Alternate transcripts expressed in response to diet reflect tissue-specific regulation of ABCA1

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Abstract **ABCA1 is essential for the transport of lipids across plasma membranes and for the maintenance of plasma HDL-cholesterol levels. The transcriptional regulation of ABCA1 is complex and is currently poorly understood. We previously generated human ABCA1 bacterial artificial chromosome transgenic mice that expressed RNA and protein, which allowed us to identify three alternate ABCA1 transcripts. Each transcript arises from different exon 1 sequences (exon1b, exon1c, and exon1d) that are spliced directly into exon 2, which contains the ATG site, and all generate full-length protein. We have now determined the tissue-specific expression of each of these transcripts in humans and mice and have shown that their patterns of expression are similar. Exon1d transcript is predominantly expressed in liver and macrophages and is preferentially increased in the liver in response to a high-fat diet. The exon1b transcript is expressed predominantly in liver, testis, and macrophages, but it is only upregulated in macrophages in response to a high-fat diet. The exon1c transcript is ubiquitously expressed and is upregulated in the brain, stomach, and other tissues in mice on a high-fat diet. Our data indicate that specific transcripts in different tissues play key roles in alterations of ABCA1-mediated changes in HDL levels and atherosclerosis in response to environmental stimuli.**—Singaraja, R. R., E. R. James, J. Crim, H. Visscher, A. Chatterjee, and M. R. Hayden. **Alternate transcripts expressed in response to diet reflect tissue-specific regulation of ABCA1.** ***J. Lipid Res.*** **2005.** 46: **2061–2071.**

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Maintenance of cholesterol homeostasis is vital for the survival of all organisms. Many genes under complex regulatory pathways contribute to preserving this balance. One of these genes is ABCA1, which functions in the reverse cholesterol pathway and is vital for the translocation of phospholipids and cholesterol across the plasma membrane to apolipoprotein A-I (1–3). Mutations in ABCA1 lead to familial hypoalphalipoproteinemia and Tangier disease, characterized by low to absent levels of plasma HDL-cholesterol (HDL-C), reduced ability to efflux cholesterol, and the accumulation of lipid-filled foam cells in several tissues (1–3).

ABCA1 protein is highly expressed in the liver, testis, adrenal, small intestine, and brain, among other tissues (4). The gene is regulated by oxysterols that modulate ABCA1 expression through the nuclear hormone receptor, liver X receptor (LXR), which heterodimerizes with the retinoid X receptor (RXR) and binds to a direct repeat with 4 intervening nucleotide (DR4) site to activate the ABCA1 promoter (5, 6). LXRα is expressed primarily in liver, intestine, kidney, and macrophages, whereas LXRβ is widely expressed (7). LXRs in turn are controlled by the transcription factors peroxisome proliferator-activated receptor γ/δ (PPARγ/δ) (8, 9). ABCA1 is also upregulated by PPARα and PPARγ activators (10).

In addition, ABCA1 is upregulated by retenoic acid receptor γ, which binds to the same DR4 element in the ABCA1 promoter as LXRα (11). cAMP-mediated upregulation of ABCA1 is also present (12–14), although the cAMP regulatory motif in the human ABCA1 promoter has not yet been identified (15). A promoter region 100–200 bp upstream of the exon 1 transcription start site that is responsive to free cholesterol has also been identified (16), and it has been shown that ABCA1 expression in fibroblasts is critically dependent on cholesterol loading (17). Interferon γ downregulates ABCA1, especially in mouse macrophages and foam cells (18), and ZNF202, a zinc finger protein, has been shown to repress ABCA1 expression in HepG2 and RAW cells (19, 20). In addition, the thyroid hormone receptor also suppresses ABCA1 transcription, mediated through the DR4 elements (21) (**Fig. 1A**).

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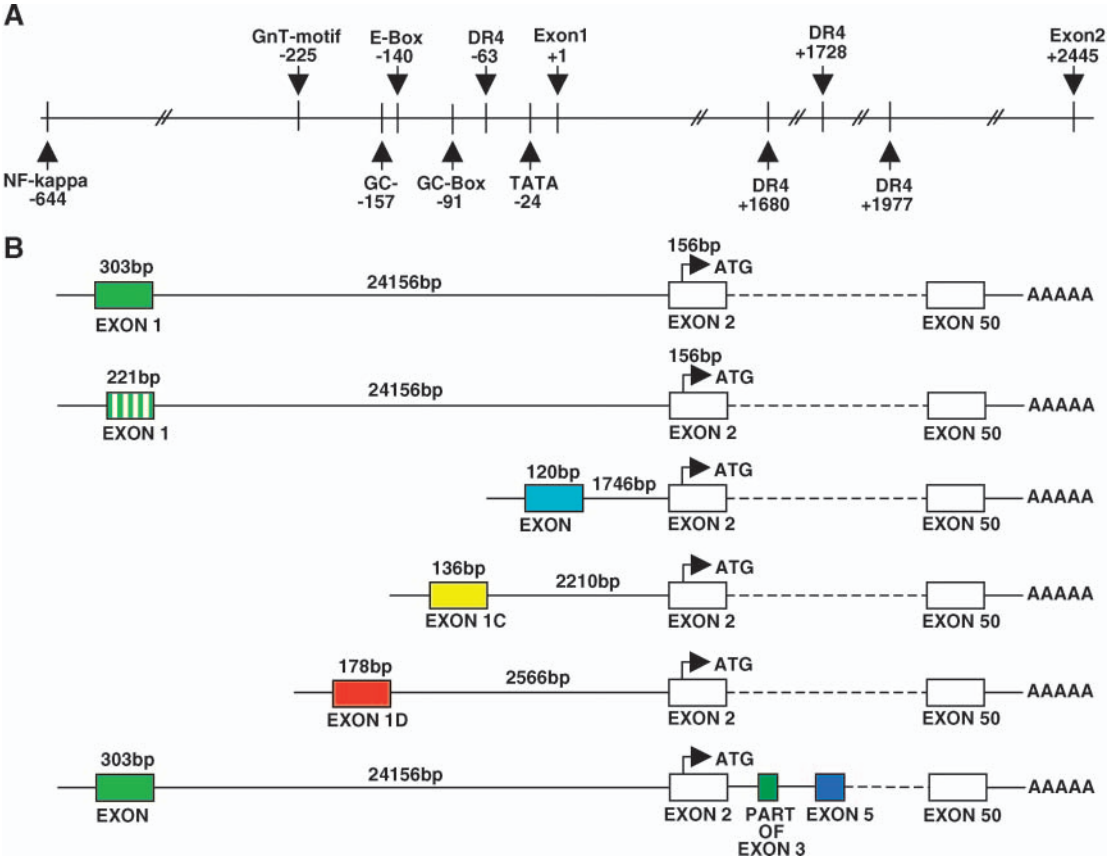

