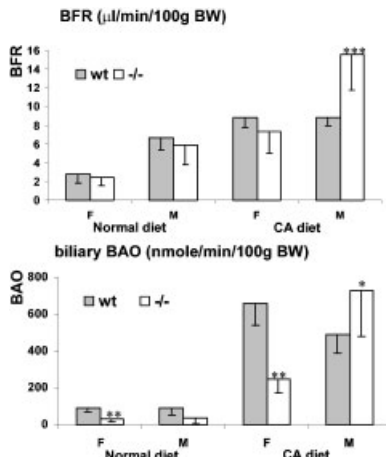


HEPATOLOGY HIGHLIGHTS

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The Sister Has a Brother

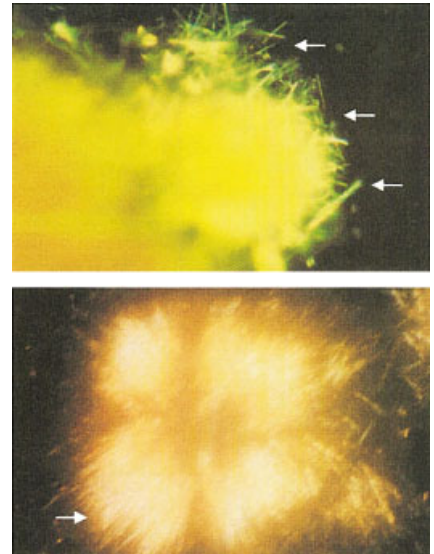


The bile salt export pump, also known as the sister of P-glycoprotein (*spgp*), is responsible for bile acid secretion. Mutations in this gene are associated with severe progressive familial intrahepatic cholestasis type 2, a fatal disease of children. However, *spgp* knockout mice exhibit only mild

nonprogressive cholestasis. The mild phenotype has been attributed to the ability of mice but not humans to detoxify hydrophobic bile acids by hydroxylation and, possibly, to these bile acids (*e.g.*, muricholic acid) being exported by a different transport system. Wang and colleagues fed a 0.5% cholic acid diet to *spgp* $^{-/-}$ mice. This led to progressive fatal illness over 5 to 9 days, whereas the diet was well tolerated in wild type mice. The deterioration was accompanied by biochemical features of cholestatic hepatitis (except for normal γ -GT) and accompanying histologic changes. Cholic acid induced selective changes in gene expression, such as up-regulation of *mrp3* and *mrp4*. As expected, cholic acid feeding markedly increased plasma and liver bile acid concentrations in knockout mice. However, surprisingly the bile acid output and bile flow rate were high in knockout mice fed cholic acid compared to normal diet and to wild type mice fed cholic acid (see figure). Thus it appears that when bile acids accumulate at high concentration in *spgp* $^{-/-}$ mice, they are secreted at a high rate into bile. The importance of this study is the demonstration that loading with cholic acid elicits the PFIC-2 phenotype and that a presumed low-affinity, high-capacity canalicular bile acid transporter exists and is possibly up-regulated by cholic acid feeding but cannot protect against the accumulation and toxicity of cholic acid. The identification of this "brother" transporter and its physiologic role remains to be determined. (See HEPATOLOGY 2003;38:1489–1499.)

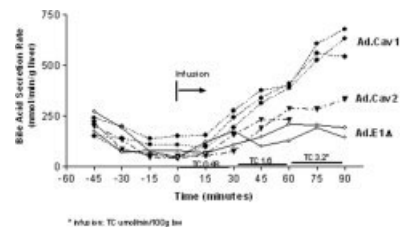
Mice Really Do Resemble People: The *Mdr2* Story

