particularly those involved in the intracellular degradation of apoB.

MATERIALS AND METHODS

Mice

Human apoB transgenic (HuBTg) mice were generated as described (24) and received by us in a mixed genetic background (50% FVB/N, 25% SJL/J, and 25% C57BL/6J). We have since bred the human apoB transgene onto the C57BL/6 (B6) strain for over 10 backcrosses and generated a congenic HuBTg strain (designated B6 HuBTg).

The offspring from an outcross between two inbred parental strains are designated F1. In this report, F1 offspring were generated by crossing male B6 HuBTg mice to females of six inbred mouse strains: 129/Sv (abbreviated 129), BALB/c (BALB), C3H/HeJ (C3H), FVB/NJ (FVB), NZB/BINJ (NZB), and SJL/J (SJL). The 129 strain used is the source of ES J9 cells (30) and has been maintained by sib-mating in our laboratory for 5 years. All other strains were purchased from the Jackson Laboratory (Bar Harbor, ME). Genetic materials from several inbred strains (FVB and SJL) were present in the HuBTg founder mouse. These strains were chosen for their potential roles in the varying plasma apoB levels observed in our mouse colonies with mixed genetic background (data not shown). Other strains were chosen based on the known differences in their plasma apoB levels when compared to the B6 strain (26, 27). F1 offspring are designated by the names of their two parental strains separated with a slash (e.g., 129/B6 F1 HuBTg). Presence of the human apoB transgene in the F1 offspring was determined by PCR as described (24).

Mice were maintained in a 12-h light/dark cycle (light cycle: 7 am–7 pm). Mice were fed rodent chow (PicoLab Rodent Chow 20, No. 5053; Purina Lab Chows, St. Louis, MO) and had free access to water. For fasting plasma samples and in vivo measurement of apoB and triglyceride secretion experiments, mice were fasted for 4 h (10 am–2 pm), retroorbitally bled, and/or subjected to experimental procedures immediately afterwards. All mice were bled at 8–16 weeks unless otherwise indicated. Age and gender of animals for any given experiment are specified in the Results section.

Measurement of plasma total triglycerides (TG) and human apoB

Blood from fasted mice was drawn via the retroorbital venous plexus. Total TG levels were determined using a colorimetric assay kit (No. 339-10, Sigma). Human apoB levels in mouse plasma samples were measured using a turbidimetric assay as described below. Anti-human apoB antibody was obtained from Sigma (No. 357-125). Human apoB calibrator (No. 992-27591) and two control human serum samples were obtained from Wako Chemicals USA (Richmond, VA). Mouse samples (2–6 μl) were mixed with the antibody reagent (200 μl) and incubated at 37°C for 5 min. The turbidity in each well was then measured at 340 nm in a microtiter plate reader (SpectraMax 250, Molecular Devices Inc.). The plasma human apoB concentration of mouse samples was determined from a calibration curve obtained from dilutions of the human apoB calibrator.

Determination of in vivo apoB and TG secretion rates (SRs)

Assessment of apoB and TG SRs in age-matched animals (12–16 weeks) was performed as described with slight modifications (31). For the determination of apoB SRs, 4 hour-fasted mice were injected intravenously with a solution containing 200 μCi [^{35}S]methionine and 500 mg/kg Triton WR 1339 (Sigma) in 0.9% NaCl. Plasma VLDL clearance is virtually completely inhibited in mice under these conditions. Blood was taken at the 0 min (just prior to injection), 60 and 120 min after the injection. Plasma samples (10 μl) were added with equal volume of 2× Laemmli sample buffer (32) and boiled for 5 min and then loaded onto 4% SDS-PAGE, dried, and exposed to X-ray film to visualize labeled apoB proteins. Both B-100 and B-48 bands were cut from dried gels and counted in scintillation fluid in a liquid scintillation counter (Beckman Inc.). To normalize total protein synthesis of animals, a small aliquot of the plasma sample (3 μl) was subjected to TCA precipitation to assess total TCA-precipitable protein counts in any given sample. In each experiment, the TCA-precipitable protein count from each sample was divided by the mean TCA-precipitable protein count of all plasma samples to yield a correction factor for each sample. Both B-100 and B-48 protein counts (per 10 μl of plasma samples) were then normalized by the correction factor yielded for the given plasma sample and multiplied by 100 to give protein count per ml of plasma (i.e., cpm/ml). The apoB SR was then calculated by subtracting normalized protein counts at the 60-min time point from normalized protein counts at the 120-min time point yielding apoB SR expressed as cpm/ml/h.

