270* Neuroendocrine characterization of the intestine of F508del CFTR mice

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We previously found hypertrophy of the gut in F508del homozygous mice.
Objectives: To characterize the neuroendocrine and innervation pattern in the intestines of wt, homo- and heterozygous F508del CFTR mice.
Methods: Specimens from the gastrointestinal tract were studied by immunocytochemistry (ICC) for VIP, galanin, NPY, CGRP, CART, VAchT as well as NOS and in situ hybridization (ISH) for VIP and CART mRNA. Enzymes involved in the formation and degradation of S-1-P and C-1-P were studied by isotope-assays. The muscle layer of the small intestine was thickened 4 times with hypertrophy of both the circular and longitudinal layer. In nerve fibres in the circular muscle VIP immunostaining was more intense in CF than in wt mice. The other neuropeptides/markers appeared unchanged. Furthermore, the submucosal VIP neurons (lacking CART) had up regulated VIP-mRNA while the myenteric ones (CART positive) had not. Instead CART-mRNA was upregulated in the myenteric VIP-neurons. This is different from the findings in a mechanical ileus model in which VIP is increased in both layers. No other differences were seen in endocrine intestinal cells (gastrin, CCK, GIP, GLP-1, serotonin, secretin and somatostatin).
Conclusion: Homozygous F508del mice have thicker enteric muscular layers compared to controls. Their VIP-containing fibres in circular muscle, and submucosal VIP neurons are more frequent and intensively immunoreactive. The relevance to MI and DIOS in CF need further studies on humans.

271* Pancreatic and biliary secretion differ in cystic fibrosis and wild-type pigs

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Objectives: The hallmark feature of pancreatic disease in cystic fibrosis (CF) is the production of viscous, low-volume and acidic fluid. Because pancreatic function studies in humans are done by sampling the jejunal fluid, it is not known whether pancreatic or biliary secretions are equally affected in CF. With a pancreatic histopathology similar to humans with CF and separate biliary and pancreatic duct openings into the intestine, the pig model offers an opportunity to examine pancreatic and biliary fluids separately.
Methods: Four WT, 3 *CFTR*$^{-/-}$, *and* 2 *CFTR*$^{\Delta F508/\Delta F508}$ newborn pigs were studied. Bile and pancreatic juice were collected from blind intestinal loops at baseline and 30 min after secretin. Bile was also sampled from the gallbladder.
Results: Compared to WT pigs, pancreatic juice volume and pH were low in CF pigs (8.4±0.1 in WT vs. 5.7±0.1 in CF). Contrary to WT, pancreatic juice volume and pH did not increase in CF pigs following secretin. Bile volume and pH were not different between WT and CF pigs at baseline, but unlike WT pigs, fluid volume did not increase in CF pigs. Gallbladder bile pH was not different between WT and CF pigs. Compared to WT, pancreatic juice protein concentration was 8 fold higher in CF pigs; bile protein concentration was 2 fold higher.
Conclusion: Pancreatic fluid was acidic, low in volume and high in protein in CF pigs. Bile volume and pH were not significantly different between WT and CF pigs, but bile volume did not increase after secretin in CF pigs. The differences between the pancreatic and biliary secretion may have important implications in the pancreaticobiliary disease pathogenesis in CF.

272* Ursodeoxycholate reduces cholate biosynthesis rate and pool size in *Cftr*$^{-/-}$ mice