Fig. 1. Regulatory elements in the ABCA1 promoter and intron 1 and a schematic diagram of previously described ABCA1 transcripts. A: Schemes of both the most upstream and the intron 1 promoter of ABCA1. ABCA1 is upregulated through the liver X receptor/retinoid X receptor (LXR/RXR) elements at position −63 of the most upstream promoter (5, 6) and at positions −4,686, −7,656, and −7,174 of the intron 1 promoter (25, 33, 34). In addition, ABCA1 is upregulated by cAMP (12–14), and the GC boxes at −91 and −157 and the E-box motif at position −140 have been shown to be essential for ABCA1 upregulation (6, 16, 23). ABCA1 is downregulated by the ZNF202 transcription factor (19, 20). Also shown are the two potential peroxisome proliferator-activated receptor elements discovered in intron 1 of ABCA1 that have not been functionally characterized. B: Diagram of the ABCA1 transcripts. The three alternative transcripts (hExon1b, hExon1c, and hExon1d) are generated from sequences in intron 1 of the ABCA1 gene, and each contains an alternative exon 1 that is spliced into the same exon 2 of ABCA1, which contains the ATG translation initiation site. Therefore, each transcript gives rise to the same ABCA1 protein. DR4, direct repeat with 4 intervening nucleotides; NF-kappa, nuclear factor κB.

Several transcription start sites have been identified for ABCA1, and levels of groups of these transcripts have been characterized (22). The farthest upstream start site generates an exon 1 that is 303 bp long, is 24,551 bp upstream of the ATG, and is 40 bp downstream from a modified TATA box (6, 23). This transcript contains binding sites for AP1, nuclear factor κB, Sp1, and sterol-regulatory element binding protein and consists of six G/C-rich regions in close proximity. It also contains an LXR binding DR4 element at position +4 of exon 1 (5). The next transcription start site occurs ~90 bp downstream of the first start site and generates a truncated 221 bp exon 1 (16). A weak TATA box is present upstream of this transcription start site. One other transcript, lacking part of exon 3 and without all of exon 4, has been described (24), but it does not generate a full-length protein (Fig. 1B).

We have previously generated ABCA1 bacterial artificial chromosome (BAC) transgenic mice (25) and described the presence of three alternate transcripts in these mice. We termed these transcripts exon1b, exon1c, and exon1d and determined that they are also similarly present in humans (25). These alternate transcripts contain different exon 1 sequences, but each splices into the same exon 2, which contains the ABCA1 translation start codon, thus generating the same full-length ABCA1 protein. They are localized downstream of TATA and CAAT sequences and have been shown to have functional LXR/RXR binding sites upstream of the start sites (25).

We and others have shown that ABCA1 protein levels are increased significantly in the liver and macrophages of mice fed a high-fat diet (4, 25). It is unclear whether specific alternate transcripts are responsible for this tissue-specific response to dietary stimulation.

In the current study, we determined the tissue distribution of the ABCA1 transcripts and gained insight into their regulation by elucidating their response to cholesterol loading. Our data indicate that specific ABCA1 transcripts are expressed in different tissues, and these transcripts demonstrate differential responsiveness to activation by external stimuli such as diet.

MATERIALS AND METHODS

Identification of alternate human and mouse *ABCA1* splice variants

Alternate transcripts in the ABCA1 gene were identified as described previously (25). Briefly, ABCA1 BAC transgenic mice and control littermate wild-type mice were killed by CO_2 inhalation, and tissues were dissected and frozen. RNA was isolated from tissue according to the manufacturer's protocol using the Trizol RNA isolation kit (Invitrogen). Mouse and human liver Marathon Ready cDNA (Clontech) was also used for the identification of splice variants. 5′ rapid amplification of cDNA ends (RACE) was performed according to the manufacturer's instructions (Invitrogen). Human *ABCA1*-specific reverse primers in exon 6 (CCCTCAGCATCTTGTCCACAGTAGAC) and exon 4 (GAAGTGTTCCTGCAGAGGGCATG) of ABCA1 (25) or reverse mouse *ABCA1*-specific primers in exon 2 (CGAATGTCAGATTCTTCCAC or CTTCGAAATGTCAGATTCTTCCAC) and the adaptor primers provided were used with the Marathon Ready cDNA samples. All amplified products were TA cloned (Invitrogen) and sequenced using an ABI Prism 3100 genetic analyzer (Applied Biosystems, Foster City, CA) sequencer. The sequence was assembled using Chromas version 1.45 (Technelysium Pty Ltd., Queensland, Australia) or Phred-Phrap (CodonCode Corp., Dedham, MA) and was compared with human *ABCA1* (gi number 9247085) or mouse *ABCA1* (gi number 11611824) genomic sequences using the Basic Local Alignment Search Tool server at the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov).

Feeding of an atherogenic diet

ABCA1 BAC transgenic and wild-type littermates were fed an atherogenic diet containing 15.75% cocoa butter, 1.25% cholesterol, and 0.5% sodium cholate (TD90221; Harlan Teklad), a control chow diet containing 0.5% sodium cholate (TD99057; Harlan Teklad), or a control chow diet containing no sodium cholate for 7 days. Both diets and water were provided ad libitum.

RT-PCR analysis of human ABCA1 splice variants

RNA was isolated from snap-frozen tissue according to the manufacturer's protocol using Trizol reagent (Invitrogen). Isolated RNA was quantified using a Pharmacia Ultrospec 3000 spectrophotometer and separated on a 1% agarose/formaldehyde gel to assess quality, and 4 μg of the isolated RNA was used for reverse transcription using Superscript II reverse transcriptase (Invitrogen) according to the manufacturer's protocol. ABCA1 splice variants were analyzed as follows. Human transcript exon1 (hExon1) was amplified using the forward primer GTAGGAGAAAGAGACGCAAAC and the reverse primer CATTCATGTTGTTCATAGGGTG designed in exon 3 of ABCA1. The PCR cycling conditions were 95°C for 4 min followed by a hold at 78°C for 5 min, during which Advantage polymerase mix (Clontech) was added as a hot start. This was followed by 35 cycles of 94°C for 45 s, 60°C for 1 min, 72°C for 2.5 min, and a 72°C extension for 7 min. Human transcript exon1b (hExon1b) was amplified using the forward primer CAAGCTCTGTCCTGAGCCAC and the same reverse primer and cycling conditions as used for transcript exon1. Human transcript exon1c (hExon1c) was detected using the same conditions and the forward primer GAGAAGGGAACTCACATTGCTTTG, and human exon1d (hExon1d) was detected using the forward primer CACGGTAGAACTTTCTACTGTG and the same reverse primer used for the other transcripts. The annealing temperature for the detection of exon1d was increased from 60°C to 64°C. The 18s rRNA was detected using the Ambion classic II 18s kit according to the manufacturer's instructions. The 18s competimer-to-primer ratio used was 1:9. All amplified products were separated on a 2% agarose gel containing ethidium bromide, and densitometric quantification was performed using the Bio-Rad GelDoc 100 (Bio-Rad Laboratories, Hercules, CA) and Quantity One software, version V.4.01 (Bio-Rad Laboratories). The relative abundance of the ABCA1 transcripts was expressed as the ratio of the quantified ABCA1 to the 18s PCR product.