TG SRs were determined concomitantly with the in vivo apoB SRs described above or similarly as above but exclusive of [^{35}S]methionine. Plasma samples from the baseline, 60- and 120-min time points were measured for TG levels. The TG SR was calculated by subtracting the TG level at the 60-min time

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point from the TG level at the 120-min time point and expressed as mg/dl/h.

Northern blot analysis

Total cellular RNA was isolated from the liver using the guanidinium thiocyanate method (33). Total cellular RNA (10 μg) was separated on 6% formaldehyde/0.8% agarose gels and then transferred to a nylon membrane. Hybridizations were carried out as described previously (34). A human apoB cDNA probe (34) was used to detect human apoB mRNA. A mouse β-actin riboprobe (Ambion Co., Austin, TX) was used in each experiment to normalize the loading of RNA samples. For quantification, autoradiograms were scanned with a densitometer.

ApoB mRNA editing

Total cellular RNA samples were subjected to RT-PCR using standard RNA-PCR reagents (Perkin Elmer). The RT-reaction was performed using a 3′ primer specific to either mouse or human apoB as previously described (35, 36). The products from the RT-reaction were then subjected to PCR amplification using a 5′ primer specific to either mouse or human apoB. For the primer extension assay, mouse or human-specific oligonucleotide 32 nucleotides 3′ to the editing site (nucleotide 6666) were end-labeled with ^{32}P-γATP (35, 36). The products were separated by 8% polyacrylamide/7 M urea gels. Gels were dried and exposed to X-ray films. The gel fragments representing either B-100 or B-48 mRNA were cut and quantitated in a liquid scintillation counter. The percent of either mouse or human apoB mRNA edited in each mouse liver sample was determined by dividing the radioactivity of the B-48 fragment by the sum of the radioactive counts for both the B-100 and B-48 fragments.

RESULTS

Varying plasma human apoB levels in six F1 HuBTg mouse strains

Male congenic B6 HuBTg mice were bred with females of various inbred mouse strains. Fasting plasma human apoB levels from the parental B6 and six F1 HuBTg mouse strains are summarized in **Table 1**. Male mice from five of the six F1 HuBTg mouse strains (BALB/B6, C3H/B6, 129/B6, FVB/B6, SJL/B6) showed significantly lower plasma apoB levels compared to the parental B6 HuBTg mice (95 ± 14 mg/dl). Male BALB/B6, C3H/B6 and 129/B6 F1 HuBTg mice had dramatically lower plasma apoB levels (61 ± 11, 64 ± 5 and 67 ± 8 mg/dl, respectively) compared to those of the male HuBTg B6 mice. To a lesser extent but statistically significant, male FVB/B6 and SJL/B6 mice (86 ± 10 and 84 ± 10 mg/dl, respectively) had lower plasma apoB levels compared to the B6 strain. In contrast, male NZB/B6 F1 HuBTg mice had plasma human apoB levels (105 ± 12 mg/dl) similar to those of the B6 HuBTg strain. Though similar trends were observed in the female counterparts of all six F1 HuBTg strains, the differences relative to the parental B6 strain were less pronounced (Table 1). These results show that varying plasma human apoB levels in the F1 HuBTg mouse strains resulted from genetic differences. These data also suggest that inbred mouse strains BALB, C3H, 129, FVB and SJL possess a functionally polymorphic allele(s) for an unidentified locus (loci) which lowers plasma human apoB levels when introduced into F1 HuBTg mice.

We note that Western blot analysis of plasma apoB from these mouse strains showed that only a trace amount of human B-48 proteins was detectable in the plasma from these mice (data not shown) as were reported in HuBTg mice with mixed genetic background (24, 25). Therefore, the human apoB levels detected by our immunoassays predominantly represent the levels of human B-100 proteins but not human B-48 proteins in the plasma.

