Regulation of the gene encoding the intestinal bile acid transporter ASBT by the caudal-type homeobox proteins CDX1 and CDX2

Li Ma,1 Moritz Jüttner,1 Gerd A. Kullak-Ublick,1,2 and Jyrki J. Eloranta1,2

1Department of Clinical Pharmacology and Toxicology, University Hospital Zurich; and 2Zurich University Research Priority Programme “Integrative Human Physiology” (ZIHP), Zurich, Switzerland

Submitted 11 March 2011; accepted in final form 16 October 2011

Bile acids are synthesized as a result of cholesterol catabolism in the liver, and they function as physiological detergents to facilitate the intestinal absorption of dietary fats and fat-soluble vitamins. The apical sodium-dependent bile acid transporter (ASBT; gene symbol SLC10A2) is a 48-kDa transmembrane protein expressed in the terminal ileum, biliary tract, and renal proximal tubule cells. At the apical membrane of ileal enterocytes, ASBT is the chief mediator of active sodium-dependent intestinal bile acid absorption (11). Bile acids are then effluxed into the portal blood via the heterodimeric organic solute transporter-α/β (OSTα/β) at the basolateral membrane. Together with the basolateral bile acid uptake system Na+-taurocholate cotransporting polypeptide (NTCP) at the sinusoidal membrane of hepatocytes and bile salt efflux pump (BSEP) at the liver canicular membrane, ASBT and OSTα/β maintain efficient enterohepatic bile acid circulation (1, 17).

Prior studies have shown that ASBT gene expression is controlled at the transcriptional level in a complex manner: known transcriptional regulators of the human ASBT promoter include the glucocorticoid receptor (GR) (22), the hepatocyte nuclear factor-1α (HNF-1α) (23, 40), and the peroxisome proliferator-activated receptor-α (PPARα) (23). Both serum (12) and certain bile acids (13) appear to elevate ASBT expression via a response element for the activator protein-1 (AP-1). In a knockout mouse model, the transcription factor Gata4 regulates the jejunal-ileal gradient of ASBT expression (4). Alterations in ASBT function or ASBT gene expression have been associated with intestinal diseases in humans: mutations in the ASBT gene can lead to bile acid malabsorption (33), whereas reduced ileal ASBT protein (22) or mRNA (45) levels have been found in patients with inflammatory bowel disease (IBD). It was recently shown that the expression levels of three bile acid transporters [ASBT, the multidrug resistance protein-3 (MRP3), and the ileal bile acid-binding protein (I-BABP)] are elevated in esophageal epithelial cells from patients who are diagnosed with Barrett’s esophagus (BE) and are again decreased or lost in esophageal adenocarcinoma at both mRNA and protein levels (16). However, the precise mechanisms, which mediate the aberrant expression of the ASBT transporter gene in BE, are not known. BE is a premalignant lesion to esophageal adenocarcinoma, where squamous epithelium at the distal end of the esophagus is replaced by an intestine-like epithelium. The molecular origins of BE are not yet fully understood. One theory suggests that bile acids in the gastroesophageal reflux contents cause tight junctions to break in squamous cells, allowing them to leak into the basal layer, thus inducing cell transdifferentiation (37). Another recent study by Wang et al. (48) suggests that the transcription factor p63 plays a role in the etiology of BE (44).

