pixlbio

CASE STUDY

Decoding biology.
Pixel by pixel.

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Modeling progressive familial intrahepatic cholestasis type 2 through CRISPR-derived PFIC2 pixHep

Progressive familial intrahepatic cholestasis (PFIC) is a heterogeneous group of autosomal recessive liver diseases, characterized by mutations in genes involved in hepatocellular bile acid secretion. Amongst these, PFIC2, caused by mutations in the ABCB11 gene, represents half of all PFIC cases. Patients typically present with symptoms such as liver failure, cirrhosis, and hepatocellular carcinoma. Despite the importance of the disease, there are currently no licensed treatments, mainly due to the lack of appropriate pre-clinical models in drug discovery. At pixlbio we have developed a novel iPSC-derived hepatocyte system that recapitulates the human PFIC2 phenotype in-a-dish, offering, for the first time, an effective pre-clinical PFIC2 disease model for hit-lead drug screening studies.

Advantages
Functional bile acid transport pathways with high ABCB11 and MRP2 expression levels
Disease circuit verified carrying the D482G mutation in the ABCB11 gene
Optimized bioassays measuring bile acid transport as end-point assay in pixHep
Suitable *in vitro* platforms for screening of compound and gene therapy systems
Standardized cell products containing iPSC-derived human hepatocytes producing reproducible and biologically relevant data

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CASE STUDY

pixlbio CRISPR-derived PFIC2 iPSCs carry the D482G mutation without any effect in pluripotency status

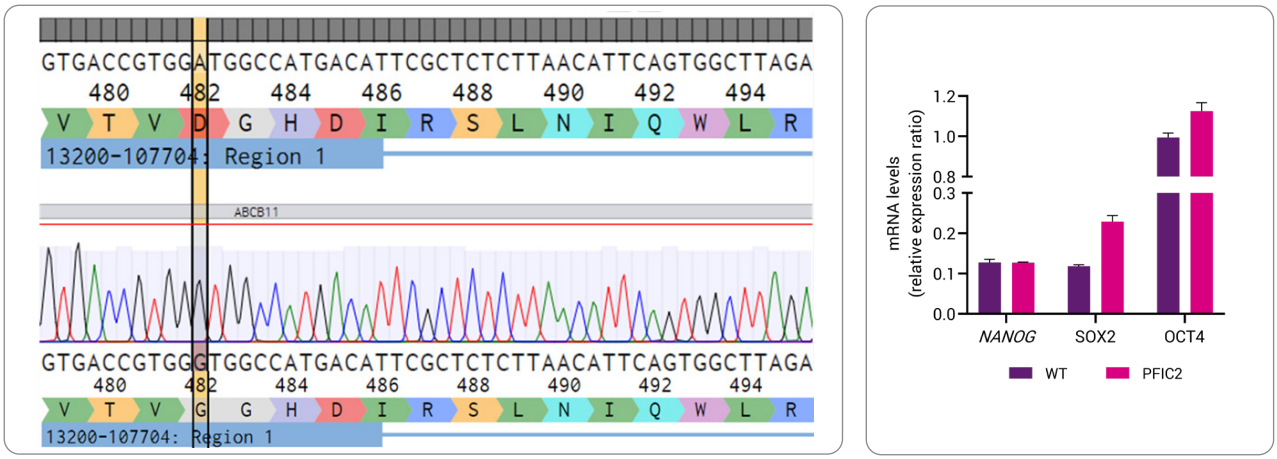

Figure 1. Sanger sequencing showing wild-type (top sequence) and mutated iPSCs (bottom sequence) carrying the D482G mutation (GAT>GTT) in the ABCB11 gene. The codon change is highlighted with yellow (left). mRNA expression levels of the key pluripotency markers NANOG, SOX2, and OCT4 in wild-type (WT) and CRISPR-derived ABCB11 iPSCs (PFIC2) (right). mRNA data were normalized to GAPDH and are presented as mean±SEM of n=3 biological replicates.