Contribution of apoB SRs to varying plasma human apoB levels

To determine whether apoB SRs are major determinants for plasma human apoB levels in various F1 mouse strains, in vivo apoB secretion experiments were carried out in age-matched animals (12–16 weeks of age). This assay was first used to address the relative amounts of mouse versus human apoB made in these HuBTg mice. Specifically, this assay compared apoB SRs between B6 HuBTg mice that produce both human and mouse apoB and non-transgenic wild-type littermates producing only mouse apoB (**Fig. 1, top**). After normalization for total protein synthesized in each mouse, we found that the

TABLE 1. Plasma apoB and TG levels

HuBTg Strain	Male n	Male Human ApoB	Female n	Female Human ApoB	*P* Value M vs. F	Male TG	Female TG	*P* Value M vs. F
		mg/dl		*mg/dl*		*mg/dl*	*mg/dl*	
B6	79	95 ± 14	17	90 ± 6	n.s.	202 ± 30	167 ± 31	<0.0001
BALB/B6	13	61 ± 11[c]	8	73 ± 8[b]	0.01	143 ± 22[c]	134 ± 7[b]	n.s.
C3H/B6	14	64 ± 5[c]	13	74 ± 7[b]	0.0004	174 ± 20[c]	161 ± 27	n.s.
129/B6	11	67 ± 8[c]	9	73 ± 10[a]	n.s.	173 ± 18[b]	143 ± 17[a]	0.03
FVB/B6	21	86 ± 9[b]	27	84 ± 9	n.s.	223 ± 32[a]	213 ± 27[c]	n.s.
SJL/B6	19	84 ± 10[b]	18	81 ± 11	n.s.	224 ± 16[b]	186 ± 19[b]	<0.0001
NZB/B6	7	105 ± 12	16	102 ± 9[a]	n.s.	233 ± 30[a]	201 ± 20[b]	0.006

Mice (8 weeks of age) were fasted for 4 h and bled for apolipoprotein and lipid profiles; n, number of mice measured for either human apoB or TG levels. All values shown are mean ± SD. Comparisons between the B6 and various F1 HuBTg mouse strains of the same gender were made with a *t* test for statistical significance. Comparisons between male and female mice were also performed with a *t* test.

P values are expressed as [a], $P < 0.05$; [b], $P < 0.01$; and [c], $P < 0.001$.

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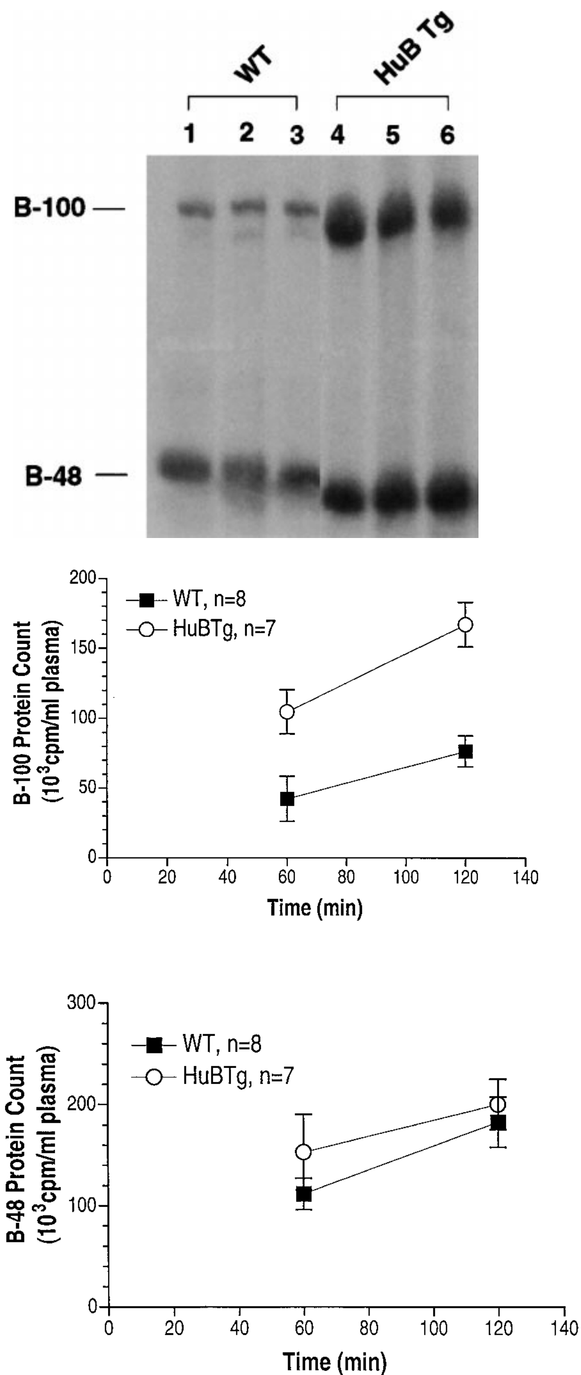