Taqman analysis of the human ABCA1 splice variants in human and mouse tissue

Human tissue samples were run on Taqman assays using four ABCA1 markers, exon1, exon1b, exon1c, and exon1d. Tissue samples from the mice were also run using Taqman assays using three ABCA1 markers, hExon1b, hExon1c, and hExon1d. For each tissue sample, reactions were run in triplicate. A housekeeping gene, marker rRNA, was also run to normalize the amount of RNA added to each reaction. No-template controls were run for each master mix to check for any contamination. For each reaction, 40 ng of total RNA was used and gene amplification was detected using the ABI Prism 7900 Sequence Detection System. The ABCA1 markers were 6-carboxyfluroescein labeled, and the rRNA marker was VIC™ labeled to allow multiplexing within the same reaction. The Ct (threshold cycle) value, which is the cycle when amplified product is detected to be above threshold (or background) by the PCR system, was determined for each sample. ΔCt values were determined by subtracting the rRNA Ct value from the ABCA1 Ct, and thus were normalized for input RNA quantity. These data were used to calculate fold changes in gene expression by first taking an average of the repeats for each sample and then using the formula:

$$2^{(-(\text{Group A } (\Delta\text{Ct Average}) - \text{Group B } (\Delta\text{Ct Average})))}$$

Probe/primer sequences used for the quantification of the transcripts were as follows: human exon1F, ACAGGCTTTGACCGATAGTAACCT; human exon1R, TTGCCGGGACTAGTTCCTTTTAT; human exon1 probe, TGCGCTCGGTGCAGCCGAAT; human exon1bF, GCTGTGCTGCCAGTTGTCAT; human exon1bR, AGGACACAGGCCTCCAAAGTT; human exon1b probe, TTGAACAAACTGATGCAGTGCTGGTTTAACTC; human exon1cF, CTAGAGGATATTGTTGGAATGAAGAAAG; human exon1cR, CGAGATAATATAAATAATTTCCCAACACAGTAC; human exon1c probe, AACTCACATTGCTTTGGCACTTAAATTAAGCCA; human exon1dF, TGTGGCTCTATGCTACTTCTTAGCA; human exon1dR, AGAAAAAGGTTCTGACTCCAAGGA; and human exon1d probe, TTCTCCATGTGCTTCCTGGAGA.

RT-PCR analysis of the mouse ABCA1 splice variants

RNA was isolated and quantitated as described above. Endogenous mouse ABCA1 splice variants were analyzed as follows. Mouse transcript exon1c (mExon1c) was detected using the forward primer GAACCATCGATTGCGTCTGACC and the reverse primer CTTCGAAATGTCAGATTCTTCCAC designed in exon 2 of mouse ABCA1 (primer set 2). The PCR cycling conditions were 95°C for 5 min, followed by 33 cycles of 96°C for 45 s, 63°C for 30 s, 72°C for 45 s, and a 72°C extension for 7 min. Mouse transcript exon1d (mExon1d) was quantified by subtracting the values of mExon1c (primer set 2) from the values obtained using the forward primer ACCAGGGTGTCAGAGGTGTC and the same reverse primer and cycling conditions used for exon1c (primer set 3), because this primer set amplifies both mExon1c and mExon1d. Mouse transcript exon1b (mExon1b) was quantified by subtracting the values of primer set 3 from the values amplified using the forward primer TTAATGACCAGCCACAG and the same conditions and reverse primers used for mExon1c (primer set 1). The 18s rRNA was detected using the Ambion classic II 18s kit and quantitated as described above.

Statistics

All statistical analyses were performed using the two-tailed *t*-test in GraphPad Prism (version 3 for Windows; GraphPad Software, San Diego, CA).

RESULTS

Tissue distribution of total human ABCA1 mRNA in BAC mice

We previously looked for the transcripts in human ABCA1 BAC transgenic mice that were responsible for protein expression (25). These studies revealed that in the BAC mice, three alternate human ABCA1 transcripts were present, each containing a novel exon 1 that was spliced into exon 2 of the ABCA1 gene with its ATG translation start site (Fig. 1B) (25). To determine whether the total human ABCA1 mRNA from the BAC transgenic mice showed the same tissue abundance and distribution as the endogenous mouse ABCA1 transcripts described previously (4), we performed semiquantitative RT-PCR using primers in exon 4 and exon 6 of the human ABCA1 gene. The liver, testes, and brain showed the highest mRNA abundance. This is in agreement with our previous data, in which these tissues showed the highest levels of protein expression (25). The kidney and heart showed the next highest levels of mRNA abundance, and the macrophages, intestine, spleen, and stomach showed the lowest abundance (**Fig. 2A**). These data are similar to previously published levels for the abundance of endogenous mouse transcripts (4). Levels of human ABCA1 transcripts in the BAC mice remained unchanged when the mice were fed a control diet with cholate (data not shown).

Human ABCA1 mRNA distribution patterns are similar in human tissue and when expressed in BAC mice

We next sought to determine whether the total human ABCA1 transcripts showed a similar tissue distribution pattern in humans as it did when expressed in the mouse background. Total human transcript levels were highest in the liver with the testis, kidney, brain, and intestine showing slightly lower levels (Fig. 2B), which is essentially simi-

