MDR1 as a potential therapeutic target for progressive familial intrahepatic type II in children.

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Introduction: Progressive Familial Intrahepatic Cholestasis type II (PFIC2) is a rare disease that primarily affects children under the age of two. It is characterized by mutations in the *ABCB11* gene that encodes for the bile salt export pump (BSEP). The loss of BSEP causes bile to be accumulated in the hepatocyte, thus leading to cholestasis and liver injury. The only curable treatment for PFIC2 is a liver transplant. *Abcb11* knockout mouse develops mild cholestasis. It is suggested that Multidrug Resistant Protein 1 (MDR1), another canalicular ABC transporter, may compensate for loss of BSEP. We established the first zebrafish *abcb11b* knockout model and showed that they accumulated bile salts within the liver, and resembled the phenotypes seen in patients with PFIC2. In both human and zebrafish that are deficient in BSEP, we found that MDR1 was mislocalized to the hepatocyte cytoplasm. In *abcb11b* zebrafish mutants treated with rapamycin, an autophagy inducer, MDR1 was localized to the canalicular membrane, coinciding with partial restoration of bile excretion.
Methods: To understand how MDR1 is mislocalized within the BSEP deficient zebrafish, we used transgenesis techniques to express MDR1 fluorescent fusion protein in the hepatocytes to visualize MDR1 localization and study the effect of MDR1 overexpression on bile excretion.. We generated *abcb11b;mdr1* mutants to determine if MDR1 is necessary for the rescuing mechanism seen when treated with rapamycin, and *abcb11b;cyp7a1* mutants to investigate if bile accumulation effects MDR1 localization.
Results: We showed that in *abcb11b;mdr1* double mutants the rapamycin treatment failed to restore bile excretion. Meanwhile, overexpression of either the zebrafish or human MDR1 induced a partial rescue of bile excretion in the hepatocytes of *abcb11b* mutants. These studies suggest that MDR1 can be prompted to serve as an alternative bile salt transporter to compensate for BSEP loss. We also showed that in *abcb11b;cyp7a1* double mutants, in which bile synthesis was largely inhibited, the canalicular localization of MDR1 was restored. These results indicate that MDR1 mislocalization within *abcb11b* mutants is due to the bile salt accumulation in hepatocytes. We currently focus on identifying the cellular mechanisms that act downstream of bile salt accumulation to cause MDR1 mislocalization. In conclusion, these studies have identified the cellular mechanism causing MDR1 mislocalization within *abcb11b* mutant zebrafish hepatocytes and introduced a new therapeutic target for future PFIC2 treatments.