Fig. 1. In vivo apoB secretion in wild-type and HuBTg mice. Age-matched, 4-h fasted male B6 HuBTg mice and their wild-type littermates were injected with Triton WR1339 and [^{35}S]methionine. Mice were subsequently bled at 60 and 120 min after injection. Plasma samples (10 μl) from each time point were separated by 4% SDS-PAGE and followed by fluorography and then exposed to X-ray films. Representative samples from 120-min time points are shown in Fig. 1, top. Both B-100 and B-48 gel bands were cut and counted. Protein counts were normalized by TCA-precipitable counts, expressed as cpm/ml, and are plotted in Fig. 1, bottom. Time (h) post-injection is plotted on the x-axis. Either B-100 protein counts (top panel) or B-48 protein counts (bottom panel) are plotted on the y-axis.

apoB-100 SR in the B6 HuBTg mice was 3-fold higher ($P < 0.0001$) than that in wild-type controls (upper panel of Fig. 1, bottom) and that B-48 SRs were similar in both groups of mice (lower panel of Fig. 1, bottom). Although production of human apoB could somehow stimulate mouse apoB secretion, it is most likely that the majority, if not all, of the increase in the B-100 SR in HuBTg mice is due to increased secretion of hepatic human apoB. As no significant difference in B-48 SRs was observed between the HuBTg and wild-type mice, the B-48 SR represents mostly mouse B-48 production. These results showed that in this assay, measurement of B-100 production was an indicator of hepatic human B-100 production, whereas B-48 SR was an indicator of mouse B-48 production. As all assays were performed in mice fasted for at least 4 h, the intestinal contribution to B-48 production was minimized (37).

The apoB secretion rates (both B-100 and B-48) for six F1 HuBTg and the parental B6 HuBTg strains are summarized in **Table 2**. In the BALB/B6 strain, male HuBTg mice had a significantly lower B-100 SR than that in male B6 HuBTg mice. The lower B-100 SR in the BALB/B6 F1

TABLE 2. In vivo apoB secretion rates

HuBTg Strain	Sex	n	B-100 SR	B-48 SR
			cpm/ml/h	*cpm/ml/h*
B6	M	42	113000 ± 29230	100200 ± 41480
BALB/B6	M	8	82100 ± 34490[a]	78980 ± 62350
C3H/B6	M	9	69410 ± 27320[c]	56940 ± 21600[a]
129/B6	M	8	68190 ± 24480[c]	82600 ± 42960
FVB/B6	M	8	89500 ± 27330[a]	98790 ± 40830
SJL/B6	M	7	115600 ± 16530	87180 ± 49860
NZB/B6	M	6	99980 ± 41230	67580 ± 28420
B6	F	9	144170 ± 30060	77600 ± 44750
BALB/B6	F	0	ND	ND
C3H/B6	F	10	83520 ± 34570[b]	38940 ± 25820[a]
129/B6	F	7	77830 ± 20130[c]	26660 ± 12850[a]
FVB/B6	F	6	144000 ± 33840	68280 ± 45230
SJL/B6	F	8	150800 ± 32460	56630 ± 24880
NZB/B6	F	8	136300 ± 35380	45340 ± 37500
			P value[d]	*P* value[d]
B6	M vs. F		0.01	n.s.
C3H/B6	M vs. F		n.s.	n.s.
129/B6	M vs. F		n.s.	0.007
FVB/B6	M vs. F		0.006	n.s.
SJL/B6	M vs. F		0.02	n.s.
NZB/B6	M vs. F		n.s.	n.s.

Age-matched mice were fasted for 4 h prior to experiments. Male B6 HuBTg mice (n = 6–8) were used as control mice for each experiment. For presentation in this table and comparison across strains and genders, SR for each mouse was normalized as follows. The mean SR of male HuBTg mice (n = 6–8) from each experiment was divided by the mean SR of all male B6 HuBTg mice (n = 42) from a total of 12 experiments to yield a correction factor that adjusts for the variance between individual experiments. SR from each mouse was then normalized against the correction factor in each experiment. For example, the mean B-100 SR of male B6 HuBTg from all experiments was 113,000 cpm/ml/h and the mean B-100 SR of male HuBTg (n = 6) for experiment #1 was 99,000 cpm/ml/h. Therefore, the correction factor for experiment #1 would be 0.88. Comparison was performed between each F1 strain versus the B6 strain of the same gender. Comparisons between male and female mice were performed with a *t* test. *P* values were derived from a *t* test for statistical significance; n.s., not statistically significant; ND, not determined.

P values are expressed as [a], $P < 0.05$; [b], $P < 0.01$; [c], $P < 0.001$.