Caudal-type homeobox-1 (CDX1) and -2 (CDX2) are transcriptional factors of the homeodomain family, and homologs of the Drosophila melanogaster protein Caudal that is essential for the early anterior-posterior development and body patterning (19). Despite the high level of amino acid identity (95%) within their DNA-binding domains, they play at least partially distinct roles in the intestine. Cdx1−/− mice exhibit a shift in the body frame (39), and Cdx2+/− heterozygous mutant mice develop colonic tumors (10). These two mouse models also show differential responses upon inflammatory challenge (7). Complete Cdx2-null mice are embryonic lethals, as evidently are Cdx1/Cdx2 double-null mice. In adult mammals, CDXs/Cdxs are expressed exclusively in the small and large intestine, playing important roles in proliferation and differentiation of intestinal epithelial cells. However, their levels have been reported to be aberrantly elevated in esophageal metaplasia, such as BE, compared with the adjacent normal tissue (36, 41). Similarly to the bile acid transporters, CDX2 expression has been reported to be decreased upon progression of esophageal metaplasia into high-grade adenocarcinoma (21, 42). A number
of CDX1 and CDX2 transcriptional target genes have been identified so far. These include marker genes for intestinal differentiation, such as sucrase isomaltase (5), mucin 2 (32), cytokeratin 20 (9), and desmocollin 2 (20), as well as genes encoding intestinal transporters, such as peptide transporter 1 (PEPT1; gene symbol SLC15A1) (35) and sodium-coupled monocarboxylate transporter 1 (SCMT1; gene symbol SLC5A8) (24). Furthermore, it has been suggested that CDX1 and CDX2 can regulate their own expression in esophageal cell lines via both auto- and crossregulation (26).

Aberrant esophageal expression of both ASBT and CDXs in BE prompted us to study whether the expression of the ASBT gene is regulated by the CDXs.

MATERIALS AND METHODS

Chemicals. Deoxyadenosine 5’-[α-32P]triphosphate (6,000 Ci/mmol) was purchased from Perkin Elmer (Schwabenbuch, Switzerland). Restriction enzymes were from Roche Diagnostics (Rotkreuz, Switzerland) and PuReTaq Ready-To-Go PCR beads from GE Healthcare (Grellbrugg, Switzerland). The oligonucleotides (Table 1) were synthesized by Microsynth (Balgach, Switzerland). Other chemicals were purchased from Sigma-Aldrich (Buchs, Switzerland), unless stated otherwise.

Cell culture. All cell lines were obtained from LGC Promochem (Molsheim, France). Human esophagus-derived cell line Het-1A was cultured in bronchial epithelial cell growth medium (BEGM) supplemented with SingleQuot kit (Lonza, Basel, Switzerland). Human esophagus OE19 cells were cultured in RPMI-1640 + 2 mM L-glutamine. Human colon-derived T84 cells were cultured in DMEM/F-12 (Invitrogen, Basel, Switzerland), Caco-2 cells in DMEM (Sigma Aldrich, Basel, Switzerland) and PuReTaq Ready-To-Go PCR beads from GE Healthcare (Glattbrugg, Switzerland). The oligonucleotides (Table 1) were synthesized by Microsynth (Balgach, Switzerland). Other chemicals were purchased from Sigma-Aldrich (Buchs, Switzerland), unless stated otherwise.

Reverse transcription and real-time PCR. Four micrograms of RNA were reverse transcribed with high-capacity cDNA reverse transcription kit (Applied Biosystems, Rotkreuz, Switzerland) before the real-time PCR analysis using the TaqMan assays Hs00156451_m1 (CDX1), Hs00230919_m1 (CDX2), Hs00166561_m1 (ASBT), Hs00167041_m1 (HNF-1α), Hs00200229_m1 (villin), or human ACTB (beta-actin) endogenous control (Applied Biosystems) on a 7900HT fast real-time PCR system (Applied Biosystems). The relative mRNA levels were calculated by the comparative threshold cycle method. Within an experiment, all tests were performed in triplicate.

Reporter gene constructs and expression vectors. The human CDX1 and CDX2 expression constructs (kind gifts from Dr. Juan Lucio Iovanna) in the pcDNA3.1 vector (Invitrogen) were created as described in Ref. 31. Mutations in the constructs were corrected to wild-type by site-directed mutagenesis (QuikChange, Stratagene, La Jolla, CA). Cloning of the human ASBT (−1,688/+525) (23) and ASBT (−830/+525) (22) promoter constructs has been previously described. The human ASBT promoter fragment −99/+525 was amplified using oligonucleotides shown in Table 1 and the promoter