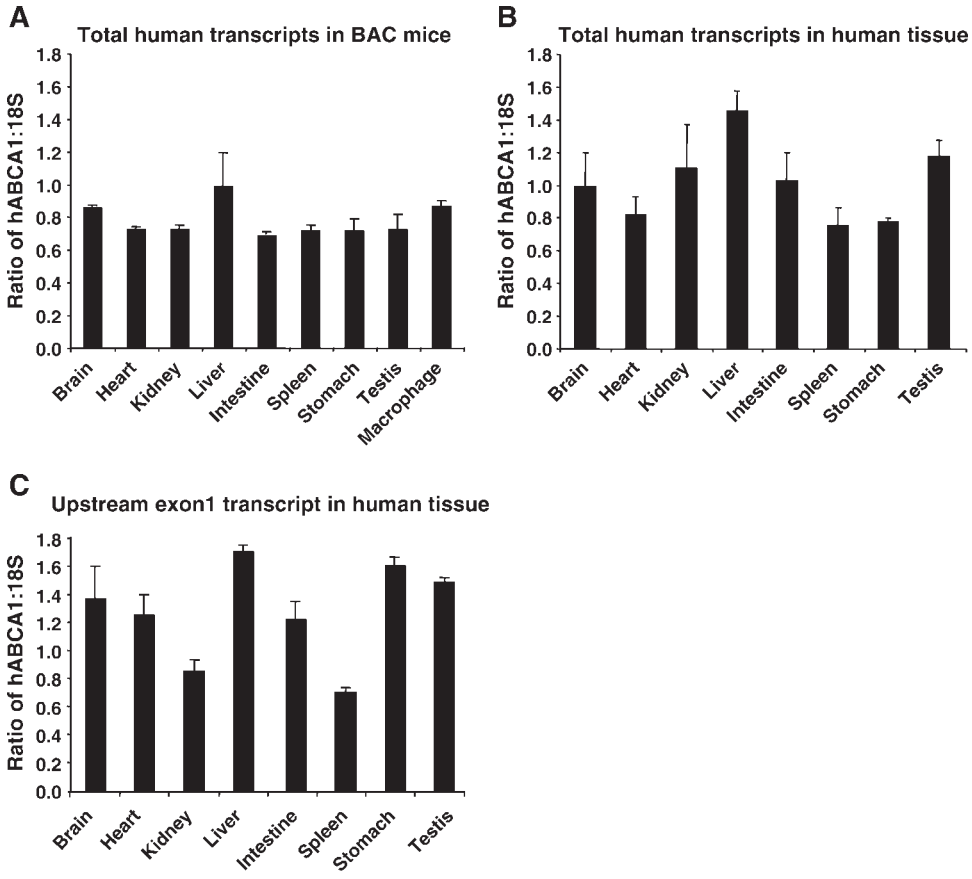

Fig. 2. Distribution of the human ABCA1 transcripts in bacterial artificial chromosome (BAC) transgenic mouse and human tissue. Because the BAC transgenic mice were generated using a human BAC, we determined the total human transcript distribution in these mice (A) and in human tissues (B). Total ABCA1 transcript distribution was assessed using PCR primers that were generated in exon 4 and exon 6 of the ABCA1 gene. Both BAC mice and human tissue showed similar tissue distribution of ABCA1 mRNA and showed the presence of ABCA1 in all tissues tested, with the highest levels observed in the liver. C: The upstream human exon 1 transcript (present in human tissues but not in the BAC mice, because the upstream exon 1 was not contained in the ABCA1 BAC) was localized in all tissues tested, with the highest levels observed in the liver, stomach, testis, and brain. Error bars represent standard deviation around the mean.

lar to the pattern of human transcript distribution in the BAC mice. Human macrophages were not tested because of the unavailability of macrophages from the human tissue bank. In addition, we assessed the tissue distribution of the previously characterized exon 1 transcript in human tissue and found that it was ubiquitously expressed and at relatively similar levels in the tissues tested (Fig. 2C). This transcript was not assessed in the BAC mice because the human ABCA1 BAC used to generate the mice did not contain either the regular promoter or exon 1.

Human transcripts hExon1b and hExon1d show tissue-specific distribution

We hypothesized that alternative transcripts in different tissues could account for these tissue-specific mRNA and protein expression patterns. To determine the tissue dis-

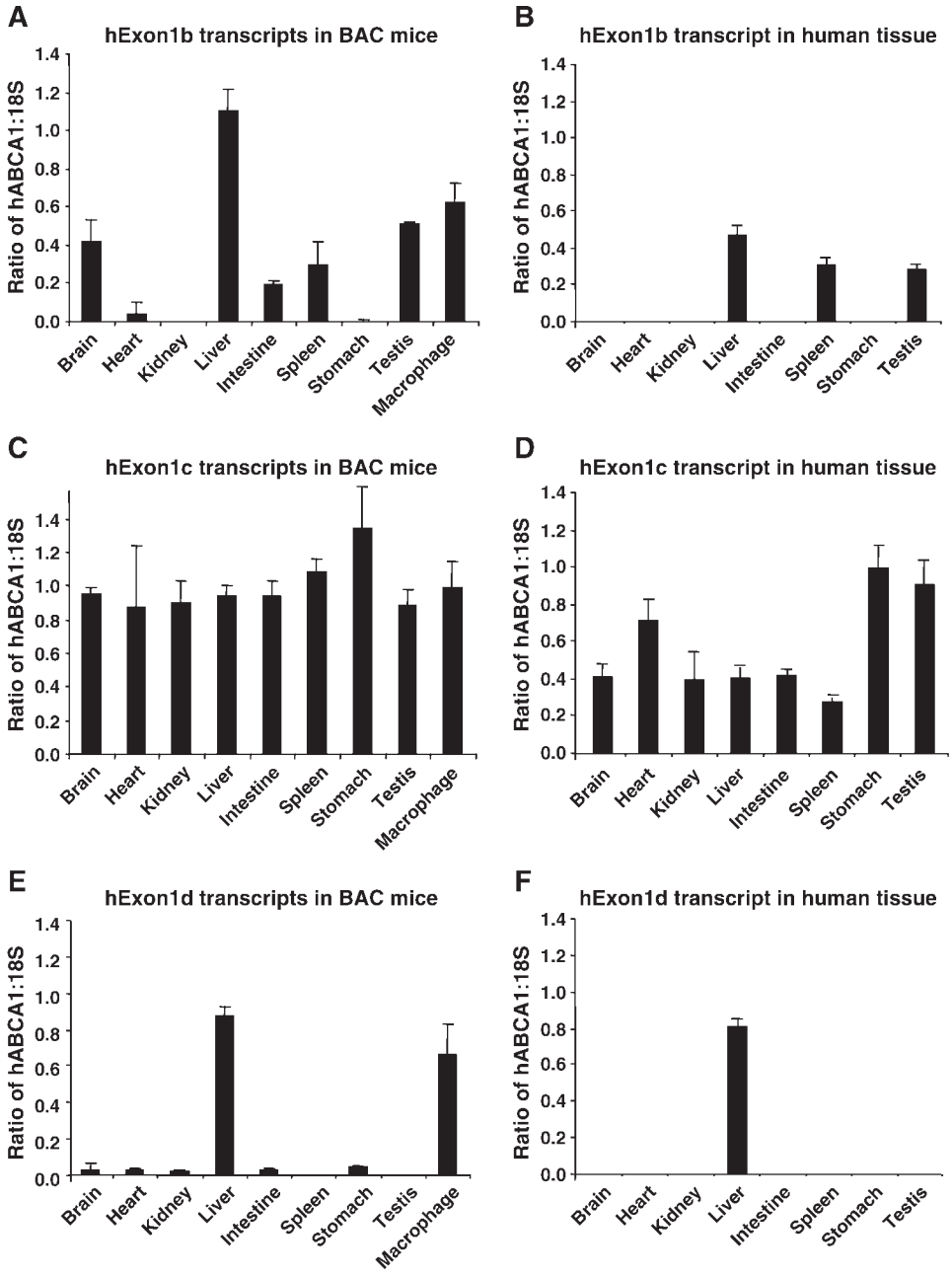

Fig. 3. Distribution of the alternate human ABCA1 transcripts in BAC mice and human tissues. RNA was isolated from BAC mouse and human tissues, and RT-PCR was performed to determine the mRNA distribution pattern of ABCA1 in various tissues. A: hExon1b showed the highest levels in the liver and was found in brain, liver, intestine, spleen, testis, and macrophage in BAC mice. B: hExon1b was quantified in various human tissues and was found in the liver, spleen, and testis, with the highest levels observed in the liver. C, D: In BAC mice (C) and in human tissues (D), hExon1c was found in all tissues tested. In the BAC mice, it was found at relatively similar levels. In humans, the stomach and testis showed the highest levels of hExon1c. E: hExon1d was found only in the liver and macrophages, with the highest levels seen in the liver of BAC mice. F: In humans, hExon1d was found solely in the liver. Error bars represent standard deviation around the mean.

