Non-alcoholic fatty liver disease (NAFLD) is now considered the most prominent cause of chronic liver disease in the Western world and its worldwide prevalence continues to increase. NAFLD includes a wide spectrum of liver abnormalities with the most severe cases involving progressive liver injury in the form of non-alcoholic steatohepatitis (NASH). Key characteristics of NASH include lipid accumulation in hepatocytes, chronic inflammation, collagen deposition and fibrosis. There are currently no FDA approved drugs for the treatment of NAFLD/NASH and there is a clear requirement for better models to understand this disease.

The single nucleotide polymorphism I148M in the gene encoding patatin-like phospholipase domain-containing protein 3 (PNPLA3) is strongly associated with adverse outcomes for NAFLD patients. PNPLA3 I148M is associated with increased hepatic inflammation, steatosis and enhanced development and severity of liver fibrosis (2). The mutation is proposed to directly affect primary human hepatic phenotype (PHH) propensity to become steatotic and the fibrogenic phenotype of hepatic stellate cells (HSC). Primary HSC I148M HSC have been shown to demonstrate profoundly more inflammatory mediators, and have higher lipid droplet content than equivalent wild-type (WT) HSC (3).

PNPLA3 I148M effects in HSC have only been explored in isolation and not in a NAFLD disease model. Using a fully human in vitro NAFLD triple co-culture model, which comprises PHH, Kupffer (HK) and HSC, we hypothesised that PNPLA3 I148M in HSC influenced NAFLD development.

Using the organ-on-a-chip technology Physiomer™, we have developed a fully human in vitro NAFLD/NASH model, utilising multiple primary human liver cell types. To demonstrate the utility of this model, we have analysed how the I148M mutation in the PNPLA3 gene can be specifically in HSC affects the disease morphology of the model. Mutations in this gene are associated with disease progression and have recently been shown to directly influence HSC disease phenotype.

**Methods and materials**

Cryopreserved human hepatocytes and human Kupffer cells were obtained from Life Technologies (USA). Hepatic stellate cells were isolated and cryopreserved by Krista Rombouts (UCL). 0.6 x 10⁶ Hepatocytes were seeded into each well on the PhysioMimix™ LC-12 plate, alongside 0.06 x 10⁶ HKPHH and 0.06 x 10⁶ HSCPHH. Cells were cultured in lean or fat DMEM containing standard supplements, including physiologically relevant concentrations of glucose, insulin, and 500 µM free fatty acids.

Fat accumulation was measured by Oil Red O staining of fixed microtissues. Staining was quantified by absorbance at 515 nm and normalised to total protein content, measured by BCA assay (Thermo Fisher). Production of IL-6, FGF-15 and albumin were all measured by ELISA (R&D Systems). Free fatty acid consumption was measured using a colourimetric assay (Almac). Cytokine profiles were also analysed in cell culture samples using Bio-Plex Pro™ Human Cytokine 18-plex (Bio-Rad).

To visualise Kupffer and stellate cells in the platform, these cells were transduced with Adenoviruses expressing mGFP and mCherry (Vector Laboratories) (BD) prior to plating into PhysioMimix LC-12 plates. Microtissues were imaged on a Nikon Eclipse Ti-E inverted fluorescent microscope. Total RNA was extracted using TRIzol (Ambion), and cDNA was逆转应醉乙型肝炎 synthesis kit (Life Technologies). Gene expression profiles were quantified in equivalent cDNA samples using Taqman assays and primer/probe dual fluorescent probes (Taqman). Mutations in PNPLA3 (I148M variant) were detected using Taqman assay rs738404 on whole genomic DNA extracted using QIAGEN DNeasy blood and tissue kit.