Identification and characterisation of a rare *MTTP* variant underlying hereditary non-alcoholic fatty liver disease.

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Conflicts of Interest

Guruprasad Aithal has served as a consultant and an advisory board member for Pfizer Inc, Inventiva Pharma, GlaxoSmithKline and KaNDy Therapeutics; he has been a consultant to Servier, Clinipace, Albireo Pharma, BenevolentAI Bio, DNDi, BerGenBio ASA, Median Technologies, FRACTYL, Amryt Pharma and AstraZeneca; and has given presentations on behalf of Roche Diagnostics and Medscape. Ioanna Ntalla is employed by Gilead Sciences Ltd. (since August 2019). All other authors declare no conflict of interests

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Abstract

Background and aims: Non-alcoholic fatty liver disease (NAFLD) is a complex trait with an estimated prevalence of 25% globally. We aimed to identify the genetic variant underlying a four-generation family with progressive NAFLD leading to cirrhosis, decompensation and development of hepatocellular carcinoma in the absence of common risk factors such as obesity and type 2 diabetes.

Methods: Exome sequencing and genome comparisons were used to identify the likely causal variant. We extensively characterised the clinical phenotype and post-prandial metabolic responses of family members with the identified novel variant in comparison to healthy non-carriers and wild-type patients with NAFLD. Variant-expressing hepatocyte-like cells (HLCs) were derived from human induced pluripotent stem cells generated from homozygous donor skin fibroblasts and restored to wild-type using CRISPR-Cas9. The phenotype was assessed using imaging, targeted RNA analysis and molecular expression arrays.

Results: We identified a rare causal variant c.1691T>C p.I564T (rs745447480) in *MTTP*, encoding microsomal triglyceride transfer protein (MTP), associated with progressive NAFLD, unrelated to metabolic syndrome and without characteristic features of abetalipoproteinemia. HLCs derived from a homozygote donor had significantly lower MTP activity and lower lipoprotein ApoB secretion compared to wild-type cells, while having similar levels of MTP mRNA and protein. Cytoplasmic triglyceride accumulation in HLCs triggered endoplasmic reticulum stress, secretion of pro-inflammatory mediators and production of reactive oxygen species.

Conclusion: We have identified and characterized a rare causal variant in *MTTP* and homozygosity for *MTTP* p.I564T is associated with progressive NAFLD without any other manifestations of abetalipoproteinemia. Our findings provide insights into mechanisms driving progressive NAFLD.

Lay Summary

- A rare genetic variant in the gene *MTTP* has been identified as responsible for development of severe non-alcoholic fatty liver disease in a four-generation family with no typical disease risk factors.

- A cell line culture created harbouring this variant gene was characterized to understand how this genetic variation leads to a defect in liver cells which results in accumulation of fat and processes which promote disease.

- This is now a useful model for studying the disease pathways and to discover new ways to treat common types of fatty liver disease.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is a complex trait encompassing a spectrum of accumulation of triglyceride-rich lipid droplets within the hepatocytes (steatosis), non-alcoholic steatohepatitis (NASH; having ballooning degeneration and inflammatory cell infiltration), varying degree and pattern of fibrosis leading to cirrhosis and its decompensation, as well as hepatocellular carcinoma (HCC). With rising incidence of obesity and type 2 diabetes, NAFLD is now the most common chronic liver disease with an estimated 25% population prevalence globally.[1]

Genome-wide association studies (GWAS) have identified a number of genetic risk variants for NAFLD including *PNPLA3* rs738409 and *TM6SF2* rs58542926, both of which have robust associations with disease phenotypes via functional pathobiological pathways.[2,3] Accretion of the PNPLA3 variant on lipid droplets sequesters coactivators, resulting in reduced lipolysis and lipophagy, and the TM6SF2 variant impairs VLDL lipidation. Accumulation of triglycerides in both contexts is associated with progressive liver disease.[2]

Microsomal transfer protein (MTP) as a heterodimer with protein disulphide isomerase (PDI) catalyses lipidation and assembly of ApoB-containing lipoproteins for secretion by hepatocytes and *MTTP* variants have been linked with susceptibility to NAFLD[4,5]. Rare,

loss-of-function mutations in *MTTP* can result in the recessive disorder abetalipoproteinemia[6], where MTP deficiency causes defective lipoprotein biosynthesis having multiple severe effects including liver steatosis and fibrosis.[5,7] However, hereditary progressive NAFLD associated with a *MTTP* variant, without any manifestations of abetalipoproteinemia, has not been previously described.

Here we have clinically characterised a large four-generation family found to have a rare *MTTP* variant located at the interface with PDI resulting in progressive NAFLD, with consequent cirrhosis, liver failure and hepatocellular carcinoma in homozygotes. We evaluated post-prandial metabolic responses in carriers of the novel *MTTP* p.I564T variant compared to non-carriers. We used hepatocyte-like cells (HLCs) derived from human induced pluripotent stem cells (hiPSCs) generated from donor skin fibroblasts from carriers and non-carriers of the *MTTP* variant, as a stable reproducible model for understanding the effect of the variant on the cellular phenotype. This has enabled us to understand how disrupted hepatic lipid homeostasis can drive steatosis and NAFLD and therefore linking genotype to phenotype in hereditary NAFLD.

Patients and methods

Further details of methods used are available in Supplementary material.

Human Samples

The clinical studies were approved by the Health Research Authority after review by the National Research Ethics Service: East Midlands Northampton Committee for the Genetics of Rare Inherited Disorders (GRID) study (Ref 12/EM/0262); North-East Committee for meal-response study (Ref: 16/NE/0251). Studies were conducted according to the Declaration of Helsinki (Hong Kong Amendment) and Good Clinical Practice (European guidelines). All participants provided written informed consent. For the meal-response analysis, participants were recruited to the study at Queens Medical Centre, Nottingham University Hospitals between 1/11/2016 and 1/6/2017. Patients with biopsy-proven NAFLD, sex and age matched (within 10 y) to family members, were

consecutively identified from a large secondary care cohort who had previously participated in research, and invited to participate. Healthy volunteers were similarly identified and invited. None had diabetes or hazardous alcohol intake and had no known liver disease and had circulating caspase-cleaved CK18 level below 99 U/L.

Clinical investigations followed standard clinical care and included 6-month follow-up as required. Variants segregating with disease were identified following exome sequencing (Illumina HiSeq2000). Genotype determination was done using Sanger sequencing (Source Bioscience Ltd) or PCR restriction fragment analysis.

In silico analysis

Models of MTP were based on Protein Data Bank sequence 617S[8] and visualised using Visual Molecular Dynamics software.[9]

Metabolite and protein analyses

Serum cholesterol, triglycerides, and ApoB, and plasma glucose were quantified using calibrated Horiba auto-analyser and reagents following validated standard manufacturer protocols (Horiba ABX) at the University of Nottingham Metabolic Analysis Facility. Serum insulin was quantified using Human Insulin specific RAI kit (Millipore). Plasma lipoproteins were separated by sequential non-equilibrium density-gradient ultracentrifugation.

Apolipoprotein B-100 (apoB-100) was determined in culture supernatants by ELISA (Sigma-Aldrich) in duplicate (twice). MTP activity was determined in lysed cells (in triplicate) at 4 dilutions using MTP Activity Assay Kit (Sigma-Aldrich). Human NFκB Pathway, Phospho-Kinase and XL Cytokine Array Kits (R&D Systems) were used to determine protein expression or secretion.

Fibroblast reprogramming, hiPSC maintenance and differentiation

Two 2mm skin punch biopsies were obtained from study participants and primary dermal fibroblasts established and skin fibroblasts were reprogrammed using CytoTune iPS 2.0 Sendai Reprogramming Kit (Invitrogen, ThermoFisher) in accordance with the

manufacturer's guidelines. Mesoderm, ectoderm and hepatocyte differentiation of hiPSCs was as described previously.[10-12]

CRISPR-Cas9 mediated correction of I564T mutation in the *MTTP*$^{(VAR/VAR)}$

For CRISPR-Cas9 editing, single-guide RNA (gaacatcctgctgtctactg) was cloned and nucleofected (Lonza) into the *MTTP*$^{(VAR/VAR)}$ parental line.[13] Clones were screened to select one with corrected alleles. hiPSCs derived from clones with corrected allele *MTTP*$^{(WT*/WT*)}$, and the parental line were differentiated to HLCs in parallel for characterisation.

Imaging and analysis of mitochondrial function and cellular reactive oxygen species

Cells were stained using Nile Red, Hoechst, DAPI or antibodies. Mitochondrial content of HLCs were visualised using 100nM MitoTracker green FM or MitoTracker deep red FM and intracellular reactive oxygen species (ROS) and mitochondrial superoxide production were assessed using 2.5μM CellROX green or 2.5μM MitoSox Red. Mitochondrial respiration was determined using the Seahorse XF96 analyser (Seahorse Bioscience, USA).

Gene expression analysis and RNA sequencing

Quantitative real-time PCR (qPCR) was carried out as described in supplemental methods. Fold changes in expression were calculated using comparative ΔΔCt method standardised against the housekeeping gene PBGD and mean of Ct values±standard error of mean (SEM) reported.[12] RNA sequencing and bioinformatics analysis were performed at the Babraham Institute (Cambridge, UK).

Statistical analysis

Statistical analyses were performed using Graph Pad Prism version 8 (La Jolla, USA) software. One-way ANOVA followed by Dunnett's multiple comparison test were used to compare data from samples grouped by a single factor. Student's T-test was used to compare the means of variables determined in two groups.

Results

Clinical presentation of family

A British four-generation family with recent Indian ancestry (Fig. 1A) was referred for genetic counselling after three individuals from the same generation developed HCC. Parent A had no history of NAFLD or metabolic syndrome; Parent B presented with NAFLD symptoms aged 80 and was diagnosed with cirrhosis aged 87. Clinical investigations found all ten children had NAFLD diagnosis as adults (C-L in Table 1) with progression to NASH, cirrhosis (in seven) and HCC (in four) suggestive of a high conversion rate between NAFLD to cirrhosis and hepatocellular carcinoma. Only one of the affected individuals had BMI>30 and instances of type 2 diabetes, hypertension or hyperlipidaemia within the family were not linked with the presence or severity of disease.

