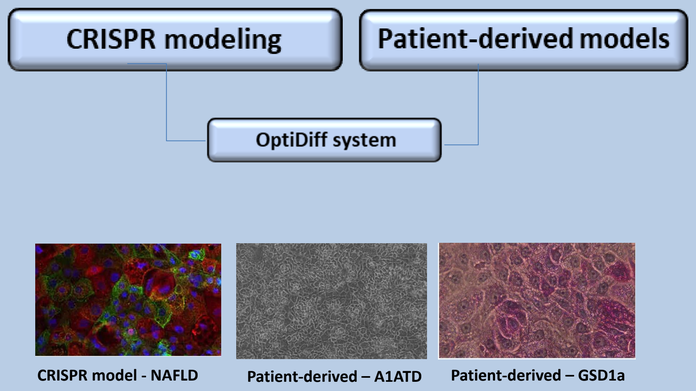
Modelling Liver Disease Using CRISPR Edited Human Induced Pluripotent Stem Cells

Vanesa Papastavrou, Gemma Gatti, William Elliott, James Heslop, Lia Panman, Carlos Gil, , & Kieran Casey
DefiniGEN Ltd, Babraham Research Campus, Cambridge , UK

DEFINIGEN
Disease Model Innovation

Disease modeling platform



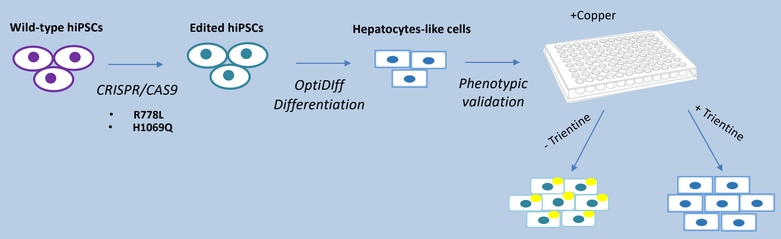


Figure 1. Schematic overview of DefiniGEN disease modelling platform: introduction of two of the most common genetic mutations within ATP7B associated with Wilson's disease, hiPSCs differentiation into hepatocytes, phenotypic validation after copper enrichment and chelator treatment.

- Wilson disease is an autosomal-recessive disorder of hepatocellular copper deposition caused by pathogenic variants in the copper-transporting gene, ATP7B.
- The most common mutation in Northern America and Europe is the missense mutation p.H1069Q and the most common mutation in East Asian populations is the missense p.R778L.
- We generated hiPSC lines with either H1069Q or R778L Knocked-in mutations within ATP7B gene. These genetically edited cells were then differentiated into hepatocytes and treated with copper to model the Wilson phenotype *in vitro*.

CRISPR MODELING

Design

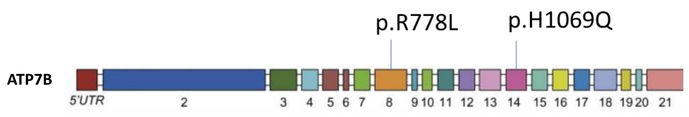


Figure 2. Schematic representation of the ATP7B gene. CRISPR/Cas9 target sites (p.H1069Q and p.R778L) are indicated at their respective locus.

Engineering

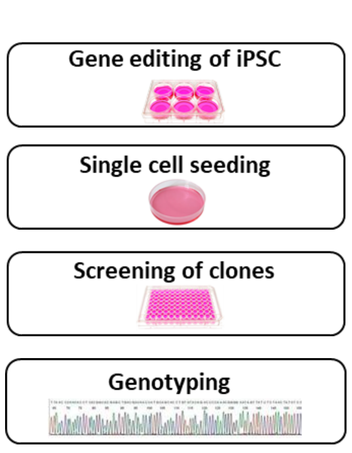


Figure 3. Schematic workflow of hiPSC line engineering using CRSPR/Cas9.