Table 1. Sequences of oligonucleotides used for cloning, EMSAs, and ChIP assays

<table>
<thead>
<tr>
<th>Oligonucleotide</th>
<th>Sequence (5'-3')</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cloning oligonucleotides</td>
<td>hASBT −99/+525 forward</td>
</tr>
<tr>
<td></td>
<td>hASBT −99/+525 reverse</td>
</tr>
<tr>
<td></td>
<td>mAsh2 −1810/+3 forward</td>
</tr>
<tr>
<td></td>
<td>mAsh2 −1810/+3 reverse</td>
</tr>
<tr>
<td></td>
<td>rAsh2 −2975/+147 forward</td>
</tr>
<tr>
<td></td>
<td>rAsh2 −2975/+147 reverse</td>
</tr>
<tr>
<td>EMSA oligonucleotides</td>
<td>CDXRE_1_top</td>
</tr>
<tr>
<td></td>
<td>CDXRE_2_top</td>
</tr>
<tr>
<td></td>
<td>CDXRE_3_top</td>
</tr>
<tr>
<td></td>
<td>CDXRE_4_top</td>
</tr>
<tr>
<td></td>
<td>CDXRE_5_top</td>
</tr>
<tr>
<td></td>
<td>CDXRE_1_top_mut</td>
</tr>
<tr>
<td></td>
<td>CDXRE_2_top_mut</td>
</tr>
<tr>
<td></td>
<td>CDXRE_3_top_mut</td>
</tr>
<tr>
<td></td>
<td>CDXRE_4_top_mut</td>
</tr>
<tr>
<td></td>
<td>CDXRE_5_top_mut</td>
</tr>
<tr>
<td></td>
<td>CDXRE_6_top_mut</td>
</tr>
<tr>
<td></td>
<td>CDX_consensus</td>
</tr>
<tr>
<td>ChIP oligonucleotides</td>
<td>hASBT_forward −736</td>
</tr>
<tr>
<td></td>
<td>hASBT_reverse −154</td>
</tr>
<tr>
<td></td>
<td>hASBT_forward intron 1</td>
</tr>
<tr>
<td></td>
<td>hASBT_reverse intron 1</td>
</tr>
</tbody>
</table>

Where applicable, restriction sites introduced are underlined and the corresponding enzymes used are given in parentheses. Only the top strands are shown for oligonucleotides used in electrophoretic mobility shift assays (EMSAs). CDXRE consensus motifs are indicated in bold, and introduced mutations are in italics. ChIP, chromatin immunoprecipitation assay.
construct ASBT(−1,688/+525) as the template. The PCR fragment was cloned into the pGEM-T vector (Promega, Dübendorf, Switzerland), and further subcloned into the pGKL3 basic vector (Promega). The mouse Asbt promoter fragment (−1,810/+3) and the rat Asbt promoter fragment (−2,975/+147) were cloned from mouse or rat, respectively, genomic DNA (Clontech) using PCR oligonucleotides shown in Table 1. Both PCR products were cloned into pGEM-T vector and further subcloned into the pGKL3 basic vector. To create the pTNT-CDX constructs for in vitro translation, the CDX1 and CDX2 cDNA fragments were cut from the pcDNA3.1 constructs with EcoRI and subcloned into the EcoRI restriction site of the expression vector pTNT (Promega). The identities of all PCR-cloned fragments were verified by DNA sequencing (Microsynth).

