The apical sodium-dependent bile salt transporter (Asbt) in sea lamprey (*Petromyzon marinus*) transports early evolved 5α bile alcohols but not modern 5β bile acids

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ASBT transports bile salts in humans and rodents, in which the major bile salts are the "modern" C24 bile acids with C5-hydrogen at the β-configuration. It is not known whether Asbt in the evolutionarily primitive sea lamprey can transport "modern" bile salts as it uses "ancient" 5α-bile alcohols, which are structurally very different bile salts. Our molecular characterization indicates that lamprey Asbt transports "ancient" 5α bile alcohols but not "modern" 5β bile acids. This finding also suggests that the bile salt enterohepatic circulation is a conserved function throughout vertebrate evolution.

In vertebrates, the liver excretes bile salts into the intestine, where they facilitate absorption of dietary lipids. In humans and rodents, bile salts are re-absorbed in the terminal ileum and transported back to liver to maintain an enterohepatic circulation[4]. In this process, the apical sodium-dependent bile acid transporter (ASBT/SLC10A2) plays a pivotal role in reabsorption of bile salt in the intestine of these species[3]. We have previously demonstrated that Asbt in the little skate (*Leucoraja erinacea*) transports both scymnol sulfate and taurocholic acid (TCA) in a sodium-dependent manner[2], indicating that Asbt plays the same role in the enterohepatic circulation of bile salts in skate as it does in mammals. However, it is not known whether the ASBT orthologs in the sea lamprey (*Petromyzon marinus,* here after lamprey) has the same function, since the structure of the lamprey bile salts are 5α-bile alcohols. In this report, we characterize Asbt at the structural and functional level in sea lamprey.

Upstream migratory sea lamprey were caught in the Kennebunk River in southern Maine and maintained at MDIBL in 11˚C fresh water tanks for up to 3 weeks with 12 hours light-dark cycle. Everted gut sac experiments were performed as we previously described in 16˚C lamprey Ringers solution (with or without sodium) supplemented with 50 μM ^{3}H-taurocholic acid (^{3}H-TCA, 2 mCi/mmol), and incubated for 1 hr with gentle agitation. The sacs were then washed in ice-cold medium and homogenized. The concentration of ^{3}H-TCA in the lysate was measured in a liquid scintillation counter. BLAST search of lamprey genome was performed to identify ASBT orthologs candidate genes. Reverse-transcription PCR (RT-PCR) was used to clone a fragment of lamprey Asbt (lpAsbt) followed by 5´ and 3´ RACE PCR to obtain the full-length cDNA. A luciferase-based ASBT-farnesoid X receptor α reporter (FXR/NR1H4) assay was utilized to detect lpAsbt transport activity for bile salts, because conjugated bile salts require a specific transporter (*e.g.,* ASBT) to cross the cell membrane, and they are ligands for human FXR[1]. Thus transactivation of FXR reflects the ability of the ASBT to transport a given bile salt into the transfected cells.

Gut sac experiment using adult lamprey intestine failed to show sodium-dependent transport activity for ^{3}H-TCA. However, molecular cloning identified an ortholog of ASBT in lamprey intestine, which encodes 363 amino acids (Genbank Acc #: JX014266) and shares 58% sequence identity to human ASBT. Real-time RT-PCR revealed that lpAsbt mRNA was expressed primarily in the kidney and intestine, less in testes, heart and brain, and was undetectable in muscle, gill and liver in adult lamprey. A similar expression pattern was also found in lamprey larva. Further analysis indicated that lpAsbt mRNA is most abundant in the distal intestine. Recombinant expression of lpAsbt in transfected COS-7 cells confirmed that lpAsbt was not able to transport ^{3}H-TCA, although Western blot assay detected expression of FLAG-tagged lpAsbt protein in these cells. However, a luciferase-based ASBT-FXR reporter assay indicated that lpAsbt transports 5α-petromyzonol sulfate (5α-PZS) and 5α-cyprinol sulfate, two "ancient" bile salts, with high affinity, while having low affinity for scymnol sulfate, and no activity for the "modern" bile salt TCA (Fig 1). Adult lamprey liver extract also transactivated FXR when lpAsbt was cotransfected in this reporter assay. In contrast, human ASBT demonstrated transport activity for all of the bile salt structures stated above, as well as several other modern bile salts, including tauroursodeoxycholic acid (TUDCA), taurochenodeoxycholic acid (TCDCA), TCA, glycocholic acid and glycoursodeoxycholic acid.

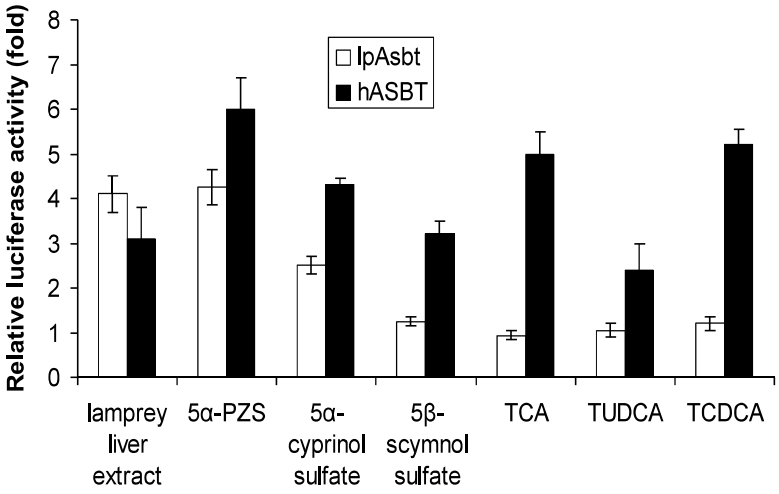

Figure 1. A dual-luciferase reporter assay demonstrates that lamprey Asbt is a bile salt transporter, although its substrate specificity is limited to just the evolutionarily "ancient" bile salt. HEK293T cells were transfected with FXR, RXR, hIBABP-Luc and Rnilla Luc, as well as control vector, or lpAsbt, or human ASBT (hASBT) vectors. Transfected cells were treated with 1 μM indicated bile salt or ~1 μM bile salts from adult lamprey liver. Luciferase readings of this analysis are relative to cells transfected with the corresponding vector control by setting its value as 1. All values represent at least three independent experiments and are expressed as means ± SD.

In summary, we demonstrated that lamprey Asbt is a bile salt transporter, although its substrate specificity was limited to the early evolved bile salts. These findings also indicated that the enterohepatic circulation of bile salts is a conserved function throughout vertebrate evolution. Further analysis and comparison of the sequence and function of lpAsbt and human ASBT will help identify ±human ASBT structural determinants for bile salt substrate specificity

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