Identification of rare MTTP variant allele associated with diagnosis

Functional variants which were unique to affected family members were identified by whole exome sequencing of twelve affected individuals (Fig. 1A) by comparison with nine unaffected South Asian controls (including spouses of F, G, H & J , three unrelated participants from the EXCEED study[14], and two unrelated South Asian individuals with cholangiocarcinoma) and databases of genetic variation identified in the general population. We identified a missense variant: genomic NC_000004.12:g.99608899T>C, NM_000253.2:c.1691T>C, protein NP_000244.2:p.Ile564Thr, in *MTTP* that was unique to affected family members (Figs. 1B and 1C) and fully segregated with disease phenotype in those individuals analysed. All six homozygous individuals developed cirrhosis and three also developed HCC, while some heterozygotes had no diagnosed disease (Table 1). Presence of both heterozygotes and homozygotes for the rare allele in 3rd generation of the family implies that individual A must also have carried the variant allele. The presence of fatty liver in wild-type individual I is suggested to be incidental relating to lifestyle factors.

This I564T variant has been previously described in combination with a second rare variant (IVS1+1G>C), manifesting as severe fatty liver in an atypical case of abetalipoproteinemia in Japan[15] but was reported to have 'mild effect' in the mother carrying I564T alone. The I564T variant is described in the NCBI database[16] as rs745447480 with allele frequency $<1.6 \times 10^5$ in an analysis of 251,024 alleles (gnomAD exomes v2.1.1) present in four non-Finnish European cases. The NCBI allele frequency aggregator population database reports 2 variants out of a 35,910 global total (both were in European population). GEM-Japan whole genome aggregation panel reports 1 allele in 15,198[17]. Other family members were subsequently tested for this variant and clinical features of abetalipoproteinemia[6] were investigated (Tables 1 & 2). Their genotype for other common functional variants at loci in *MTTP* (rs745447480, rs3816873, rs2306985), *PNPLA3* (rs738409) and *TM6SF2* (rs58542926) associated with NAFLD was also determined. None of 83 NAFLD patients from the Trivandrum cohort[18] had the *MTTP* p.I564T variant allele.

Mapping of the I564T variant onto the crystal structure[8] located it to heterodimer interaction interface within a hydrophobic pocket on the MTP subunit surface having surrounding polar and charged residues. (Fig. 1C & D). Mutation analysis predicts medium mutation sensitivity and substitution to threonine, a polar residue, will likely destabilise this hydrophobic pocket, promote interactions with the surrounding polar/charged residues and thereby cause local conformational variations (PolyPhen=1; GERP=5.120; CADD=20.4, Mutation Assessor=0.76, REVEL=0.519)[19]. A plausible impact on dimer formation and MTP functionality is therefore expected. In contrast, substitutions E98D, N166S, I128T and H297Q arising from common SNPs, map to the protein surface exposed to the solvent in the complex structure (Fig. 1D) and mutation analysis predicts low mutation sensitivity and high tolerance of substitutions at each of these positions.

Postprandial responses in affected individuals

The phenotypic impact of the *MTTP* p.I564T variant was assessed through investigation of metabolic responses to fat consumption. Family members were invited and responses

in five participants were compared to that in age and gender matched healthy volunteers and NAFLD patients (Fig. 2A; Supplementary Table 1). The level of ApoB, the protein constituent assembled into chylomicron and very low density lipoproteins (VLDL) via the activity of MTP, was notably lower in the MTP564-TT homozygote F than in other participants including two MTP564-IT heterozygotes (participants K and Q) and the MTP564-TT liver-transplant recipient J (Fig. 2B). Subsequent testing of six further heterozygotes also showed levels within the normal range. Testing of a stored, pre-transplant serum sample from individual J and clinical data revealed that levels were also markedly lower before receiving a replacement liver where the gene is likely restored.

We also compared levels of serum ApoB-100, the isoform associated with VLDL, in the MTP564-TT homozygotes F and J, with that of wild-type MTP564-II control, participant 1, supporting the proposal that expression of the variant form in the liver results in reduced VLDL secretion (Fig. 2C). Levels of total cholesterol both before and after the meal were also noticeably lower in the MTP564-TT homozygote F (Supplementary Fig. 1) compared to all other study participants due to only very low levels of HDL-cholesterol being present (<0.4mmol/L). Clinical data confirms this observation (Table 1) and the same phenotype was noted in another MTP564-TT homozygote, H; however levels in homozygote J, pre-transplant, were normal. Participant F also reported post-prandial gastrointestinal discomfort and diarrhoea following the study meal.

The levels of circulating triglyceride in F were lower than in the healthy and disease controls (participants 1 and 2, Fig. 3A) but were similar to levels in participant 3 who possessed two variant alleles for *TM6SF2* rs58542926 (TM6SF2-KK). TM6SF2 is also involved in VLDL lipoprotein secretion and the variant associated with impaired function and decreased serum LDL-cholesterol.[20,21] In contrast, the MTP564-TT homozygote with a liver transplant showed a similar triglyceride response to her two matched controls (Fig. 3B). Investigation of VLDL and chylomicron lipoprotein associated triglycerides (Fig. 3C-F) revealed that both components were again lowered in participant F (MTP564-TT) and 3 (TM6SF2-KK) compared to the controls while appeared normal the transplanted MTP564-TT, J. Other family members had no notable defects in lipoprotein triglycerides

(Supplementary Fig. 2). Furthermore, VLDL-cholesterol levels were similarly blunted in participants F (MTP564-TT) and 3 (TM6SF2-KK) but not in other family members (Supplementary Fig. 3) suggesting that transplant hepatocytes and MTP564-IT heterozygote hepatocytes are functioning effectively in VLDL secretion.

Of note, lipoprotein-associated lipid levels are also lower in participant 7, a NAFLD patient who is homozygous for the *PNPLA3* rs738409 variant (PNPLA3-MM) which has been linked to a relative reduction in large VLDL secretion[22] (Supplementary Figs. 2A and 3F). Circulating free fatty acids, glucose and insulin levels in the family members were unremarkable (Supplementary Fig. 4).

Generation of wild-type and MTTP$^{(VAR/VAR)}$ hiPSCs for disease modelling

To elucidate the mechanisms driving hepatic steatosis in homozygous MTP564-TT patients, we generated hiPSCs and differentiated them into hepatocytes to create an in vitro model of the variant. Fibroblasts derived from participant 1, genotyped as MTP564-II (also PNPLA3-148-IM; TM6SF2-167-EE), and family member J, MTP564-TT (also PNPLA3-148-IM; TM6SF2-167-EE), were expanded up to passage 4 and then reprogrammed into hiPSCs: *MTTP$^{(WT/WT)}$* and *MTTP$^{(VAR/VAR)}$*, respectively (Supplementary Figs. 5-7). Neither carry the TM6SF2 variant that could confound the observed phenotype. Reprogrammed fibroblasts displayed the typical features of hiPSCs forming dense cell colonies, with well-defined colony boarders, containing cells with a high nuclear to cytoplasm ratio. To confirm their pluripotent status, Oct3/4 and NANOG expression was assessed and their ability to differentiate into endoderm, mesoderm and ectoderm determined. We confirmed the karyotype as normal, without any major chromosomal abnormalities.

MTP levels are lower and lipoprotein secretion is impaired in *MTTP$^{(VAR/VAR)}$* HLCs compared to *MTTP$^{(WT/WT)}$* HLCs

To prevent bias due to differing differentiation efficiency of hiPSC lines, we differentiated cells from the study donors 1 and J into HLCs to compare morphology and gene expression profiles.[12] Both cell lines appeared morphologically similar during all stages of differentiation and generated a monolayer of HLCs by day 21 (Fig. 4A). Gene expression

was similar at each of the developmental time points including definitive endoderm, foregut endoderm, and hepatoblast cells. Expression of genes associated with a mature hepatocyte phenotype was not significantly different between the two cell lines (Supplementary Fig. 7). Analysis of mRNA expression patterns for both cell lines primarily matched 'liver bulk tissue' then 'hepatocyte' (Supplementary Table 2) and both showed high similarity to HepG2, HUH7 and HEP3B cell lines.

Nile Red and Oil-Red-O staining revealed phenotypical differences with apparent significant sequestration of lipid via development of discrete lipid droplets and microvesicular steatosis throughout the cytoplasm in the *MTTP*$^{(VAR/VAR)}$ HLCs after 48h of culture (Fig. 4B,C). Quantification showed levels were >4-fold higher in *MTTP*$^{(VAR/VAR)}$ HLCs, (Fig. 4E), consistent with the proposed reduced VLDL secretion in cells expressing MTP564-TT, restricting removal of intracellular triglycerides.

Importantly however, although both immunocytochemistry and mRNA expression analysis suggested a trend towards lower MTP levels compared to cells expressing the wild-type allele this was not statistically significant (Fig. 4D,F,G). To assess VLDL export capabilities of the cell lines, and thus functioning of variant MTP in lipoprotein biosynthesis, levels of secreted ApoB-100 were determined. There was significantly less, but detectable, ApoB-100 in the media from *MTTP*$^{(VAR/VAR)}$ compared to *MTTP*$^{(WT/WT)}$ HLCs (Fig. 4H) confirming the clinical phenotype and supporting the suggestion that the MTP variant in these patients affects lipid trafficking.

Increased generation of ROS and altered mitochondrial respiration in *MTTP*$^{(VAR/VAR)}$ HLCs

Hepatic free fatty acids can be converted to triglyceride for storage as cytoplasmic droplets or secreted as VLDL, or else directly metabolised via mitochondrial β-oxidation. Therefore, impaired MTP functionality restricting lipid secretion, thus increasing the availability of fatty acids, may impact on mitochondrial activities. Using mitochondrial stress testing measuring the oxygen consumption rate in live cells revealed *MTTP*$^{(VAR/VAR)}$ HLCs had significantly higher basal and maximal mitochondrial respiration compared to mitochondria from the wild-type cell line (Figure 4I). Of importance, increased β-

oxidation would generate additional ROS, which can be a major driver of oxidative stress and cellular dysfunction. Both mitochondrial superoxide production and cytoplasmic ROS were significantly higher in the *MTTP*$^{(VAR/VAR)}$ HLCs compared to *MTTP*$^{(WT/WT)}$ (Fig. 4J,K and Supplementary Fig. 8) consistent with increased fatty acid metabolism.

