Review

JHEP|Reports

Genetic predisposition similarities between NASH and ASH: Identification of new therapeutic targets

Cristiana Bianco,[1] Elia Casirati,[2] Francesco Malvestiti,[2] Luca Valenti[1,2,*]

Summary

Fatty liver disease can be triggered by a combination of excess alcohol, dysmetabolism and other environmental cues, which can lead to steatohepatitis and can evolve to acute/chronic liver failure and hepatocellular carcinoma, especially in the presence of shared inherited determinants. The recent identification of the genetic causes of steatohepatitis is revealing new avenues for more effective risk stratification. Discovery of the mechanisms underpinning the detrimental effect of causal mutations has led to some breakthroughs in the comprehension of the pathophysiology of steatohepatitis. Thanks to this approach, hepatocellular fat accumulation, altered lipid droplet remodelling and lipotoxicity have now taken centre stage, while the role of adiposity and gut-liver axis alterations have been independently validated. This process could ignite a virtuous research cycle that, starting from human genomics, through omics approaches, molecular genetics and disease models, may lead to the development of new therapeutics targeted to patients at higher risk. Herein, we also review how this knowledge has been applied to: a) the study of the main *PNPLA3* I148M risk variant, up to the stage of the first in-human therapeutic trials; b) highlight a role of MBOAT7 downregulation and lysophosphatidyl-inositol in steatohepatitis; c) identify IL-32 as a candidate mediator linking lipotoxicity to inflammation and liver disease. Although this precision medicine drug discovery pipeline is mainly being applied to non-alcoholic steatohepatitis, there is hope that successful products could be repurposed to treat alcohol-related liver disease as well.

© 2021 The Author(s). Published by Elsevier B.V. on behalf of European Association for the Study of the Liver (EASL). This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Keywords: alcoholic liver disease; cirrhosis; fatty liver disease; genetics; IL32; interleukin-32; MBOAT7; non-alcoholic fatty liver disease; precision medicine; PNPLA3; steatohepatitis; therapy

Received 23 November 2020; received in revised form 9 March 2021; accepted 15 March 2021; available online 30 March 2021

Introduction

Non-alcoholic and alcohol-related fatty liver disease (namely NAFLD and AFLD[1,2]) are already undoubtedly, and will continue to be, leading drivers of progressive liver disease and hepatocellular carcinoma (HCC) worldwide.[3–6] Severe alcohol abuse leads to accelerated disease progression with higher rates of HCC, liver-related deaths and a poorer prognosis.[7,8] On the contrary, NAFLD is most frequently related to metabolic dysfunction (MAFLD: metabolic dysfunction-associated fatty liver disease)[1,2] and is associated with increased risk of cardiometabolic disease and cancer. The exact impact of alcohol abuse on the risk of chronic liver disease in the general population is difficult to assess, however alcohol-related cirrhosis is responsible for one-third of liver transplantations in the USA and about 20% in Europe; on the other hand, NAFLD is estimated to affect 25% of the global population, and its prevalence is expected to increase.[9,10] For these reasons, fatty liver disease (FLD) will likely pose a significant threat to public health in the near future. Excess fat accumulation in hepatocytes is the hallmark of these conditions, and it has recently been implicated in the development of liver injury and disease.[11] Although the main environmental triggers of fat accumulation differ between alcoholic fatty liver disease (AFLD) and NAFLD, they are frequently superimposed, and the pathogenesis of inflammation and progressive liver damage share many mechanisms.[12] Throughout the manuscript, we will therefore commonly refer to these disorders as FLD. The pathogenic background shared among FLDs results in a phenotype which reflects metabolic aberrations, usually characterised by short-circuits in the hepatic lipid metabolism that mediate the enhancement of lipid storage in the liver parenchyma.[11,13] The metabolic switching converts the liver to a lipid storing organ, a choice that is imposed by the necessity to counteract the accumulation of cytoplasmic free fatty acids (FFAs). The latter is mainly caused by the continuous uptake of lipids originating from the inflamed adipose tissue and by enhanced *de novo* lipogenesis (DNL), which is activated by impaired cellular redox potential and/or hyperinsulinemia.[11,13,14] In this scenario, triglyceride (or triacylglycerol TAG) storage may be seen as a protective mechanism to counteract lipotoxicity in hepatocytes.[15] During alcohol

[1]*Precision Medicine – Department of Transfusion Medicine and Hematology, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy;* [2]*Department of Pathophysiology and Transplantation, Università degli Studi di Milano, Milan, Italy*

* Corresponding author. Address: Department of Pathophysiology and Transplantation, Università degli Studi di Milano, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Translational Medicine - Department of Transfusion Medicine and Hematology, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, via F Sforza 35, 20122, Milan, Italy; Tel.: +390250320278, fax: +390250320296.
E-mail address: luca.valenti@unimi.it (L. Valenti).

abuse, hepatic lipid accumulation is promoted through four main mechanisms (summarised in Fig. 1): i) increased production of NADH secondary to the oxidation of ethanol, which promotes FFA and TAG synthesis and inhibits mitochondrial β-oxidation; ii) increased uptake of FFA coming from chylomicrons secreted by the intestinal mucosa; iii) ethanol-mediated inhibition of AMP-activated protein kinase, with the consequent increase of DNL and reduction of lipolysis due to the downregulation of the peroxisome proliferator-activated receptor a (PPAR-α) and the upregulation of the sterol regulatory element-binding protein 1c (SREBP-1c); iv) mitochondrial and microtubular damage induced by acetaldehyde and subsequent reduction of NADH oxidation, which determines accumulation of very low-density lipoproteins.[16–18]

The progression of liver damage accelerates when, especially at times of acute insults during the natural history of the disease, excess fat and lipotoxicity lead to inflammation, hepatocellular damage and fibrogenesis, a condition referred to as "steatohepatitis" (*i.e.* non-alcoholic steatohepatitis NASH and acute alcohol-related steatohepatitis ASH respectively).[19,20] Unfortunately, classical biomarkers cannot accurately stratify the risk of progressive liver disease, and there are no approved drugs to treat FLD. Indeed, the first drugs reaching late stage development for NASH either failed or were burdened by an unfavourable side effect profile.[21] Innovative approaches for biomarker discovery and therapeutic target identification are therefore urgently needed. While recent attempts mostly exploited previous knowledge related to biomarkers and candidate drugs from other hepatic and metabolic diseases, human genetics is unravelling – through unbiased approaches – the specific determinants of FLD[22] and related biological pathways.[23] At the same time, genetics is highlighting the sources of heterogeneity and possible ways to personalise treatment. Nowadays, molecular genetics is providing new tools to directly manipulate a wide array of potential disease pathways in the liver.[24,25] In this review, we will discuss the common genetic mechanisms underlying both NASH and ASH, and how they can be used to guide the design of innovative therapeutic approaches.

Key points

- Alcohol-related and non-alcoholic steatohepatitis share the main genetic determinants.
- New drugs targeting steatohepatitis can be developed by integrating genetic, transcriptomic, proteomic and lipidomic data with experimental models.
- Genetic-based drug discovery can highlight targets to treat both non-alcoholic and alcoholic steatohepatitis.
- Derangements in genes that regulate hepatic fat accumulation and hepatocellular lipid droplet remodelling are key in the pathogenesis of steatohepatitis.
- The *PNPLA3* I148M variant is the main common genetic determinant of steatohepatitis.
- *PNPLA3* silencing has a beneficial impact on experimental steatohepatitis.
- Downregulation of *MBOAT7* impairs the synthesis of arachidonic acid-containing phosphatidyl-inositol and determines the accumulation of lysophosphatidyl-inositol, leading to steatosis and promoting steatohepatitis.
- IL-32 is induced by lipotoxicity in hepatocytes and acts as a mediator of inflammation and steatohepatitis, representing a candidate biomarker and therapeutic target.

AFLD and NAFLD share genetic predisposition

The predisposition to develop progressive FLD has a strong inherited component.[11,22,26] Remarkably, NAFLD and AFLD, as well as other liver diseases where hepatic fat accumulation plays a major role (*e.g.* for chronic hepatitis C),[27] share most of the main genetic determinants (shown in Fig. 2).[22]

The main genetic determinant of FLD is the rs738409 C>G variant, encoding the I148M protein variant of patatin like phospholipase domain containing 3 (*PNPLA3*). This single-nucleotide polymorphism has been strongly associated with both NAFLD[28,29] and AFLD, including progression to cirrhosis,[30,31] HCC,[32] and severe ASH.[33,34] During insulin-resistance, *PNPLA3* is induced by insulin in hepatocytes, hepatic stellate cells (HSCs) and adipocytes under the control of SREBP-1c,[35] whose activation is also promoted by ethanol feeding in murine models.[18] PNPLA3 protein localises on lipid droplets (LDs), it has lipase activity and is involved in the remodelling of phospholipids and TAGs.[36,37] The I148M variant induces a loss of function, but the mutated protein evades ubiquitination and accumulates on LDs; by sequestering ABHD5/CGI-58, the variant also inhibits the activity of adipose tissue triglyceride lipase (ATGL/PNPLA2), the main LD-TAG-lipase in the liver.[38] The result is the impairment of lipid turnover, the enlargement of LDs and lipotoxicity.[27] In addition, the I148M variant may have independent effects on inflammation and fibrosis by impeding retinol release and rewiring transcriptional circuits in HSCs in response to liver damage.[37,39,40]

A few other variants have been demonstrated to play a role in FLD. rs58542926 C>T encoding the *TM6SF2* E167K variant causes hepatic fat accumulation, NASH, and fibrosis,[41,42] but is also associated with HCC development in AFLD.[43] TM6SF2 is a Golgi membrane protein. The loss of function induced by the risk variant impairs very low-density lipoprotein secretion, with consequent TAG accumulation and heightened susceptibility to liver damage.[41,42,44] Although upregulation of TM6SF2 may improve hepatic damage induced by FLD, it is not a viable therapeutic option, as it would increase circulating lipoproteins and cardiovascular disease.[41,42,44] Glucokinase regulator (*GCKR*) variation is associated with FLD and fibrosis.[45–47] The GCKR protein acts as an inhibitor of glucokinase.[48] The rs1260326 C>T variant encodes P446L, which lacks the ability to inhibit glucokinase in response to fructose-6-phosphate and to restrain glucose uptake, thereby favouring glycolysis, DNL and hepatic fat accumulation.[49] Possibly because the impact of the variant on FLD depends on glucose levels, this was not reported to influence AFLD. Furthermore, GCKR cannot be targeted to reduce liver fat because this would lead to hyperglycaemia.[47] On the other hand, genetic variation of membrane bound O-acyltransferase domain-containing 7 (*MBOAT7*) has been linked to predisposition to hepatic fat accumulation in both to AFLD and NAFLD.[31,50,51] Hydroxysteroid 17-beta dehydrogenase 13 (*HSD17B13*) variants (rs143404524 and rs72613567) have more recently been implicated in the protection against ASH and NASH, cirrhosis and HCC.[52–54] The protection against liver damage was larger in *PNPLA3* I148M variant carriers.[52] HSD17B13 is involved in qualitative LD remodelling through conversion of retinol to retinoic acid in hepatocytes, but it has also been predicted to metabolise several lipid species.[53] The protective variants have been shown to result in loss-of-function of enzymatic activity,[53] suggesting

