Characterization of cholestasis in a knock-in Bsep^{E297G} PFIC2 mouse model

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INTRODUCTION

Progressive Familial Intrahepatic Cholestasis type 2 (PFIC2) is a rare pediatric liver disorder arising from genetic mutations in the bile salt export pump (BSEP, *ABCB11*)[1]. E297G is the most prevalent mutation found in PFIC2 patients, which when inherited biallelically or in trans with another defective *ABCB11* allele presents with compromised bile acid transport, subsequent hepatic accumulation, and toxicity[2]. A PFIC2 mouse model homozygous for the Bsep^{E297G} mutation was generated, as well as a polyclonal antibody that recognizes both human and mouse Bsep protein. Characterization of this mouse model demonstrates the recapitulation of key translational aspects of the human disease and offers advancements for the evaluation of novel PFIC2 therapeutic interventions.

METHODS

CRISPR/Cas9-mediated gene editing was used to create *Bsep*^{E297G} knock-in mice (C57BL/6N). Female wildtype (WT), heterozygous (HET), and homozygous (HOM) mice were phenotypically characterized starting at 6 weeks. The animals used in the pharmacology study were fed chow with or without 0.01% A4250 (Odevixibat) for 14 days. The mice were assessed for clinically relevant markers associated with progressive cholestasis, liver damage, and PFIC2 pathophysiology. Parameters included serum, biliary, and hepatic bile acid concentration, bile acid composition, liver enzymes, gene expression profiles, and liver histopathology. Antibody production was carried out against mouse *Bsep* recombinant protein in NZW rabbits and antisera were affinity purified for all applications.