Increased NFκB signalling, inflammation, ER stress and secretion of pro-inflammatory mediators in *MTTP*$^{(VAR/VAR)}$ HLCs

Impaired lipid trafficking and lipoprotein assembly incurred as a consequence of reduced MTP functionality is likely to cause a range of cellular responses including ER stress and inflammation. Analysis of mRNA revealed that expression of ER stress mediators: spliced X-Box Binding protein-1 (SxBP1); activating transcription factor 6 (ATF6); binding immunoglobulin protein (BIP); and the ER stress transducer, inositol requiring enzyme 1 (IRE1) were significantly higher in *MTTP*$^{(VAR/VAR)}$ HLCs (Fig. 5A).

MTTP$^{(VAR/VAR)}$ HLCs expressed significantly higher levels of NFκB (Fig. 5B), suggesting greater ER stress and active pro-inflammatory response.

These observations were confirmed by assessing changes in intracellular and extracellular signalling (Fig. 5C-E). The observed lipid accumulation in the novel MTP564-TT variant strain was associated with increases in NFκB pathway components, indicative of activation, and phosphorylation of pro-inflammatory and pro-apoptotic pathway mediators including RelA/p65 complex, ASC, p53, FADD, and CD95. This increased pro-inflammatory signalling coincided with increased secretion of pro-inflammatory mediators including CCL3/4, BAFF, CD30, IL-1, IL-10 and IL- 23, DBP and leptin while showing decreased RBP4 and PDGF. Notably, *MTTP*$^{(VAR/VAR)}$ HLCs had more than 10-fold lower expression of phosphorylated-eNOS compared to *MTTP*$^{(WT/WT)}$ HLCs. There were no significant differences in protein phosphorylation in the other 44 proteins assayed. However, there was a trend towards decreased ERK1/2 phosphorylation in *MTTP*$^{(VAR/VAR)}$ HLCs.

$MTTP^{(VAR/VAR)}$ HLCs show increased expression of ECM remodelling and lipid metabolising genes.

Bioinformatics analysis of mRNA sequencing data to assess genome wide changes in gene expression revealed 472 genes differentially expressed (>1x log2-fold) between $MTTP^{(VAR/VAR)}$ and $MTTP^{(WT/WT)}$ cultured HLCs. These fulfil diverse cellular functions including glycolysis, lipid oxidation, oxidative phosphorylation and complement activation (Supplementary Fig. 9) and support previous observations of increased ROS generation and altered mitochondrial activity. Gene Ontology (GO) terms associated with the changing genes (Supplemental Tables 3-5) were mostly implicated in extracellular matrix (ECM) remodelling, ECM organisation and degradation, ECM receptor interactions, and proteoglycan modifications, suggesting that ECM remodelling may be initiated during hepatosteatosis.

Confirmation that the homozygous rs745447480 variant in the $MTTP^{(VAR/VAR)}$ HLCs results in significantly lower MTP lipid transfer activity.

A third cell line, $MTTP^{(WT*/WT*)}$, was generated from $MTTP^{(VAR/VAR)}$ in which the MTP564-TT in was gene-edited to wild-type 564-II using CRISPR-Cas9 transfection and selection of a corrected cloned (Supplemental Fig. 10). The resultant differentiated cell line displayed the same characteristics as the $MTTP^{(WT/WT)}$ cell line (Fig. 6 and Supplemental Fig. 11). This enables us to rule out possibility of other genetic variants harboured by the patient or healthy volunteer influencing the observed in vitro phenotype. MTP lipid transfer activity of the original patient-derived HLCs, $MTTP^{(VAR/VAR)}$, was compared to that of the gene-edited HLCs to establish the impact of the SNP rs745447480. For equivalent cellular protein quantity, MTP activity was significantly lower in the $MTTP^{(VAR/VAR)}$ HLCs compared to the edited derivative $MTTP^{(WT*/WT*)}$ having only 61% of the level determined in the wild-type cells. This is distinct from other described variants which abolish MTP activity and may explain the observed apparent phenotype of impaired lipid trafficking. To assure that the observed phenotype does not reflect compensatory activity of TM6SF2 in the same pathway, TM6SF2 mRNA expression levels were determined. This indicated that TM6SF2 expression, normalised to undifferentiated cells, is not higher in

MTTP$^{(VAR/VAR)}$ compared to *MTTP*$^{(WT*/WT*)}$ and therefore suggests that the observed phenotype is not due to increased TM6SF2 (Supplemental Fig. 12).

Discussion

We have identified and characterised a rare *MTTP* variant (p.I564T) as causative for the Mendelian trait associated with an inherited form of NAFLD, in a four-generation family. Our investigation has revealed a variant resulting in decreased ApoB-containing lipoprotein secretion in homozygotes (but not heterozygotes), in contrast to other variants causing abetalipoproteinemia where ApoB is undetectable (Fig. 2, Table 1)[6,7,23-25]. Although other carriage of this variant have been reported, no phenotypic characteristics related to these are previously described[15], none of the GWAS so far including that using UK biobank[26] and largest cross-ancestry GWAS[27] have identified particular *MTTP* variant (p.I564T) in association with NAFLD. Protein modelling (Fig. 1C) suggests the substitution moderately affects the protein structure and likely impacts upon the interaction with PDI in the formation of a normal heterodimeric enzyme, but is unlikely to abolish all functionality as in abetalipoproteinemia. Presentation of homozygote cases is clearly distinct from abetalipoproteinemia [23,28] supporting suggested subtle phenotype whereby impact is limited to liver lipid imbalance. This provides potential for treatment through reduced dietary fat intake and makes it an attractive model for cellular consequences of lipid accumulation.

Our phenotyping studies demonstrated distinct VLDL secretion responses following meal challenge and ApoB levels in the two MTTP-564TT family members: while these biomarkers were substantially low in untreated individual F, these were in the normal range in the liver transplant recipient J (Figure 2b; Table 1). Previously, the *MTTP* -493 variant (rs1800591) G allele linked with reduced MTP function has been associated with NAFLD susceptibility in a meta-analysis of 11 case-control studies.[29] Also an association study in non-diabetic patients with NASH found GG homozygotes had significantly higher plasma triglycerides, intestinal and hepatic large VLDL and oxidised LDL than GG/GT group.[30] All of the five MTP564-IT heterozygous individuals showed ApoB and lipoprotein

levels within the normal range consistent with reports that a single copy of MTTP is sufficient.[31]

Functional analysis of a childhood case with compound heterozygosity for MTTP c.619-5_619-2del and p.L435H, having severe liver fibrosis but no typical abetaliporoteinemia symptoms, found that p.L435H abolished MTP activity while the intronic variant resulted in 26% of transcripts being normally spliced allowing limited MTTP expression.[32] This suggested that the residual expression and resulting MTP activity was sufficient for substantial biological activity covering majority of necessary functionality, except for liver functions. Consistent with this, our observed modest reduction in activity to 61% would thus be predicted to have a subtle effect only on liver homeostasis. The modest impact on protein function in vitro is compatible with the less severe, non-abetalipoproteinemia clinical phenotypes described. The observed 'normal' levels of expression in HLCs may suggest that the I564M change effects either translation, protein stability/turnover or enzymatic function. Our in silico models suggested that the impact is on heterodimer assembly, stability, interaction, or activity. The latter would be entirely compatible with the unchanged protein levels observed in HLCs.

Disruption of ApoB biosynthesis and associated VLDL secretion has been widely described with a spectrum of consequences linked to characterised pathologies.[33] ApoB missense variants are also associated with development of fibrosis and HCC linked to NAFLD[34]. Furthermore, rare variants in MTTP were found to be associated with increased hepatic fat in the UK Biobank cohort[5]. The underlying mechanisms are inherently linked to nutritional intake with diets high in fats (increasing hepatic fat content) and carbohydrate (increasing hepatic de novo lipogenesis) resulting in hyperlipidemia. Hepatic lipid balance is dependent on secretion of VLDL, which is limited by availability/activity of MTP, so any variants with altered activities are likely to have metabolic effects. The low frequency of the MTTP p.I564T variant reported in large datasets[16] means that identification, recruitment and analysis of further carriers to strengthen the study would be very difficult.

Overall, VLDL secretion may increase with hepatic steatosis related to metabolic syndrome.[35] However, decreased VLDL secretion has been reported in carriers of PNPLA3 G allele[22]. VLDL secretion is also lowered in *TM6SF2* T carriers[20,21,36] which affects the same pathway. We considered key genetic risk factors, PNPLA3 and TM6SF2 variants, likely to influence disease phenotype since polygenic scores have been proposed for NAFLD[37]. TM6SF2 p.E167K is of particular interest since it acts in the same pathway as MTP so similarities in phenotypes would be expected. In our study, postprandial secretion of VLDL, the predominant postprandial lipoprotein associated with hyperlipidemia[38], was lower in participants homozygous for *TM6SF2* or *PNPLA3*, but fasting ApoB levels were normal. Whereas in *MTTP* p.I564T homozygotes we observed both a reduced level of circulating ApoB and VLDL-associated lipids. We have specifically considered the possibility of functional redundancy between TM6SF2 and MTP and potential additive effects of the variants in vivo and in vitro. First, the clinical characterisation in Table 1 shows the disease phenotype is not linked to TM6SF2 carriage as 3 affected siblings do not carry the TM6SF2 variant. Second, the post-prandial lipid data is consistent with reduction in circulating lipids due to lack of either wild type MTTP or lack of wild type TM6SF2, whereas heterozygotes retain functionality.

In addition to demonstrating the functional consequences of the *MTTP* p.I564T variant, the HLCs derived from hiPSCs provide a disease model for early stage NAFLD, beyond triglyceride accumulation. As the donors are wild type for TM6SF2, the cell phenotype reflects only the impact of the MTTP variant. Studies have shown a link between the amount of steatosis, fibrosis development and liver disease mortality[39] with lipid metabolism acting as the initiator of progression to NASH.[40] Although triglyceride sequestration may be protective, but when fatty acid storage and disposal routes reach capacity, alternative pathways resulting in lipotoxicity can occur. Components of these pathways, such as ACC-1/2, FXR/FGF19/FXR4, and SCD-1, are thus being tested as therapeutic targets.[41] Increased mitochondrial fatty acid β-oxidation may provide a protective response but uncontrolled results in the generation of ROS which can be a major driver of oxidative stress and cellular dysfunction (Figure 4I, Figure 6).