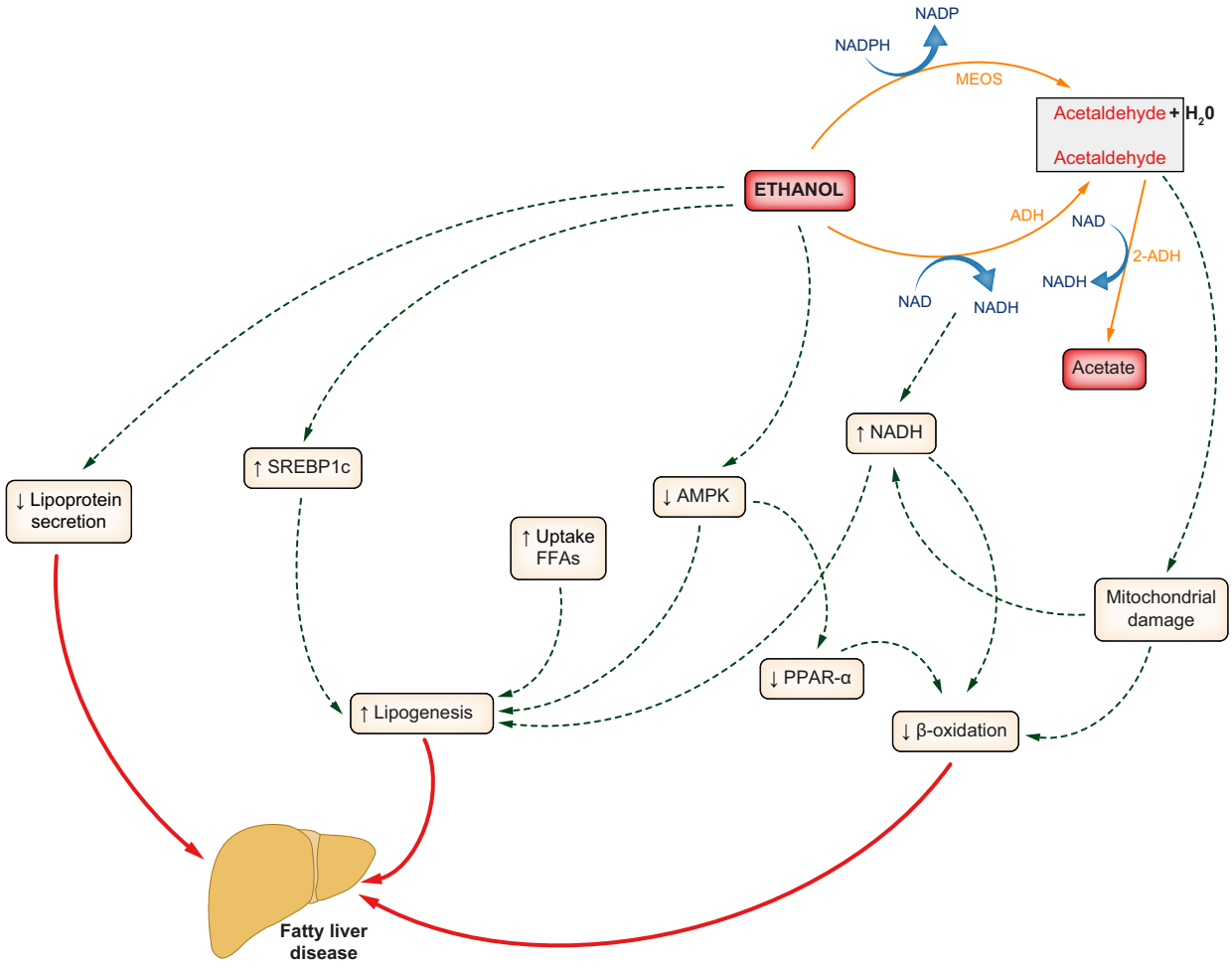

Fig. 1. Ethanol metabolism and related mechanisms promoting hepatic lipids accumulation. During alcohol consumption, ethanol is oxidised to acetaldehyde (by the constitutive pathway involving the NAD-dependent ADH) or metabolised at the level of the microsomal system through an inducible NADPH-dependent pathway involving cytochrome P450 (MEOS). Both lead to the formation of acetaldehyde, which is subsequently metabolised to acetic acid by the 2-ADH. ADH, alcohol dehydrogenase; 2-ADH, 2-alcohol dehydrogenase; AMPK, AMP-activated kinase; FFAs, free fatty acids; PPAR-α, peroxisome proliferator-activated receptor a; SREBP-1c, sterol regulatory element-binding protein 1c.

that HSD17B13 inhibition may be considered as a therapeutic strategy. The rs2642438 mitochondrial amidoxime reducing component 1 (*MARC1*) variant (M187K) has also been identified as protective against FLD progression[55] and cirrhosis.[56,57]

Once more, these data underline the common pathogenesis of FLD, highlighting possible common approaches for risk stratification and therapeutic intervention. In addition, the impact of variants in *PNPLA3, TM6SF2, MBOAT7* and *GCKR* on hepatic inflammation and fibrosis was proportional to their effect on hepatic fat accumulation, suggesting that genetically determined hepatic fat levels predispose individuals to FLD and drive progression to fibrosis and HCC.[47,58] Exploiting polygenic risk scores (PRS) as lifelong proxies of exposure to increased hepatic fat content and of qualitative alterations of hepatic fat, it was suggested that these variants have a causal role in determining progressive liver disease,[47] and HCC.[59] This "Mendelian randomization" framework exploits naturally occurring genetic variants, that have functional effects and are randomly inherited at conception, as experimental instruments to estimate the impact of the manipulation of the encoded proteins on human health. Since steatosis is the necessary condition for FLD onset and progression, hepatic fat is naturally the main therapeutic target (Table 1). Indeed, to date, pharmacological approaches targeting hepatic fat have generated the best results for the treatment of NASH.[60]

The list of genetic FLD determinants is increasing by the day. Recently, an exome-wide association study of alanine aminotransferases in the population identified variants in apolipoprotein E (*APOE*) and glycerol-3-phosphate aminotransferase (*GPAM*) regulating hepatic lipid metabolism as risk factors for FLD and cirrhosis.[61] Furthermore, in a case-control study, variants in the leptin receptor (*LEPR*), regulating appetite and liver fibrogenenesis, and in pygopus homolog-1 (*PYGO1*), potentially involved in LD remodelling, have been associated with clinical NAFLD.[62] Several candidate genes involved in alcohol metabolism, ethanol-induced oxidative stress, inflammation and fibrosis have also been investigated as modifiers of AFLD risk, but additional data are needed to validate these findings.[63,64]

Table 1. Examples of genetic pathways underlying fatty liver disease and correspondent potential therapeutic strategies.

Pathway	Genes	Therapeutic strategy	Development stage	Selected references
Hepatocellular fat accumulation	*PNPLA3, TM6SF2, GCKR, GPAM, PYGO1, MBOAT7, APOB*	Inhibition of lipogenesis (ACC, SCD1, DGAT1/2 inhibitors), stimulation of b-oxidation (TR-β agonists, PPAR agonists), anti-obesity drugs (targeting GLP1R), insulin sensitizers, glucose lowering drugs in T2D*; any drug reducing hepatic fat	Preclinical, Phase I-IV	11, 47, 61, 62
Lipoproteins remodeling and cholesterol metabolism	*PCSK7, PCKS9, APOE*	PCSK9/7 silencing or neutralization, statins	PCKS9 approved for hypercholesterolemia	61, 69, 81, 135
Lipid droplet remodeling and lipotoxicity	*PNPLA3, ABHD5; MBOAT7*	PNPLA3 silencing Modulation of LPI metabolism, hepatic GPR55 antagonism IL32 silencing or neutralization	Preclinical, Phase I Hypothetical Hypothetical	11, 25, 47, 74, 76 107
Oxidative and ER stress	*HFE, MARC1, SOD2, UCP2, SERPINA1*	Iron depletion, vitamin E, sylibinin	Phase II	55, 115, 136, 137
Hepatic retinol metabolism, inflammation and fibrogenesis	*PNPLA3, HSD17B13, MERTK, LEPR*	Retinoid receptors modulation, HSD17B13 silencing or direct inhibition, modulation of MERTK activity	Hypothetical	37, 39, 52-54, 62, 79, 80
Bile acids – FGF19 – b-Klotho – FXR axis	*NR1H4, KLB*	FXR agonists, FGF19 partial agonists, bile acids reuptake inhibitors, pre/pro-biotics, lubiprostone	Preclinical, Phase I-III	126, 129

ABHD5, abhydrolase domain containing 5; ACC, acetyl-CoA carboxylase; *APOB*, apolipoprotein B; DGAT, diacylglycerol O-acyltransferase; FGF19, fibroblast growth factor 19; FXR, farnesoid X receptor; *GCKR*, glucokinase regulator; GLP1R, glucagon-like peptide 1 receptor; GPR55, G protein-coupled receptor 55; *HFE*, homeostatic iron regulator; *HSD17B13*, hydroxysteroid 17-beta dehydrogenase 13; IL32, interleukin 32; *KLB*, klotho beta; LPI, lysophosphatidylinositol; *MARC1*, mitochondrial amidoxime reducing component 1; *MBOAT7*, membrane bound O-acyltransferase domain containing 7; *NR1H4*, nuclear receptor subfamily 1 group H member 4; PCSK, proprotein convertase subtilisin/kexin; *PNPLA3*, patatin like phospholipase domain containing 3; PPAR, peroxisome proliferator activated receptor; SCD1, stearoyl-CoA desaturase; *SERPINA1*, serpin family A member 1; *SOD2*, superoxide dismutase 2; T2D, type 2 diabetes; *TM6SF2*, transmembrane 6 superfamily member 2; TR-b, thyroid hormone receptor b; *UCP2*, uncoupling protein 2.

* Stimulation of lipid secretion is not a viable option due to the major impact on circulating lipids and on cardiovascular risk profile in highly susceptible individuals.[42]

A new discovery paradigm: From human to molecular genetics and into the clinic

Directly targeting variant proteins responsible for the development of progressive steatohepatitis is an attractive approach for the treatment of FLD. Indeed, drug target discovery based on human genetics has a 4-fold higher probability of success (regulatory approval) than other methods.[65] This new discovery cycle (Fig. 3) would start from human genetics, highlighting causal genetic variants robustly associated with the trait of interest. The subsequent linkage of genetic variation with transcriptomic, proteomic and – especially relevant here – lipidomic data,[66,67] coupled with basic studies examining the direct impact

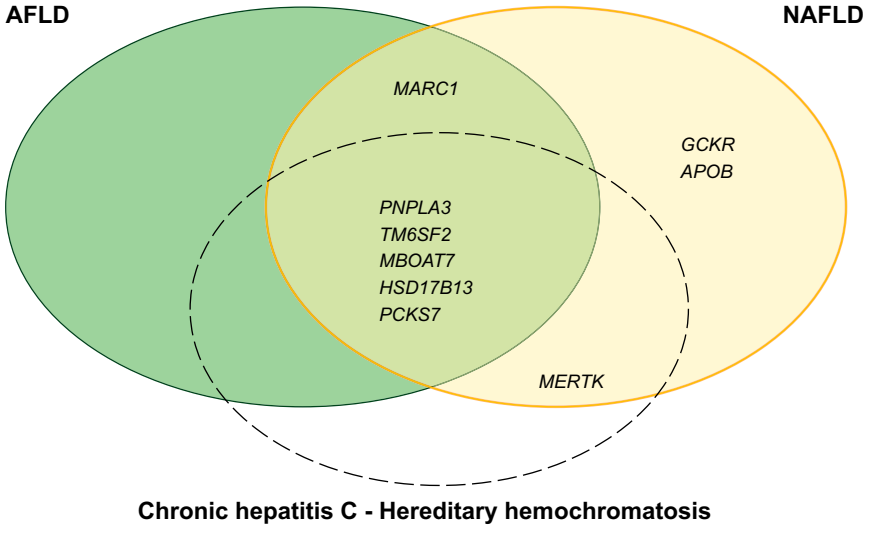

Fig. 2. Alcohol-related and non-alcoholic steatohepatitis share major genetic determinants. Among the main genetic determinants which predispose to the development of progressive FLD, it is possible to distinguish a central core of genes that include modulators of fatty acid metabolism, lipid storage and secretion. These genes highlight the common genetic background, shared between AFLD and NAFLD. Conversely, other genes are specifically associated with only 1 type of liver injury such as *GCKR* and *APOB* in NAFLD/MAFLD. AFLD, alcoholic fatty liver disease; *APOB*, apolipoprotein B; FLD, fatty liver disease; *GCKR*, glucokinase regulator; *HSD17B13*, hydroxysteroid 17-beta dehydrogenase 13; MAFLD, metabolic dysfunction-associated fatty liver disease; *MARC1*, mitochondrial amidoxime reducing component 1; *MBOAT7*, membrane bound O-acyltransferase domain containing 7; NAFLD, non-alcoholic fatty liver disease; *PCSK7*, proprotein convertase subtilisin/kexin Type 7; *PNPLA3*, patatin like phospholipase domain containing 3; *TM6SF2*, transmembrane 6 superfamily member 2.

of variants in experimental models *in vitro* and *in vivo*, would highlight the mechanisms underlying the association. Genome editing techniques are proving to be powerful tools to model the impact of genetic variation in *in vitro* and *in vivo* models.[68–70] This may enable the selection of the most appropriate, if any, therapeutic target, to be tested first in models and, if successful, in patients (Fig. 3). In parallel, this process would lead to a progressive improvement in risk stratification, and in a more accurate selection of those who are more likely to benefit from the eventual therapeutic innovations (Fig. 3).[22] Recent advances in genotyping and sequencing technologies, enabling the genome-wide characterisation of large cohorts of well-phenotyped individuals, alongside the computational power to link the different "omics" approaches are rendering this revolution possible.