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Objectives: Ursodeoxycholate (UDCA) is used as treatment for cystic fibrosis liver disease (CFLD). It is hypothesized that the therapeutic action of UDCA is either mediated via choleretic activity or via effects on bile salt (BS) metabolism. However, the effects of UDCA on biliary BS composition and the enterohepatic circulation of bile salts in CF conditions are unclear.
Methods: *Cftr*$^{-/-}$ and control mice were either fed UDCA enriched chow (0.5% wt/wt) for 3 weeks. We evaluated the effects on the biliary BS composition and on the biosynthesis rate (SR) and pool size (PS) of cholate (CA), the mayor hydrophobic BS.
Results: In non UDCA treated *Cftr*$^{-/-}$ mice the fractional biliary CA content was significantly higher compared to controls (61% vs. 46%, resp.; $p < 0.01$). However, the biliary UDCA enrichment was ~50% lower in *Cftr*$^{-/-}$ mice compared to controls (3% vs. 6%, resp.; $p < 0.01$). Both the SR (16±1 vs.10±2 μmol·100g^{-1} BW^{-1}·day, resp.; $p < 0.01$) and PS (28±3 vs. 18±1 μmol·100g^{-1}·BW^{-1}, resp.; $p < 0.01$) were significantly higher in *Cftr*$^{-/-}$ mice compared to controls. After UDCA the biliary BS composition consisted for more than ~80% of UDCA in both *Cftr*$^{-/-}$mice and controls. Both in *Cftr*$^{-/-}$ mice and controls UDCA treatment drastically diminished the SR (−85% and −81%, resp. each $p < 0.01$) and PS (−87% and −92%, resp. each $p < 0.01$).
Conclusion: *Cftr*$^{-/-}$ mice have a more hydrophobic biliary BS composition compared to controls. In *Cftr*$^{-/-}$ mice the SR and the PS of CA are increased compared to controls, leading to a more cytotoxic BS profile. UDCA reduces the hydrophobic BS profile of *Cftr*$^{-/-}$ mice. This favorable effect of UDCA on bile salt metabolism could contribute to the assumed beneficial effects of UDCA in CFLD.

273 TGF-β and SMAD proteins participate in the fibrotic process of liver disease in cystic fibrosis

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Focal biliary fibrosis may occur early in cystic fibrosis (CF). TGF-βs are cytokines that regulate proliferation and differentiation of a wide spectrum of cells. TGF-βs signal through transmembrane threonine-kinase receptors, activating intracellular mediators, called SMADs, which modulate the transcription of target genes. The aim of this study was to investigate the contribution of TGF-β cytokines to the hepatic fibrosis in CF.
Methods: LD was demonstrated in 12 CF patients, who had a mean age of 12.5 years and underwent percutaneous liver biopsy with the biopsy needle directed to the site of the lesion with the aid of CT scan. Hepatic tissue mRNA levels of TGF-β1, TGF-β2, TGF-β3, CTGF, ALK-5 and Smad-2, -3, -4, -7 were estimated by quantitative real-time RT-PCR.
Results: Strong correlation between the expression of TGF-βs, ALK-5, Smads and CTGF at the fibrosing process was observed (TGF-β3/TGF-β1 p: 0.0114, Smad-2/ALK-5 p: <0.0001, Smad-3/ALK-5 p: 0.0011, Smad-3/Smad-2 p: 0.0030, Smad-7/Smad-4 p: 0.0102, Ctgf/Smad-7 p: 0.0006, ALK-5/TGF-β1 p: 0.0001, ALK-5/TGF-β3 p: 0.0006, Smad-2/TGF-β1 p: 0.0001, Smad-2/TGF-β3 p: 0.0045, Smad-3/TGF-β3 p: 0.0114, Smad-7/TGF-β1 p: 0.0002, Smad-7/TGF-β3 p: 0.0139, Smad-7/ALK-5 p: 0.0001, Smad-7/Smad-2 p: 0.0001, Smad-7/Smad-3 p: 0.0026, Ctgf/TGF-β1 p: 0.0034, Ctgf/TGF-β2 p: 0.0261, Ctgf/ALK-5 p: 0.0114, Ctgf/Smad-2 p: 0.0005). Significant correlation between fibrosis and inflammation grade was also demonstrated (p: 0.0072).
Conclusion: The pathway of TGF–SMADs contributes to the fibrosing process of CFLD. There is an increase of the degree of inflammation in association with fibrosis. Thus, the measurement of tissue cytokines may be helpful in the assessment of the degree of the reactive fibrosing process in CFLD.