INTRODUCTION OF E297G VARIANT DISRUPTS BSEP PROTEIN MATURATION AND LOCALIZATION

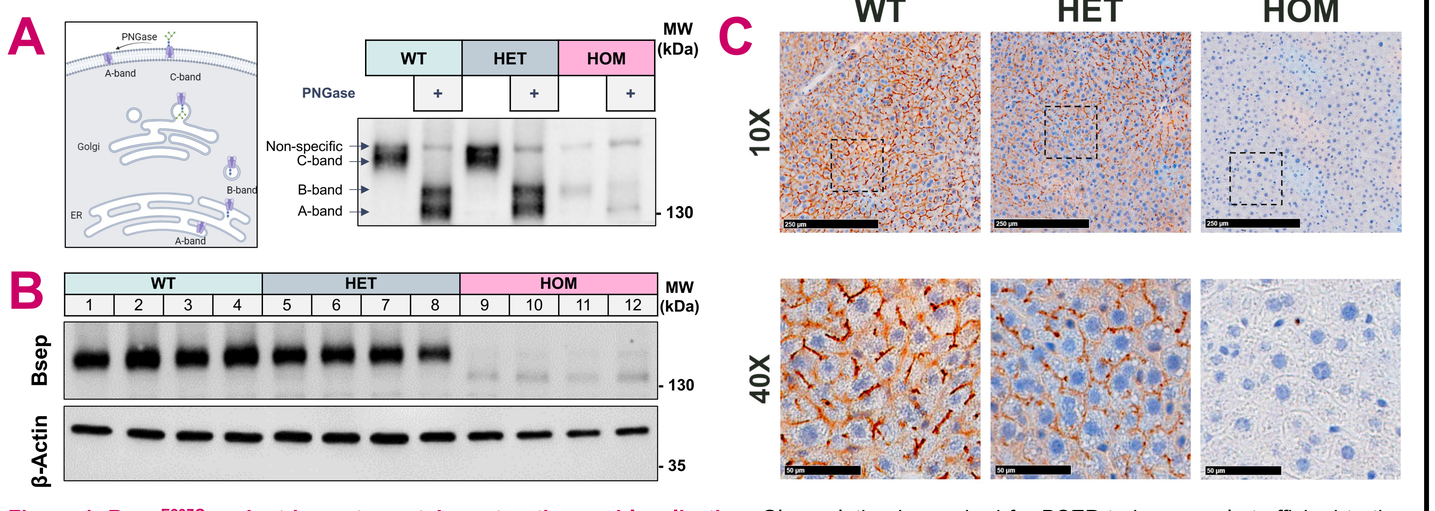

Figure 1. Bsep^{E297G} variant impacts protein maturation and localization. Glycosylation is required for BSEP to be properly trafficked to the membrane. These modifications are added in a stepwise process to generate B-band (immature, partially glycosylated), C-Band (fully glycosylated mature protein) from the unmodified form A-Band. **(A)** Application of PNGase removes all glycosylation modifications and converts mature C-band in WT and HET animals to unmodified A-band, while it converts B-band in HOMs to A-band demonstrating a defect in glycosylation of Bsep^{E297G}. **(B)** Western blot of liver tissue lysates from 12-week-old female WT, HET, and HOM Bsep^{E297G} mice demonstrating the appearance of the immature B band in HOM animals. **(C)** Immunohistochemistry for Bsep in frozen liver cryo-sections from WT, HET and HOM mice. Scale bar, 250 µm (10X, top) and 50 µm (40X, bottom).

CHOLESTATIC AND HEPATOTOXIC BIOMARKERS ARE INCREASED IN PFIC2 MOUSE MODEL

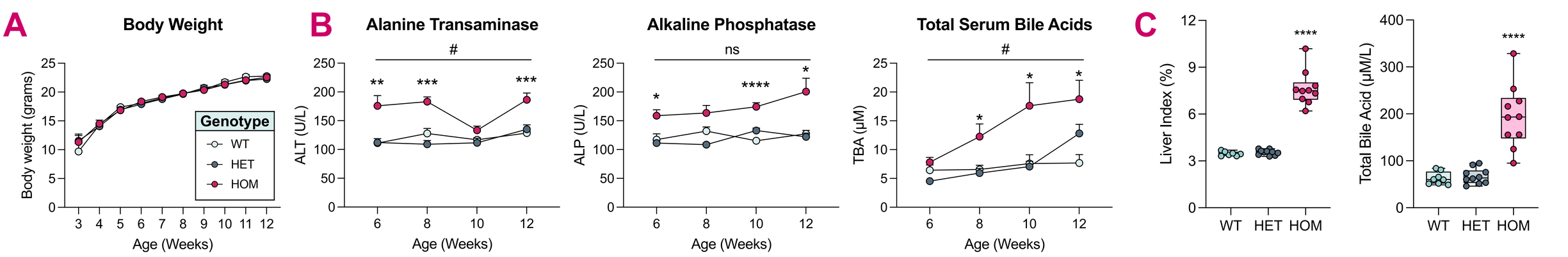

Figure 2. Bsep^{E297G} mice demonstrate evidence of cholestasis and liver injury. **(A)** Body weight of female WT, HET and HOM mice from 3 weeks to 12 weeks of age. **(B)** Compared to WT and HET animals, HOMs demonstrate a progressive increase in serum ALT, ALP and total serum bile acids from 6 weeks of age to 12 weeks of age. **(C)** Liver index and total liver bile acids in female HOM mice are elevated at 12 weeks of age.