The most striking example of this paradigm is being offered by the *PNPLA3* I148M variant, as the main inherited and common risk factor for severe steatohepatitis, cirrhosis and HCC related to FLD. Since fat accumulation in I148M carriers is due to alterations in lipid turnover rather than DNL,[71] therapies targeting hepatic lipogenesis have limited efficacy in mutation carriers.[72,73] Conversely, directly silencing the hepatic expression of the *PNPLA3* risk variant using novel therapeutics, such as oligonucleotides, may be an effective strategy (Fig. 3, outer circle in green).[74,75] This technology has already been approved to treat severe forms of dyslipidaemia and metabolic disorders.[75] Indeed, down-modulation of expression of *PNPLA3* – associated with a linked genetic variant (E434K) – has a beneficial impact on liver injury in I148M risk variant carriers and was not associated with unfavourable phenotypes.[76] *PNPLA3* silencing has been tested *in vitro* and in animal models. In mice, downregulation of PNPLA3 improved liver fat levels.[38] Injection of antisense oligonucleotides against hepatic *Pnpla3* in mice fed steatogenic and steatohepatitis-inducing diets reduced fat, inflammation and fibrosis, with a more pronounced benefit in animals bearing the I148M protein variant.[25] The beneficial effect of PNPLA3 downregulation on hepatocellular fat and HSCs transactivation could also be achieved with small molecules, such as momelonib, which is active on the JAK1/2 and TGF-β/SMAD pathways.[77] Even though this compound could lead to off-target effects and may not be suitable for chronic administration in a non-neoplastic condition, the aforementioned study provides proof-of-principle that small molecules may achieve the goal of suppressing PNPLA3. Meanwhile, phase I clinical studies have been registered, in which the safety, tolerability and pharmacokinetics/dynamics of escalating doses of liver-targeted PNPLA3-antisense oligos (*e.g.* NCT04142424, NCT04483947) will be tested in obese individuals and patients with NASH who are homozygous for the *PNPLA3* I148M variant.

Another potential approach to counteract the detrimental impact of the *PNPLA3* I148M variant may be represented by the inhibition of HSD17B13.[52] However, HSD17B13 activity remains undefined. While the protective genetic variant has been associated with downregulation of hepatic *PNPLA3* expression, it may involve regulation of retinol metabolism,[53] with potentially widespread biological effects. In addition, experimental evidence

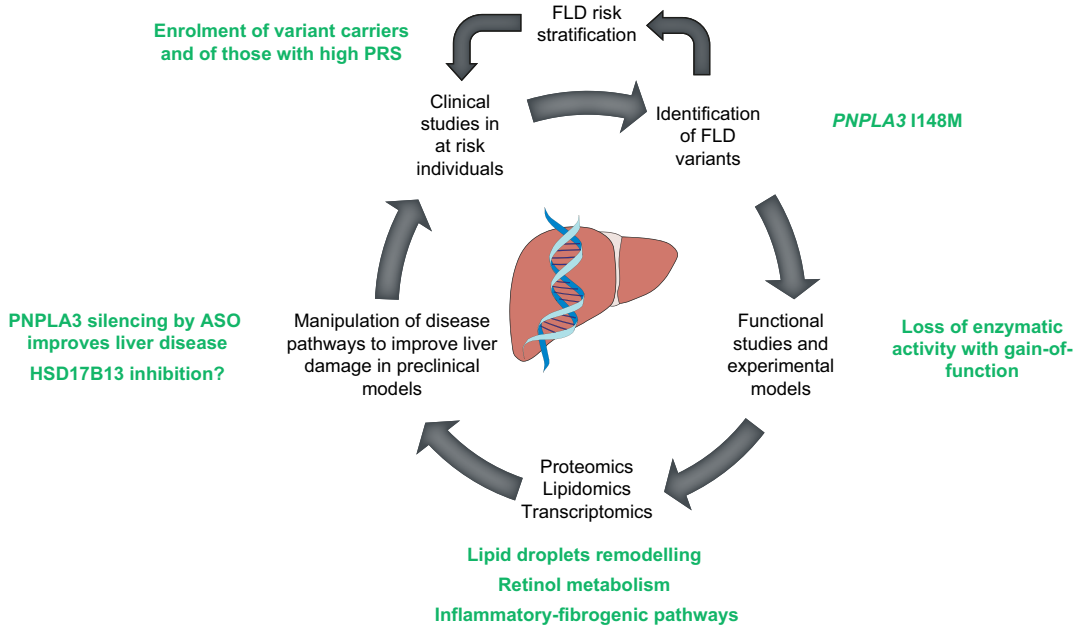

Fig. 3. The new discovery paradigm: From human to molecular genetics and into the clinic. FLD risk stratification results from a cyclic interplay between clinical studies in at risk individuals and the manipulation of the associated pathways to improve liver damage. The direct target discovery approach – based on human genome level data – aims to identify high-impact FLD variants. This strategy may lead to a progressive improvement in risk stratification, coupling the information carried by the characterisation of risk variants with information derived from several bioinformatic "omics" approaches. The following characterisation of the disease mechanisms in experimental models leads to novel therapeutics. Among all the efforts devoted to the pursuit of a personalised medicine approach, this represents an optimal strategy for the modulation of disease pathways during pre-clinical studies and then in clinical trials. The example of *PNPLA3* I148M variant is illustrated in the outer circle of the figure (in green). The modulation of genes involved in lipid droplet remodelling and lipotoxicity employing ASOs, as in the case of *PNPLA3* risk variant, may be a successful blueprint. ASO, anti-sense oligonucleotides; FLD, fatty liver disease; HSD17B13, hydroxysteroid 17-beta dehydrogenase 13; *PNPLA3*, patatin like phospholipase domain containing 3; PRS, polygenic risk score.

related to *PNPLA3* and *MERTK* variants suggests that impaired retinol metabolism may be involved in triggering inflammation and fibrogenesis during steatohepatitis.[37,39,40,78–80]

It is worth noting that enrolment or stratification based on *PNPLA3* I148M, the major genetic risk variants for FLD, and ethnicity, may improve the outcomes of clinical trials in steatohepatitis, independently of the drug target. Indeed, this would enable the selection of a more homogeneous subset of patients in terms of pathophysiology and disease progression. On the other hand, given the different representation of genetic risk variants, targeted drugs may be more effective in specific ethnic groups, *e.g.* individuals of Hispanic rather than African ancestry for PNPLA3-targeted drugs.

Promising therapeutic targets that have emerged from genetic studies are summarised in Table 1. Owing to their novelty, we will focus on data emerging during the last year on the role of lipotoxicity and in particular of MBOAT7 and interleukin-32 (IL-32) in the pathogenesis of FLD, with a final note on the gut-liver axis and bile acid metabolism. However, other promising targets include a) modulation of cholesterol metabolism, which is associated with the induction of steatohepatitis,[81] particularly in patients negative for the *PNPLA3* variant [72]; b) oxidative and endoplasmic reticulum (ER) stress, whose involvement in steatohepatitis and progressive liver disease is supported by several genetic loci, likely including the latest protective association detected at *MARC1*.[55]

The possible beneficial impact of drugs targeting FLD genes on ASH remains to be proven, since hepatic damage is thought to be partly mediated by ethanol metabolites (*e.g.* acetaldehyde).

From quantitative to qualitative alterations of liver fat: The case of *MBOAT7* and LPI

Thus, human genetics and complementary epidemiological evidence are consistent with the notion that hepatic fat accumulation plays a causal role in progressive liver disease.[22,47,60] However, initial data are beginning to shed light on the qualitative alterations in liver fat (from lipidomics) and activation of intracellular pathways (from transcriptomics) that come with worsening FLD and are involved in disease progression. The most notable recent example is provided by the identification of the rs641738 C>T variant of *MBOAT7* as a risk factor for FLD,[31,50,51] and the discovery of the mechanism underlying the association.[50,58,67] Indeed, this discovery pinpointed a role for a specific lipid species (namely lysophosphatidyl-inositol LPI), revealing new research avenues.

The rs641738 C>T *MBOAT7* variant increases the risk of the full spectrum of FLD, but it was first identified as a genetic determinant of the susceptibility towards alcohol-related cirrhosis,[31,50,51] and is a general modifier of liver disease progression.[82,83] The impact on the full spectrum of FLD, from hepatic fat accumulation, to NASH, fibrosis and HCC has been confirmed by a recent large meta-analysis considering over a million individuals of various ethnicities.[51] This risk allele has also been associated with an increased risk of HCC in patients with NAFLD without advanced fibrosis,[58] and an additional rare likely pathogenic variant has recently been detected in NAFLD-HCC.[84]

MBOAT7 encodes LPI acyltransferase 1 (also known as LPIAT1). It is an ER membrane protein with 6 transmembrane domains.[85] MBOAT7 is involved in phospholipid acyl-chain remodelling in the so-called Land's cycle, incorporating arachidonic acid (AA) and other polyunsaturated fatty acids (PUFAs) into LPI and other lysophospholipids. Indeed, biallelic loss-of-function mutations in *MBOAT7* cause an early onset and severe neurological phenotype with cognitive impairment.[86] This phenotype is associated with impaired neuronal and myelin development, and fully recapitulated in *Mboat7* knock-out mice, which showed a marked deficit of incorporation of AA into LPI.[86,87] MBOAT7 belongs to a family of membrane-bound acyltransferases that catalyse the transfer of acyl-CoA to several lipid substrates.[88] The catalytically active site is in the ER lumen and comprises a conserved asparagine and a preserved histidine at position 321 and 356 of the protein.[85,89]

In human hepatocytes, the rs641738 C>T variant causes the downregulation of hepatic MBOAT7 both at the level of mRNA expression and protein synthesis (about 50% lower in carriers of the risk allele, who make up more than one-third of the general population),[50,68] possibly due to linkage with variation of the *MBOAT7* 3'-untranslated region.[58] In keeping with the main enzymatic activity of MBOAT7, the result is impaired remodelling of plasmatic and hepatic phosphatidylinositol (PI) species, and in particular the reduction of AA-containing PI, in patients carrying the risk variant, without major changes in the composition of other phospholipids.[50,67,68,90,91] This phenotype was fully recapitulated by knocking out *MBOAT7* in experimental models in human hepatocytes and in mouse livers.[68,90–92]

However, the relevance of MBOAT7 downregulation is not limited to carriage of the rs641738 risk variant. Importantly, downregulation of hepatic MBOAT7 is also associated with liver damage and adiposity-insulin resistance independently of the genetic background.[68,92] Furthermore, MBOAT7 downregulation during insulin resistance was also observed in the adipose tissue,[68,92] and confirmed in animal and *in vitro* models. Hepatic *Mboat7* transcription was curtailed in response to the rise in circulating insulin and the activation of Akt-dependent insulin signalling in mice, in response to refeeding and insulin injection, and in primary hepatocytes.[68]

MBOAT7 downregulation has a causal role in the pathogenesis of FLD. Indeed, the silencing of hepatic MBOAT7 or hepatocellular specific deletion led to fat accumulation in hepatocytes both *in vivo* and *in vitro*.[68,90–92] The impact was comparable to the effect of the rs641738 genetic risk variant.[68] The resultant impairment in LPI metabolism led to a reduction of AA-containing PI and the accumulation of saturated LPI, which is converted to TAG by the alternative pathway through diacylglycerol, contributing to LD formation (Fig. 4).[68,90] This process is associated with upregulation of lipogenesis with SREBP-1c.[68,93] Moreover, the deficiency of AA-containing PI upregulated CDP-diacylglycerol synthase, causing accelerated PI synthesis, and promoted PI degradation into diacylglycerol by phospholipase C, triggering a vicious cycle that generates TAG leading to steatosis.[90] All in all, these new discoveries suggest that MBOAT7 downregulation represents a physiological mechanism regulated by insulin that accompanies hepatic DNL in post-prandial conditions, facilitating the incorporation of fatty acids into TAG and LD in a non-toxic form. However, during chronic hyperinsulinemia related to insulin resistance this process may become maladaptive, because it sustains hepatic lipogenesis leading to FLD, in particular in carriers of the *MBOAT7* risk variant who experience more severe enzymatic deficiency. Future studies are warranted to examine whether replenishing AA-containing PI may attenuate liver damage in experimental models and in patients with dysmetabolism (See Fig. 4).