tribution of the three alternate human transcripts in the BAC mice, we performed semiquantitative RT-PCR and Taqman assays on various tissues isolated from the BAC transgenic mice. We found that the highest levels of human transcript hExon1b was expressed in the liver, with much lower levels in the brain, intestine, spleen, testis, and macrophages (**Fig. 3A**) of BAC mice. In human tissues, a similar pattern was observed, with highest levels of hExon1b expressed in the liver. The spleen and testis showed lower levels of this transcript in humans (Fig. 3B).

In contrast, human transcript hExon1c was found in all tissues tested at relatively equivalent levels both in the BAC mice (Fig. 3C) and in human tissues (Fig. 3D).

Human transcript hExon1d was seen specifically in the liver and macrophages of the BAC mice at equivalent levels (Fig. 3E). In human tissues, transcript hExon1d was

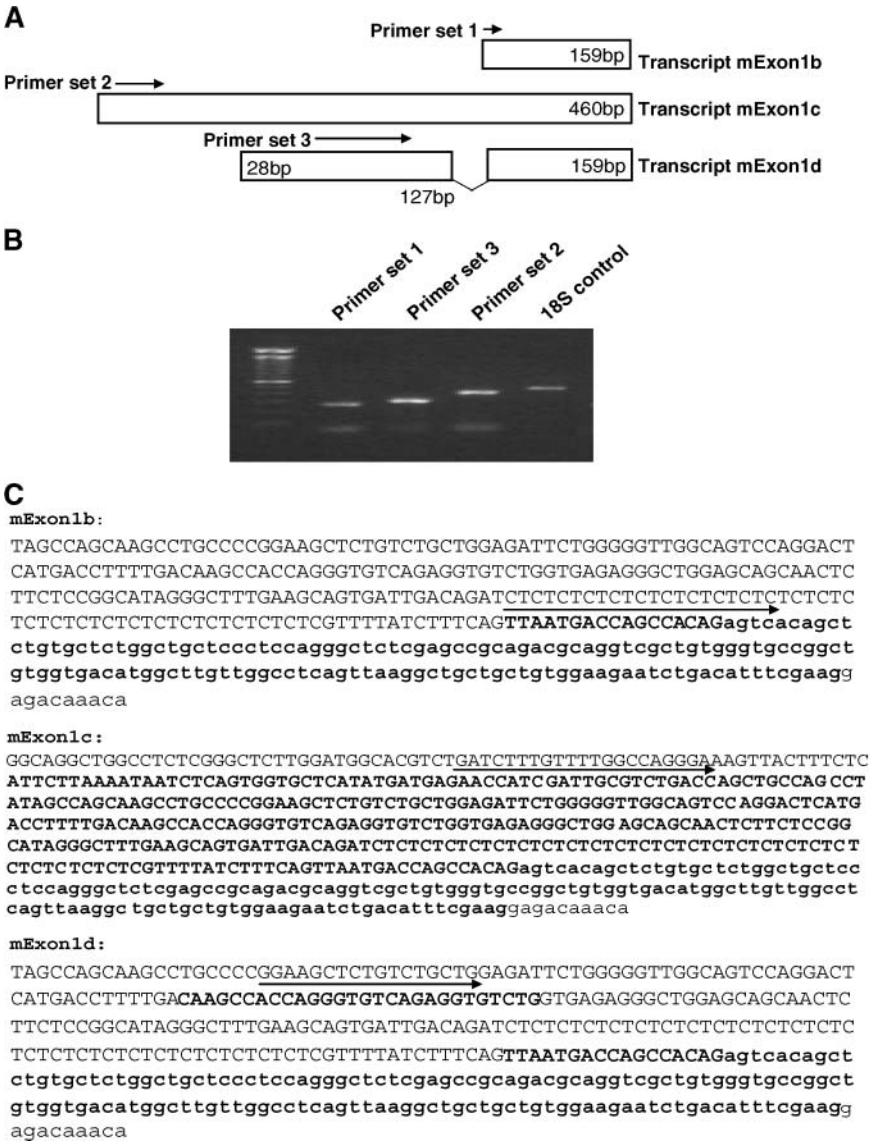

Fig. 4. Schemes and sequences of the endogenous alternate mouse transcripts. 5′ rapid amplification of cDNA ends was performed on RNA isolated from liver tissue from wild-type mice, and the presence of three alternate transcripts was discovered. A: These transcripts arise in intron 1 of the mouse ABCA1 gene. Transcript mExon1b extends the length of the previously described mouse exon 2, and expressed sequence tags have been described containing this exon. mExon1c also results in an extended exon 2 by ~300 bp. mExon1d consists of an alternate exon 1 sequence that splices into exon 2 of the mouse sequence. As in humans, exon 2 in mice contains the ATG translation initiation site. B: Alternate endogenous mouse ABCA1 transcripts were quantified using RT-PCR. mExon1c levels were assessed using primer set 2, which generates a 460 bp fragment. mExon1d levels were assessed by subtracting values of primer set 2 from primer set 3 (184 bp product). mExon1b levels were quantitated by subtracting values for primer set 3 from primer set 1 (156 bp product). C: The sequences of the transcripts are provided, with the primers used for amplification indicated. Boldface, lowercase letters indicate the previously described exon 2. Boldface, uppercase letters indicate the new exonic sequences.

observed only in the liver (Fig. 3F). Macrophages were not tested because of their unavailability in the human tissue bank. No differences were observed in the levels or distribution of hExon1b, hExon1c, and hExon1d between the mice fed a chow diet with and without cholate (data not shown).

The endogenous mouse ABCA1 mRNA transcripts show similar tissue distribution to that seen with human mRNA

To determine whether the mouse ABCA1 gene produces alternate transcripts that are similarly distributed in the mouse, we performed 5′ RACE analysis on mouse RNA; as in humans, three alternate transcripts were present in the mouse (**Fig. 4**). To determine the tissue distribution of these transcripts in mice, and also to determine whether these mouse transcripts showed similar distribution to the human ABCA1 alternate transcripts, we performed distribution analysis of the mouse transcripts in various tissues. First, we found that the total mouse ABCA1 mRNA showed similar tissue distribution to both the total human transcripts in the mouse background and the endogenous human transcripts in human tissues. Essentially, mRNA levels were the highest in the liver, brain, testis, and kidney. Lower levels were observed in the heart, intestine, macrophages, spleen, and stomach (**Fig. 5A**). These levels remained unchanged when the mice were fed a control chow diet with cholate (data not shown).