We show that as lipid accumulation increases, hepatocytes have increased ER stress, activate pro-inflammatory signalling pathways including NFκB, P53, eNOS and secrete pro-inflammatory mediators. This coincides with increased production of reactive oxygen species, superoxide production and alterations to mitochondrial respiration driving the disease progression leading to cirrhosis and hepatocellular carcinoma as seen among the family members. Similar findings were reported in cardiomyocytes derived in an MTTP pR46G variant model.[42] Excessive lipid accumulation in hepatocytes can serve as substrates for the generation of lipotoxic species. One of the major consequences of hepatic lipid metabolism is mitochondrial β-oxidation and esterification to form triglycerides which can serve as a protective mechanism against lipotoxicity in hepatocytes. However, if lipid accumulation is in excess of the β-oxidation capacity, such as in NAFLD, toxic intermediates can accumulate which induce metabolic stress and subsequent inflammation and cell death. Changes in expression of ECM remodelling-associated genes, suggestive of its occurrence during steatosis may contribute to drive progression to fibrosis, which is clinically observed later.

We conclude that the main feature of the *MTTP* p.I564T variant is impaired ApoB secretion and hepatic lipid accumulation due to decreased lipid transfer activity distinct from classical abetalipoproteinemia phenotype where MTP expression is abolished. Identification and characterization of a rare disease such as hereditary NAFLD is of medical significance in Indian populations where high rates of founder events have been reported[43]. In addition, HLC modelling supports this, providing additional details of signalling, inflammatory and metabolic cellular pathways involved, highlighting pathophysiology driving NAFLD progression and possible therapeutic targets.

Data Availability

Study data are available on request. The three EXCEED exome sequences are available in the European Genome-phenome Archive using accession number EGAD00001007649. Access to sensitive genetic data and cell lines will be restricted to research facilities with institutional data and material transfer agreements to protect participant anonymity.

Abbreviations

ECM: extracellular matrix

ELISA: enzyme-linked immunosorbent assay

GO: Gene Ontology

GWAS Genome-wide association studies

HCC: hepatocellular carcinoma

hiPSCs: human induced pluripotent stem cells

HLCs: hepatocyte-like cells

MTP: microsomal triglyceride transfer protein

NAFLD: Non-alcoholic fatty liver disease

PCR: polymerase chain reaction

PDI: protein disulphide isomerase

ROS: reactive oxygen species

SEM: standard error of mean

VLDL: very low density lipoproteins

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Tables

Table 1. Clinical characteristics of family members.

Person (Fig 1A) *MTTP* p.Ile564Thr	Sex	Liver Disease Diagnosis (method)	Age Diagnosed	Type 2 diabetes	BMI	Hypertension	Liver biochemistry & lipid blood analyses at diagnosis	Other subsequent investigations, treatments and comorbidities	*PNPLA3* p.I148M	*TM6SF2* p.E167K	*MTTP* p.I128T	*MTTP* p.Q297H
B IT	♀	Cirrhosis & ascites (USS)	88	x	<30	✓	ALT=26, AP=120, Chol=3.5, TG=1.29,	Cardiovascular-related death aged 90.	MM	EK	TT	HH
C TT	♀	HCC & cirrhosis (CT/Biopsy)	57	✓	<30	✓	ALT=38, AP=129, Normal lipids,	Liver resection ablation, right hemihepatectomy, HCC recurred. Liver-related death.	IM	EE	TT	HH
D TT	♂	Cirrhosis (MRI)	60	x	<30	✓	Bilirubin=312µmol/L, Normal lipids,	Liver screen=normal. Died of gallbladder sepsis aged 72.	IM	EE	TT	HH
E IT	♂	FL (USS)	57	✓	30	✓	ALT=30, AP=75, Chol=2.5, TG=0.75, HDL=1.2, LDL=1.0	Stable for 18y: TE=5.8kPa; CAP=341; ApoB=0.94	IM	EE	nd	HH
F TT	♂	Cirrhosis (USS)	56	x	21	✓	ALT=23, AP=125, Chol=2.4, TG=0.58, HDL=1.7, LDL=0.4,	No retinitis pigmentosa. Vagotomy and pyloroplasty; Mild Bone marrow failure;	IM	EK	TT	HH
G T*[1]	♂	HCC & cirrhosis (Biopsy)	57	x	<30	x	Normal lipids	Multifocal HCC: Chemotherapy and Hepatectomy. Died aged 61.	nd	nd	nd	nd
H TT	♂	HCC, cirrhosis, Portal hypertension	51	x	<30	✓	Chol=2.2, TG=0.2, HDL=1.6, LDL=0.5,	Liver transplant x2. Died due to cardiac complications aged 63.	IM	EK	TT	nd
I II	♀	FL (USS)	50	x	<30	x		Diet/Lifestyle changed: recovered/stable >5y:TE=4.0kPa; CAP=223 ApoB=1.02	IM	EE	TT	HH
J TT	♀	Cirrhosis (MRI) HCC	50 54	x	25	✓	ALT=52, AP=87, ApoB=0.32, Normal lipids;	Liver transplant age 56: recovered/stable >5y:TE=4.6kPa CAP=214; BMI=20.3; Duodenal Biopsy: no evidence of abetalipoproteinemia	IM	EE	TT	HH
K IT	♀	NASH (USS) (TE:CAP=380)	48	x	24	✓	ALT=79, AP=108; Normal lipids; ApoB=1.0;	Diet/Lifestyle changed: recovered/stable 10y:TE=6.2kPa CAP=298. Duodenal Bx: no evidence of abetalipoproteinemia	IM	EK	nd	HH
L TT	♀	Cirrhosis (USS)	59	x	18	✓	Elevated ALT & AST Normal lipids	Stable for 10y: TE=10.3kPa: CAP=276, ALT=104, AP=87, AST=73, Cardiac arrhythmia, osteoporosis, Vitamin D deficiency	IM	EE	TT	HH
M II	♂	Healthy	x	x	26	x	Normal lipids	TE=4.6kPa; CAP=253; Vitamin D deficiency	I I	EK	IT	QH
N IT	♂	NASH (USS)	40	x	28	x	TE=3.6kPa CAP=373; lipids and ALT elevated	Lifestyle & diet modified – stable 7y	IM	EK	nd	QH
O IT	♂	FL (USS)	38	x	27	x	lipids elevated	Lifestyle & diet modified – recovered; Stable for 19y:TE=5.8kPa; CAP=341)	I I	EK	IT	QH
Q I T	♂	FL (USS)	28	x	19	x	Normal lipids, Duodenal Biopsy: evidence of duodenal lipid	Improved after 10y:TE=6.3kPa CAP=199.	MM	EE	IT	HH

For MTTP p.E98D all tested were EE; for MTTP p.N166S all tested were NN. 1likely T carrier based on pedigree. USS: abdominal ultrasonographic steatosis score; Bx: biopsy; FL: fatty liver; HCC: hepatocellular carcinoma; ALT: alanine transaminase U/L; AP: alkaline phosphatase U/L; Chol: cholesterol mmol/L; TG: triglycerides mmol/L; HDL: high-density lipoprotein mmol/L; LDL: low-density lipoprotein mmol/L; ApoB: apolipoprotein B g/L; TE: transient elastography; CAP: Controlled Attenuation Parameter dB/m; nd, not determined.

Table 2. Clinical characteristics of individuals investigated in generation IV.

MTTP genotype p.I564T	Sex male/female	Liver Disease Diagnosis (method)	Age at testing	Type 2 diabetes	BMI	Hypertension	Liver biochemistry & lipid blood analyses at diagnosis
I T	♂	NASH (USS)	32	x	20	x	ALT=53, AP=68, Chol=7, TG=3.14, VitD<15; TE=7.4kPa CAP=352
I T	♂	FL (USS)	37	x	18	x	Normal LFTs (ALT=14), Normal Lipids; HbA1c=9.5;
I T	♂	FL (USS)	40	x	28	x	ALT=74, Chol=6, TG=2.12; TE=3.6kPa CAP=373
I T	♀	healthy (USS)	29	x	20	x	Normal LFTs (ALT=13), Normal Lipids ApoB=0.95, TE=3.5kPa CAP=122
I T	♂	healthy (TE)	32	x	27	x	TG=2.57, Chol=3.7, ApoB=1.04. TE=5.3 kPa
I T	♂	healthy (USS)	31	x	18	x	Normal LFTs (ALT=19), Normal lipids,
I T	♀	healthy (USS)	37	x	19	x	Normal LFTs (ALT=10), Chol=4.2, TG=1.77; ApoB=0.96; TE=4.2kPa CAP=291
I T	♀	healthy (USS)	39	x	21	x	Normal LFTs (ALT=23), Normal Lipids;
I T[1]	♀	healthy (USS)	41	x	20	x	Normal LFTs (ALT=27), Chol=4.6, TG=2.15; ApoB=1.02, TE=3.1kPa CAP=231
I T[1]	♂	healthy (USS)	44	x	<30	x	Normal LFTs (ALT=27), Chol=3.5, TG=2.4; TE=5.5kPa CAP=281
I T	♂	healthy (USS)	49	x	<30	x	Normal LFTs (ALT=14), Chol=4.88, TG=0.98, ApoB=1.09, TE=5.1kPa CAP=245
I T	♀	FL (USS) healthy (USS)	35 38	x	21	x	Normal LFTs (ALT=12), Chol=6, TG=1.5; ApoB=1.31, TE=4.2kPa CAP=325. Recovered after 3y: TE=6.3kPa CAP=278
I T	♂	FL (USS) healthy (USS)	38 42	x	27	x	Normal LFTs (ALT=39), Chol=2.9, TG=0.98; Recovered after 4y: TE=5.8kPa CAP=243
I I[1]	♂	FL (USS) healthy (USS)	14 19	x	21	x	Normal LFTs (ALT=15), Normal Lipids Recovered after 5y: TE=6.9kPa CAP=216
I I[1]	♀	healthy (USS)	17	x	20	x	Normal LFTs (ALT=15), Normal Lipids TE=6.4kPa CAP=216
I I[1]	♀	healthy (USS)	20	x	<30	x	Normal LFTs (ALT=9), Normal Lipids

[1]deduced from pedigree. USS: abdominal ultrasonographic steatosis score; NASH: non-alcoholic steatohepatitis; FL: fatty liver; ALT: alanine transaminase U/L; AP: alkaline phosphatase U/L; Chol: cholesterol mmol/L; TG: triglycerides mmol/L; LFTs: liver function tests; ApoB: apolipoprotein B g/L (research laboratory data); TE: transient elastography; CAP: Controlled Attenuation Parameter dB/m.

Figure Legends

Fig. 1. Identification of a pathogenic variant in large family with non-alcoholic fatty liver disease.