Of note, increasing the hepatocellular fat content may not be the only mechanism by which MBOAT7 downregulation promotes liver disease. This hypothesis is supported by the strong association

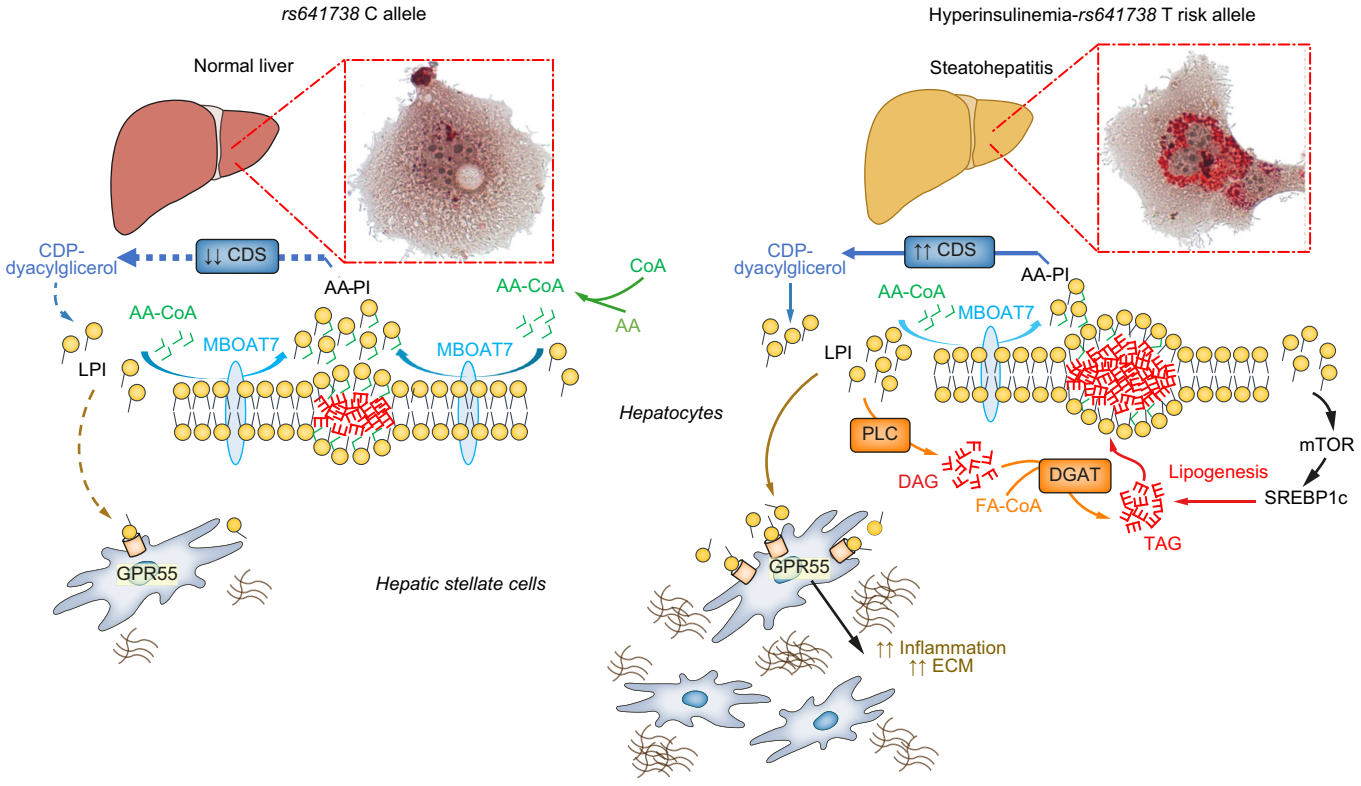

Fig. 4. MBOAT7 downregulation and LPI accumulation leads to steatohepatitis. MBOAT7 localises to the endoplasmic reticulum, where it catalyses the transfer of AA to LPI resulting in AA-PI, that is incorporated into cell membranes. In carriers of the rs641738 risk variant or during hyperinsulinemia, MBOAT7 is downregulated, resulting in a reduction of AA-PI and accumulation of LPI that is converted into TAG by the alternative synthesis pathway through the intermediate DAG, a process promoted by SREBP1c. Deficiency of AA-PI upregulates CDS, enhancing LPI synthesis. In HSCs, LPI binds to GPR55 triggering inflammatory and profibrotic gene expression and ECM deposition. AA, arachidonic acid; AA-PI, AA-containing PI; CDS, CDP-diacylglycerol-synthase; DAG, diacylglycerol; ECM, extracellular matrix; HSCs, hepatic stellate cells; LPI, lysophosphatidyl-inositol; MBOAT7, membrane bound O-acyltransferase domain containing 7; PI, phosphatidylinositol; SREBP-1c, sterol regulatory element-binding protein 1c; TAG, triacylglycerol.

between *MBOAT7* and hepatic fibrosis that has emerged from human genetic data.[31,50,51,58,82,83] Experimental models confirmed that downregulation of *MBOAT7* promoted hepatic fibrogenesis. In hepatic spheroid models composed of hepatocytes and HSCs, downregulation of MBOAT7 induced profibrotic and proinflammatory cytokines, leading to the activation of HSCs, to the expression of fibrogenic genes and to collagen accumulation.[9] Similarly, genes implicated in inflammation and fibrosis were overexpressed in *Mboat7*-deficient mice, which exhibited hepatic fat accumulation and impaired LPI remodelling, and were overall more susceptible to fibrotic NASH.[68,90–92] Although carriage of the *MBOAT7* risk variant is associated with histological inflammation on liver biopsy in patients at risk of NASH,[50] and hepatic *Mboat7* downregulation may facilitate lipotoxicity-induced acute inflammation in experimental models,[92] some evidence suggests that *MBOAT7* downregulation can trigger hepatic fibrosis independently of inflammation.[68,90,91] A possible mechanism of liver injury and fibrogenesis might be related to the accumulation of LPI. Indeed, circulating levels of LPI are increased in patients with advanced liver fibrosis compared to healthy controls, while AA-containing PI is reduced.[92] Moreover, in high-fat diet-fed mice, LPI administration induced inflammatory and fibrotic genes, especially when Mboat7 was downregulated.[92]

Recent evidence implicated the G protein-coupled receptor 55 (GPR55) in the pathogenesis of NASH. GPR55 is a cannabinoid receptor,[94] whose main endogenous ligand is LPI.[95] Fondevila *et al.* found that LPI increased GPR55 expression and promoted hepatic lipid accumulation in *in vitro* and *in vivo* models; moreover, GPR55 was overexpressed in patients with FLD and NASH.[96] Hepatic injury induced by LPI seemed to be partly mediated by GPR55, since the inhibition of the receptor reduced hepatic lipid content and profibrotic gene expression in mice.[96] Accordingly, activation of the LPI/GPR55 axis increased the expression of profibrotic genes and extracellular matrix production in HSCs, and stimulated cell proliferation in a GPR55-dependent fashion *in vitro*.[96] Therefore, MBOAT7 downregulation may directly promote liver damage by increasing LPI concentration and inducing liver fibrosis via GPR55 (Fig. 4). Downregulation or antagonism of hepatic GPR55 may therefore represent another potential therapeutic strategy for steatohepatitis.

Lipotoxicity and IL-32 at the interface between fat accumulation and progressive liver disease

While the factors modulating the fate of lipids in the liver during steatohepatitis are only beginning to be understood, there is already robust evidence that dysmetabolism and insulin resistance lead to lipotoxicity and inflammation, contributing to liver damage,[97] and representing the main trigger (together with excess alcohol) for the phenotypic expression of genetic risk

variants of FLD.[98] Incidentally, these genetic epidemiology data are consistent with the major impact of weight loss[99] and the possible utility of anti-obesity drugs in the treatment of NASH.[100] Besides acting as an energy store, adipose tissue also exerts its function as an endocrine organ by releasing cytokines, hormones and growth factors. Collectively defined as adipokines, these mediators are required for the regulation of metabolism, immune response and homeostasis at a systemic level.[101] Excess adiposity and the subsequent infiltration of adipose tissue with immune cells, ultimately result in altered adipokines, promoting metabolic complications and liver damage.[101] Furthermore, chronic inflammation and insulin resistance induce FFA release from the adipose tissue, facilitating hepatocellular fat accumulation, while inflammatory mediators drive the deposition of fibrotic tissue, the hallmark of the transition to severe liver disease.[102]

Among the plethora of inflammatory markers associated with obesity and FLD, IL-32 is emerging as a master regulator of obesity-driven inflammation, bridging fat excess and lipotoxicity with liver damage. Indeed, circulating IL-32 levels correlate with adiposity and decrease after bariatric surgery.[103] Similarly, *IL32* mRNA levels are upregulated in the adipose tissue of obese individuals, and correlate with the enhanced expression of pro-inflammatory mediators, including IL-1β, IL-6 and IL-10, suggesting that IL-32 acts as a master regulator orchestrating obesity-driven inflammation.[103] In human visceral adipocytes, lipopolysaccharide and tumour necrosis factor-α (TNF-α) administration induces IL-32 transcription. IL-32α increased the expression of IL-1β, TNF-α and extracellular matrix-remodelling genes, whereas IL-32 silencing yielded the opposite effects.[103] Accordingly, serum levels of IL-32 are higher in patients with type 2 diabetes and correlate with body mass and fasting blood sugar.[104]

IL-32 is also highly expressed in the liver and induced during liver disease.[105,106] We recently showed that IL-32 is the most robustly upregulated transcript in obese individuals with severe NAFLD, in particular in carriers of the *PNPLA3* I148M risk variant, and can be induced by lipotoxicity in hepatocytes.[107] IL-32β was the most expressed isoform in hepatocytes, and hepatic IL-32 was coregulated with a set of inflammatory genes and chemokines.[107] Accordingly, it was recently shown that *PNPLA3* I148M induces metabolic reprogramming, leading to a shift in TAG/phospholipid composition and activating inflammatory pathways.[108] Moreover, in individuals without inflammatory diseases, circulating IL-32 correlated with the hepatic transcript, and was higher in patients with severe NAFLD, improving the diagnostic accuracy of non-invasive biomarkers.[107] Dali-Youcef *et al.* reported that hepatic IL-32 was upregulated in isolated steatosis and even more so in NASH, which was paralleled by an increase in inflammatory cytokines.[109] Furthermore, IL-32 abrogated insulin-dependent AKT phosphorylation in primary human hepatocytes, implying that IL-32 may be causally involved in insulin resistance,[109] and it may also facilitate intracellular lipid accumulation by modulating cholesterol efflux.[110]

Notably, IL-32 and TNF-α mutually increase the transcription of each other, triggering a positive feedback loop that drives low-grade inflammation in lipid-storing organs by inducing IL-1β, IL-6 and IL-10 secretion.[111] The *IL32* promoter contains binding sites for fatty acid-responsive transcription factors, consistent with upregulation during lipotoxicity [107]; IL-32 has been implicated in triggering endothelial inflammation in response to a post-prandial increase in FFAs.[112] Mechanistically, IL-32α was shown to promote STAT3 localisation at the *IL6* promoter by mediating STAT3 phosphorylation by PKCε.[113] Hepatic STAT3 signalling is enhanced in patients carrying the *PNPLA3* I148M variant[107] and in mice overexpressing mutant *PNPLA3*.[108] The role of STAT3 in insulin resistance is well established: STAT3 activates *SOCS3* transcription, which negatively regulates insulin signalling by interacting with the insulin receptor and insulin receptor substrate-1.[114] Moreover, both IL-6 and ceramides amplify binding of STAT3 to the hepcidin promoter, leading to dysregulation of iron metabolism, an additional contributor to liver disease progression.[115]

Overall, IL-32 levels correlate with obesity, insulin resistance, and steatohepatitis, suggesting that it contributes directly and indirectly to liver damage by bridging excessive intracellular fat levels with the chronic inflammation underlying these conditions. A working model is shown in Fig. 5. This hypothesis needs to be proven in experimental models of NASH, which is rendered more difficult by the fact that mice do not bear any *IL32* gene orthologue. In addition, IL-32β, which is mostly expressed in hepatocytes, is not secreted through canonical pathways; therefore, the mechanism leading to the release of IL-32 into the extracellular space, as well as the signalling pathways activated by this atypical cytokine, are yet to be clarified.[116] Circulating IL-32 may thus represent a new liver disease biomarker and a therapeutic candidate, which can be targeted by hepatic gene silencing or neutralisation by monoclonal antibodies. However, no information is yet available on IL-32 expression during ASH.