PFIC2 MOUSE LIVER BILE ACID COMPOSITION IS ALTERED DUE TO CHANGES IN GENE EXPRESSION

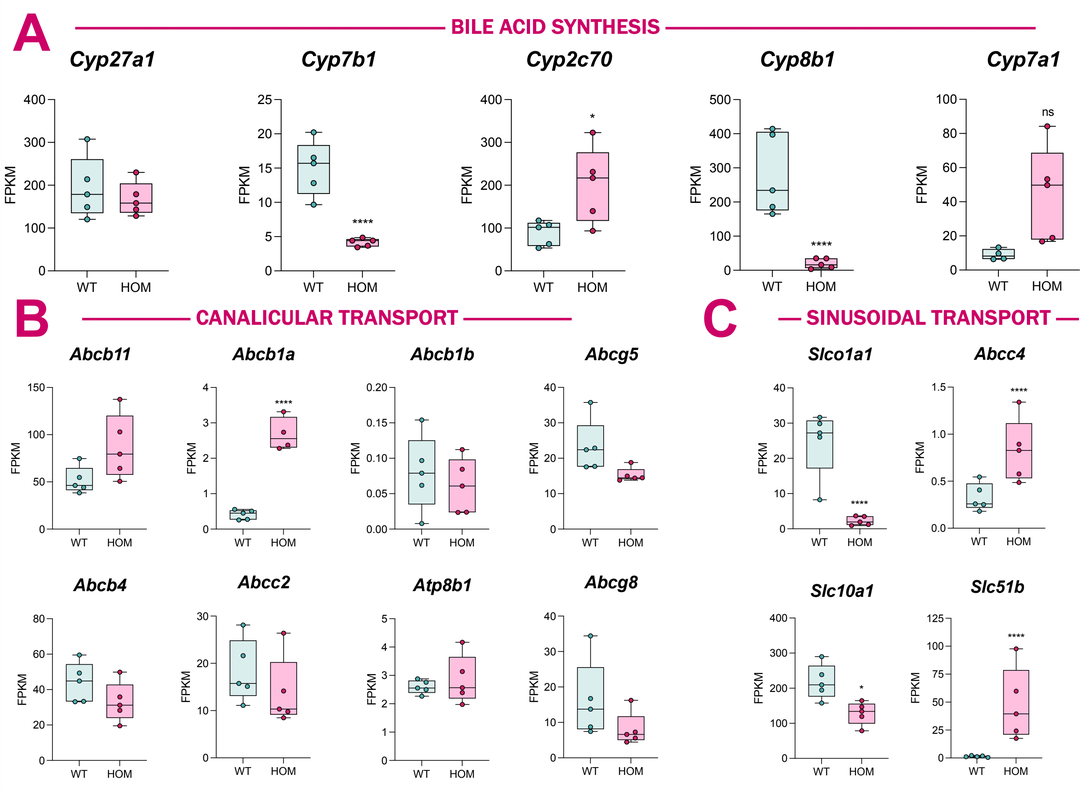

Figure 3. Bsep^{E297G} mice present with altered gene expression profile consistent with a counter-regulatory response to cholestasis. Total RNA was extracted from frozen liver tissue and used for RNA-Seq analysis on an Illumina HiSeq1000 system (Novogene Co). **(A)** Changes in primary bile acid synthesis pathways in WT and HOM mice **(B)** Changes in canalicular transporter gene expression in WT and HOM mice. **(C)** Changes in expression of sinusoidal bile acid transporter genes. *p<0.05, **p<0.001, ****p<0.0001 with Benjamini-Hochberg correction.

