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APOLIPOPROTEIN B MESSENGER RNA EDITING IS DEVELOPMENTALLY REGULATED IN PIG SMALL INTESTINE: NUCLEOTIDE COMPARISON OF APOLIPOPROTEIN B EDITING REGIONS IN FIVE SPECIES

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Apolipoprotein B (apo B) mRNA undergoes a posttranscriptional tissue-specific editing reaction which changes codon 2153 from glutamine (CAA) in apoB-100 mRNA to an in-frame stop codon (UAA) in apoB-48 mRNA. Small intestinal apo B mRNA was found to be predominantly (>90%) unedited in fetal (40-day gestational age) piglets but >95% edited in neonatal, suckling and adult animals. By contrast, both fetal, neonatal and adult pig liver contained >99% unedited, apo B-100 mRNA. The nucleotide sequence spanning the edited region in apo B mRNA was found to be highly conserved. We speculate that the regulation of apo B mRNA editing may be developmentally modulated in pig small intestine.

Apolipoprotein B (apo B) is an essential structural component of triglyceride-rich and low density lipoprotein (LDL). Plasma apo B exists in two forms, classified on a centile scale as apo B-100 and apo B-48. In humans, apo B-100 is synthesized in the liver as a mature protein of 4563 amino acids whereas apo B-48, which contains the amino-terminal 2152 amino acids of apo B-100, arises as a result of a novel form of RNA editing in the small intestine (1,2). This editing mechanism changes codon 2153 in apo B-100 mRNA from glutamine (CAA) to an in-frame translational stop codon (UAA) in apo B-48 mRNA.

By contrast with human liver, the rat liver synthesizes both apo B-100 and apo B-48 (3,4). We and others have shown that apo B mRNA editing accounts for this pattern of apoB synthesis in rat liver (5,6) and is responsive to alterations in hepatic lipogenesis (7) and thyroid hormone status (5,8). Recent work suggests that apo B mRNA editing is also developmentally regulated in rat small intestine and liver (9). We have recently demonstrated that apo B mRNA editing is developmentally regulated in human fetal small intestine (10). Furthermore, the progressive

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increase in apo B mRNA editing was associated with a corresponding pattern of apo B synthesis and secretion (10). The current studies were undertaken to examine apo B mRNA editing in fetal, neonatal and adult pig small intestine since this animal demonstrates many similarities to humans in regard to lipoprotein metabolism (11,12).

Materials and Methods

Tissues:

Small intestine and liver of forty day gestational age (fetal), 2 day (newborn), 1-2 week (suckling), and adult (maternal) female domestic swine were surgically removed and washed extensively in ice-cold sterile saline. Portions were snap-frozen in liquid-nitrogen and RNA extracted as described (10,13). Human intestinal and hepatic RNA was prepared as previously described (10).

Primer extension assay:

Analysis of apo B mRNA editing was performed on total RNA with modifications to the method of Driscoll et al (14). Briefly, total RNA (5ug) was denatured at 75 degrees C for 10 minutes and annealed to 32p-end labeled oligonucleotide BT1(5'AATCATGTAAATCATAACTATCTTTAATATACTGA,5'end at 6674, complementary to human apoB cDNA) at 45 degrees C overnight. The annealed products were extended in the presence of 500uM each dATP, dCTP, dTTP and dideoxy GTP (Pharmacia), using 10 units of MMLV reverse transcriptase (BRL) at 42 degrees C for 90 minutes. Products were analyzed by 8% polyacrylamide-urea gel electrophoresis and subjected to autoradiography at -70 degrees C. The ratio of edited to unedited apo B mRNA was determined by laser densitometric scanning.

ApoB DNA Sequence Flanking the Edited Base:

A 514 basepair (bp) fragment flanking nucleotide 6666 in apo B cDNA from nucleotides 6272 to 6786 was amplified from pig and mouse genomic DNA using Taq DNA polymerase with human primers PCR 12 (5'GCCCCAAGAATTTACAATTGTTGC,5'end at nucleotide 6272) and PCR 10 (5'CACGGATATGATAGTGCTCAT,5'end at nucleotide 6786). The amplified fragment was then cloned into M13 [vector mp19] for DNA sequencing. The sequence information was confirmed by repeat analysis using a complementary homologous internal primer (5'CAAGAATTTTCAATGTTGC,5' end at 6765). Intestinal apo B cDNA flanking the edited base was also sequenced following reverse transcription and polymerase chain reaction amplification of total RNA. Sequencing was carried out by dideoxy chain termination (15) using both Klenow and sequenase methods (sequenase DNA sequencing kit, United Biochemical Corporation). Nucleotide sequences were analyzed using the Beckman Microgenie program.

