Cell Metabolism
Perspective

Leveraging Human Genetics to Identify Potential New Treatments for Fatty Liver Disease

Stefano Romeo,[1,2,3,*] Arun Sanyal,[4,*] and Luca Valenti[5,*]
[1]Department of Molecular and Clinical Medicine, University of Gothenburg, Gothenburg, Sweden
[2]Clinical Nutrition Unit, Department of Medical and Surgical Sciences, Magna Graecia University, Catanzaro, Italy
[3]Cardiology Department, Sahlgrenska University Hospital, Gothenburg, Sweden
[4]Division of Gastroenterology and Hepatology, Department of Medicine, Virginia Commonwealth University, Richmond, VA, USA
[5]Department of Pathophysiology and Transplantation, Università degli Studi di Milano, Translational Medicine, Department of Transfusion Medicine and Hematology, Fondazione IRCCS Ca' Granda, Pad Marangoni, Milan, Italy
*Correspondence: stefano.romeo@wlab.gu.se (S.R.), arun.sanyal@vcuhealth.org (A.S.), luca.valenti@unimi.it (L.V.)
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Fatty liver disease (FLD), including its more severe pathologies, namely steatohepatitis, hepatocarcinoma, and cirrhosis, is the most common cause of chronic liver disease worldwide and is projected to become the leading cause of hepatocellular carcinoma and end-stage liver disease. FLD is heterogeneous with multiple etiologies and diverse histological phenotypes, so therapies will ultimately need to be individualized for relevant targets. Inherited factors contribute to FLD, and most of the genetic variation influencing liver disease development and progression is derived from genes involved in lipid biology, including *PNPLA3, TM6SF2*, *GCKR, MBOAT7*, and *HSD17B13*. From this point of view, we focus in this perspective on how human molecular genetics of FLD have highlighted defects in hepatic lipid handling as a major common mechanism of its pathology and how this insight could be leveraged to treat and prevent its more serious complications.

Introduction

Nonalcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease in most parts of the world (Younossi et al., 2016) and is projected to become the leading cause of hepatocellular carcinoma and end-stage liver disease, requiring consideration for liver transplantation (Charlton et al., 2011). The burden of end-stage liver disease due to NAFLD is further expected to increase 2- to 3-fold over the next two decades (Estes et al., 2018). These sobering statistics have fueled substantial efforts to develop pharmacological therapeutics to treat the disease. Implicit in these efforts is the concept that NAFLD, especially its aggressive phenotype, nonalcoholic steatohepatitis (NASH), is a relatively homogeneous, "one size fits all" condition. However, there are several lines of evidence indicating that the condition is heterogeneous and that therapies will ultimately need to be individualized for relevant targets within relevant populations. These ideas are considered in greater detail below.

NAFLD Has Multiple Etiologies

By definition, NAFLD does not result from the consumption of alcohol. Unfortunately, this characterization does not specify what causes hepatic fat accumulation and liver damage. It is now appreciated that a number of etiologies can directly cause or aggravate NAFLD. These factors range from hereditary conditions such as lipodystrophy, hypobetalipoproteinemia, and cholesterol ester storage disease to drugs such as amiodarone and tamoxifen, as well as small intestinal bacterial overgrowth and jejunoileal bypass. However, the most common risk factors remain excess adiposity and insulin resistance with its associated factors, such as type 2 diabetes, excessive intake of specific nutrients (e.g., food-industry-added fructose), and low physical activity (Chalasani et al., 2018). Even within such populations, there are subsets of individuals with specific genetic predispositions who are more likely to develop aggressive disease (Romeo et al., 2008; Sookoian et al., 2009). It is further recognized that many individuals consume alcohol in amounts greater than the strict cut-offs used in clinical trials to define the "nonalcoholic" nature of the condition, but not enough to attribute their liver disease solely to alcohol. It is therefore common to have multiple etiologies and risk factors for fatty liver disease (FLD) in the same individual.

In its simplest form, NAFLD presents as a fatty liver with predominantly macrovesicular steatosis. The other phenotype is steatohepatitis. In its classic form, steatohepatitis includes steatosis, hepatocellular ballooning, and lobular inflammation with a mainly centrilobular distribution (Kleiner et al., 2005). However, frequently, the individual features are not fully expressed or typically distributed, and this histological picture has been referred to as borderline NASH (Kleiner et al., 2005). Recent data indicate that (1) the natural course of borderline NASH is intermediate between that of NAFLD and definite NASH (Kleiner et al., 2016), and (2) disease activity may fluctuate in predisposed individuals based on the modification of environmental triggering factors (Pelusi et al., 2019b). In childhood, NASH often occurs in a predominantly periportal region introducing another layer of heterogeneity to the condition (Schwimmer et al., 2005). The molecular basis for this occurrence remains unknown. In each of the broad phenotypes, there is further heterogeneity in terms of the severity of disease activity and fibrosis. Portal inflammation, possibly due to fluctuations in disease activity in response to changing environmental triggers, has also been reported to be associated with disease progression and adds to the histological variability of the condition.

Several lines of evidence indicate that inherited factors contribute to NAFLD (Eslam et al., 2018). First, some evidence has been obtained from twin studies, leading to the estimation that 25%–50% of hepatic fat variability is accounted for by heritable factors, while the estimate is even higher for the presence of NAFLD (Loomba et al., 2015; Makkonen et al., 2009). Second, population studies have clearly shown a strong interethnic variability in the susceptibility to NAFLD, which is higher in Hispanics, intermediate in Europeans, and lower in African-Americans (Guerrero et al., 2009). Third, family studies have shown that the risk of NAFLD increases with the number of parents affected and that cases of FLD progressing to advanced fibrosis tend to cluster in families (Caussy et al., 2017; Long et al., 2019). The identification of the specific major genetic determinant of the disorder by the first genome-wide association study (the I148M variant in PNPLA3) led to the discovery that this inherited variant accounts for a large fraction of the interethnic variability in NAFLD susceptibility (Romeo et al., 2008), while other inherited variants have been robustly demonstrated to contribute to NAFLD (Eslam et al., 2018).