(A) Pedigree. Clinical features are described in Table 1. Diagnosis is indicated by shading: black, hepatocellular carcinoma (HCC); dark grey, cirrhosis; light grey, non-alcoholic steatohepatitis (NASH); Dashed lines indicate no investigations; * Exome sequenced. Blue letters indicates residue 564 in MTP. (B) Variant rs745447480 sequencing. (C) local environment of I564 on MTP–PDI interface (hydrophobic pocket (white), polar (green) and charged (red/blue) residues); (D) 564T variant and common nonsynonymous variants (side-chains: C=cyan; O=red; N=blue) in model derived from PDB ID:617S[8], a heterodimer of Protein Disulfide Isomerase (PDI), blue, and MTTP gene product (MTP), grey.

Fig. 2. Meal-response study to investigate metabolism in family members and matched controls.

(A) Study Design. (B) ApoB levels. Participants are grouped according to age and gender matching to family members F, J, K, Q and M (Supplementary Table 1). Participants are grouped according to age and gender-matched (white bars are healthy volunteers (HV); grey bars are NAFLD patients). MTP residue 564 is indicated and PNPLA3 or TM6SF2 in parentheses if homozygous for variant rs738409 or rs58542926. (C) Serum ApoB-100. HV, healthy volunteers; NAFLD, non-alcoholic fatty liver disease.

Fig. 3. Triglyceride levels in study participants.

Total serum triglycerides: (A), Participant F and matched controls and (B), J and matched controls. VLDL-triglyceride: (C), Participant F and matched controls and (D), J and matched controls. Chylomicron-triglyceride: (E) Participant F and matched controls and (F), J and matched controls. *MTTP* genotypes are shown for family members. *PNPLA3* p.I148M and *TM6SF2* p.E167K genotype are indicated in parentheses.

Fig. 4. Characterisation of MTP-564T homozygote variant, *MTTP*$^{(VAR/VAR)}$ and wild-type hepatocyte-like cells (HLCs).

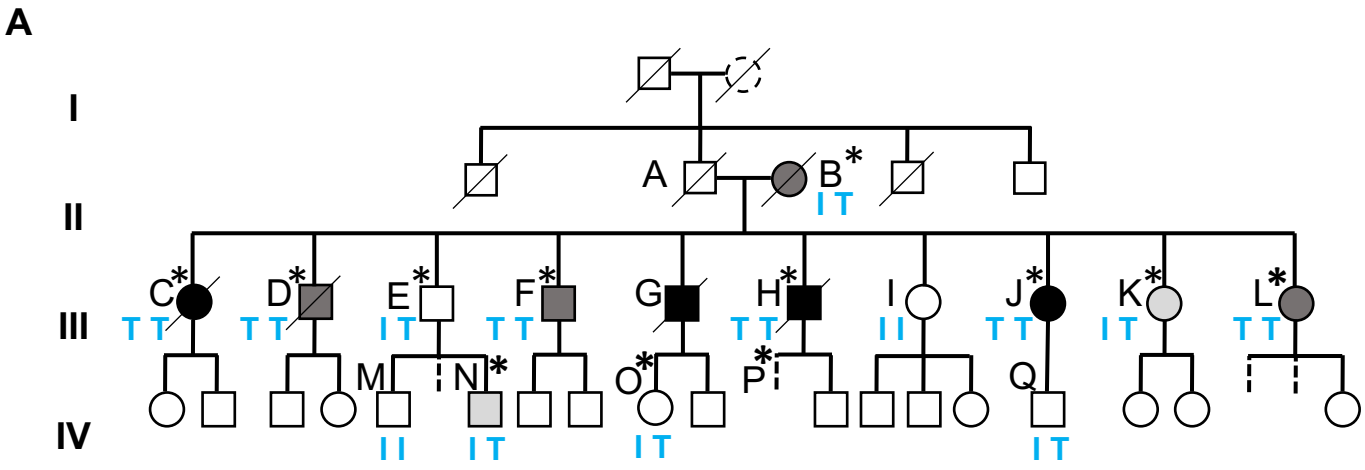
(A) Light microscopy image of terminally differentiated hiPSC-derived HLCs. (B) Oil-Red-O staining of HLCs (light microscopy). (C) Nile Red staining of lipids ±DAPI staining (fluorescence microscopy). (D) MTP protein expression immunocytochemistry ±DAPI staining. Quantification of Nile Red fluorescence (E), and MTP staining (F) in HLCs. (G) Expression of MTP determined by Q-PCR. (H) ApolipoproteinB-100 secretion by HLCs (ELISA). (I) Basal and maximal mitochondrial respiratory rates in *MTTP*$^{(WT/WT)}$ (●) and *MTTP*$^{(VAR/VAR)}$ (□) HLCs. Quantification of cellular superoxide (J) and reactive oxygen species (K) from fluorescence microscopy. Mean±standard error. $P<0.05$ is significant (T-test, paired, 2-tailed).

Fig. 5. Phenotypic characterisation of *MTTP*$^{(VAR/VAR)}$ hiPSC-derived HLCs.

(A) Expression of inflammation related and ER-stress related genes. (B) Expression of *NFκB*-associated intracellular signalling proteins in *MTTP*$^{(VAR/VAR)}$ HLCs, relative to expression in *MTTP*$^{(WT/WT)}$. (C) Phosphorylated proteins in *MTTP*$^{(VAR/VAR)}$ HLCs, normalised to *MTTP*$^{(WT/WT)}$. (D) Secreted proteins from *MTTP*$^{(VAR/VAR)}$ HLCs, normalised to *MTTP*$^{(WT/WT)}$.

Fig. 6. Restoration of activities by gene editing of *MTTP*$^{VAR/VAR}$ 564-TT to 564-II.