Using semiquantitative RT-PCR, we determined the distribution of the three alternate transcripts in the mouse and discovered that mouse transcript mExon1b was found in the liver, intestine, testis, and macrophages and was found at similar levels in these tissues (Fig. 5B). This transcript shows essentially similar tissue distribution to the human hExon1b transcript. Mouse transcript mExon1c was found in all tissues tested at similar levels (Fig. 5C), showing identical distribution to the human hExon1c transcript.

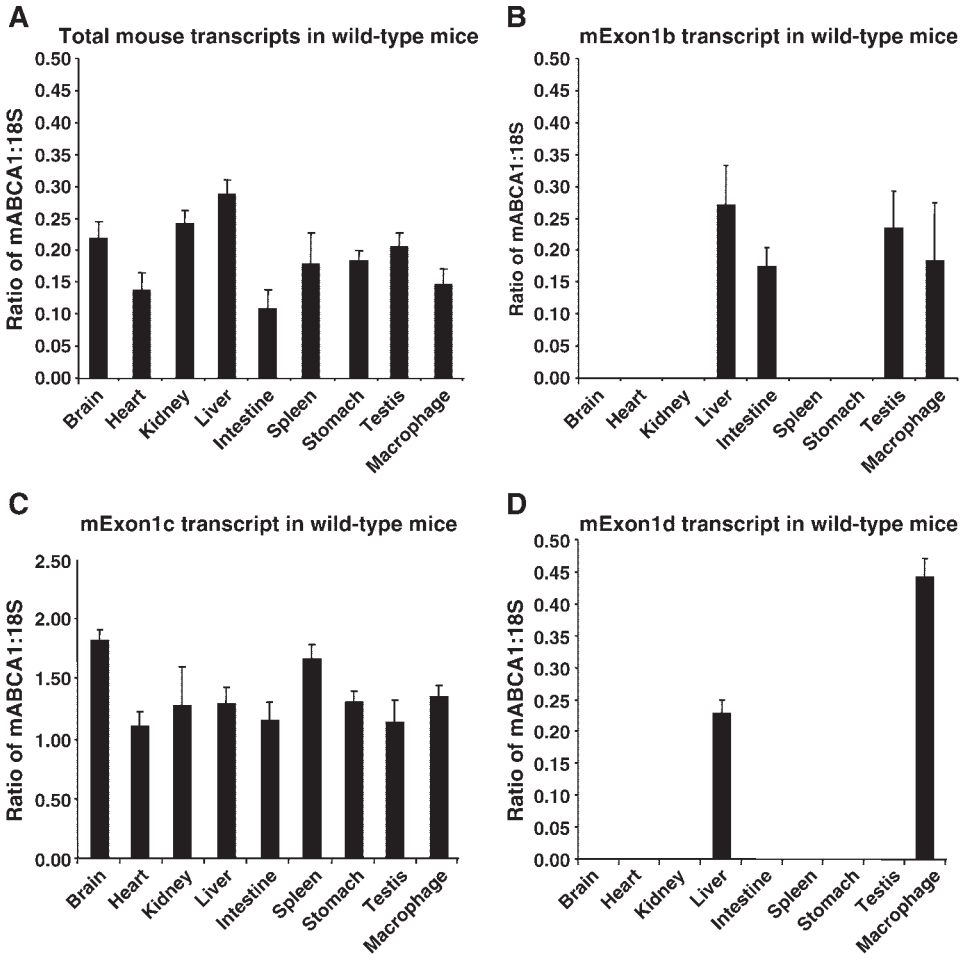

Fig. 5. Distribution of endogenous mouse transcripts in wild-type mice. The tissue distribution and abundance of mouse ABCA1 transcripts were quantified to determine differences between the expression of the human and mouse transcripts. A: Total mouse transcripts were quantified using mouse ABCA1 primers designed in exon 3 and exon 5 of the mouse ABCA1 gene. Mouse ABCA1 mRNA was most abundant in the liver and was also found at high levels in the brain, testis, and kidney. B: mExon1b transcript was found in the liver, intestine, testis, and macrophages of mice, with the highest levels observed in the liver and testis. C: mExon1c was distributed in all tissues tested and at relatively similar levels in most of the tissues. D: mExon1d was found solely in the liver and macrophages and was most abundant in macrophages. Error bars represent standard deviation around the mean.

The mouse transcript mExon1d was found in the liver and macrophages, with highest levels in the macrophages (Fig. 5D), showing a similar tissue distribution to the human transcript hExon1d. Thus, the alternate endogenous ABCA1 transcripts exon1b, exon1c, and exon1d show similar tissue distribution patterns between the mouse and human.

Specific human and mouse ABCA1 transcripts in the liver and macrophages are significantly upregulated in response to feeding of a high-fat diet

We next sought to determine which transcripts are responsible for the increases in ABCA1 mRNA and protein levels observed when mice are fed a high-fat diet (25). Because ABCA1 expression is known to be strongly regulated by oxysterols, and because feeding of high-fat diets has previously been shown to increase ABCA1 protein in the liver and macrophages and to increase plasma HDL-C levels (25), we wished to determine the response of total human transcripts in the mouse background to high-fat feeding. When the mice were placed on a high-fat diet for 7 days, there was a significant upregulation of total human transcripts in the liver (49.9 ± 16.5%; n = 6, $P = 0.002$) and in macrophages (56.2 ± 16.1%; n = 6, $P = 0.003$) (**Fig. 6A**) of BAC mice. Increases were also observed in the brain, intestine, and testis, although these values did not reach significance.

We next determined the response of endogenous mouse ABCA1 transcripts to a high-fat diet. Endogenous total mouse ABCA1 transcripts were significantly upregulated in the liver, kidney, intestine, spleen, testis, and macrophages of the mice. The highest level of upregulation was observed in the liver (36.4 ± 12.1%; n = 6, $P < 0.0001$) (Fig. 6B).

We then determined the relative contribution of each of the three alternate transcripts of both human and endogenous mouse ABCA1 to the increase in total ABCA1 mRNA and protein expression after feeding of a high-fat diet in wild-type and BAC mice. Both the human transcript hExon1b (94.3 ± 20.8%; n = 6, $P < 0.0001$) (**Fig. 7A**) and the mouse transcript mExon1b (122.2 ± 15.8%; n = 6, $P = 0.0002$) (Fig. 7B) were specifically upregulated only in the macrophages of the mice. Our previous data showed an ~70% increase in ABCA1 protein levels in macrophages from BAC mice fed a high-fat diet compared with those on a chow diet (24). Thus, it is likely that the promoter of transcript exon1b is involved in the response to fat feeding in macrophages.