The gut-liver axis and bile acid metabolism

Finally, candidate genetic studies are beginning to provide independent validation of the role of the gut-liver axis and bile acid metabolism in NASH. Here, the interaction between the host and microbiome genes should be considered. Indeed, both ASH and NASH are associated with altered microbial composition,[117,118] albeit with some differences.[119,120] This process leads to dysbiosis and over-representation of pathogenic bacteria and metabolites, causing mucosal inflammation and alterations to the gut-vascular barrier.[121,122] This alteration of the microbiome is accompanied by altered remodelling of bile acids, with a reduction of secondary bile acids that are more potent farnesoid X receptor (FXR) agonists.[123] In experimental models, FXR agonism reversed gut-vascular barrier disruption during high-fat diet feeding, thereby protecting against NASH.[124]

Although evidence is still at an early stage, genetic variants that modulate the gut-liver axis recapitulate the impact of drugs that modulate this pathway. First, genetic variation at the *NR1H4* locus encoding FXR (rs35724) was linked to increased expression of hepatic FXR and of key targets involved in bile acid signalling via fibroblast growth factor receptor-19 (FGF19). FGF19 – the main enterokine released by the intestine in response to FXR – regulates bile acid metabolism in the liver by binding to FGF receptor-4 (FGFR4) and the β-klotho (KLB) coreceptor.[125] The rs35724 variant was associated with increased FGFR4 and target engagement on sterol synthesis, namely cytochrome P39a1 (CYP39A1).[126] Indeed, during steatohepatitis, reduced levels of FXR and FGF19 can lead to the accumulation of bile acids in the intestine and the liver, where their synthesis from cholesterol is not inhibited and export to the bile is downregulated, thereby promoting inflammation and carcinogenesis.[127]

In keeping with the beneficial impact of FXR agonists on liver fibrosis in patients with NASH,[128] the gain-of-function *NR1H4* variant was associated with protection against severe NAFLD and fibrosis.[126] *Vice versa*, a genetic variant in *KLB* associated with

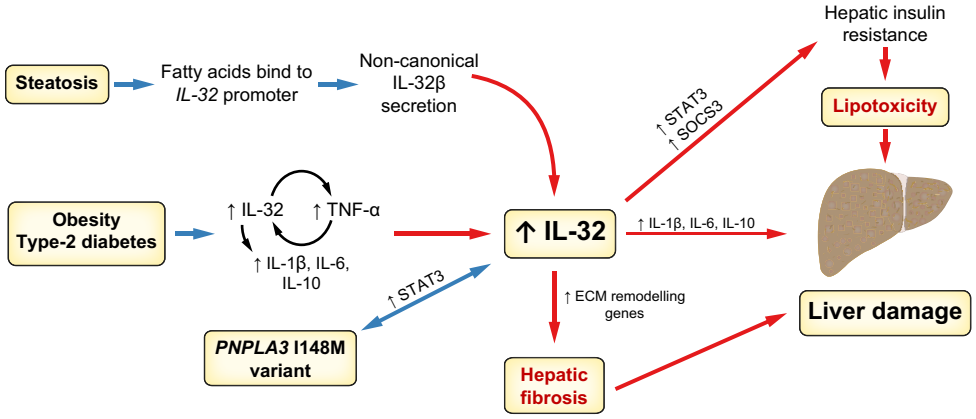

Fig. 5. Potential mechanisms linking IL-32 with liver damage in fatty liver disease and steatohepatitis. IL-32 levels are elevated in obese and diabetic patients, suggesting a link between this cytokine and low-grade chronic inflammation and insulin resistance. More importantly, IL-32 is highly induced during liver damage, and its levels correlate with disease severity, progression to NASH and carriage of the *PNPLA3* I148M variant. Acting as a master regulator of other pro-inflammatory cytokines, IL-32 promotes inflammation and liver damage by increasing the secretion of IL-1β, IL-6 and IL-10, and may also drive hepatic fibrogenesis by promoting the transcription of ECM-remodelling genes. Moreover, the *IL-32* promoter is targeted by fatty acid-responsive transcription factors and thus steatosis promotes non-canonical IL-32β secretion in the liver. Both IL-32 and carriage of *PNPLA3* I148M upregulate STAT3, leading to lipotoxicity and ultimately liver damage. ECM, extracellular matrix; IL-interleukin-; NASH, non-alcoholic steatohepatitis; *PNPLA3*, patatin like phospholipase domain containing 3.

reduced protein expression sensitised obese children to liver damage.[129] Strikingly, genetic predisposition to increased FXR activity also recapitulated the main adverse effect of both FXR and FGF19 agonists, namely the increase in circulating cholesterol – worsening the cardiovascular risk profile.[126,128,130] The highlighted underlying mechanism, *i.e.* increased cholesterol

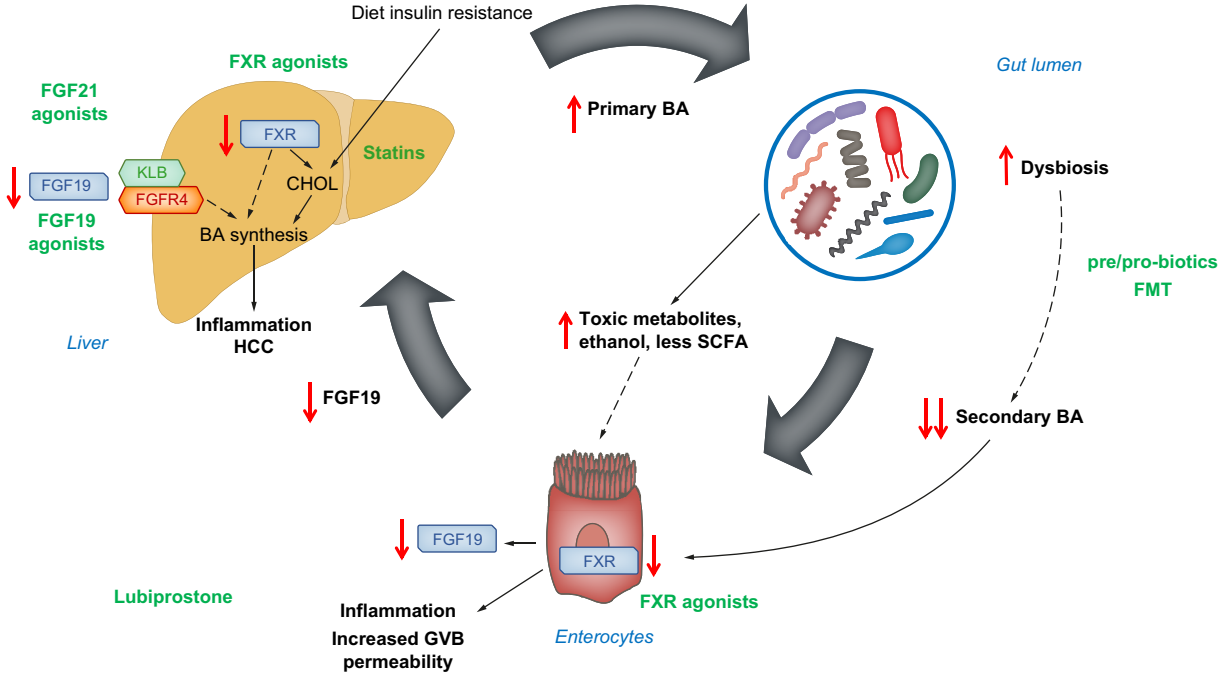

Fig. 6. Human genetics supports the involvement of the gut-liver axis in NASH. NASH is associated to microbiota dysbiosis, and the over representation of pathogenic bacterial species leads to impairment in the gut-vascular barrier and bile acid management. Reduction in secondary bile acids results in hampered FXR signalling, facilitating intestinal inflammation and further impairment of the gut-blood barrier, which can be rescued by FXR agonists. FXR activation results in the release of FGF19, an enterokine that regulates hepatic bile acid metabolism by interacting with FGFR4 receptor and KLB co-receptor. Reduction of FXR in steatohepatitis negatively affects bile acid metabolism, and therefore bile acids accumulate in the liver promoting inflammation and carcinogenesis. Notably, rs35724 falling in the *NR1H4* locus is protective against liver damage, but increases cardiovascular risk by increasing cholesterol synthesis; on the other hand, a *KLB* variant reducing protein levels promotes liver damage in obese patients. Pathways altered during NASH are indicated by red arrows, with potential therapeutic approaches in green. BAs, bile acids; CHOL, cholesterol; FGF, fibroblast growth factor; FGFR, FGF receptor; FMT, faecal microbiota transplant; FXR, farnesoid X receptor; HCC, hepatocellular carcinoma; KLB, klotho beta; NASH, non-alcoholic steatohepatitis; SCFAs, short-chain fatty acids.

synthesis, also provided the rationale for the beneficial and possibly synergic impact of co-treatment with statins.[128,131]

The role of this "FXR-FGF19-KLB-bile acids-microbiome" pathway in the pathogenesis of NASH is presented in Fig. 6. The recent demonstration that the presence of FXR is necessary for modulation of the bile acid pool and consequently of the intestinal microbiome in response to FXR,[132] and that modulation of specific bacterial species may predict the beneficial impact of therapy with FGF19 agonists on liver damage,[133] collectively suggest that these processes are best described as an interconnected cycle (See Fig. 6).

Conclusions

In summary, human genetics offers a new approach to the development of therapeutics for ASH and NASH that may have predicted the outcomes, pinpointing both the strengths and weaknesses, of drug approaches undergoing evaluation in clinical trials. Furthermore, molecular genetics is highlighting new drug targets and has already led to the design of the first ever precision medicine approaches for a common liver disease, which are entering the clinical research arena. These aim at targeting the genetic risk variants to cure steatohepatitis by suppressing a contributing cause. Given the common genetic pathophysiology of ASH and NASH, there is also the potential and hope that some of the novel approaches developed for NASH can be repurposed to treat AFLD and ASH, but additional studies are needed to prove this hypothesis. Most importantly, this class of drugs may be particularly effective in patients with FLD and steatohepatitis,[1,2] with dysmetabolism associated with moderate alcohol consumption, who cannot be diagnosed with NASH, but who are at high risk of disease progression.[134]

Abbreviations

AA, arachidonic acid; ASH, alcoholic steatohepatitis; DAG, diacylglycerol; DNL, *de novo* lipogenesis; ER, endoplasmic reticulum; FFAs, free fatty acids; FGF19, fibroblast growth factor 19; FLD, fatty liver disease; FXR, farnesoid X receptor; GCKR, glucokinase regulator; GPR55, G protein-coupled receptor 55; HCC, hepatocellular carcinoma; HSC, hepatic stellate cells; HSD17B13, hydroxysteroid 17-beta dehydrogenase 13; IL-, interleukin-; LDs, lipid droplets; LPI, lysophosphatidyl-inositol; *HFE*, homeostatic iron regulator; *MARC1*, mitochondrial amidoxime reducing component 1; MBOAT7, membrane bound O-acyltransferase domain-containing 7; NASH, non-alcoholic steatohepatitis; *PNPLA3*, patatin like phospholipase domain containing 3; PPAR, peroxisome proliferator-activated receptor; PRS, polygenic risk score; PUFAs, polyunsaturated fatty acids; SREBP, sterol response element binding protein; TAG, triacylglycerol; TNF-α, tumour necrosis factor-α.