Transient plasmid transfections and luciferase reporter assays. To study the effect of CDX overexpression on endogenous ASBT mRNA expression, OE19 cells were seeded on 12-well plates at the density of 250,000 cells/well the day before transfections. Cells were transfected with 2.5 μg of either pcDNA3.1 vector control or 2.5 μg of either CDX expression construct, using the plasmids pTNT-CDX1 and pTNT-CDX2 as templates, were generated with TnT T7 coupled reticulocyte lysate system (Promega) prepared using the NE-PER extraction kit (Pierce, Lausanne, Switzerland). Six micrograms of LS174T nuclear extracts were used for each well. In the top strand and a 5′ signed to have a 5′out overhang in the bottom strand when annealed, allowing radioactive labeling by fill-in reactions. Fifty nanograms of annealed oligonucleotides were labeled in 20-μl of reaction buffer, 250 nM dGTP/dCTP/dTTP, and 1 μCi of [α-32P]-dATP. Unincorporated nucleotides were removed using Microspin G-25 columns (GE Healthcare). Six micrograms of LS174T nuclear extracts prepared using the NE-PER extraction kit (Pierce, Lausanne, Switzerland) or 1.5 μl of in vitro translated CDX1/CDX2 proteins each, generated with TnT T7 coupled reticulocyte lysate system (Promega) using the plasmids pTNT-CDX1 and pTNT-CDX2 as templates, were used per DNA-binding reaction. Protein-DNA complexes were formed in the binding buffer [20 mM Tris·HCl, pH 8.0; 60 mM KCl; 2 mM MgCl2 12% (vol/vol) glycerol; 0.3 mM DTT; 87.5 ng/μg of one of the following antibodies: anti-CDX1 (CDX1 Ab1), anti-CDX1 (ab24000, Abcam, Cambridge, UK) (CDX1 Ab2), anti-CDX2 (71–83, Sigma) (CDX2 Ab1), anti-CDX2 (MU392A-100, Biogenex, Fremont, CA) (CDX2 Ab2). Two ampiclons were assayed for immunoprecipitated samples, using the oligonucleotide primers listed in Table 1, containing either the ASBT promoter region (−736/−154) or a region from the first intron of the ASBT gene. After the initial denaturation stage at 94°C for 3 min, the PCR cycling conditions were 94°C for 20 s, 60°C for 30 s, and 72°C for 1 min. For the ASBT promoter region, 5 μl of each PCR product were taken after 38 cycles, and for the ASBT intron fragment, 5 μl of each PCR product were taken after 30 cycles. The ASBT promoter and intronic PCR fragments were resolved on 1.5% agarose gels, containing GelRed gel stain (Chemie Brunschwig, Basel, Switzerland).

Human tissue collection and processing. Signed, informed consent forms were collected for all human subjects. The study protocol and consent forms were approved by the Ethical Committees of the sample collections sites. A total of 35 patients were included in the study. BE tissue was collected at the Department of Gastroenterology, Qingdao Municipal Hospital, China; Department of Gastroenterology, Shanghai General Hospital of Chinese People’s Armed Police Forces, Shanghai, China; Department of Gastroenterology, General Hospital of Chinese People’s Armed Police Forces, Beijing, China in 2011. BE were diagnosed endoscopically and histologically on the basis of typical features such as the presence of specialized columnar epithelium and goblet cells. Histology was performed by an experienced local pathologist. Of the 35 patients, 22 (63%) were men and 13 (37%) were women. The mean age ± SD. Differences among patients, 29/35 (83%) had detectable CDX1 mRNA expression, 35/35 (100%) had detectable CDX2 expression, and 20/35 (57%) had detectable ASBT expression. The BE samples were homogenized by ultrason. RNAs were isolated using the TRizol reagent (Invitrogen) and were quantified with a NanoDrop ND-1000 spectrophotometer (Witec, Littau, Switzerland). Ileal biopsies were obtained from 48 patients, 17 (35%) had detectable ASBT expression. The BE samples were homogenized by ultrasound. RNAs were isolated using the TRizol reagent (Invitrogen) and were quantified with a NanoDrop ND-1000 spectrophotometer (Witec, Littau, Switzerland). Ileal biopsies were obtained from 48 healthy patients during routine surveillance colonoscopies in the endoscopy unit of the Division of Gastroenterology and Hepatology at the University Hospital Zurich between 2004 and 2008. Of the 48 patients, 17 (35%) were men and 31 (65%) women. The mean age ± SD of the cohort was 54.3 ± 13.7 yr, and the median age was 58.5 yr. Ileal samples were collected in RNAlater (Ambion, Austin, TX) and syringed into TRizol reagent before RNA isolation. RNAs were quantified with a NanoDrop ND-1000 spectrophotometer.
with Tukey’s post hoc test. P values of <0.05 were considered significant. For the correlation of mRNA expression levels in human tissue material, logarithmic values of mRNA levels were analyzed using linear regression. All statistical analysis was carried out using GraphPad Prism (GraphPad Software, San Diego, CA).