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HuBTg mice (~72%, Table 2) parallels lower plasma human apoB levels (~64%, Table 1) compared to those in the B6 strain. These results show that the lowering of plasma human apoB levels in the BALB/B6 mice was due mainly to a decrease in the hepatic B-100 SR. It is likely that the BALB strain possesses a polymorphic allele(s) (relative to the B6 strain) for a lower apoB SR. This conclusion is supported by the trend toward a lower B-48 secretion rate as well in the BALB/B6 F1 HuBTg mice. These alleles may act either dominantly or codominantly resulting in a lower human apoB level phenotype in the BALB/B6 F1 mice. Overall, these results demonstrate clearly that there are genetic factor(s) regulating apoB levels via the hepatic apoB secretion rate.

In addition to the BALB/B6 strain, both male and female mice of the C3H/B6 and 129/B6 strains had significantly lower B-100 SRs than that of the B6 strain (Table 2). As in the case of the BALB/B6 strain, the lower B-100 SRs corresponded well with the lower plasma human apoB levels in these F1 strains. ApoB-48 SRs were, in general, lower in these F1 mice as well. In the FVB/B6 strain, male, but not female, F1 HuBTg mice had lower B-100 SRs compared to the B6 HuBTg mice. The B-100 SRs in the FVB/B6 mice also correlated well with the plasma apoB levels relative to the B6 strain.

In the SJL/B6 F1 strain, the B-100 SR was similar to that in the B6 strain, but plasma human apoB levels were lower than those in the B6 strain suggesting a faster clearance rate for apoB-100-containing lipoproteins. Finally, the NZB/B6 F1 strain appeared to have similar plasma human apoB levels and B-100 SRs compared to those in the B6 strain.

Evidence for genetic control of plasma TG levels and in vivo TG secretion rates and their relationships to plasma apoB levels and apoB SRs

The correlation between plasma apoB and TG levels in the F1 HuBTg strains was assessed. As shown in Table 1, varying plasma TG levels were also observed in the F1 HuBTg strains tested. The lower plasma TG levels in male BALB/B6, C3H/B6, and 129/B6 F1 mice paralleled their lower plasma apoB levels compared to those in the parental B6 strain. In contrast, higher plasma TG levels in male FVB/B6, SJL/B6, and NZB/B6 were accompanied with lower or similar plasma apoB levels relative to the parental B6 strain.

To further assess the relationship between plasma TG levels and in vivo TG SRs, we determined TG SRs concurrently with apoB SRs in these mouse strains. The results are summarized in **Table 3**. In male mice of three F1 strains, BALB/B6, SJL/B6, NZB/B6, the TG SRs correlated well with their plasma TG levels compared to those in the parental B6 strain (Table 1). In the BALB/B6 strain, TG and apoB SRs were both lower than in the B6 parental strain. However, compared to the parental B6 strain, a higher TG SR was accompanied with an unchanged B-100 SR in both the SJL/B6 and the NZB/B6 strains. These results suggest disassociation in the regulation of in vivo TG and apoB-100 secretion rates in these

TABLE 3. In vivo TG secretion rates

Strain	Male n	Male TG SR	Female n	Female TG SR	P Value (M vs. F)
		mg/dl/h		*mg/dl/h*	
B6	49	265 ± 88	7	158 ± 38	0.003
BALB/B6	8	187 ± 26[a]	0	ND	ND
C3H/B6	12	241 ± 62	11	193 ± 70	n.s.
129/B6	12	289 ± 109	6	155 ± 27	0.01
FVB/B6	10	231 ± 99	5	192 ± 95	n.s.
SJL/B6	10	328 ± 51[a]	11	212 ± 44[a]	<0.0001
NZB/B6	10	343 ± 104[a]	6	166 ± 107	0.005

Age-matched mice were fasted for 4 h prior to experiments. Male B6 HuBTg mice (n = 6–8) were used as control mice for each experiment. All SR data were normalized against the B6 HuBTg strain from each experiment as described in Table 2. Values shown are mean ± SD; ND, not determined. Comparison between F1 strain versus B6 strain of the same gender was performed with a *t* test. Comparisons between male and female mice were also performed with a *t* test; n.s., not statistically significant.

P value expressed as [a], $P < 0.05$.

two F1 strains. In general, female mice had lower plasma TG levels and TG SRs. It appeared that strains BALB, SJL and NZB possess polymorphic allele(s) regulating TG SRs in a dominant or codominant fashion relative to the B6 allele(s). Gender differences were also observed in the correlation between plasma TG levels and TG SRs in two of the F1 strains (BALB/B6 and NZB/B6). Taken together, these data (Tables 1–3) showed that gender differences in the regulation of plasma apoB levels, TG levels, apoB SRs, and TG SRs varied among the F1 strains tested.