pixlbio CRISPR-derived LDLR iPSCs successfully differentiate to pixHep

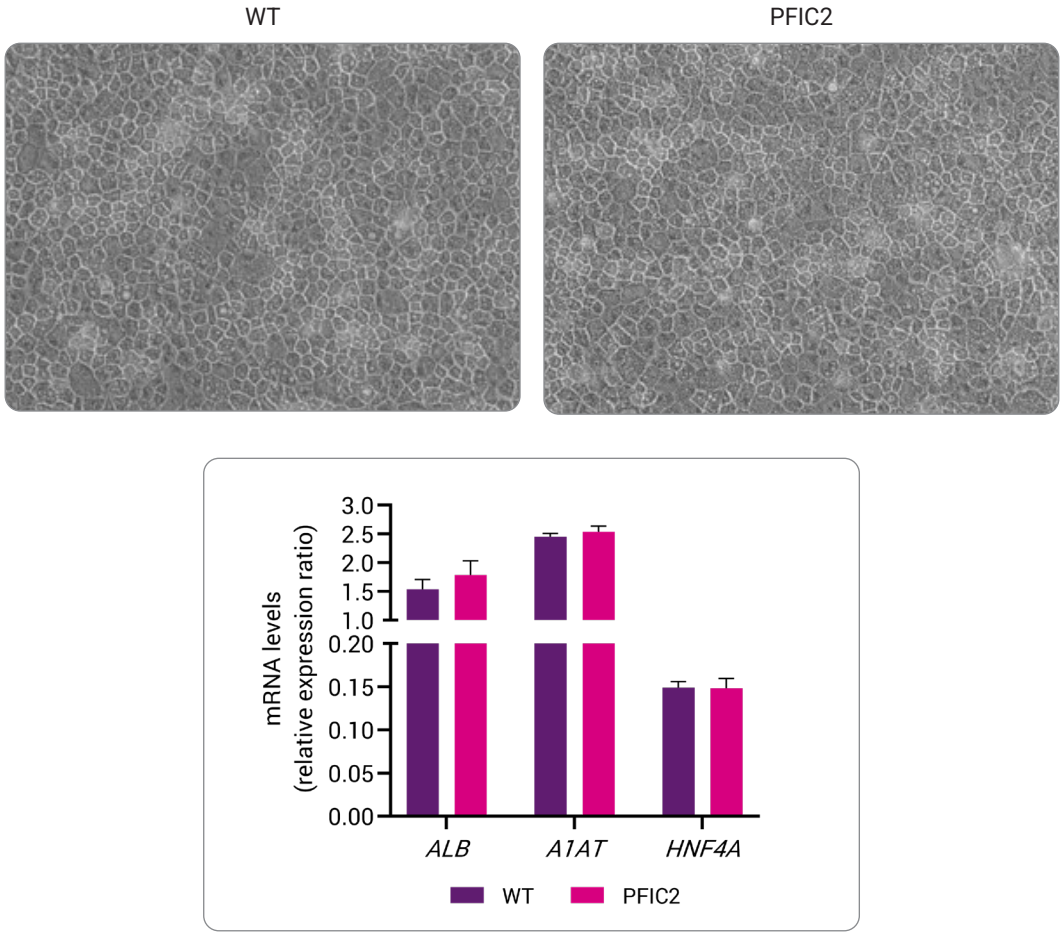

Figure 2. Representative images demonstrating the characteristic hepatocyte cobblestone morphology in wild-type (WT)(left) and PFIC2 pixHeps (middle). mRNA expression levels of the hepatocyte maturity markers albumin (ALB), alpha-1-antitrypsin (A1AT), and hepatocyte nuclear factor 4A (HNF4A) in wild-type iPSCs (WT) and PFIC2 pixHeps. mRNA data were normalized to PPIA and are presented as mean±SEM of n=2 biological replicates.

pixlbio CRISPR-derived PFIC2 pixHep demonstrate reduced ABCB11 mRNA and protein expression