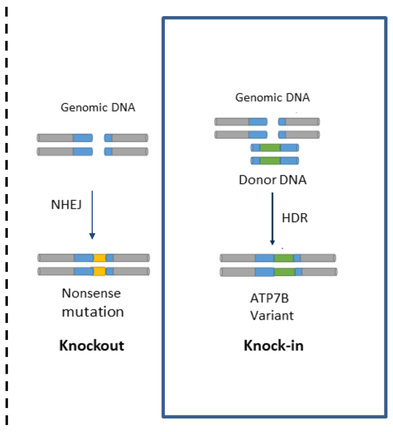


Figure 4. Schematic representation of the CRISPR/Cas9 techniques used to genetically manipulate hiPSCs. ATP7B gene variants were generated using homology-direct repair (HDR).

QC

Mycoplasma test	Morphology	Key pluripotency markers	Disease confirmation

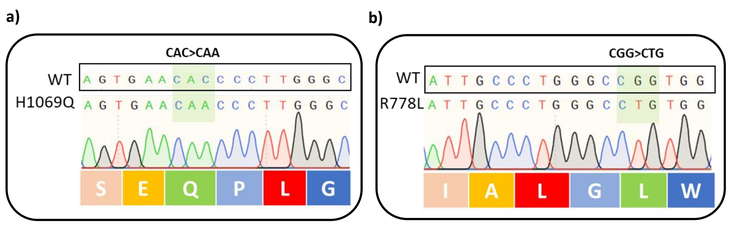


Figure 5. Sanger sequencing of the CRISPR edited ATP7B gene confirming disease mutations.
a) ATP7B homozygous knock-in clone with H1069Q mutation (His to Gln at position 1069).
b) ATP7B homozygous knock-in clone with R778L mutation (Arg to Leu at position 778).

OptiDIFF PLATFORM

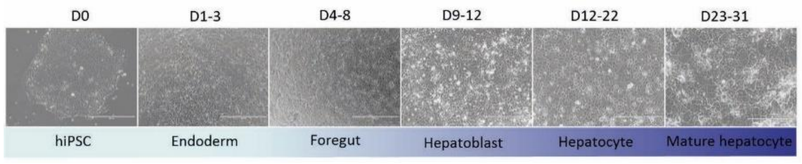


Figure 6. Representative images of the various steps of the hepatocyte differentiation protocol that recapitulates the embryonic hepatic development.

Key hepatocyte marker analysis

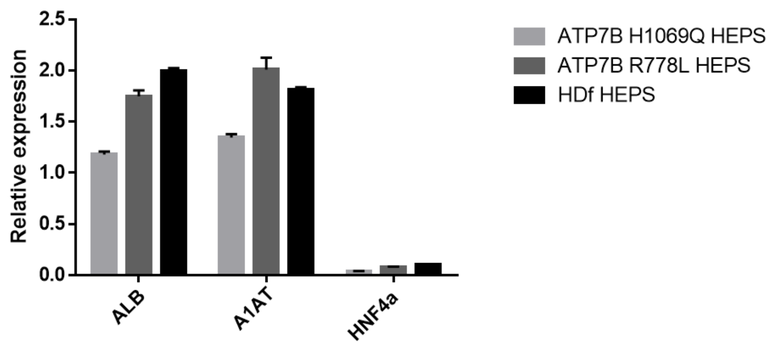


Figure 7. Gene expression of key hepatic markers: *ALB* (Albumin), *A1AT* (Alpha-1 Antitrypsin) and *HNF4a* (Hepatocyte Nuclear Factor-4), 17-days post-thaw. Data presented in comparison to PHH (Primary Human Hepatocytes).