Results

Apo B mRNA editing was analyzed in samples of pig small intestine and liver from fetal, maternal and newborn animals, the latter animals studied following consumption of either low-fat or fat-enriched diets (Fig 1). Under the conditions of the primer extension assay, the primer anneals to apo B mRNA and extends to the first upstream cytidine, located at nucleotide 6666. If nucleotide 6666 of apo B-100 mRNA remains unedited, a product of

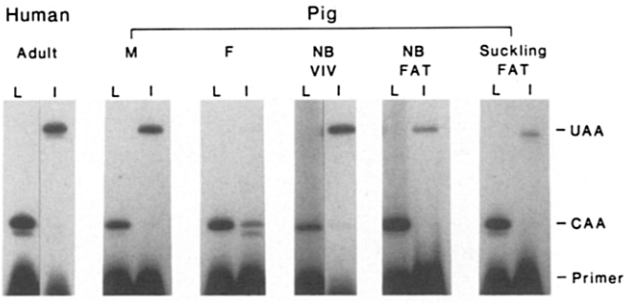

Figure 1.

Developmental regulation of pig intestinal apo B mRNA editing determined by RNA primer extension.

Total RNA from pig liver (L) and small intestine (I) of maternal (M), 40 day fetal (F), newborn (NB) animals-the latter fed either a low-fat (VIVONEX, VIV) or fat-rich diet (FAT) - and suckling animals were used to quantitate unedited (CAA) and edited (UAA) apo B mRNA. Total RNA from human adult liver (L) and small intestine (I) was assayed in parallel and shown for comparison. A representative example of 2 individual assays, each conducted in duplicate, is shown.

43 bases is produced (CAA) whereas, if nucleotide 6666 is edited to a U, the primer extends to the next upstream C at nucleotide 6655 with an extension product of 54 bases (UAA), conditions identical in both human and pig (Fig. 1). Apo B mRNA editing was detectable (approximately 8% UAA) in fetal pig small intestine at 40 days gestation, the only time-point examined. Attempts to biosynthetically label the apo B species, in-vivo, in this setting were unsuccessful (data not shown). Newborn, suckling, and maternal intestinal apoB mRNA all contained predominantly edited (UAA) transcript (95%) with no differences in the newborn attributable to dietary fat. In this setting we have previously shown that only apo B-48 is synthesized (12). By contrast, there was no detectable edited transcript from either fetal, newborn, or adult liver, all containing >99% unedited apo B mRNA (CAA). The results of primer extension were confirmed by direct sequencing of intestinal apo B cDNA between nucleotides 6565-6765 which showed an identical sequence to genomic DNA with the exception of nucleotide 6666.

A species comparison of the DNA and translated amino acid sequence spanning the apo B mRNA editing region - corresponding to nucleotides 6408-6765 and residues 2092-2185 of human apoB cDNA - is shown in Figures 2A and 2B respectively. The pig, rabbit, rat, and mouse sequences are aligned with the human sequence to illustrate the conservation in this region. The human, rabbit, rat, and part of the mouse sequences have been previously characterized (1,5,16,17). This region is highly conserved among the five species (Table 1) and the homology is greater at the

```
A       6482                                                6522
Human   CCCACAGCAAGCTAATGATTATCTGAATTCATTCAATTGGGAGAGACAAGTTTCACATGC
Pig     ----------T--------------G-A------G------A----------TGAG---
Rabbit  ----------T--------------G-A-----------------------CAG---
Rat     T--T-----GAT-C--------------G---CTG-C--------------AG-TGG---
Mouse   T--T-----GAT-C--C----------G---CTG-C--------------AG-TGG---

        6542                                                6582
Human   CAAGGAGAAACTGACTGCTCTCACAAAAAAGTATAGAATTACAGAAAATGATATACAAAT
Pig     ---AA------AT-G--A-T---TGG--G-T--------------------G---G---
Rabbit  ------------A---A--T----------T----A---------G-------------C
Rat     ------A---T-A---T--T---TGG----C--------------T------G---T---
Mouse   ------A---A-A---T--T---TGG----T--------------T------G---T---

        6602                                                6642
Human   TGCATTAGATGATGCCAAAATCAACTTTAATGAAAAACTATCTCAACTGCAGACATATAT
Pig     ---------CA--------------C--------------A----G--A--A------G-
Rabbit  ------G---A----------------A-----------G--------T---------G-
Rat     ---C------AG---------------G----------C--------TG-------CGC
Mouse   ---CA-----AG---------------C----------C--------TG-------CGC

        6662                                                6702
Human   GATACAATTTGATCAGTATATTAAAGATAGTTATGATTTACATGATTTGAAAATAGCTAT
Pig     ----------------------------A-----------------T--G-C------
Rabbit  ----------------------------A--T----C----------T----------
Rat     ----------------------G-----A-------GC---G--C--A----G-A----
Mouse   ----------------------------A-------CC------C--A----G-A----

        6722                                                6762
Human   TGCTAATATTATTGATGAAATCATTGAAAAATTAAAAAGTCTTG
Pig     -----GG-------------------C--C---G----T-----
Rabbit  A----G------A---C-------G-----------T-----
Rat     ----C-G---------AG-----------GC------TG----
Mouse   ----G-G---------CG-----------G-------TG----
```