Financial support

MyFirst Grant AIRC n.16888, Ricerca Finalizzata Ministero della Salute RF-2016-02364358, Ricerca corrente Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, the European Union (EU) Programme Horizon 2020 Programme "Photonics" under grant agreement "101016726" for the project "REVEAL: Neuronal microscopy for cell behavioural examination and manipulation", the Innovative Medicines Initiative 2 joint undertaking of European Union's Horizon 2020 research and innovation programme and EFPIA for the project LITMUS- "Liver Investigation: Testing Marker Utility in Steatohepatitis" under grant agreement No. 777377), Fondazione IRCCS Ca' Granda "Liver BIBLE" PR-0391, Fondazione IRCCS Ca' Granda core COVID-19 Biobank (RC100017A) to LV.

Conflict of interest

LV has received speaking fees from MSD, Gilead, AlfaSigma and AbbVie, served as a consultant for Gilead, Pfizer, Astra Zeneca, Novo Nordisk, Intercept, Diatech Pharmacogenetics and Ionis Pharmaceuticals, and received research grants from Gilead.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

LV study conception design, CB, EC, and LV: first manuscript drafting all authors were involved in manuscript writing and revision.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhepr.2021.100284.

References

[1] Eslam M, Sanyal AJ, George J, International Consensus P. MAFLD: a consensus-driven proposed nomenclature for metabolic associated fatty liver disease. Gastroenterology 2020;158:1999–2014. e1991.
[2] Valenti L, Pelusi S. Redefining fatty liver disease classification in 2020. Liver Int 2020;40:1016–1017.
[3 Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease-meta-analytic assessment of prevalence, incidence and outcomes. Hepatology 2016;64:73–84.
[4] Estes C, Anstee QM, Arias-Loste MT, Bantel H, Bellentani S, Caballeria J, et al. Modeling NAFLD disease burden in China, France, Germany, Italy, Japan, Spain, United Kingdom and United States for the period 2016–2030. J Hepatol 2018;69:896–904.
[5] **Goldberg D**, **Ditah IC**, Saeian K, Lalehzari M, Aronsohn A, Gorospe EC, et al. Changes in the prevalence of hepatitis C virus infection, nonalcoholic steatohepatitis, and alcoholic liver disease among patients with cirrhosis or liver failure on the waitlist for liver transplantation. Gastroenterology 2017;152:1090–1099. e1091.
[6] **Dyson J**, **Jaques B**, Chattopadyhay D, Lochan R, Graham J, Das D, et al. Hepatocellular cancer: the impact of obesity, type 2 diabetes and a multidisciplinary team. J Hepatol 2014;60:110–117.
[7] Younossi Z, Henry L. Contribution of alcoholic and nonalcoholic fatty liver disease to the burden of liver-related morbidity and mortality. Gastroenterology 2016;150:1778–1785.
[8] Haflidadottir S, Jonasson JG, Norland H, Einarsdottir SO, Kleiner DE, Lund SH, et al. Long-term follow-up and liver-related death rate in patients with non-alcoholic and alcoholic related fatty liver disease. BMC Gastroenterol 2014;14:166.
[9] Cholankeril G, Ahmed A. Alcoholic liver disease replaces hepatitis C virus infection as the leading indication for liver transplantation in the United States. Clin Gastroenterol Hepatol 2018;16:1356–1358.
[10] Adam R, Karam V, Cailliez V, O'Grady JG, Mirza D, Cherqui D, et al. 2018 annual report of the European liver transplant registry (ELTR)–50-year evolution of liver transplantation. Transpl Int 2018;31:1293–1317.
[11] **Romeo S**, **Sanyal A**, **Valenti L**. Leveraging human genetics to identify potential new treatments for fatty liver disease. Cell Metab 2020;31:35–45.
[12] Valenti L, Fracanzani AL, Fargion S. The immunopathogenesis of alcoholic and nonalcoholic steatohepatitis: two triggers for one disease? Semin Immunopathol 2009;31:359–369.
[13] Purohit V, Gao B, Song BJ. Molecular mechanisms of alcoholic fatty liver. Alcohol Clin Exp Res 2009;33:191–205.
[14] Donnelly KL, Smith CI, Schwarzenberg SJ, Jessurun J, Boldt MD, Parks EJ. Sources of fatty acids stored in liver and secreted via lipoproteins in patients with nonalcoholic fatty liver disease. J Clin Invest 2005;115:1343–1351.

[15] Yamaguchi K, Yang L, McCall S, Huang J, Yu XX, Pandey SK, et al. Inhibiting triglyceride synthesis improves hepatic steatosis but exacerbates liver damage and fibrosis in obese mice with nonalcoholic steatohepatitis. Hepatology 2007;45:1366–1374.

[16] You M, Considine RV, Leone TC, Kelly DP, Crabb DW. Role of adiponectin in the protective action of dietary saturated fat against alcoholic fatty liver in mice. Hepatology 2005;42:568–577.

[17] Nakajima T, Kamijo Y, Tanaka N, Sugiyama E, Tanaka E, Kiyosawa K, et al. Peroxisome proliferator-activated receptor alpha protects against alcohol-induced liver damage. Hepatology 2004;40:972–980.

[18] Ji C, Chan C, Kaplowitz N. Predominant role of sterol response element binding proteins (SREBP) lipogenic pathways in hepatic steatosis in the murine intragastric ethanol feeding model. J Hepatol 2006;45:717–724.

[19] Singh S, Allen AM, Wang Z, Prokop LJ, Murad MH, Loomba R. Fibrosis progression in nonalcoholic fatty liver vs nonalcoholic steatohepatitis: a systematic review and meta-analysis of paired-biopsy studies. Clin Gastroenterol Hepatol 2015;13:643–654. e641-e649; quiz e639-640.

[20] Pelusi S, Cespiati A, Rametta R, Pennisi G, Mannisto V, Rosso C, et al. Prevalence and risk factors of significant fibrosis in patients with nonalcoholic fatty liver without steatohepatitis. Clin Gastroenterol Hepatol 2019;17:2310–2319. e2316.

[21] Drenth JPH, Schattenberg JM. The nonalcoholic steatohepatitis (NASH) drug development graveyard: established hurdles and planning for future success. Expert Opin Investig Drugs 2020:1–11.

[22] **Trepo E**, **Valenti L**. Update on NAFLD genetics: from new variants to the clinic. J Hepatol 2020;72:1196–1209.

[23] Sookoian S, Pirola CJ, Valenti L, Davidson NO. Genetic pathways in nonalcoholic fatty liver disease: insights from systems biology. Hepatology 2020;72:330–346.

[24] Cespiati A, Youngson NA, Tourna A, Valenti L. Genetics and epigenetics in the clinic: precision medicine in the management of fatty liver disease. Curr Pharm Des 2020;26:998–1009.

[25] Linden D, Ahnmark A, Pingitore P, Ciociola E, Ahlstedt I, Andreasson AC, et al. Pnpla3 silencing with antisense oligonucleotides ameliorates nonalcoholic steatohepatitis and fibrosis in Pnpla3 I148M knock-in mice. Mol Metab 2019;22:49–61.

[26] Stickel F, Hampe J. Genetic determinants of alcoholic liver disease. Gut 2012;61:150–159.

[27] Dongiovanni P, Donati B, Fares R, Lombardi R, Mancina RM, Romeo S, et al. PNPLA3 I148M polymorphism and progressive liver disease. World J Gastroenterol 2013;19:6969–6978.

[28] Romeo S, Kozlitina J, Xing C, Pertsemlidis A, Cox D, Pennacchio LA, et al. Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. Nat Genet 2008;40:1461–1465.

[29] Valenti L, Al-Serri A, Daly AK, Galmozzi E, Rametta R, Dongiovanni P, et al. Homozygosity for the PNPLA3/adiponutrin I148M polymorphism influences liver fibrosis in patients with nonalcoholic fatty liver disease. Hepatology 2010;51:1209–1217.

[30] Tian C, Stokowski RP, Kershenobich D, Ballinger DG, Hinds DA. Variant in PNPLA3 is associated with alcoholic liver disease. Nat Genet 2009;42:21–23.

[31] Buch S, Stickel F, Trepo E, Way M, Herrmann A, Nischalke HD, et al. A genome-wide association study confirms PNPLA3 and identifies TM6SF2 and MBOAT7 as risk loci for alcohol-related cirrhosis. Nat Genet 2015;47:1443–1448.

[32] Trepo E, Nahon P, Bontempi G, Valenti L, Falleti E, Nischalke HD, et al. Association between the PNPLA3 (rs738409 C>G) variant and hepatocellular carcinoma: evidence from a meta-analysis of individual participant data. Hepatology 2014;59:2170–2177.

[33] Atkinson SR, Way MJ, McQuillin A, Morgan MY, Thursz MR. Homozygosity for rs738409:G in PNPLA3 is associated with increased mortality following an episode of severe alcoholic hepatitis. J Hepatol 2017;67:120–127.

[34] Atkinson S, Way M, McQuillin A, Morgan MY, Thursz M. GS03–a genome-wide association study identifies PNPLA3 and SLC38A4 as risk loci for alcoholic hepatitis. J Hepatol 2016;64:S134.

[35] Huang Y, He S, Li JZ, Seo YK, Osborne TF, Cohen JC, et al. A feed-forward loop amplifies nutritional regulation of PNPLA3. Proc Natl Acad Sci U S A 2010;107:7892–7897.

[36] Mitsche MA, Hobbs HH, Cohen JC. Patatin-like phospholipase domain-containing protein 3 promotes transfer of essential fatty acids from triglycerides to phospholipids in hepatic lipid droplets. J Biol Chem 2018;293:6958–6968.

[37] **Pirazzi C**, **Valenti L**, **Motta BM**, Pingitore P, Hedfalk K, Mancina RM, et al. PNPLA3 has retinyl-palmitate lipase activity in human hepatic stellate cells. Hum Mol Genet 2014;23:4077–4085.

[38] BasuRay S, Wang Y, Smagris E, Cohen JC, Hobbs HH. Accumulation of PNPLA3 on lipid droplets is the basis of associated hepatic steatosis. Proc Natl Acad Sci U S A 2019;116:9521–9526.

[39] Pingitore P, Dongiovanni P, Motta BM, Meroni M, Lepore SM, Mancina RM, et al. PNPLA3 overexpression results in reduction of proteins predisposing to fibrosis. Hum Mol Genet 2016;25:5212–5222.

[40] Bruschi FV, Claudel T, Tardelli M, Caligiuri A, Stulnig TM, Marra F, et al. The PNPLA3 I148M variant modulates the fibrogenic phenotype of human hepatic stellate cells. Hepatology 2017;65:1875–1890.

[41] Kozlitina J, Smagris E, Stender S, Nordestgaard BG, Zhou HH, Tybjaerg-Hansen A, et al. Exome-wide association study identifies a TM6SF2 variant that confers susceptibility to nonalcoholic fatty liver disease. Nat Genet 2014;46:352–356.

[42] Dongiovanni P, Petta S, Maglio C, Fracanzani AL, Pipitone R, Mozzi E, et al. Transmembrane 6 superfamily member 2 gene variant disentangles nonalcoholic steatohepatitis from cardiovascular disease. Hepatology 2015;61:506–514.