PHENOTYPIC VALIDATION

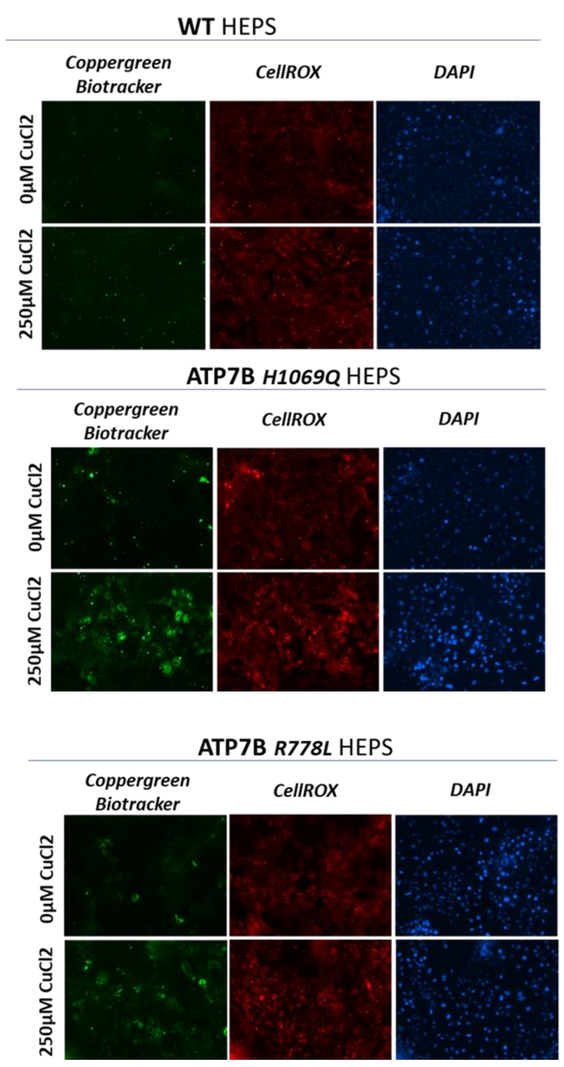


Figure 8. Simultaneous imaging of copper levels and oxidative stress in WT, ATP7B H1069Q and ATP7B *R778L* HLCs treated with copper (CuCl2 0-250 µM)**.** Copper levels and reactive oxygen species were measured using fluorescence induced by copper green dye and CellRox Orange, respectively. Fluorescence intensity measured by ImageJ software and normalized to number of nuclei (3 different fields)**.**

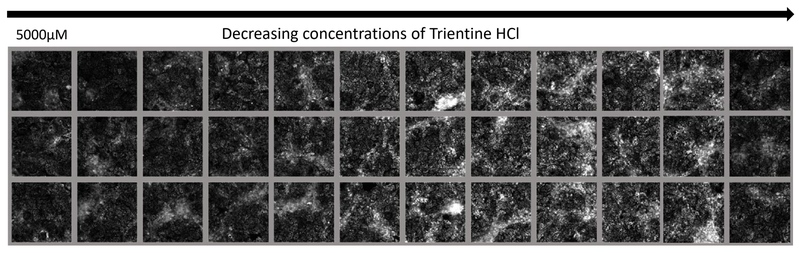


Figure 9. Effect of escalating doses of the Cu^{2+} chelator: Trientine Hydrochloride in oxidative stress. CellROX orange staining

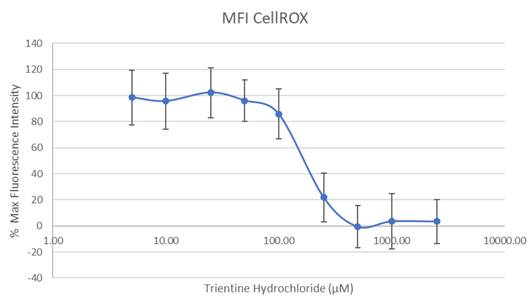
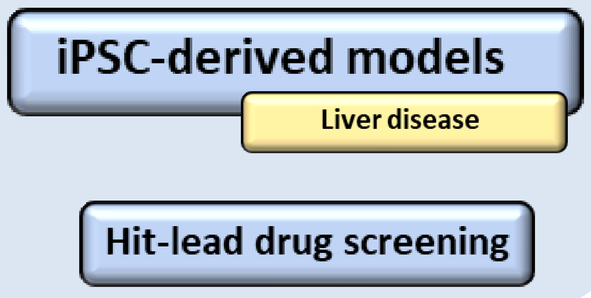


Figure 10. Dose response observed between 100 and 500 µM Trientine Hydrochloride Ave. No CuCl2 set as 0% intensity.

Future work

DefiniGEN will continue to focus on the development of iPSC-derived models of liver disease and the use of these models to provide hit-lead drug screening services for the pharmaceutical sector.



DefiniGEN Ltd, Babraham Research Campus, Moneta Building B280, Cambridge CB22 3AT, Tel: +44 (0) 1223 497106
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