Figure 2A.
Aligned nucleotide sequences of the apo B mRNA editing regions in five species. The numbering corresponds to the published human apoB-100 sequence (16). The complete human sequence is provided on the top line, and nucleotide differences from the human sequences in the other four species compared are aligned beneath. Hyphens represent nucleotides identical to the human sequence. The nucleotides corresponding to the apo B mRNA editing codon is highlighted.

```
B       2092                                                2132
Human   PQQANDYLNSFNWERQVSHAKEKLTALTKKYRITENDIQIALDDAKINFNEKLSQLQTYM
Pig     ---V----ST-S-----LS--K-HSDFMED-------VR----N----L----T-----V
Rabbit  ---V----ST--------S------TF--N-K-------T---N----L----------V
Rat     ---IH----ASD-----AG------SFMEN----D--VL----S----L-------E--A
Mouse   ---IHH---ASD-----AG----I-SFMEN----D--VL--I-S-----------E--A

        2152                                2182
Human   IQFDQYIKDSYDLHDLKIAIANIIDEIIEKLKSL
Pig     ---------N-----F-T---R------AT--I-
Rabbit  ---------NF----F-----S---Q-M----I-
Rat     -------R-N--AQ---RT--Q---R------M-
Mouse   ---------N--P----RT--E---R------M-
```

Figure 2B.
Aligned amino acid sequences of apo B mRNA editing regions in five species. The numbering corresponds to the published human apo B-100 sequence (16). The complete human amino acid sequence is provided on the top line, and amino acid residue differences are indicated below. Hyphens represent amino acid residues identical to the human sequence. The single letter code for amino acids is employed.

Table 1

Nucleotide Homology Surrounding the Apo B mRNA Editing Regions of Five Species

	Human	Pig	Rabbit	Rat	Mouse
Human	-	85.6	89.4	80.1	80.6
Pig	85.6	-	85.2	77.3	77.8
Rabbit	89.4	85.2	-	79.7	81.3
Rat	80.1	77.3	79.7	-	94.4
Mouse	80.6	77.8	81.3	94.4	-

The comparisons are expressed as percent homology between pairs of sequences. The homologies between nucleotide 6408-6765 were calculated using the Microgenie Compare/Align program.

nucleotide than at the amino acid level. The overall nucleotide homology was higher between human, pig, and rabbit than between human, rat and mouse. However, the nucleotide homology between rat and mouse sequences exceeded 94%. The minimum requisite sequence of 26bp required for apo B mRNA editing in vitro is identical in the pig to that described for other species and appears enriched in adenosine and uridine compared to other regions of apo B mRNA (17).

Discussion

This study demonstrates that pig intestinal apo B-48 is generated by a conserved RNA editing mechanism. This process is relatively inactive in fetal small intestine, but was not detectable in any sample of hepatic RNA. Although newborn piglets were found to have an indistinguishable pattern of intestinal apo B mRNA editing to that found in adult animals, the exact timing of the developmental switch remains uncertain. Additionally, it remains unclear as to whether intestinal apo B-100 mRNA is actively translated in the fetal pig. Our recent studies of human fetal small intestinal apo B mRNA editing suggest a progressive increase in editing during the second trimester, with active synthesis and secretion of both apo B isoforms (10). The timing of human fetal small intestinal apo B mRNA editing contrasts with recent observations in the rat where small intestinal apo B mRNA editing reaches adult levels just prior to birth (9). Furthermore, rat hepatic apo B mRNA editing demonstrated a developmental profile distinct from either of these patterns, achieving adult levels 24 days after birth (9). The evidence thus

points to the existence of both species-specific and tissue-specific factors in the developmental regulation of apo B mRNA editing.

Although the molecular mechanisms underlying this developmentally regulated process remain unknown, our recent observations point to the likely involvement of both metabolic and hormonal factors (5,7-8). In particular, we speculate that the temporal emergence of intestinal apo B mRNA editing may be related to the functional commitment of the small intestine as a lipogenic organ. However, the nature of the presumed advantage of apo B mRNA editing in the developing gut remains unclear. The conservation of nucleotide sequence spanning the RNA editing region implies a functional definition of the stringency requirements for apo B mRNA editing. On the other hand recent studies, using site-specific mutagenesis, have demonstrated a relatively lax sequence requirement immediately flanking nucleotide 6666 (18). Thus the precise structural requirements for apo B mRNA editing have yet to be defined.

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