Human and mouse transcript exon1d showed a significant increase in expression most specifically in the liver of fat-fed BAC mice [human, 107.9 ± 15.8%; n = 6, $P = 0.0001$ (Fig. 7C); mouse, 263.5 ± 35.5%; n = 6, $P < 0.0001$ (Fig. 7D)]. In addition, transcript hExon1d also showed a mild but significant increase in macrophages (19.5 ± 17.0%; n = 6, $P = 0.009$) (Fig. 7C) in the BAC mice. In keeping with this, our previous data showed a 3-fold increase in ABCA1 protein levels in the livers of mice on a high-fat diet (4). Thus, it is likely that the exon1d promoter plays a major role in the liver's response to a high-fat diet in mouse and human.

Transcript hExon1c was increased in the brain (23.5 ± 10.7%; n = 6, $P = 0.0001$) of the BAC mice and showed relatively little increase in all other tissues tested (Fig. 7E). Mouse transcript mExon1c was significantly upregulated in the brain, heart, kidney, liver, stomach, and macrophages (Fig. 7F). All human ABCA1 transcript data were confirmed by Taqman analysis (data not shown), with similar levels of expression and upregulation observed using both techniques.

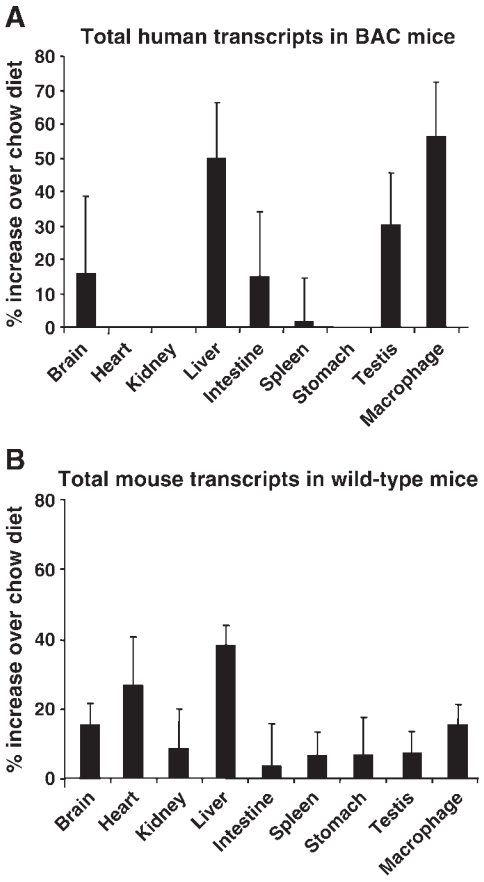

Fig. 6. Response of total ABCA1 transcripts to feeding of a high-fat diet. Transcripts were quantitated for response to feeding of a high-fat diet in mice. A: Overall, total human transcripts showed a significant increase in liver and macrophages of BAC transgenic mice. Small increases in levels of total ABCA1 transcripts were observed also in the brain, intestine, and testis, although these were nonsignificant. B: Total endogenous mouse transcripts were quantified, and significant levels of upregulation were observed in the testis, kidney, intestine, liver, macrophages, spleen, and stomach of wild-type mice. Error bars represent standard deviation around the mean.

When the data were analyzed by tissue, on a chow diet relatively equivalent levels of exon1b, exon1c, and exon1d were found in the liver in both mouse and human tissue (**Table 1**). However, upon high-fat feeding, levels of the exon1d transcript were specifically upregulated significantly in mouse liver, indicating a role for the promoter of this transcript in lipid sensing in the liver. In the macrophages, all three transcripts were also expressed in both humans and mice. However, the exon1b transcript was significantly upregulated in response to a high-fat diet, indicating a role for this promoter in responding to lipid loading in macrophages. Thus, specific transcripts in liver and