RESULTS

Modulation of endogenous ASBT mRNA expression levels by CDX knockdowns or overexpression. To investigate whether endogenous human ASBT mRNA levels are regulated by CDX1 and CDX2, we performed knockdown experiments with pools of siRNAs specifically targeting CDXs. For this purpose, we chose the human colon cancer cell line T84, which endogenously expresses both CDX1, CDX2, and ASBT, all at detectable levels. By targeting the endogenous CDX1 or CDX2 with specific siRNAs, ASBT mRNA was significantly decreased compared with its expression in control cells (Fig. 1A). Upon CDX1 siRNA treatment, there was an 80% loss of endogenous CDX1 mRNA, and no decrease of the endogenous CDX2 mRNA level (Fig. 1B). Conversely, upon CDX2 siRNA treatment, there was a significant loss of endogenous CDX2 mRNA, but endogenous CDX1 mRNA expression level remained unchanged (Fig. 1C). The siRNA knockdowns thus worked in a gene-specific manner. In a converse approach, we transiently overexpressed CDX1 and CDX2 in human esophagus-derived OE19 cells. As shown in Fig. 1D, endogenous mRNA levels of ASBT were significantly increased upon exogenous expression of CDX1 and CDX2 in the esophageal cell line.

CDX1 and CDX2 transactivate the ASBT promoter. To further study the possible direct role for CDX1 and CDX2 in the regulation of the ASBT promoter, we transiently transfected two human esophagus-derived cell lines and three human colon-derived cell lines with expression plasmids for human CDX1 and CDX2 together with the ASBT (−1,688/+525) promoter-luciferase construct. Both CDXs increased the ASBT promoter activity in the two esophageal cell lines, OE19 (Fig. 2A) and Het-1A (Fig. 2B). Potent CDX-mediated activation of the ASBT promoter was also observed in intestinal T84 (Fig. 2C), Caco-2 (Fig. 2D), and LS174T cells (Fig. 2E). A comparative study with the human, mouse, and rat ASBT/Asbt promoter-luciferase constructs transfected into Caco-2 and murine IEC-4.1 cells revealed an increased activity of the mouse Asbt promoter by both CDX1 and CDX2 in both cell lines, whereas the rat Asbt promoter was significantly activated by both CDX1 and CDX2 in IEC-4.1 cells but only by CDX2 in Caco-2 cells (Fig. 1, F and G).

In silico analysis of the ASBT promoter region. Next, we performed an in silico analysis on the proximal 850 base pairs of the human ASBT promoter (NCBI reference sequence: NT_009952.14) to identify putative CDX response elements (CDXREs). The consensus core motif for CDX DNA-binding is 5′-TTTA/C-3′ or 5′-G/AATAA-3′ (19). We identified nine putative CDXREs within the proximal ASBT (−850/+1) promoter region by MatInspector software (8) and visual inspection (Fig. 3).

The region containing the predicted CDX binding sites mediates ASBT promoter activation. To study whether the proximal promoter region containing the predicted CDXREs can mediate the CDX-dependent activation, we used two ASBT promoter deletion constructs in transient transfections in

Fig. 1. Modulation of endogenous apical sodium-dependent bile acid transporter (ASBT) mRNA expression levels by CDX1 and CDX2. A–C: ASBT mRNA expression levels are reduced upon knockdown of endogenous CDX1 and CDX2 expression in T84 cells. Cells were transfected with control nontargeting siRNA, siCDX1, or siCDX2 at a final concentration of 50 nM twice at a 24-h interval and harvested after 48 h. The ASBT, CDX1, and CDX2 mRNA expression levels are normalized to those obtained for beta-actin. A: knockdown of either CDX1 or CDX2 leads to a significant decrease in ASBT mRNA expression. B: CDX1 siRNAs significantly reduce CDX1 but not CDX2 expression. C: CDX2 siRNAs significantly reduce CDX2 but not CDX1 expression. D: exogenous expression of CDX1 and CDX2 significantly increases endogenous ASBT mRNA levels in human esophagus-derived OE19 cells. Significant difference: **P < 0.01; ***P < 0.001.
Caco-2 cells. The construct ASBT (−830/+525), which contained all the predicted CDXREs, was potently activated by both CDX1 and CDX2, whereas the construct ASBT (−990/+525), lacking all predicted CDXREs, exhibited significantly reduced activation (Fig. 4). The activation by CDXs was not entirely abolished, suggesting that there may be further CDXREs located in the 5′-UTR of the ASBT gene. We further performed individual site-directed mutagenesis on all predicted CDXREs, but disruption of any single element was not sufficient to efficiently reduce the CDX-mediated transactivation of the ASBT promoter in reporter assays, implying that several of the predicted CDXREs are involved in this regulation (data not shown).