(A) Light microscopy showing terminally differentiated hiPSC-derived hepatocyte-like cells (HLCs) from *MTTP*$^{(VAR/VAR)}$ and gene-edited derivative *MTTP*$^{(WT*/WT*)}$. (B) MTP enzyme activity in HLCs. Mean±standard error; significance level $P<0.05$ (T-test).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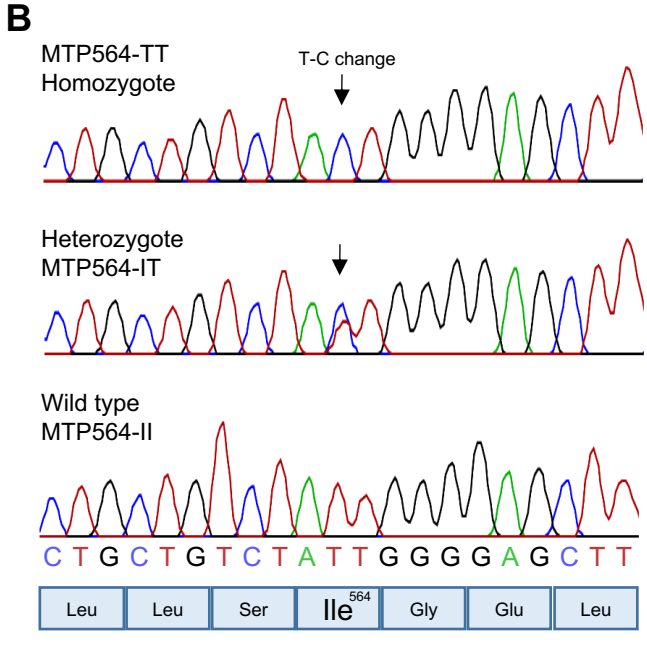
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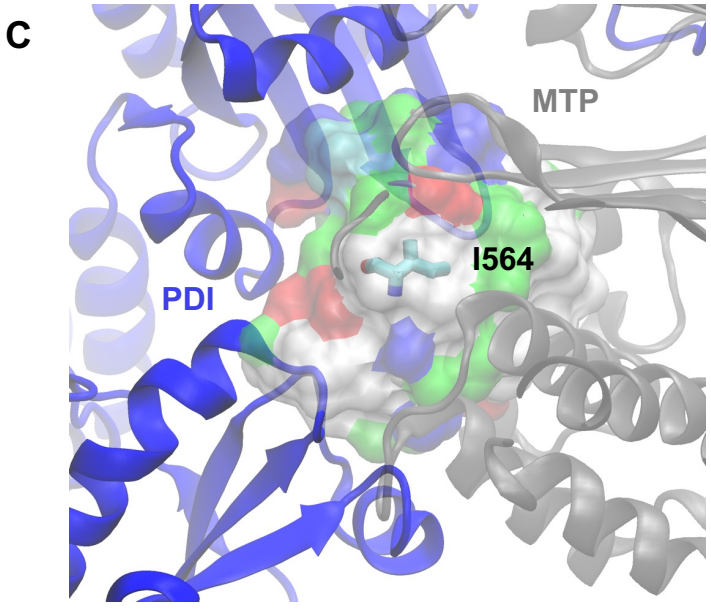
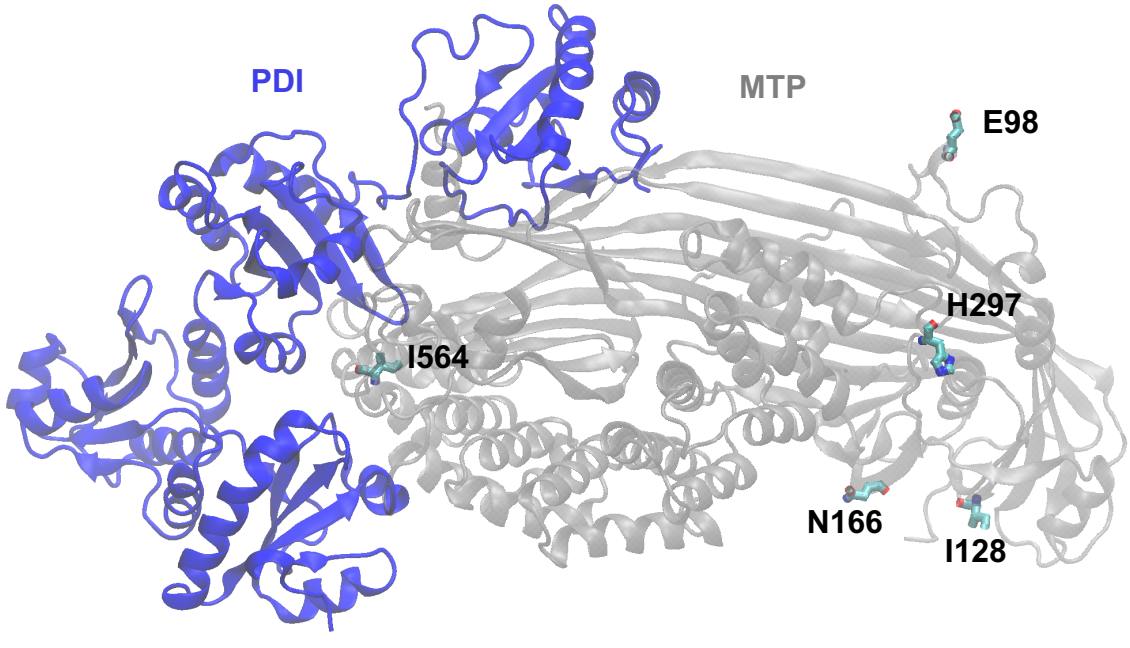
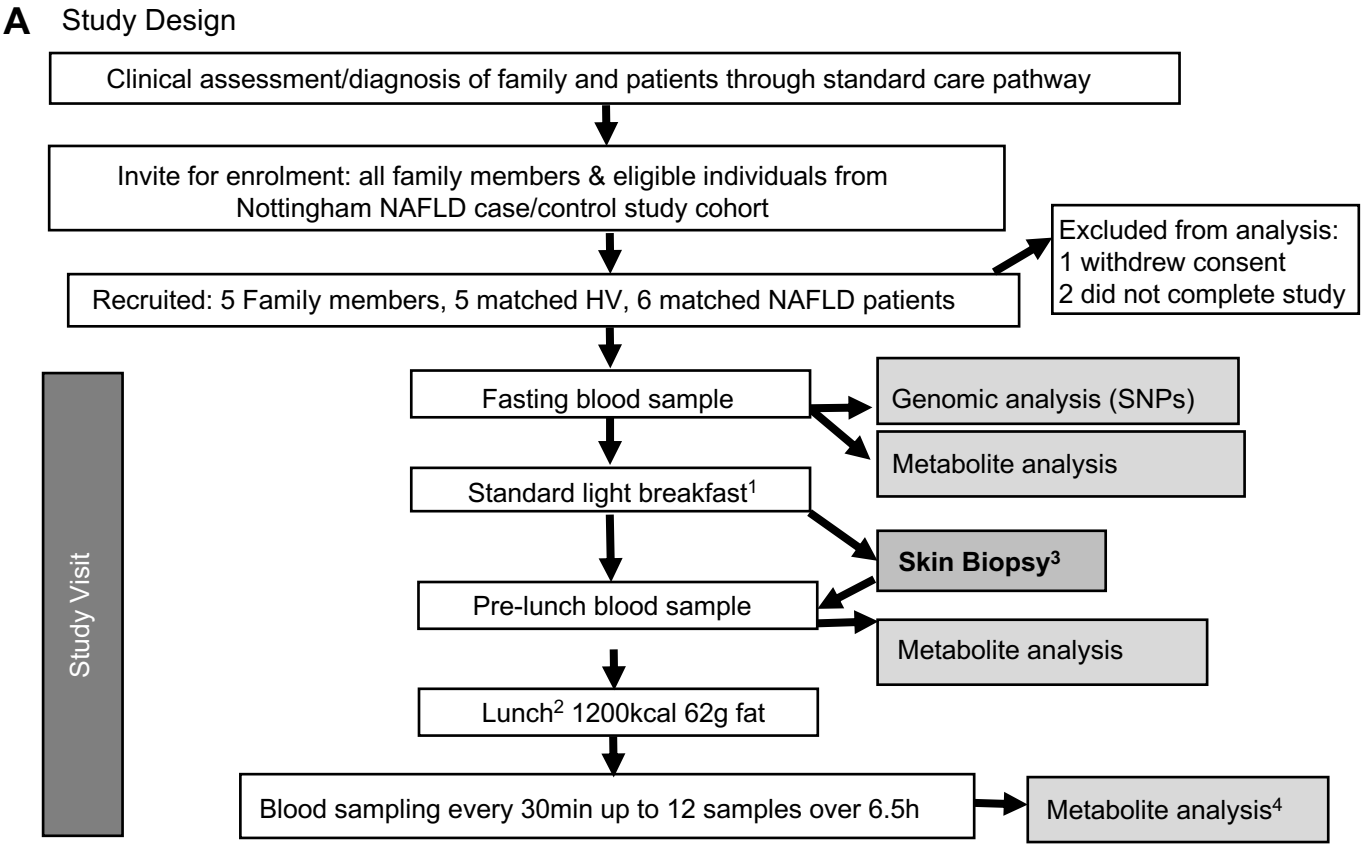
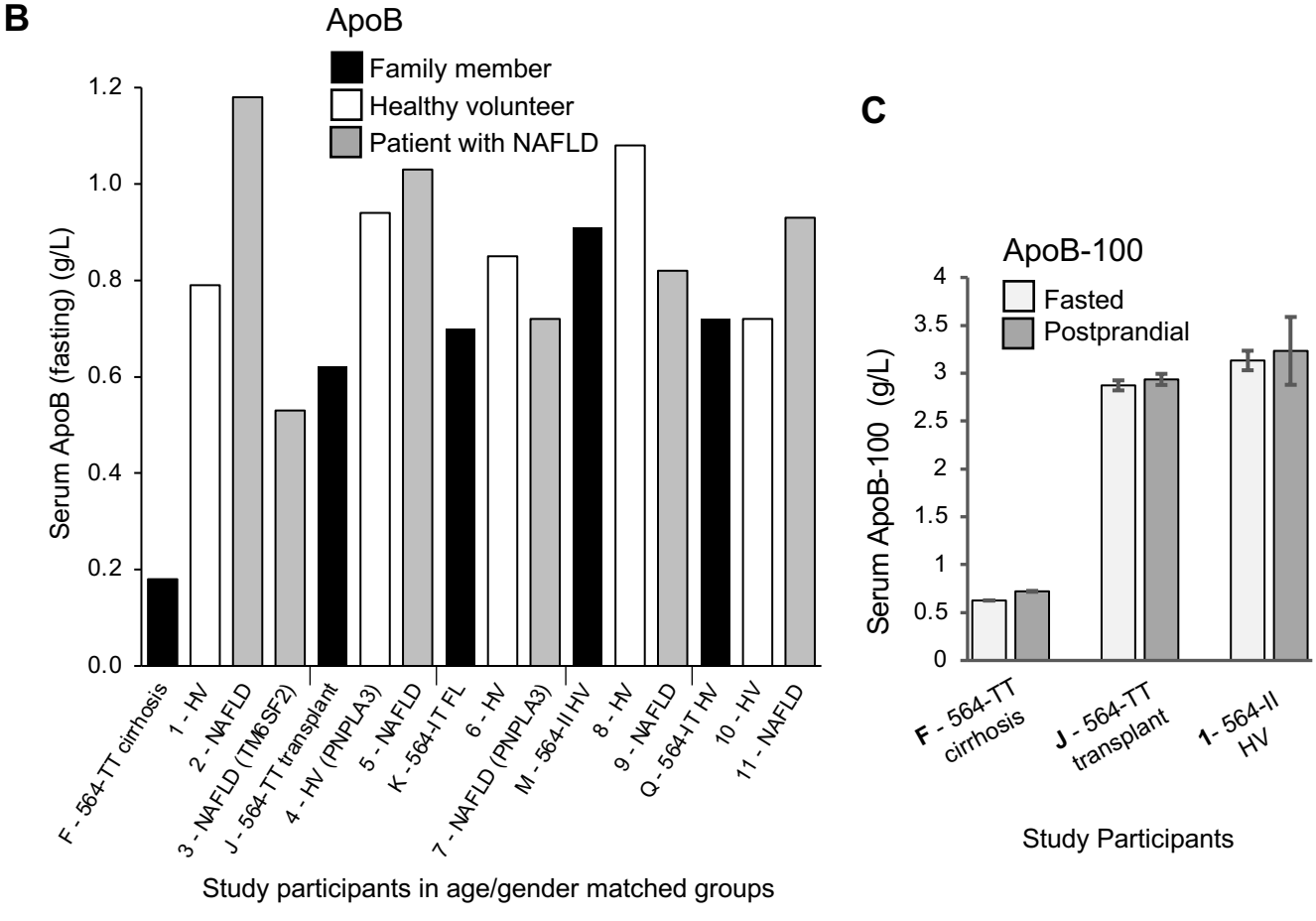
Glu
Leu