[43] Wang Y, Kory N, Cohen JC, Hobbs HH. PNPLA3, CGI-58, and inhibition of hepatic triglyceride hydrolysis in mice. Hepatology 2019;69:2427–2441.

[44] Prill S, Caddeo A, Baselli G, Jamialahmadi O, Dongiovanni P, Rametta R, et al. The TM6SF2 E167K genetic variant induces lipid biosynthesis and reduces apolipoprotein B secretion in human hepatic 3D spheroids. Sci Rep 2019;9:11585.

[45] Speliotes EK, Yerges-Armstrong LM, Wu J, Hernaez R, Kim LJ, Palmer CD, et al. Genome-wide association analysis identifies variants associated with nonalcoholic fatty liver disease that have distinct effects on metabolic traits. Plos Genet 2011;7:e1001324.

[46] Petta S, Miele L, Bugianesi E, Camma C, Rosso C, Boccia S, et al. Glucokinase regulatory protein gene polymorphism affects liver fibrosis in non-alcoholic fatty liver disease. PLoS One 2014;9:e87523.

[47] Dongiovanni P, Stender S, Pietrelli A, Mancina RM, Cespiati A, Petta S, et al. Causal relationship of hepatic fat with liver damage and insulin resistance in nonalcoholic fatty liver. J Intern Med 2018;283:356–370.

[48] Peter A, Stefan N, Cegan A, Walenta M, Wagner S, Konigsrainer A, et al. Hepatic glucokinase expression is associated with lipogenesis and fatty liver in humans. J Clin Endocrinol Metab 2011;96:E1126–E1130.

[49] Beer NL, Tribble ND, McCulloch LJ, Roos C, Johnson PR, Orho-Melander M, et al. The P446L variant in GCKR associated with fasting plasma glucose and triglyceride levels exerts its effect through increased glucokinase activity in liver. Hum Mol Genet 2009;18:4081–4088.

[50] **Mancina RM**, **Dongiovanni P**, Petta S, Pingitore P, Meroni M, Rametta R, et al. The MBOAT7-TMC4 variant rs641738 increases risk of nonalcoholic fatty liver disease in individuals of European descent. Gastroenterology 2016;150:1219–1230. e1216.

[51] Teo K, Abeysekera KWM, Adams L, Aigner E, Anstee QM, Banales JM, et al. rs641738C>T near MBOAT7 is associated with liver fat, ALT, and fibrosis in NAFLD: a meta-analysis. J Hepatol 2020;74:20–30.

[52] Abul-Husn NS, Cheng X, Li AH, Xin Y, Schurmann C, Stevis P, et al. A protein-truncating HSD17B13 variant and protection from chronic liver disease. N Engl J Med 2018;378:1096–1106.

[53] Ma Y, Belyaeva OV, Brown PM, Fujita K, Valles K, Karki S, et al. HSD17B13 is a hepatic retinol dehydrogenase associated with histological features of non-alcoholic fatty liver disease. Hepatology 2019;69:1504–1519.

[54] Stickel F, Lutz P, Buch S, Nischalke HD, Silva I, Rausch V, et al. Genetic variation in HSD17B13 reduces the risk of developing cirrhosis and hepatocellular carcinoma in alcohol misusers. Hepatology 2020;72:88–102.

[55] Emdin CA, Haas ME, Khera AV, Aragam K, Chaffin M, Klarin D, et al. A missense variant in Mitochondrial Amidoxime Reducing Component 1 gene and protection against liver disease. Plos Genet 2020;16:e1008629.

[56] Emdin CA, Haas M, Ajmera V, Simon TG, Homburger J, Neben C, et al. Association of genetic variation with cirrhosis: a multi-trait genome-wide association and gene-environment interaction study. Gastroenterology 2021;160:1620–1633.

[57] **Innes H**, **Buch S**, Hutchinson S, Guha IN, Morling JR, Barnes E, et al. Genome-wide association study for alcohol-related cirrhosis identifies risk loci in MARC1 and HNRNPUL1. Gastroenterology 2020;159:1276–1289. e1277.

[58] Donati B, Dongiovanni P, Romeo S, Meroni M, McCain M, Miele L, et al. MBOAT7 rs641738 variant and hepatocellular carcinoma in non-cirrhotic individuals. Sci Rep 2017;7:4492.

[59] **Bianco C**, **Jamialahmadi O**, **Pelusi S**, Baselli G, Dongiovanni P, Zanoni I, et al. Non-invasive stratification of hepatocellular carcinoma risk in non-alcoholic fatty liver using polygenic risk scores. J Hepatol 2020.

[60] Pelusi S, Valenti L. Hepatic fat as clinical outcome and therapeutic target for nonalcoholic fatty liver disease. Liver Int 2019;39:250–256.
[61] Jamialahmadi O, Mancina RM, Ciociola E, Tavaglione F, Luukkonen PK, Baselli G, et al. Exome-wide association study on alanine aminotransferase identifies sequence variants in the GPAM and APOE associated with fatty liver disease. Gastroenterology 2021;160:1634–1646.
[62] Anstee QM, Darlay R, Cockell S, Meroni M, Govaere O, Tiniakos D, et al. Genome-wide association study of non-alcoholic fatty liver and steatohepatitis in a histologically-characterised cohort. J Hepatol 2020;73:505–515.
[63] **Mancina RM**, **Ferri F**, Farcomeni A, Molinaro A, Maffongelli A, Mischitelli M, et al. A two gene-based risk score predicts alcoholic cirrhosis development in males with at-risk alcohol consumption. Appl Clin Genet 2019;12:1–10.
[64] Nezi V, Deutsch M, Gazouli M, Alexopoulou A, Paparrigopoulos T, Liappas IA, et al. Polymorphisms of the CD14 genes are associated with susceptibility to alcoholic liver disease in Greek patients. Alcohol Clin Exp Res 2013;37:244–251.
[65] Nelson MR, Tipney H, Painter JL, Shen J, Nicoletti P, Shen Y, et al. The support of human genetic evidence for approved drug indications. Nat Genet 2015;47:856–860.
[66] **Luukkonen PK**, **Zhou Y**, Sadevirta S, Leivonen M, Arola J, Oresic M, et al. Hepatic ceramides dissociate steatosis and insulin resistance in patients with non-alcoholic fatty liver disease. J Hepatol 2016;64:1167–1175.
[67] Luukkonen PK, Zhou Y, Hyotylainen T, Leivonen M, Arola J, Orho-Melander M, et al. The MBOAT7 variant rs641738 alters hepatic phosphatidylinositols and increases severity of non-alcoholic fatty liver disease in humans. J Hepatol 2016;65:1263–1265.
[68] Meroni M, Dongiovanni P, Longo M, Carli F, Baselli G, Rametta R, et al. Mboat7 down-regulation by hyper-insulinemia induces fat accumulation in hepatocytes. EBioMedicine 2020;52:102658.
[69] Dongiovanni P, Meroni M, Baselli G, Mancina RM, Ruscica M, Longo M, et al. PCSK7 gene variation bridges atherogenic dyslipidemia with hepatic inflammation in NAFLD patients. J Lipid Res 2019;60:1144–1153.
[70] Shen B, Zhang W, Zhang J, Zhou J, Wang J, Chen L, et al. Efficient genome modification by CRISPR-Cas9 nickase with minimal off-target effects. Nat Methods 2014;11:399–402.
[71] Margherita Mancina R, Matikainen N, Maglio C, Soderlund S, Lundbom N, Hakkarainen A, et al. Paradoxical dissociation between hepatic fat content and de novo lipogenesis due to PNPLA3 sequence variant. J Clin Endocrinol Metab 2015:jc20144464.
[72] Dongiovanni P, Petta S, Mannisto V, Margherita Mancina R, Pipitone R, Karja V, et al. Statin use and nonalcoholic steatohepatitis in at risk individuals. J Hepatol 2015;63:705–712.
[73] Scorletti E, West AL, Bhatia L, Hoile SP, McCormick KG, Burdge GC, et al. Treating liver fat and serum triglyceride levels in NAFLD, effects of PNPLA3 and TM6SF2 genotypes: results from the WELCOME trial. J Hepatol 2015;63:1476–1483.
[74] Valenti L, Dongiovanni P. Mutant PNPLA3 I148M protein as pharmacological target for liver disease. Hepatology 2017;66:1026–1028.
[75] Alkhouri N, Reddy GK, Lawitz E. Oligonucleotide-based therapeutics: an emerging strategy for the treatment of chronic liver diseases. Hepatology 2021;73:1581–1593.
[76] Donati B, Motta BM, Pingitore P, Meroni M, Pietrelli A, Alisi A, et al. The rs2294918 E434K variant modulates patatin-like phospholipase domain-containing 3 expression and liver damage. Hepatology 2016;63:787–798.
[77] Schwartz BE, Rajagopal V, Smith C, Cohick E, Whissell G, Gamboa M, et al. Discovery and targeting of the signaling controls of PNPLA3 to effectively reduce transcription, expression, and function in pre-clinical NAFLD/NASH settings. Cells 2020;9.
[78] **Mondul A**, **Mancina RM**, **Merlo A**, Dongiovanni P, Rametta R, Montalcini T, et al. PNPLA3 I148M variant influences circulating retinol in adults with nonalcoholic fatty liver disease or obesity. J Nutr 2015;145:1687–1691.
[79] Petta S, Valenti L, Marra F, Grimaudo S, Tripodo C, Bugianesi E, et al. MERTK rs4374383 polymorphism affects the severity of fibrosis in non-alcoholic fatty liver disease. J Hepatol 2016;64:682–690.
[80] Cai B, Dongiovanni P, Corey KE, Wang X, Shmarakov IO, Zheng Z, et al. Macrophage MerTK promotes liver fibrosis in nonalcoholic steatohepatitis. Cell Metab 2020;31:406–421. e407.
[81] Wang X, Cai B, Yang X, Sonubi OO, Zheng Z, Ramakrishnan R, et al. Cholesterol stabilizes TAZ in hepatocytes to promote experimental non-alcoholic steatohepatitis. Cell Metab 2020;31:969–986. e967.
[82] Thabet K, Asimakopoulos A, Shojaei M, Romero-Gomez M, Mangia A, Irving WL, et al. MBOAT7 rs641738 increases risk of liver inflammation and transition to fibrosis in chronic hepatitis C. Nat Commun 2016;7:12757.
[83] Thabet K, Chan HLY, Petta S, Mangia A, Berg T, Boonstra A, et al. The membrane-bound O-acyltransferase domain-containing 7 variant rs641738 increases inflammation and fibrosis in chronic hepatitis B. Hepatology 2017;65:1840–1850.
[84] **Pelusi S**, **Baselli G**, Pietrelli A, Dongiovanni P, Donati B, McCain MV, et al. Rare pathogenic variants predispose to hepatocellular carcinoma in nonalcoholic fatty liver disease. Sci Rep 2019;9:3682.
[85] **Caddeo A**, **Jamialahmadi O**, Solinas G, Pujia A, Mancina RM, Pingitore P, et al. MBOAT7 is anchored to endomembranes by six transmembrane domains. J Struct Biol 2019;206:349–360.
[86] Johansen A, Rosti RO, Musaev D, Sticca E, Harripaul R, Zaki M, et al. Mutations in MBOAT7, encoding lysophosphatidylinositol acyltransferase I, lead to intellectual disability accompanied by epilepsy and autistic features. Am J Hum Genet 2016;99:912–916.
[87] Lee HC, Inoue T, Sasaki J, Kubo T, Matsuda S, Nakasaki Y, et al. LPIAT1 regulates arachidonic acid content in phosphatidylinositol and is required for cortical lamination in mice. Mol Biol Cell 2012;23:4689–4700.
[88] Matsuda S, Inoue T, Lee HC, Kono N, Tanaka F, Gengyo-Ando K, et al. Member of the membrane-bound O-acyltransferase (MBOAT) family encodes a lysophospholipid acyltransferase with broad substrate specificity. Genes Cells 2008;13:879–888.
[89] **Gijon MA**, **Riekhof WR**, Zarini S, Murphy RC, Voelker DR. Lysophospholipid acyltransferases and arachidonate recycling in human neutrophils. J Biol Chem 2008;283:30235–30245.
[90] Tanaka Y, Shimanaka Y, Caddeo A, Kubo T, Mao Y, Kubota T, et al. LPIAT1/MBOAT7 depletion increases triglyceride synthesis fueled by high phosphatidylinositol turnover. Gut 2021;70:180–193.
[91] Thangapandi VR, Knittelfelder O, Brosch M, Patsenker E, Vvedenskaya O, Buch S, et al. Loss of hepatic Mboat7 leads to liver fibrosis. Gut 2021;70:940–950.
[92] Helsley RN, Venkateshwari V, Brown AL, Gromovsky AD, Schugar RC, Ramachandiran I, et al. Obesity-linked suppression of membrane-bound O-Acyltransferase 7 (MBOAT7) drives non-alcoholic fatty liver disease. Elife 2019;8.
[93] Xia M, Chandrasekaran P, Rong S, Fu X, Mitsche MA. Hepatic deletion of Mboat7 (Lpiat1) causes activation of SREBP-1c and fatty liver. J Lipid Res 2020. https://doi.org/10.1194/jlr.RA120000856.
[94] Baker D, Pryce G, Davies WL, Hiley CR. In silico patent searching reveals a new cannabinoid receptor. Trends Pharmacol Sci 2006;27:1–4.
[95] Oka S, Nakajima K, Yamashita A, Kishimoto S, Sugiura T. Identification of GPR55 as a lysophosphatidylinositol receptor. Biochem Biophys Res Commun 2007;362:928–934.
[96] Fondevila MF, Fernandez U, Gonzalez-Rellan MJ, Da Silva Lima N, Buque X, Gonzalez-Rodriguez A, et al. The L-alpha-lysophosphatidylinositol/GPR55 system induces the development of non-alcoholic steatosis and steatohepatitis. Hepatology 2021;73:606–624.
[97] Tilg H, Moschen AR. Evolution of inflammation in nonalcoholic fatty liver disease: the multiple parallel hits hypothesis. Hepatology 2010;52:1836–1846.
[98] Stender S, Kozlitina J, Nordestgaard BG, Tybjaerg-Hansen A, Hobbs HH, Cohen JC. Adiposity amplifies the genetic risk of fatty liver disease conferred by multiple loci. Nat Genet 2017;49:842–847.
[99] Vilar-Gomez E, Martinez-Perez Y, Calzadilla-Bertot L, Torres-Gonzalez A, Gra-Oramas B, Gonzalez-Fabian L, et al. Weight loss through lifestyle modification significantly reduces features of nonalcoholic steatohepatitis. Gastroenterology 2015;149:367–378. e365; quiz e314-365.
[100] Armstrong MJ, Gaunt P, Aithal GP, Barton D, Hull D, Parker R, et al. Liraglutide safety and efficacy in patients with non-alcoholic steatohepatitis (LEAN): a multicentre, double-blind, randomised, placebo-controlled phase 2 study. Lancet 2016;387:679–690.
[101] Marra F, Bertolani C. Adipokines in liver diseases. Hepatology 2009;50:957–969.
[102] Valenti L, Bugianesi E, Pajvani U, Targher G. Nonalcoholic fatty liver disease: cause or consequence of type 2 diabetes? Liver Int 2016;36:1563–1579.
[103] Catalan V, Gomez-Ambrosi J, Rodriguez A, Ramirez B, Valenti V, Moncada R, et al. Increased interleukin-32 levels in obesity promote adipose tissue inflammation and extracellular matrix remodeling: effect of weight loss. Diabetes 2016;65:3636–3648.