CDX1 and CDX2 bind to six of the nine predicted CDX response elements in vitro. To study the potential in vitro interaction between CDX1 and CDX2 with their predicted binding motifs within the ASBT promoter, we performed EMSAs using nuclear extracts derived from LS174T cells, in which both CDX1 and CDX2 are endogenously expressed, using both wild-type and mutant predicted CDX response element as radiolabeled probes. Two DNA-protein complexes were formed on the CDXREs 2, 3, 4, 7, 8, and 9, which were abolished by addition of either CDX1 (the faster complex) or CDX2 antibodies (the slower complex) (Fig. 5A). Addition of unrelated antibodies raised against nuclear receptor transcription factors PPARα or RXRα did not affect CDX-DNA com-

---

Fig. 2. CDX1 and CDX2 transactivate the human ASBT (A–E), mouse (mAstb; F and G), and rat (rAstb; F and G) Astb promoters. Two hundred nanograms of CDX1 or CDX2 expression plasmids were transfected into OE19, Het-1A, T84, Caco-2, and LS174T cells, together with 400 ng ASBT (−1,688/+525) promoter luciferase construct. Reporter activities were measured 36 h later. A: OE19 cells. B: Het-1A cells. C: T84 cells. D: Caco-2 cells. E: LS174T cells. F: Caco-2 cells. G: IEC-4.1 cells. Significant difference: **P < 0.01; ***P < 0.001; ns, not significant.
CDX REs were introduced into the oligonucleotides and
plex formation. When mutations designed to interfere with
CDX binding were introduced into the oligonucleotides and
used as the radioactively labeled probes, the formation of the
two CDX-DNA complexes was abolished or strongly reduced.
The other putative CDX REs within the proximal ASBT pro-
moter (CDX REs 1, 5, and 6) predicted in silico failed to form
specific complexes with CDXs in EMSAs (data not shown). To
further confirm the specificity of CDX1 and CDX2 binding to
the CDX REs within the ASBT promoter, we performed EMSA
competition experiments (Fig. 5B), using the CDX consensus
binding site as a radiolabeled probe and increasing molar excesses of unlabeled competitor oligonucleotides harboring the ASBT promoter CDX REs. The binding of recombinant in vitro translated CDXs to their consensus binding site was competed off in a dose-dependent manner, upon addition of competitor oligonucleotides containing each wild-type CDX RE of the ASBT promoter. Mutated CDX elements were incapable of, or significantly less efficient in, competing for CDX binding. These data indicate that CDXs are capable of specifically binding to CDX-responsive elements in the proximal ASBT promoter sequence in vitro.

CDX1 and CDX2 interact with the proximal ASBT promoter region within living cells. To confirm the interaction between CDX1 and CDX2 with the ASBT promoter also within living cells, ChIP assays were performed using nuclear proteins extracted from T84 cells in which CDX1, CDX2, and ASBT are all endogenously expressed. As shown in Fig. 6, top, two different CDX1 and two CDX2 antibodies were efficient in precipitating the promoter region ASBT (−736/−154), whereas the nonspecific mouse IgG antibodies failed to precipitate the ASBT promoter region. As an additional specificity control, we amplified a 221-bp region from the first intron of the ASBT gene using the same ChIP samples as templates. None of the CDX antibodies was able to precipitate this intronic region of the ASBT gene (Fig. 6, bottom).