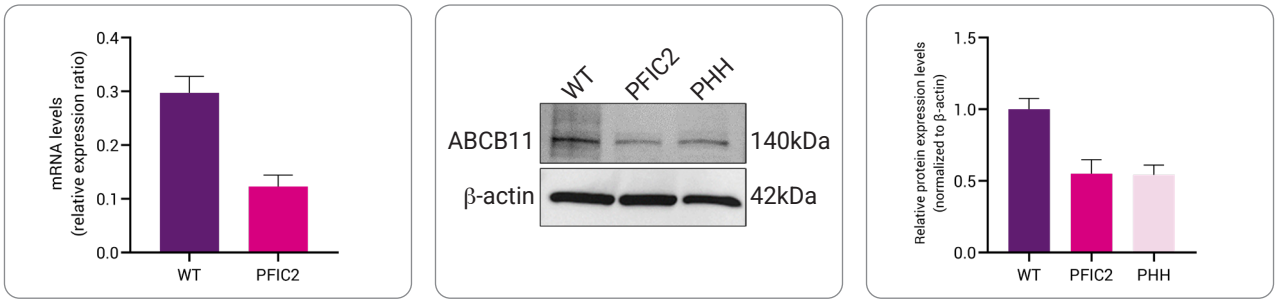

Figure 3. mRNA expression levels of ABCB11 in wild-type (WT) and PFIC2 pixHeps cultured in transwell culture system (left). Protein expression levels of ABCB11 in transwell-cultured WT iPSCs and PFIC2 pixHeps in comparison to primary human hepatocytes (PHH) (middle). mRNA data were normalized to 18S rRNA, and protein data to β-actin. All data are presented as mean±SEM of n=3-4 biological replicates.

pixlbio CRISPR-derived PFIC2 pixHep exhibit disrupted ABCB11 localization

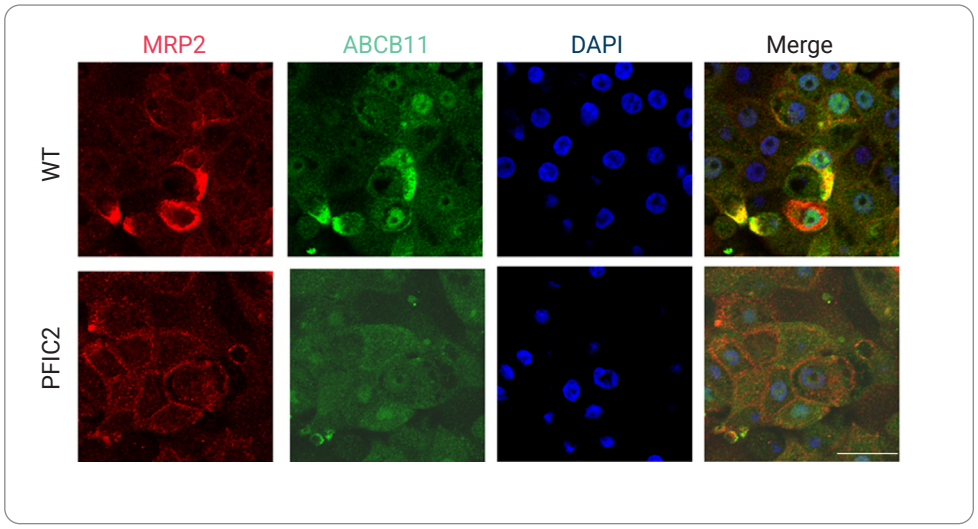

Figure 4. Immunocytochemistry analysis of MRP2 (red) and ABCB11 (green) in wild-type (WT) and PFIC2 pixHeps cultured in transwell culture system. Scale bar: 50 µm.

pixlbio CRISPR-derived PFIC2 pixHep demonstrate reduced bile acid transport

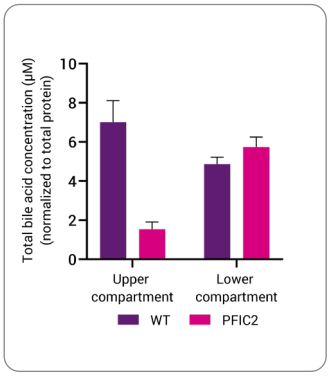

Figure 5. Taurocholic acid (TCA) quantification in the upper and lower transwell compartments of WT and PFIC2 pixHeps following 48 hours of TCA addition (10 µM) to the lower compartment, indicating dysfunctional ABCB11 activity in the PFIC2 cells. Data are presented as mean±SEM of n=4 biological replicates