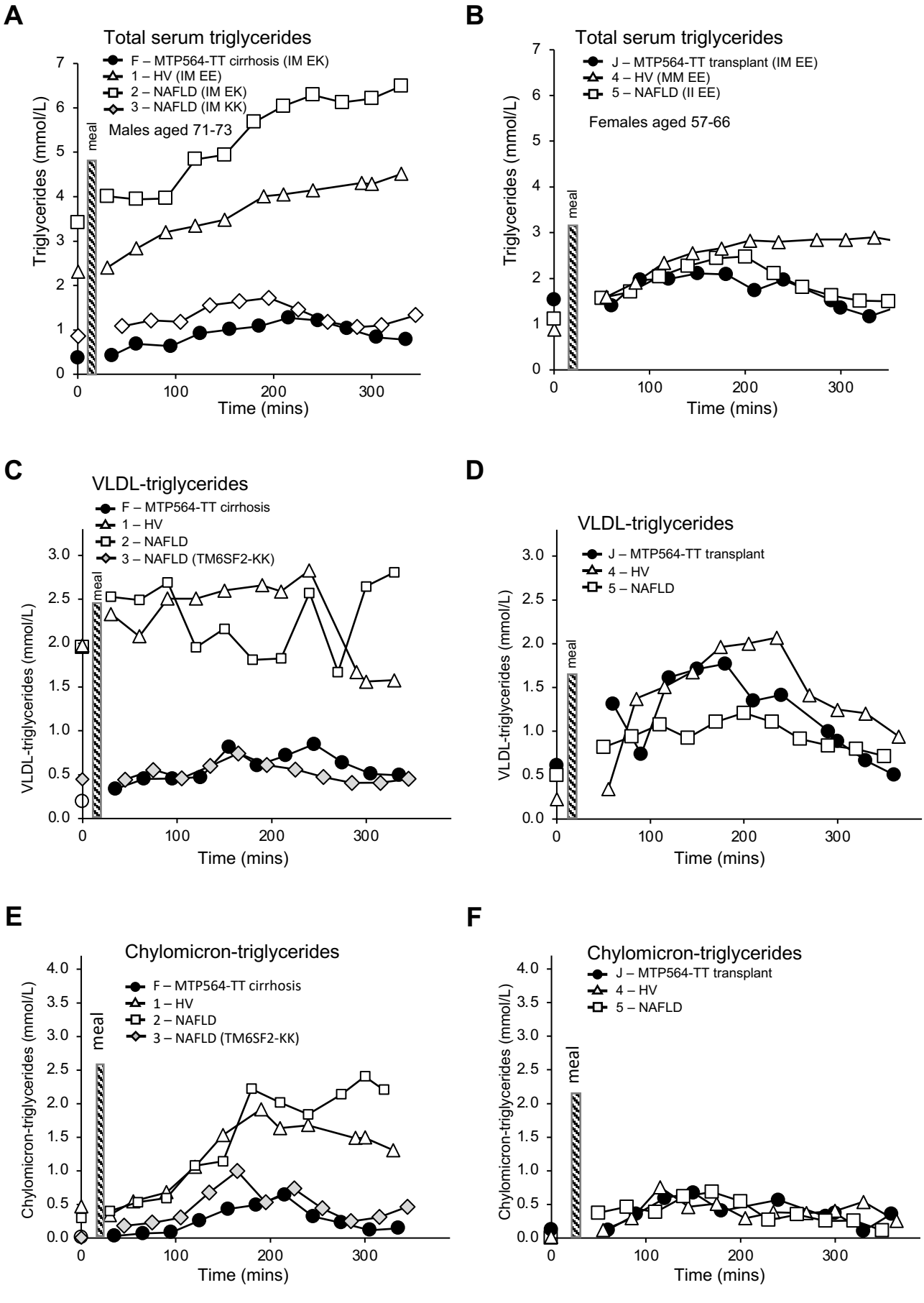

C
MTP
I564
PDI

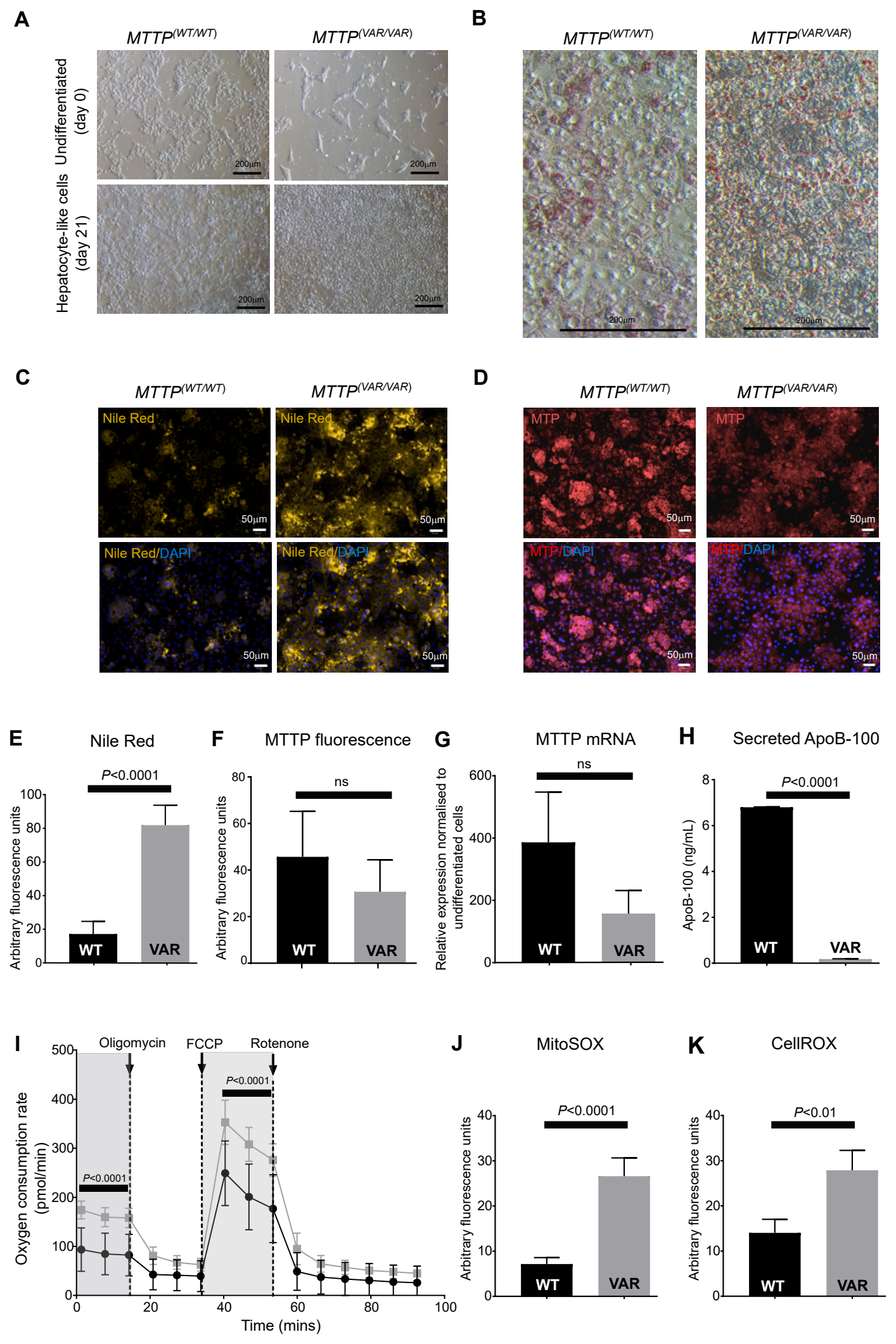

PDI
MTP
E98
H297
I564
N166
I128

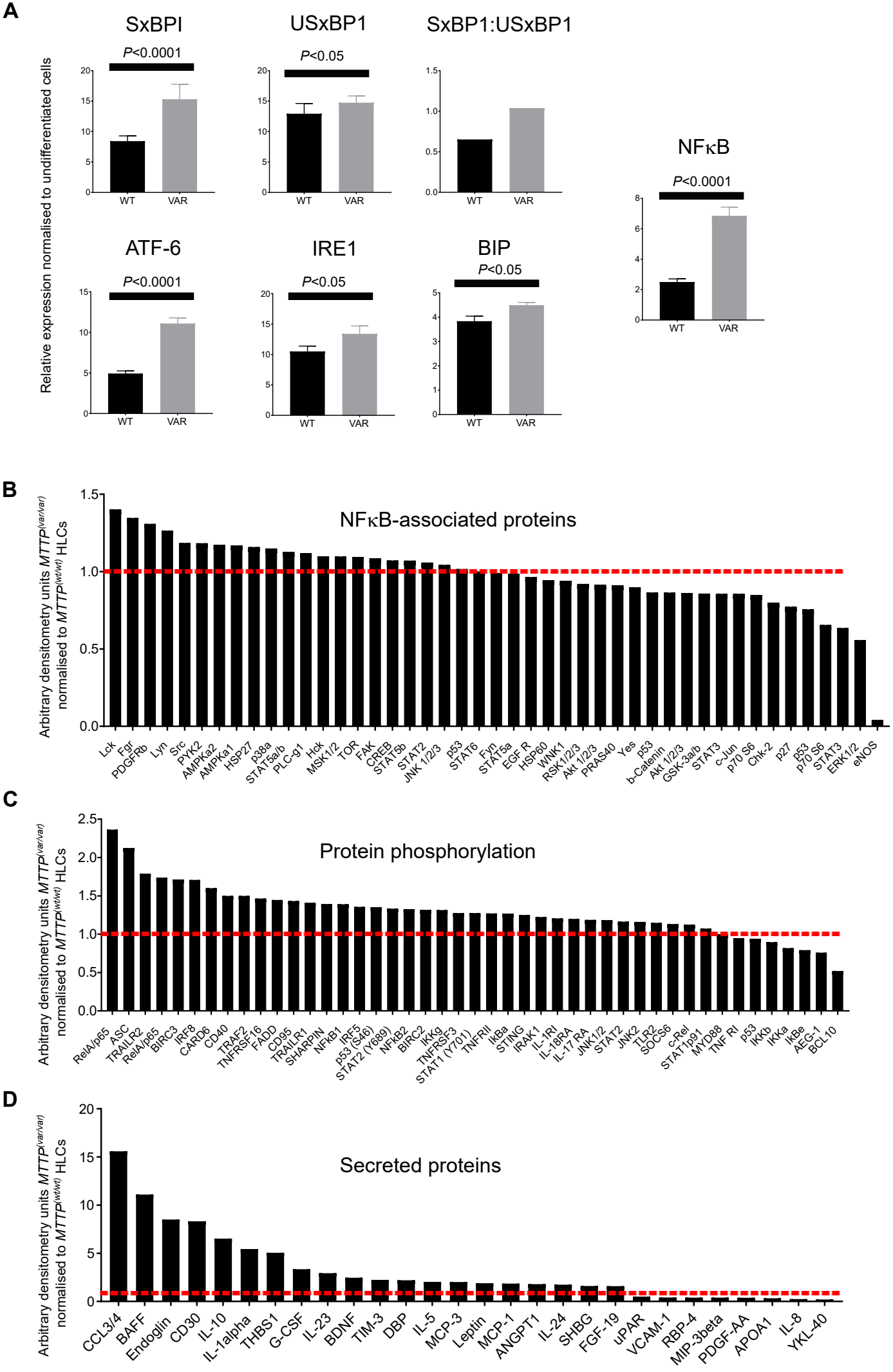


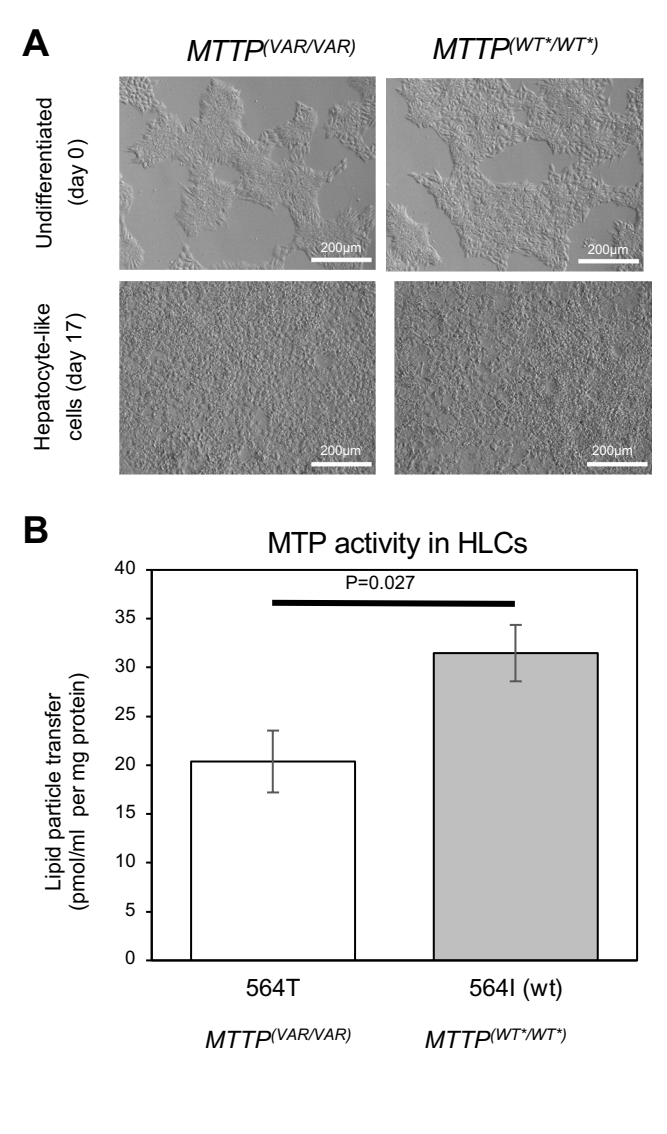
[1]milk and cornflakes.
[2]80-175min between breakfast start and pre-lunch sample.
[3]from participants J and 1 used to derive cell lines.
[4]see supplemental methods




A
Total serum triglycerides
F – MTP564-TT cirrhosis (IM EK)
1 – HV (IM EE)
2 – NAFLD (IM EK)
3 – NAFLD (IM KK)
Males aged 71-73
meal
Triglycerides (mmol/L)
Time (mins)
B
Total serum triglycerides
J – MTP564-TT transplant (IM EE)
4 – HV (MM EE)
5 – NAFLD (II EE)
Females aged 57-66
meal
Triglycerides (mmol/L)
Time (mins)
C
VLDL-triglycerides
F – MTP564-TT cirrhosis
1 – HV
2 – NAFLD
3 – NAFLD (TM6SF2-KK)
meal
VLDL-triglycerides (mmol/L)
Time (mins)
D
VLDL-triglycerides
J – MTP564-TT transplant
4 – HV
5 – NAFLD
meal
VLDL-triglycerides (mmol/L)
Time (mins)
E
Chylomicron-triglycerides
F – MTP564-TT cirrhosis
1 – HV
2 – NAFLD
3 – NAFLD (TM6SF2-KK)
meal
Chylomicron-triglycerides (mmol/L)
Time (mins)
F
Chylomicron-triglycerides
J – MTP564-TT transplant
4 – HV
5 – NAFLD
meal
Chylomicron-triglycerides (mmol/L)
Time (mins)

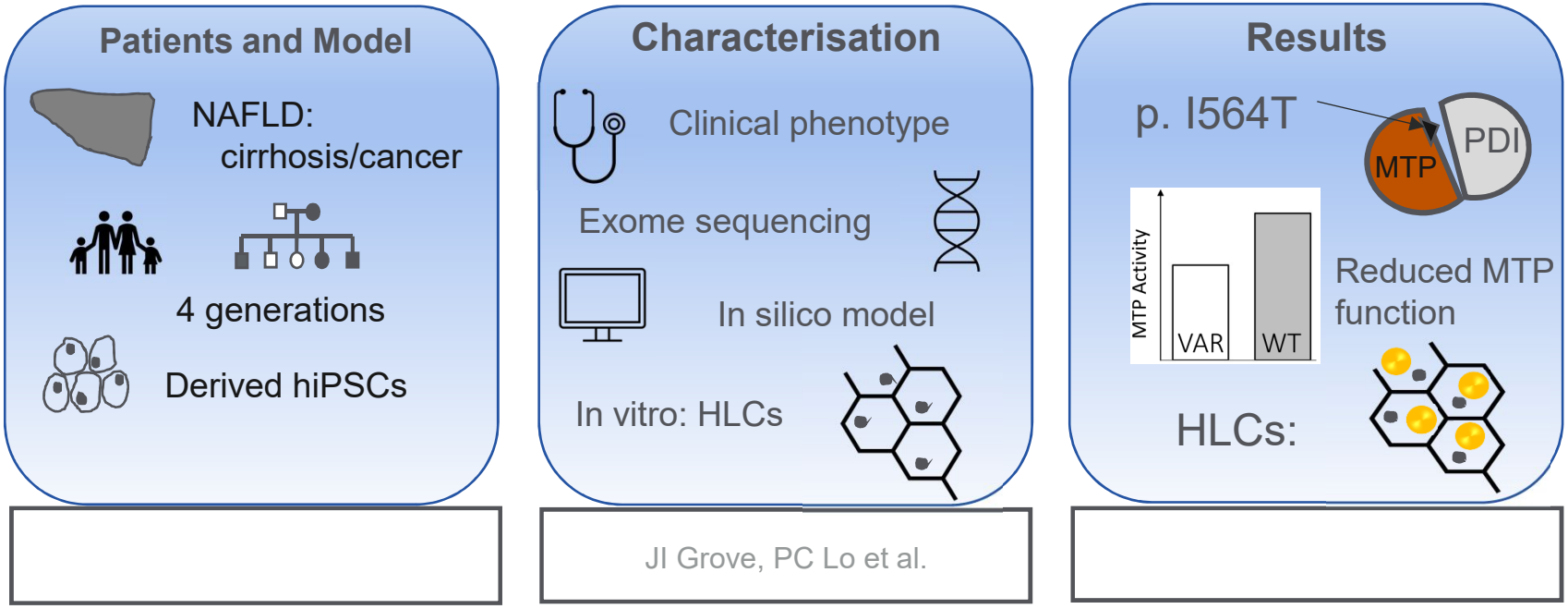

A
MTTP(WT/WT)
MTTP(VAR/VAR)
Undifferentiated (day 0)
Hepatocyte-like cells (day 21)
200μm
B
MTTP(WT/WT)
MTTP(VAR/VAR)
200μm
C
MTTP(WT/WT)
MTTP(VAR/VAR)
Nile Red
Nile Red/DAPI
50μm
D
MTTP(WT/WT)
MTTP(VAR/VAR)
MTP
MTP/DAPI
50μm
E
Nile Red
P<0.0001
Arbitrary fluorescence units
WT
VAR
F
MTTP fluorescence
ns
Arbitrary fluorescence units
WT
VAR
G
MTTP mRNA
ns
Relative expression normalised to undifferentiated cells
WT
VAR
H
Secreted ApoB-100
P<0.0001
ApoB-100 (ng/mL)
WT
VAR
I
Oligomycin
FCCP
Rotenone
P<0.0001
P<0.0001
Oxygen consumption rate (pmol/min)
Time (mins)
J
MitoSOX
P<0.0001
Arbitrary fluorescence units
WT
VAR
K