Correlation analysis of CDX, ASBT, and HNF-1α expression levels in human Barrett’s esophagus tissue and ileal tissue. To study whether CDXs or the known transactivator of the ASBT promoter, HNF-1α (34), may be chief factors in maintaining the expression of ASBT mRNA in human Barrett’s esophagus tissue, we measured their expression in RNAs isolated from esophageal biopsies derived from BE patients. In total, tissue was obtained from 35 patients, out of which 20, who expressed mRNAs of all genes of interest, were included in the analysis. ASBT mRNA levels significantly correlated with those of CDX1 (Fig. 7A), CDX2 (Fig. 7B), and HNF-1α.
healthy ileum (Fig. 7C). We further observed a strong and significant correlation between the mRNA expression levels of CDX1 and CDX2 (Fig. 7D). In addition to the BE tissue, we also investigated the potential correlation between CDX, HNF-1α, and ASBT expression levels in ileal tissue derived from 48 healthy subjects. In the ileum, ASBT mRNA levels were not significantly correlated with those of either CDX1 (Fig. 7E) or CDX2 (Fig. 7F), although there was a significant correlation between ASBT and HNF-1α mRNA expression levels (Fig. 7G). Similar to the BE tissue, significant correlation between the mRNA expression levels of CDX1 and CDX2 was observed in the healthy ileum (Fig. 7H).

**DISCUSSION**

CDX1 and CDX2 play important roles in early gastrointestinal development, and also in gastrointestinal diseases in adult humans. Their ectopic overexpression in esophageal and stomach metaplasia suggests that they may play a role in the pathogenesis of these diseases (38). Alternatively, CDX expression could be the consequence rather than the cause of metaplasia. Given the elevated and ectopic expression of both CDXs and ASBT in BE, we studied whether CDXs may act as regulators of the ASBT promoter. Here, we have demonstrated in human esophageal and intestine-derived cell lines that the human ASBT promoter is a direct target for transcriptional activation by the transcription factors CDX1 and CDX2. Our finding adds the intestinal bile acid transporter, ASBT, to the list of genes regulated by CDXs. Other intestinal transporters regulated by CDXs include PEPT1 (35) and SMCT1 (24). Furthermore, our study adds another component to the already known complexity of transcriptional regulation of ASBT gene expression. It is currently not known whether the CDXs interact with the previously identified regulators of the ASBT promoter, such as GR (22), HNF-1α (23, 40), and PPARα (23). In this context, it is interesting to note that it has been previously proposed that HNF-1α and CDX2 have been proposed to cooperate with each other in the regulation of the intestinal genes sucrase isomaltase (5) and calbindin-D9k (43).

The correlation between the mRNA expression levels of CDXs and the bile acid transporter ASBT was confirmed in BE biopsy material. The role of bile acids in the molecular pathogenesis of esophageal metaplasia still remains somewhat unclear, although bile acids have been shown to directly augment CDX1 (26) and CDX2 (6, 27) expression. It may thus be that, secondary to gastrointestinal reflux, the bile acids that enter the esophagus may lead to elevated ASBT expression via induction of its transactivators CDXs. Excess level of bile acids are toxic and have shown to be carcinogenic to the gastrointestinal tract (14) and liver (28, 29, 46), and bile acids are the major component of the duodenal juice, which can cause severe esophageal mucosal damage (25). Dvorak et al. demonstrated ASBT, the ileal bile acid-binding protein (IBABP), and the multidrug-resistant protein 3 (MRP3) are elevated in BE and decreased in esophageal adenocarcinoma at both the mRNA and protein levels (15). It has been proposed that bile acid transporter expression in gastrointestinal metaplasia of the esophagus serves as a protective mechanism by the host to remove excess bile acids secondary to chronic biliary reflux. In contrast, in healthy human ileal tissue, CDX expression did not correlate with ASBT expression, indicating that this regulatory mechanism may be more relevant in BE than in healthy ileum. We note that correlation has been previously reported between ASBT and CDX2 in ileal tissue of patients suffering from chronic diarrhea (2).