Mdr2 is an ABC transporter gene responsible for phospholipid secretion in bile. Mutations in this gene are responsible for progressive familial intrahepatic cholestasis type 3. The lack of phospholipids appears to unmask the toxicity of bile acids on the biliary tree, which leads to segmental strictures and fibro-obliteration of bile ducts. This human disease is accompanied by intrahepatic and gallbladder microlithiasis, but this has not been described in mice. To establish the pathogenesis of this cholelithiasis, Lammert and colleagues examined the physical chemistry of bile in *Mdr2* $^{-/-}$ mice compared with wild type mice. Needle-shaped cholesterol crystals and stones (see figure) were observed in knockout mice in a progressive age-dependent fashion. Stone formation begins at 12 weeks in females and 15 weeks in males. The atypical crystal structure may have some relationship to the qualitative differences in the composition of bile acids and trace phospholipids contained in bile of knockout animals. Intrahepatic duct stones were observed in older female knockout mice after the earlier appearance of fibro-obliteration and segmental strictures of bile ducts, suggesting that the intrahepatic stones are caused by the strictures (stasis) in the presence of abnormal bile. This is an important study because it is the first documentation that *Mdr2* $^{-/-}$ mice develop gallstones. This is the second animal model of spontaneous gallstone formation without dietary manipulation (*e.g.*, cholesterol feeding); the other is the deer mouse. The *Mdr2* knockout is a fascinating model that resembles primary sclerosing cholangitis and recurrent pyogenic cholangitis; it should be very useful in testing treatment strategies for low phospholipid-associated cholelithiasis. (See HEPATOLOGY 2004; 39:117–129.)



Caveolin Caveats

Caveolins are membrane proteins that bind cholesterol and other lipids, forming detergent resistant foci in plasma membrane and intracellular vesicles-



(caveolae). Caveolae may play a significant role in intracellular signaling (sequestering receptor complexes) and intracellular trafficking. To gain insight into the role of caveolins in cholesterol and bile acid secretion, Moreno and colleagues infected mice with recombinant human caveolin 1 (Cav-1) and 2 (Cav-2) adenoviruses to overexpress caveolins. In normal liver, Cav-1 and Cav-2 were predominantly localized to the sinusoidal membrane, although some was identified in the canalicular membrane. The overexpressed human Cav-1 was found mainly at the plasma membrane, whereas human Cav-2 was associated with the Golgi complex. Cav-1 overexpression led to a 40% increase in plasma high density lipoprotein cholesterol that was not due to changes in its receptor. In both Cav-1 and Cav-2 overexpressing mice (compared with vector controls), bile flow increased by 30% to 40%, accompanied by a 40% to 70% increase in biliary bile salt output and similar increases in phospholipid and cholesterol secretion, which were probably secondary to bile salt secretion. There was no effect on bile salt pool size or composition. Surprisingly, Cav-1 overexpression markedly increased the bile acid secretory maximum capacity (see figure). This was not due to changes in expression or localization of Ntcp or Bsep. This interesting, well-executed study suggests a role for caveolins and possibly caveolae in the regulation of Bsep. However, many questions need to be addressed. Does Bsep bind to Cav-1, and can the two be coimmunoprecipitated? How does overexpression of caveolin affect membrane retrieval of Bsep? What accounts for the apparent discrepancy between Cav-2 increasing biliary bile acid and lipid output but not bile acid transport maximum? Do caveolin knockout mice exhibit changes in bile secretion? Does overexpression of caveolin mimic a physiologic regulatory process? What effect does overexpression have on other canalicular transport processes? (See HEPATOLOGY 2003;38:1477–1488.)