[104] Fadaei R, Bagheri N, Heidarian E, Nouri A, Hesari Z, Moradi N, et al. Serum levels of IL-32 in patients with type 2 diabetes mellitus and its relationship with TNF-alpha and IL-6. Cytokine 2020;125:154832.
[105] Moschen AR, Fritz T, Clouston AD, Rebhan I, Bauhofer O, Barrie HD, et al. Interleukin-32: a new proinflammatory cytokine involved in hepatitis C virus-related liver inflammation and fibrosis. Hepatology 2011;53:1819–1829.
[106] Kim DH, Park ES, Lee AR, Park S, Park YK, Ahn SH, et al. Intracellular interleukin-32gamma mediates antiviral activity of cytokines against hepatitis B virus. Nat Commun 2018;9:3284.
[107] Baselli GA, Dongiovanni P, Rametta R, Meroni M, Pelusi S, Maggioni M, et al. Liver transcriptomics highlights interleukin-32 as novel NAFLD-related cytokine and candidate biomarker. Gut 2020;69:1855–1866.
[108] Banini BA, Kumar DP, Cazanave S, Seneshaw M, Mirshahi F, Santhekadur PK, et al. Identification of a metabolic, transcriptomic and molecular signature of PNPLA3-mediated acceleration of steatohepatitis. Hepatology 2021;73:1290–1306.
[109] Dali-Youcef N, Vix M, Costantino F, El-Saghire H, Lhermitte B, Callari C, et al. Interleukin-32 contributes to human nonalcoholic fatty liver disease and insulin resistance. Hepatol Commun 2019;3:1205–1220.
[110] Xu Z, Dong A, Feng Z, Li J. Interleukin-32 promotes lipid accumulation through inhibition of cholesterol efflux. Exp Ther Med 2017;14:947–952.
[111] Shoda H, Fujio K, Yamaguchi Y, Okamoto A, Sawada T, Kochi Y, et al. Interactions between IL-32 and tumor necrosis factor alpha contribute to the exacerbation of immune-inflammatory diseases. Arthritis Res Ther 2006;8:R166.
[112] Gorzelak-Pabis P, Wozniak E, Wojdan K, Chalubinski M, Broncel M. Single triglyceride-rich meal destabilizes barrier functions and initiates inflammatory processes of endothelial cells. J Interferon Cytokine Res 2020;40:43–53.
[113] Kang JW, Park YS, Lee DH, Kim JH, Kim MS, Bak Y, et al. Intracellular interaction of interleukin (IL)-32alpha with protein kinase Cepsilon (PKCepsilon) and STAT3 protein augments IL-6 production in THP-1 promonocytic cells. J Biol Chem 2012;287:35556–35564.
[114] Shi H, Tzameli I, Bjorbaek C, Flier JS. Suppressor of cytokine signaling 3 is a physiological regulator of adipocyte insulin signaling. J Biol Chem 2004;279:34733–34740.
[115] Valenti L. Uncovering the genetics of cirrhosis: new plots for the usual suspects. Liver Int 2020;40:281–282.
[116] Nakayama M, Niki Y, Kawasaki T, Takeda Y, Ikegami H, Toyama Y, et al. IL-32-PAR2 axis is an innate immunity sensor providing alternative signaling for LPS-TRIF axis. Sci Rep 2013;3:2960.
[117] Loomba R, Seguritan V, Li W, Long T, Klitgord N, Bhatt A, et al. Gut microbiome-based metagenomic signature for non-invasive detection of advanced fibrosis in human nonalcoholic fatty liver disease. Cell Metab 2017;25:1054–1062. e1055.
[118] Addolorato G, Ponziani FR, Dionisi T, Mosoni C, Vassallo GA, Sestito L, et al. Gut microbiota compositional and functional fingerprint in patients with alcohol use disorder and alcohol-associated liver disease. Liver Int 2020;40:878–888.
[119] Lang S, Demir M, Duan Y, Martin A, Schnabl B. Cytolysin-positive Enterococcus faecalis is not increased in patients with non-alcoholic steatohepatitis. Liver Int 2020;40:860–865.
[120] Lang S, Demir M, Schnabl B. Targeting pathobionts for the treatment of alcohol-associated liver disease. Liver Int 2021;41:239–240.
[121] Marra F, Svegliati-Baroni G. Lipotoxicity and the gut-liver axis in NASH pathogenesis. J Hepatol 2018;68:280–295.
[122] Miele L, Marrone G, Lauritano C, Cefalo C, Gasbarrini A, Day C, et al. Gut-liver axis and microbiota in NAFLD: insight pathophysiology for novel therapeutic target. Curr Pharm Des 2013;19:5314–5324.
[123] Puri P, Daita K, Joyce A, Mirshahi F, Santhekadur PK, Cazanave S, et al. The presence and severity of nonalcoholic steatohepatitis is associated with specific changes in circulating bile acids. Hepatology 2018;67:534–548.
[124] Mouries J, Brescia P, Silvestri A, Spadoni I, Sorribas M, Wiest R, et al. Microbiota-driven gut vascular barrier disruption is a prerequisite for non-alcoholic steatohepatitis development. J Hepatol 2019;71:1216–1228.
[125] Gadaleta RM, Moschetta A. Metabolic Messengers: fibroblast growth factor 15/19. Nat Metab 2019;1:588–594.
[126] Grimaudo S, Dongiovanni P, Pipitone R, Baselli G, Camma C, Cabibi D, et al. FXR rs35724 G>C variant modulates cholesterol levels, carotid atherosclerosis and liver damage in non-alcoholic fatty liver. Dig Liver Dis 2019;51:e26.
[127] Jia W, Xie G. Probiotics, bile acids and gastrointestinal carcinogenesis. Nat Rev Gastroenterol Hepatol 2018;15:205.
[128] **Younossi ZM**, **Ratziu V**, Loomba R, Rinella M, Anstee QM, Goodman Z, et al. Obeticholic acid for the treatment of non-alcoholic steatohepatitis: interim analysis from a multicentre, randomised, placebo-controlled phase 3 trial. Lancet 2019;394:2184–2196.
[129] **Dongiovanni P**, **Crudele A**, Panera N, Romito I, Meroni M, De Stefanis C, et al. beta-Klotho gene variation is associated with liver damage in children with NAFLD. J Hepatol 2020;72:411–419.
[130] Harrison SA, Rinella ME, Abdelmalek MF, Trotter JF, Paredes AH, Arnold HL, et al. NGM282 for treatment of non-alcoholic steatohepatitis: a multicentre, randomised, double-blind, placebo-controlled, phase 2 trial. Lancet 2018;391:1174–1185.
[131] Harrison SA, Neff G, Guy CD, Bashir MR, Paredes AH, Frias JP, et al. Efficacy and safety of aldafermin, an engineered FGF19 analog, in a randomized, double-blind, placebo-controlled trial of patients with nonalcoholic steatohepatitis. Gastroenterology 2021;160:219–231.
[132] Gadaleta RM, Garcia-Irigoyen O, Cariello M, Scialpi N, Peres C, Vetrano S, et al. Fibroblast Growth Factor 19 modulates intestinal microbiota and inflammation in presence of Farnesoid X Receptor. EBioMedicine 2020;54:102719.
[133] Loomba R, Ling L, Dinh DM, DePaoli AM, Lieu HD, Harrison SA, et al. The commensal microbe veillonella as a marker for response to an FGF19 analog in nonalcoholic steatohepatitis. Hepatology 2021;73:126–143.
[134] Bianco C, Romeo S, Petta S, Long MT, Valenti L. MAFLD vs NAFLD: let the contest begin! Liver Int 2020;40:2079–2081.
[135] **Grimaudo S**, **Bartesaghi S**, Rametta R, Marra F, Margherita Mancina R, Pihlajamaki J, et al. PCSK9 rs11591147 R46L loss-of-function variant protects against liver damage in individuals with NAFLD. Liver Int 2021;41:321–332.
[136] Fares R, Petta S, Lombardi R, Grimaudo S, Dongiovanni P, Pipitone R, et al. The UCP2-866 G > A promoter region polymorphism is associated with nonalcoholic steatohepatitis. Liver Int 2015;35:1574–1580.
[137] Al-Serri A, Anstee QM, Valenti L, Nobili V, Leathart JB, Dongiovanni P, et al. The SOD2 C47T polymorphism influences NAFLD fibrosis severity: evidence from case-control and intra-familial allele association studies. J Hepatol 2012;56:448–454.