In both BE and healthy ileal tissue, the mRNA levels of HNF-1α significantly correlated with ASBT mRNA expression levels in both BE and healthy ileum biopsies. This finding is also consistent with previous reports that Hnf-1α-null mice have severely reduced Asbt expression (34) and that ileal HNF-1α protein levels correlate with ASBT mRNA levels in humans (3). Furthermore, we have demonstrated that CDX1 and CDX2 mRNA expression levels significantly correlate with each other in both human BE and ileal tissue, supporting the prior suggestion that they can mutually regulate each other’s expression (26).

It is interesting to note that the CDX-mediated activation was conserved in the mouse and rat Asbt promoters. Consistent with this, our preliminary in silico analysis revealed several putative CDXREs in both rodent Asbt promoters (data not shown), even though their overall homology with the human ASBT promoter is low. The level of activation of the rodent Asbt promoters appeared lower than for the human ASBT promoter. However, we note the limitation of the experimental setup in that human CDX expression constructs were used in these experiments, and these may have somewhat different coactivator requirements from the rodent counterparts. It will be interesting to study whether the intestinal Asbt expression and consequent bile acid absorption is reduced in Cdx mutant mice, although it should be noted that complete Cdx1/Cdx2-null mice cannot be analyzed for this purpose since they are not viable until birth.

Based on our present study, we conclude that CDX-mediated promoter activation may lead to aberrant esophageal expression of the bile acid uptake system ASBT and consequently to an increase in epithelial bile acid uptake activity by the mucosa in Barrett’s esophagus. Furthermore, our findings may provide an explanation for the correlation between the decrease in both ASBT and CDX expression in high-grade esophageal dysplasia.

**ACKNOWLEDGMENTS**

We thank Christian Hiller for excellent technical assistance. Leif Kühler and Diana Jung are acknowledged for assistance in cloning the rodent Asbt promoter constructs. Walter Bodmer and Carol Chan are acknowledged for kindly providing a CDX1 antibody, Juan Lucio Iovanna for the kind gift of CDX expression plasmids, and Anthony Jevnikar for the generous donation of the IEC-4.1 cells. Furthermore, we are very grateful to Xiangjun Jiang, Department of Gastroenterology, Qiongdao Municipal Hospital, China; Zhanju Liu, Department of Gastroenterology, Shanghai Tenth People’s Hospital, Tongji University School of Medicine, Shanghai, China; Jun Wan, Division of AJP-Gastrointest Liver Physiol • doi:10.1152/ajpgi.00102.2011 • www.ajpgi.org
Gastroenterology, Chinese PLA General Hospital, Beijing, China, and Haifeng Liu, Department of Gastroenterology, General Hospital of Chinese People’s Armed Police Forces, Beijing, China for generous donation of tissue samples. Carsten Wagner and our team members are acknowledged for helpful discussions.

**GRANTS**

This study was supported by the Swiss National Science Foundation (grant 320030_120463) to G. A. Kullak-Ublick and J. J. Eloranta.

**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**AUTHOR CONTRIBUTIONS**


Fig. 7. Correlation analysis of CDX1 (A), CDX2 (B), and HNF-1α (C) mRNA expression levels, with ASBT mRNA expression levels in BE biopsies from 20 subjects using linear regression analysis. D: correlation analysis of CDX1 and CDX2 mRNA levels with each other in BE tissue. R² values and P values: A, R² = 0.7789, P < 0.0001; B, R² = 0.7861, P < 0.0001; C, R² = 0.5513, P < 0.0001; D, R² = 0.7965, P < 0.0001. Beta-actin was used as housekeeping gene for normalization in esophageal material. Correlation analysis of CDX1 (E), CDX2 (F), and HNF-1α (G) mRNA expression levels with ASBT mRNA expression levels in ileal biopsies from 48 healthy subjects using linear regression analysis. H: correlation analysis of CDX1 and CDX2 mRNA levels in ileal tissue. R² values and P values: E, R² = 0.0000141, P = 0.9936; F, R² = 0.02726, P = 0.2621; G, R² = 0.1732, P = 0.0033; H, R² = 0.2019, P = 0.0014. Villin was used as the housekeeping gene for normalization in ileal material. Logarithmically transformed values of CDX1, CDX2, HNF-1α, and ASBT mRNA levels were used.