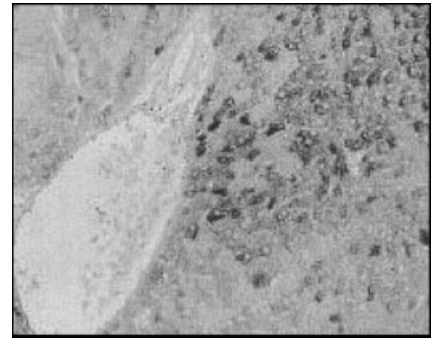
Pufa Proves Particularly Protective

Sterol regulatory element-binding proteins (SREBPs) are transcription factors that regulate fatty acid and cholesterol synthesis. They exist as membrane proteins in the endoplasmic reticulum bound to SREBP cleavage-activating protein (SCAP). When cholesterol binds to SCAP, it retains SREBPs in the endoplasmic reticulum; in the absence of cholesterol, SCAP escorts SREBPs to the Golgi, where resident proteases cleave the SREBPs releasing active or mature forms that translocate to the nucleus. SREBP-1c controls fatty acid, and SREBP2 controls cholesterol synthesis genes. Leptin-deficient ob/ob mice have increased hepatic triglyceride and SREBP-1c messenger RNA. Polyunsaturated fatty acids (PUFAs) are known to decrease SREBP-1 expression. Therefore, Sekiya and colleagues used fish oil to study the effect of PUFAs on hepatic steatosis and SREBP-1 in ob/ob mice. PUFA feeding markedly decreased mature SREBP-1 protein but had no effect on precursor membrane form, messenger RNA, or SREBP-2 expression. Decreased mature SREBP-1 was accompanied by decreased expression of its target genes (fatty acid synthesis), decreased hepatic triglycerides, and serum alanine aminotransferase. Using a transgenic animal that constitutively expresses mature SREBP-1, fish oil still decreased hepatic triglyceride, suggesting

that it has additional effects independent of down-regulation of SREBP-1c. However, eicosapentaenoic acid feeding did not down-regulate steatosis in these transgenic animals. PUFAs also ameliorated insulin resistance, possibly by serving as peroxisomal proliferator activated receptor (PPAR) α ligands. Previous work showed that double SREBP-1/leptin-deficient mice are protected from fatty liver but still have decreased insulin sensitivity, so SREBP-1 down-regulation by PUFAs does not account for PUFA-induced decreased insulin resistance. This work suggests that PUFA therapy may be beneficial for the prevention or treatment of nonalcoholic steatohepatitis, both by inhibiting SREBP-1 activation and hepatic steatosis and by ameliorating insulin resistance. This suggestion must be tempered by the fact that others have shown that PUFAs worsen alcoholic liver injury. In addition, it will be of interest to elucidate the mechanism by which PUFAs decrease the production of mature SREBP-1c while sparing SREBP-2. (See HEPATOLOGY 2003;38:1529–1539.)

You See UCP Too?

Uncoupling proteins (UCPs) are inner mitochondrial membrane proteins that uncouple mitochondria. This uncoupling has two effects: wasting energy and decreasing superoxide production by decreasing



mitochondrial membrane potential. Thus loss of UCP function results in increased superoxide generation and, conversely, UCP overexpression limits reactive oxygen species and is cytoprotective. Horimoto and colleagues tested the hypothesis that UCP2 knockout mice would exhibit increased reactive oxygen species (ROS), which would affect liver regeneration. Following partial hepatectomy, UCP2 $-/-$ mice exhibited delayed regeneration due to lower DNA synthesis. As a measure of ROS, the authors measured hepatic malondialdehyde (lipid peroxidation) levels; this was higher at baseline in UCP2 $-/-$ liver but further increased only minimally after hepatectomy. The consistently higher oxidative stress in UCP2 $-/-$ was associated with higher levels of p21 and phospho-p38, both of which inhibit cell cycle progression. In wild type mice, partial hepatectomy was associated with an increased expression of UCP2 protein from 24 to 72 hours. Immunohistochemistry revealed that the increased UCP2 expression was mainly in the periportal hepatocytes (see figure). Thus the findings support the hypothesis that UCP2 expression increases after partial hepatectomy, which may limit ROS formation. In the absence of UCP2, the sustained increase in ROS may delay growth by altering cell cycle regulation. More work is needed to establish the trigger for increased UCP2 expression and to link the effects of UCP2 absence on cell cycle with ROS levels. (See HEPATOLOGY 2004;39:386–392.)