Human apoB mRNA levels and apoB mRNA editing in three HuBTg F1 strains with concomitantly lower plasma apoB levels and hepatic apoB-100 secretion rates

To determine whether the decreased apoB-100 secretion rates in the three F1 strains with concomitantly lower plasma apoB levels were due to a decrease in human apoB mRNA levels, Northern blot analyses were performed. As shown in **Fig. 2** and **Table 4**, human apoB mRNA levels in the BALB/B6 (130 ± 47%), C3H/B6 (110 ± 16%), and 129/B6 strains (97 ± 14%) were similar to those in the B6 strain (100 ± 19%). These results suggest that the regulation of apoB-100 SRs in these F1 mouse strains is most likely not transcriptional.

To determine whether or not lower apoB-100 SR was the result of increased hepatic apoB mRNA editing activity in these three F1 HuBTg strains (BALB/B6, C3H/B6, and 129/B6), both mouse and human apoB mRNA editing activity was assessed. The results are summarized in **Table 5**. In male mice, no significant differences in mouse apoB editing activity were observed among any of the three F1 HuBTg strains and the parental B6 HuBTg strain. However, approximately 10% more human apoB mRNA was edited to apoB-48 mRNA in male C3H/B6 ($P = 0.045$) mice compared to that in the male B6 mice. No significant differences in the editing of mouse apoB mRNA was observed between the B6 and the other two F1 HuBTg strains (i.e., BALB/B6 and 129/B6).

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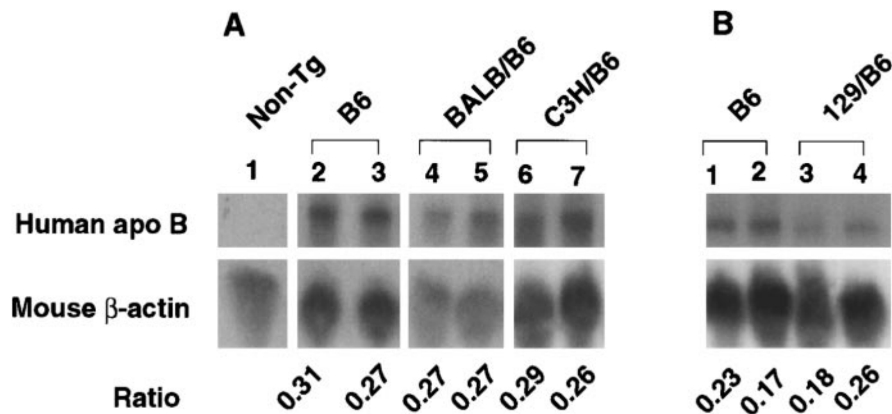

Fig. 2. Human apoB mRNA levels in the B6 and F1 HuBTg mice. Total cellular RNA isolated from livers of 4-h fasted mice were separated by 0.8% agarose/6% formaldehyde gels and then blotted to nylon membranes. Representative samples are shown in this figure. RNA from male mice are shown in Fig. 2A: a non-Tg control mouse (lane 1), B6 HuBTg (lanes 2–3), BALB/B6 HuBTg (lanes 4–5), and C3H/B6 HuBTg mice (lanes 6–7). RNA from female mice are shown in Fig. 2B: B6 HuBTg (lanes 1–2) and 129/B6 HuBTg mice (lanes 3–4). The blots were hybridized to ^{32}P-labeled human apoB and mouse β-actin probes and then washed under stringent conditions to prevent cross-hybridization of mouse apoB to the human apoB probe. Washed membranes were then exposed to X-ray film. The amount of apoB mRNA in each sample was normalized with mouse β-actin. The ratio of apoB to mouse β-actin in each sample is shown beneath each lane.

ApoB mRNA editing was also assessed in female B6 and 129/B6 HuBTg mice. Like their male counterparts, no significant difference in the editing activity was observed between the two strains. However, significantly lower RNA editing activity was observed in female mice compared to their counterparts in both strains. In some strains, significant differences were observed between mouse apoB and human apoB editing (Table 5).

Although strain and gender differences in the RNA editing activity were observed, these differences did not correlate well with apoB-48 SRs (Table 2). Therefore, these results suggest that varying RNA editing activity in some of the F1 HuBTg strains most likely does not play a significant role in the lower apoB-100 SRs observed in these strains.