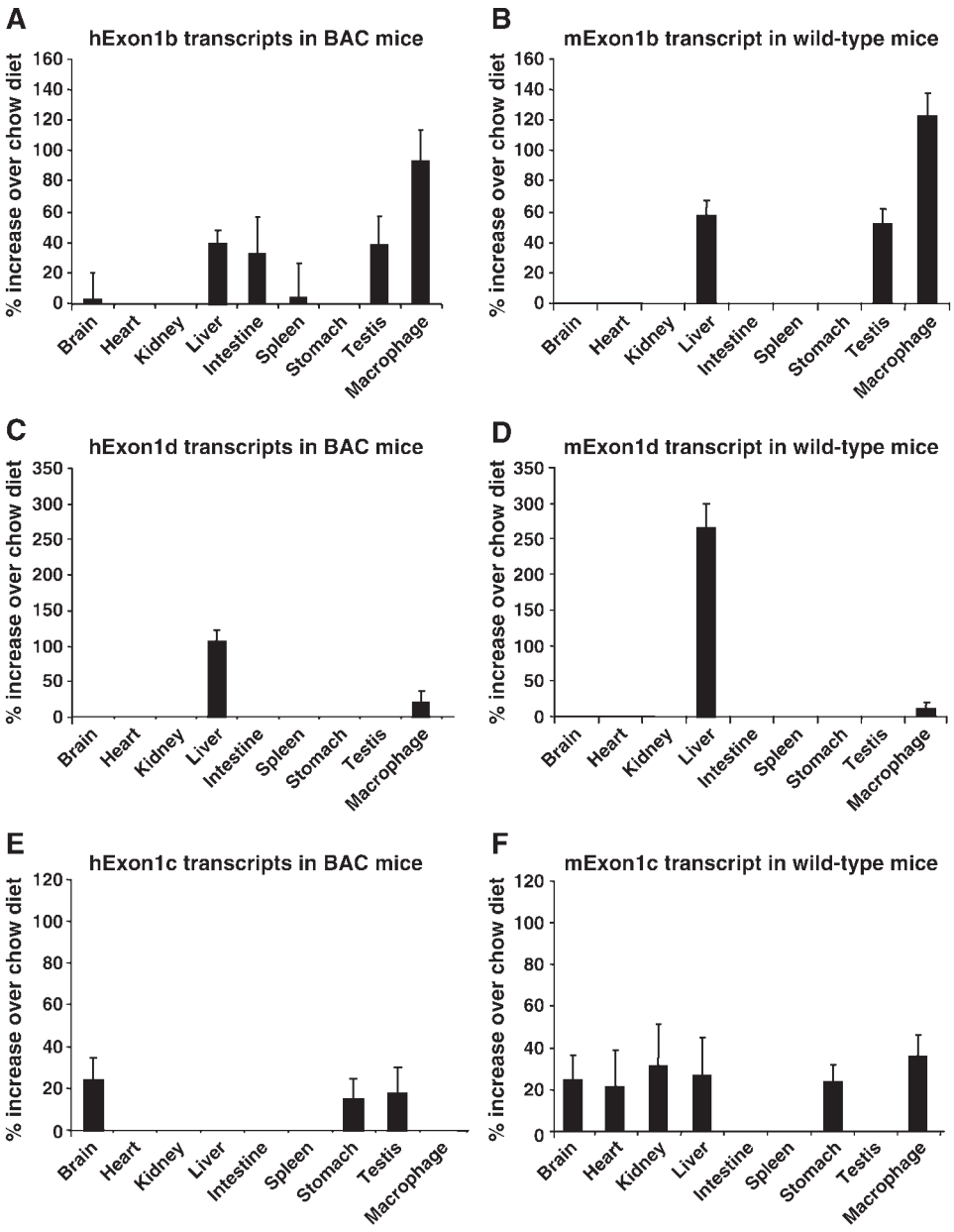

Fig. 7. Response of alternate ABCA1 transcripts exon1b, exon1d, and exon1c to a high-fat diet. A, B: After 7 days of feeding of a high-fat diet, both the human transcript hExon1b (A) and the mouse transcript mExon1b (B) showed dramatic increases specifically in macrophages. The testis and liver also showed mild and insignificant increases in exon1b transcripts. C: hExon1d showed high levels of upregulation in the liver of the BAC transgenic mice. In addition, small but significant levels of upregulation of this transcript were observed in the macrophages. D: As with hExon1d, the mExon1d transcript showed significant upregulation specifically in the liver of the wild-type mice. E: hExon1c transcripts were significantly upregulated in the brain and testis of the BAC mice. Minor increases were also observed in the stomach. F: mExon1c was upregulated in several tissues in the wild-type mice, including the brain, kidney, liver, macrophages, and stomach. Error bars represent standard deviation around the mean.

macrophages play a major role in upregulating ABCA1 expression in response to a fat diet.

DISCUSSION

Intracellular cholesterol levels are under precise and exquisite control and reflect the net balance between lipid biosynthesis, efflux from cells, and influx into the cells of cholesterol associated with lipoproteins. The regulation of these processes is stringently controlled, as excess cholesterol is toxic to cells, and a number of mechanisms have evolved to rid cells of free cholesterol. Lipids play important roles in cell membrane assembly and function, post-Golgi protein sorting (26), signal transduction and generation of cell surface polarity (27), and the activation of

TABLE 1. Distribution of human alternative transcripts in BAC mouse and human tissues

Transcript	Brain	Heart	Kidney	Liver	Intestine	Spleen	Stomach	Testis	Macrophages
hExon1b									
BAC mice	+	−	−	+	+	+	−	+	+
Human	−	−	−	+	−	+	−	+	nd
hExon1c									
BAC mice	+	+	+	+	+	+	+	+	+
Human	+	+	+	+	+	+	+	+	nd
hExon1d									
BAC mice	−	−	−	+	−	−	−	−	+
Human	−	−	−	+	−	−	−	−	nd

nd, not determined.

immune responses (28). Although the complex regulation mechanisms for maintaining cholesterol homeostasis remain unclear, impairment of such control mechanisms results in diseases such as atherosclerosis.

ABCA1 has a crucial involvement in the efflux of lipids from cells. The expression levels of this gene are exquisitely controlled. In this study, we determined the tissue distribution and abundance of each of the three alternate ABCA1 transcripts (25) and identified mouse ABCA1 transcripts that show similar distribution and regulation patterns to the human transcripts. We also determined the response of each of these transcripts to feeding of a high-fat diet. In addition, we found that the feeding of a diet containing cholate did not alter the levels or distribution of the ABCA1 transcripts.

This study identifies transcripts that are specifically expressed at different levels in the liver and macrophages, implying different relative contributions of these transcripts to the expression of ABCA1 mRNA and protein in different tissues, all of which are essential for the maintenance of body sterol levels and atherosclerosis. For example, the liver contained all three transcripts, with exon1b and exon1d being most abundant. All three transcripts were also found in macrophages. However, when the mice were fed a high-fat diet, exon1b was dramatically upregulated in macrophages but showed no change in the liver. In contrast, transcript exon1d was significantly upregulated in the liver, different from macrophages. These data correlate well with our previous data showing significant upregulation of ABCA1 protein in the liver and macrophages of fat-fed BAC mice (4). Because expression of ABCA1 in macrophages alone is not sufficient to increase plasma HDL-C (29, 30) levels but increasing ABCA1 in the liver results in increased plasma HDL-C (31, 32), the action of ABCA1 transcript exon1d likely contributes directly to the increased plasma HDL-C through the expression of hepatic ABCA1 mRNA and protein. In addition, because the expression of ABCA1 in macrophages resulted in significant decreases in atherosclerotic lesions, even in the face of low plasma HDL-C (30), it is likely that the action of ABCA1 transcript exon1b reduces foam cell formation. Thus, it is likely that ABCA1's function in macrophages, where it is thought to increase lipid efflux and reduce foam cell formation, thereby preventing atherosclerosis, is predominantly influenced by the expression of the exon1b transcript in both humans and mice. It is also likely that exon1d transcripts are the principal source of the increased ABCA1 expression in liver responding to excess cholesterol and therefore have a vital function in the maintenance of plasma and liver lipid levels, particularly HDL, in both species.

Because these alternate transcripts respond differently to the same stimulus, they are likely driven by different promoter elements. We had previously identified three functional LXR elements in the intron 1 region of human ABCA1, upstream of the transcription start sites for all three transcripts (25). We and others (33, 34) also identified upstream CAAT and TATA promoter sequences. In addition, although not functionally confirmed, we have identified two putative PPAR elements that occur upstream of the hExon1b and hExon1d transcripts. These PPAR response elements contain direct repeats separated by a DR1 element and are similar in sequence to the PPRE consensus sequence AGGTCA (35, 36).

Three PPAR subtypes exist, PPARα, PPARγ, and PPARδ, with each showing different tissue distribution patterns and being activated by different factors. All three PPARs bind to the same PPAR element, and the activation of specific PPARs depends on ligand availability, the phosphorylation status of PPARs, and the recruitment of coactivators and corepressors. Thus, it is conceivable that specific PPAR activation may result in their activation of ABCA1 transcripts specifically in one tissue over another or in the activation of one isoform over another, with different functional consequences.

Because high levels of ABCA1 are atheroprotective, considerable interest exists in developing therapeutic compounds aimed at increasing ABCA1 levels. Because our BAC mouse model has previously shown that reductions in lesions are mediated by increased ABCA1 levels and are independent of HDL-C levels (37), therapeutic compounds targeting specific promoter elements associated with transcripts specific for macrophages could give rise to advantageous phenotypes, such as abrogation of atherosclerosis, whereas compounds specifically increasing ABCA1 levels in the liver will result in beneficial effects associated with increased HDL-C levels.

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