THERAPEUTIC TREATMENT WITH ODEVIXIBAT AMELIORATES BILE ACID PHENOTYPE IN PFIC2 MOUSE

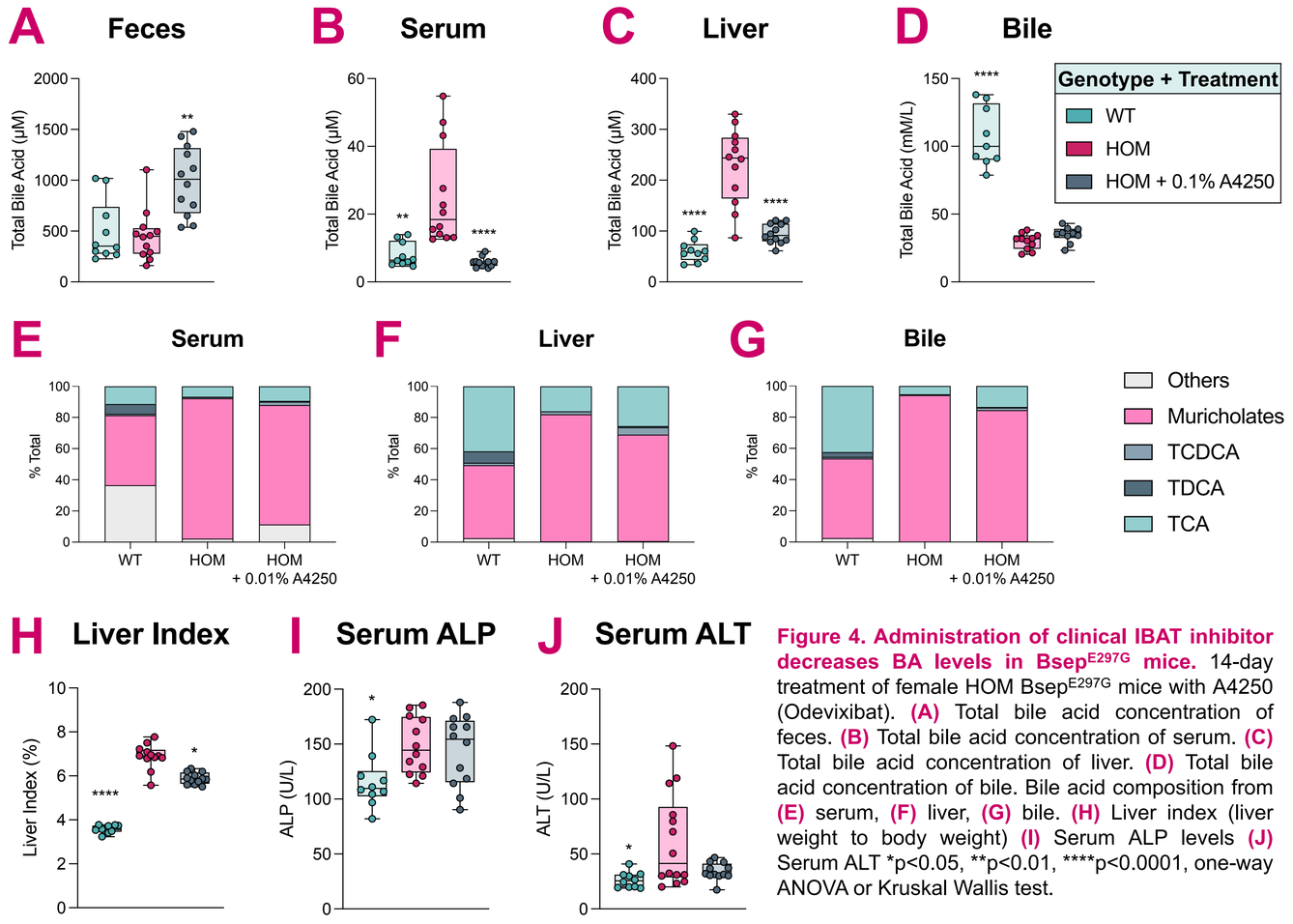

Figure 4. Administration of clinical IBAT inhibitor decreases BA levels in Bsep^{E297G} mice. 14-day treatment of female HOM Bsep^{E297G} mice with A4250 (Odevixibat). **(A)** Total bile acid concentration of feces. **(B)** Total bile acid concentration of serum. **(C)** Total bile acid concentration of liver. **(D)** Total bile acid concentration of bile. Bile acid composition from **(E)** serum, **(F)** liver, **(G)** bile. **(H)** Liver index (liver weight to body weight) **(I)** Serum ALP levels **(J)** Serum ALT *p<0.05, **p<0.01, ****p<0.0001, one-way ANOVA or Kruskal Wallis test.

SUMMARY AND CONCLUSIONS

	Bsep$^{E297G/E297G}$	Bsep^{KO}	PFIC2
Cholestasis	✓	✓	✓
Serum BAs	↑	↑	↑
Bile composition	↑ PL; ↑Ch	↑ PL; ↑Ch	ND
Failure to thrive	✗	✓	✓
ALP and ALT	↑	↑	↑
GGT	NC	NC	NC
Hepatomegaly	✓	✓	✓
Liver failure	✗	✗	✓
Pruritus	ND	ND	✓
Sexual dimorphism	✓	✓	✗
IBAT responsive	✓	ND	✓

PL, phospholipid; Ch, cholesterol; NC, no change; ND, not determined

The *Bsep*^{E297G} homozygous mouse serves as a translational model of PFIC2 which exhibits several of the key pathophysiological hallmarks of the human disease. Homozygous E297G animals have defective BSEP protein maturation and localization, and as a result demonstrate progressive increase in serum markers for cholestasis and hepatotoxicity. Moreover, liver bile acids are increased, and biliary bile acids are decreased, indicating disruption of hepatobiliary homeostasis. Bile acid composition shifts towards a more hydrophilic state, similar to what is reported in the BSEP KO mouse[3]. Our novel PFIC2 mouse responds to IBAT inhibitor highlighting this model provides a powerful *in vivo* platform to evaluate potential disease modifying therapeutics for the treatment of PFIC2. This model can further an understanding of the relationship between deficits in BSEP and the resultant cholestasis that develops from that deficiency.

References

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3. Fuchs, C. D. et al. Metabolic preconditioning protects BSEP/ABCB11−/− mice against cholestatic liver injury. J Hepatol 66, 95–101 (2017).