DISCUSSION

In this report, various inbred mouse strains were crossed with a congenic human apoB transgenic mouse of B6 background to determine genetic contributions to the variance of plasma apoB levels. F1 HuBTg offspring were characterized by plasma apoB and TG levels, and in vivo apoB and TG secretion rates. By comparing these measurements to those of the parental B6 HuBTg strain, different phenotypes resulting from genetic differences were defined in six F1 HuBTg strains (**Table 6**). Several inbred strains were identified as possessing polymorphic alleles (relative to the B6 strain) regulating plasma apoB levels mainly via hepatic apoB-100 secretion rates. Three of these F1 strains, BALB/B6, C3H/B6, and 129/B6, appear to be informative candidates for further genetic studies to identify factors regulating hepatic apoB secretion and hence plasma apoB levels. In addition, genetic effects on TG secretion and TG levels in these F1 mouse strains were also demonstrated, hence it is likely that independent factors affecting TG secretion could be investigated as well.

In the BALB/B6, C3H/B6, and 129/B6 F1 mouse strains, the hepatic apoB-100 secretion rate appeared to

TABLE 4. Hepatic human apoB mRNA levels in HuBTg mouse strains

HuBTg Strain	Male n	Female n	Human ApoB mRNA (% of the B6 strain)
B6	5	3	100 ± 19
BALB/B6	5	0	130 ± 47
C3H/B6	5	0	110 ± 16
129/B6	2	6	97 ± 14

Data were pooled from both male and female mice as no gender difference was observed. Values shown are mean ± SD. Human apoB mRNA levels are expressed as a percentage relative to the B6 strain. Comparison was performed between F1 strain versus B6 strain (*t* test). No significant difference was observed between any of the F1 strains versus the B6 strain.

TABLE 5. Mouse and human apoB mRNA editing in the liver

HuBTg Strain	Sex	Mouse ApoB		Human ApoB	
		n	% RNA Edited	n	% RNA Edited
B6	M	5	81 ± 3	4	72 ± 7
BALB/B6	M	5	83 ± 3	5	81 ± 4
C3H/B6	M	5	83 ± 2	4	82 ± 4[a]
129/B6	M	6	71 ± 10	5	71 ± 4
B6	F	4	74 ± 2	4	56 ± 4
129/B6	F	5	77 ± 11	5	62 ± 6

Age-matched mice were fasted for 4 h prior to experiments. Values shown are mean ± SD. Comparison was performed between the F1 strain and the parental B6 strain within each gender using a *t* test.
$P < 0.05$.

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TABLE 6. Lipoprotein phenotypes of F1 vs. parental B6 HuBTg mice

HuBTg Strain	Plasma Human ApoB	B-100 SR	B-48 SR	Plasma TG	TG SR
B6	↔	↔	↔	↔	↔
BALB/B6	↓	↓	↔	↓	↓
C3H/B6	↓	↓	↓	↓*	↔
129/B6	↓	↓	↔*	↓	↔
FVB/B6	↓*	↓*	↔	↑	↔
SJL/B6	↓*	↔	↔	↑	↑
NZB/B6	↔	↔	↔	↑	↑*

Data from male mice shown in Tables 1–3 are summarized in this table. The asterisk indicates gender differences.

be a major determinant of plasma apoB levels (Table 6). In comparison to the parental B6 HuBTg strain, these F1 HuBTg strains had concomitantly lower plasma apoB levels and apoB-100 secretion rates. We also assessed possible mechanisms regulating the hepatic apoB-100 secretion rate in these F1 HuBTg mouse strains. Northern blot analysis showed no differences in the human apoB mRNA levels in these strains suggesting that the regulation for the varying apoB-100 secretion rates is most likely not transcriptional. Both apoB secretion rates and apoB mRNA editing (mouse and human) were unchanged in either BALB/B6 or 129/B6 HuBTg mice compared to B6 HuBTg mice. In the C3H/B6 HuBTg mice, apoB-48 secretion rates were lower despite an increase in human apoB mRNA editing compared to the B6 strain. These results suggest that the lowering of plasma human apoB-100 levels was unlikely due to a shift from apoB-100 to apoB-48 secretion. Although highly unlikely, we cannot rule out the possibility that there may be potential strain differences in responsiveness to treatment with Triton WR-1339. Nevertheless, these F1 HuBTg strains can serve as a powerful tool for further genetic analysis of factors regulating hepatic apoB-100 secretion rates.

