Elevated biliary cholesterol in spite of hepatic ABCG5/G8 deficiency: biliary lipid secretion in conditional knock-out mice

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Background: Gallstone disease caused by biliary cholesterol supersaturation and subsequent nucleation of cholesterol crystals is one of the most common gastrointestinal diseases worldwide. Cholesterol is secreted into bile by the hepatocanicular heterodimer ABCG5/G8 (Fig. 1). Besides its function in the liver, Abcg5/g8 is also expressed in enterocytes, where it transports plant sterols and cholesterol back into the intestinal lumen. The aim of this study was to analyze the biliary lipid composition under the condition that ABCG5/8 is selectively inactivated in the liver but not in the intestine.

Methods: Using BAC-based recombination, we generated conditional Abcg5/g8 knock-out mice that allow tissue-specific deletion of the first two exons of Abcg5 and the first exon of Abcg8 by cre-mediated recombination. Abcg5/g8 flox mice were crossed to albumin-cre mice expressing cre in the liver under the control of the albumin promoter (Abcg5/g8-HPK, Fig. 2).

Results: All animals developed normally and showed no gross abnormalities (Fig. 3). Expression of Abcg5 and Abcg8 was not detectable in the liver but unaffected in the intestine (Fig. 4). Total biliary lipids in hepatic bile were significantly reduced in Abcg5/g8-Hep-KO compared to wild-type controls (1.1 ± 1.0 vs. 1.5 ± 1.0 g/dL), while cholesterol (10.0 ± 1.0 vs. 3.4 ± 0.4 mol %) and the cholesterol saturation index (CSI, 2.3 ± 0.3 vs. 0.9 ± 0.1) were significantly elevated (Fig. 5). Analyzing the bile composition during the first hour of the acute bile fistula, we demonstrated that bile acid output was significantly decreased (67.0 ± 6.8 vs. 124.6 ± 18.7 μmol/hr/kg), while both cholesterol (10.26 ± 1.2 vs. 4.8 ± 0.8 μmol/hr/kg) and phospholipids (33.1 ± 4.6 vs. 27.7 ± 3.7 μmol/hr/kg) were elevated (Fig. 5). The expression of the alternative cholesterol transporter Abcg1 as well as the expression of the bile acid transporter Abcb11 were significantly elevated in the liver of Abcg5/g8-Hep-KO compared to wild-type mice (Fig. 6). The rate-limiting enzyme of the bile acid synthesis (Cyp7a1) and the nuclear bile acid receptor Fxr were downregulated in Abcg5/g8-Hep-KO livers.

Conclusion: Our results show that the liver-specific deletion of Abcg5/g8 leads paradoxically to biliary cholesterol supersaturation and a markedly increased CSI in the conditional knock-out mice. Further studies are needed to determine the mechanistic changes of biliary lipid secretion at the level of the hepatocanicular membrane as well as adaptive responses.