CellROX
P<0.01
Arbitrary fluorescence units
WT
VAR


A
SxBPI
P<0.0001
USxBP1
P<0.05
SxBP1:USxBP1
NFκB
P<0.0001
ATF-6
P<0.0001
IRE1
P<0.05
BIP
P<0.05
Relative expression normalised to undifferentiated cells
WT
VAR
B
NFκB-associated proteins
Arbitrary densitometry units MTTP(var/var) normalised to MTTP(wt/wt) HLCs
Lck
Fgr
PDGFRb
Lyn
Src
PYK2
AMPKa2
AMPKa1
HSP27
p38a
STAT5a/b
PLC-g1
Hck
MSK1/2
TOR
FAK
CREB
STAT5b
STAT2
JNK 1/2/3
p53
STAT6
Fyn
STAT5a
EGF R
HSP60
WNK1
RSK1/2/3
Akt 1/2/3
PRAS40
Yes
p53
b-Catenin
Akt 1/2/3
GSK-3a/b
STAT3
c-Jun
p70 S6
Chk-2
p27
p53
p70 S6
STAT3
ERK1/2
eNOS
C
Protein phosphorylation
Arbitrary densitometry units MTTP(var/var) normalised to MTTP(wt/wt) HLCs
RelA/p65
ASC
TRAILR2
RelA/p65
BIRC3
IRF8
CARD6
CD40
TRAF2
TNFRSF16
FADD
CD95
TRAILR1
SHARPIN
NFκB1
IRF5
p53 (S46)
STAT2 (Y689)
NFKB2
BIRC2
IKKg
TNFRSF3
STAT1 (Y701)
TNFRII
IκBa
STING
IRAK1
IL-1RI
IL-18RA
IL-17 RA
JNK1/2
STAT2
JNK2
TLR2
SOCS6
c-Rel
STAT1p91
MYD88
TNF RI
p53
IKKb
IKKa
IκBe
AEG-1
BCL10
D
Secreted proteins
Arbitrary densitometry units MTTP(var/var) normalised to MTTP(wt/wt) HLCs
CCL3/4
BAFF
Endoglin
CD30
IL-10
IL-1alpha
THBS1
G-CSF
IL-23
BDNF
TIM-3
DBP
IL-5
MCP-3
Leptin
MCP-1
ANGPT1
IL-24
SHBG
FGF-19
uPAR
VCAM-1
RBP-4
MIP-3beta
PDGF-AA
APOA1
IL-8
YKL-40


A
MTTP(VAR/VAR)
MTTP(WT*/WT*)
Undifferentiated (day 0)
Hepatocyte-like cells (day 17)
200µm
200µm
200µm
200µm
B
MTP activity in HLCs
P=0.027
Lipid particle transfer (pmol/ml per mg protein)
40
35
30
25
20
15
10
5
0
564T
564I (wt)
MTTP(VAR/VAR)
MTTP(WT*/WT*)

Identification and characterisation of a rare MTTP variant underlying hereditary non-alcoholic fatty liver disease.

Hepatocyte-like cell model harbouring rs745447480 has reduced MTP lipoprotein secretion activity and develops cytoplasmic triglyceride accumulation affecting cell pathways, providing insights into disease mechanisms.



JI Grove, PC Lo et al.