Characterization of plasma TG levels and TG secretion rates in these F1 mouse strains revealed different phenotypes (Table 6). Furthermore, no correlation was observed between TG secretion rates and apoB-100 secretion rates in the six F1 strains studied. Instead, in the SJL/B6 and the NZB/B6 strain, a similar apoB-100 secretion rate was accompanied by a higher TG secretion rate (Table 6) suggesting a disassociation between the TG and the apoB-100 secretion rates. These data also suggest a distinct genetic control of either apoB or TG secretion rate in these two mouse strains. Finally, Table 6 also suggests different density properties of apoB-containing lipoproteins in some of these mouse strains. For example, mice from both the FVB/B6 and the SJL/B6 strains had lower plasma apoB and higher TG levels; they likely possess more buoyant apoB-containing lipoprotein particles than those of the B6 strain.

Numerous studies in cultured hepatic cells have shown that apoB secretion is predominantly regulated by post-translational mechanisms (see review in ref. 28 and references therein). Lipid substrate availability plays a key role in regulating assembly and secretion of apoB-containing lipoproteins (28). Increased availability of newly synthesized triglycerides can stimulate the interaction of apoB and MTP (38), facilitate completion of apoB translocation across the ER, and thereby target the apoB molecule for assembly and secretion (28). Both free cholesterol and cholesteryl ester contents have also been shown to play a role in regulating hepatic apoB secretion (28). Despite the overproduction of human apoB in the livers of HuBTg mice, there was a 3-fold increase in the apoB-100 secretion (Fig. 1). These data suggest that lipid substrate availability is unlikely to be the limiting factor for strain differences in the HuBTg mice. Western blot analysis of mouse plasma samples also showed that strain differences in mouse apoB-100 paralleled those of human apoB-100 (data not shown). These data further support the fact that the presence of the human apoB transgene simply exaggerates the phenotype of plasma apoB-100 and does not interfere with the normal cellular machinery required for apoB secretion. Despite the evidence that apoB secretion can be regulated by the biosynthetic pathways involved in lipoprotein metabolism, numerous studies suggest that regulation occurs mainly at the step of intracellular apoB degradation (see review, 29, and references therein). Specific protease(s) and/or other factor(s) have been proposed to be involved in ER and post-ER degradation (29). To date, no such factors have been identified. It is possible that one or more of the unknown factors is a major determinant for apoB secretion and may be responsible for the varying hepatic apoB-100 secretion rates in the F1 HuBTg strains identified in this report.

In humans, defects affecting plasma apoB levels due to oversecretion of hepatic apoB are evident in patients with familial combined hyperlipidemia (FCHL) (39, 40). Although FCHL appears to be inherited in an autosomal dominant manner (41), the genetic basis for this disease is heterogeneous (see review, 42, and references therein). Genetic studies show that neither the apoB gene itself nor the LDL receptor gene account for FCHL (43, 44). These studies also suggest major gene effects for plasma triglyceride levels (45) and for apoB levels (46, 47). A novel FCHL major gene located in chromosome 1q21–23 has recently been identified in 31 Finnish FCHL families (48). Concurrent to the human studies, a novel FCHL locus (*Hyplip1*) on mouse chromosome 3, syntenic to human chromosome 1q21–23, was also linked to the FCHL phenotype in the mutant mouse strain HcB-19/Dem (49). This novel locus, however, is likely to be limited to only a subset of Finnish FCHL pedigrees (42). In addition, the candidate-gene approach has identified several known genes that are associated with FCHL phenotypes in some but not other FCHL pedigrees. These genes are likely to function as "modifier" genes for the FCHL phenotypes (42). These genes include the AI-CIII-AIV cluster, lipoprotein lipase, lecithin:cholesterol acyltransferase, fatty acid binding protein 2 (see review, 42, and references therein). Overall, mounting evidence suggests that the FCHL phenotype results from the effects of unknown major genes that increase the secretion of apoB-containing lipoproteins along with a number of modifier genes that also influence the levels of plasma lipids in these patients.

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Genetic mapping of the strains identified in this paper is underway in our laboratory and a genome scan analysis of mice derived from crosses between the B6 and 129 strains has excluded the linkage of the *Hyplip1* locus to plasma human apoB levels in these mice (L-S. Huang and S. C. Chua, unpublished data). Therefore, genetic factors identified through this particular cross and crosses between the B6 and other strains identified in this paper are likely to uncover novel candidate genes for evaluating human FCHL pedigrees.

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