A caveat of these findings is that NAFLD susceptibility is driven by genes in the presence of risk factors associated with excess energy intake resulting in obesity and insulin resistance. In other words, there is an interaction between a specific genetic background (e.g., carriers of the *PNPLA3* genetic variant) and these environmental factors. This interaction has been shown for PNPLA3 as well as for the other major genetic risk variants (Pirazzi et al., 2012; Romeo et al., 2010; Stender et al., 2017). This finding indicates that the weight of genetic variants in disease susceptibility increases with the increase in the environmental risk factor.

A body of evidence supports a role for the gut microbiome in the susceptibility to NAFLD. A specific signature in the microbiome is present in individuals with NASH compared to the microbiomes of those with simple steatosis or healthy controls (Mouzaki et al., 2013; Yuan et al., 2019; Zhu et al., 2013). The diversity in the microbiome may increase fat deposition, induce changes in metabolism, and trigger inflammation contributing to chronic liver disease (Laparra and Sanz, 2010; Smith et al., 2013; Spencer et al., 2011) Large studies are needed to determine whether manipulation of the gut microbiome is a viable therapeutic strategy to treat NAFLD.

Proteomic and Metabolomic Signatures Defining NAFLD

It is interesting to note that despite similar drug intake and systemic exposure, drugs so far tested in clinical trials to treat NASH improve liver histology in only a minority of patients (Neuschwander-Tetri et al., 2015; Ratziu et al., 2016; Sanyal et al., 2010). This phenomenon raises the question of whether there is molecular heterogeneity underlying the disease state where specific targets are relevant in only some but not all individuals. Several lines of evidence support this possibility. It is already well established that subsets of the overall population of patients with NAFLD carry specific gene variants that enhance or reduce disease risk. The *PNPLA3* I148M variant was first identified to be related to the development of steatohepatitis and to be associated with more advanced disease and development of hepatocellular cancer (Dongiovanni et al., 2013; Pirazzi et al., 2012; Romeo et al., 2008). The *PNPLA3* I148M variant is also a response variable for treatment with ω-3 fish oils and statins, hampering their beneficial effect (Dongiovanni et al., 2015b; Scorletti et al., 2015). The pathogenesis of NAFLD and steatohepatitis in those with this mutation may be distinct from others with this condition, with the mechanism being more related to reduced lipid turnover and release from lipid droplets than enhanced *de novo* lipogenesis (Luukkonen et al., 2019; Mancina et al., 2015; Pirazzi et al., 2012; Wang et al., 2019), but additional studies are required to confirm that these features are independent of higher hepatic fat content.

Recently, in a diet-induced animal model of NAFLD that has been validated to reproduce many facets of human disease (Asgharpour et al., 2016; Cazanave et al., 2017; Sanyal and Pacana, 2015), it has been shown that the gene expression profile changes with disease progression, with greater expression of metabolic pathways related to lipid metabolism soon after the onset of adiposity and development of steatosis (Cazanave et al., 2017). These changes were further amplified as the disease progressed to steatohepatitis but then decreased as fibrosis developed. As the disease progressed, there was an increasing expression of inflammatory, fibrogenic, and oncogenic pathways. Importantly, these changes were related to cross-sectional human datasets including early- and late-stage diseases and were found to be concordant. There are also specific pathways linked to the severity of disease activity and stage. This heterogeneity in molecular profiles within otherwise histologically similar individuals is also reflected in the circulating metabolome, with markers of lipogenesis increasing early in the disease, and increased activation of inflammatory eicosanoids and oxidized lipids occurring with disease progression (Puri et al., 2009). Choline deficiency has also been noted in a minority of individuals (Guerrerio et al., 2012). Similarly, there are specific proteomic signatures associated with varying histological phenotypes of NAFLD (Rodríguez-Suárez et al., 2010).

Together, these data provide evidence for heterogeneity at many levels within the population of patients with NAFLD, which has specific implications for disease development, progression, and potential response to specific therapeutic agents. The data further provide a strong rationale for generating additional data to substratify the population based on relevant targets (e.g., presence of *PNPLA3* I148M concerning the inherited variants) and natural history to be able to match the right drug to the right patient.

Fatty Liver Due to Excess Nutrients or Alcohol: Same or Different Disease?

NAFLD was first described as a new clinical entity (“NASH”) in 1980 (Ludwig et al., 1980). In this case series from a Mayo clinic study, physicians reported a total of twenty individuals with histological features commonly associated with alcohol toxicity, including steatosis, oxidative damage of hepatocytes, and lobular inflammation with frequent evolution to fibrosis and cirrhosis in individuals not drinking alcohol. Patients were most frequently obese women with type 2 diabetes. The question as to whether this yet-to-be-understood disease was the same or different from alcoholic liver disease (ALD) suddenly arose.

It became subsequently clear that some of the pathophysiological mechanisms underlying inflammation (so-called “steatohepatitis”) are remarkably similar between NAFLD and ALD

(Valenti et al., 2009). These mechanisms include the activation of hepatocytes and liver macrophages (Kupffer cells) via pattern recognition receptors by bacterial products, stress molecules, and oxidized fatty acids, with the subsequent release of inflammatory mediators and inflammatory and fibrogenic cell recruitment (Tilg and Moschen, 2010; Valenti et al., 2009).

Strikingly, in recent years, genetic association studies have highlighted that the genetic determinants of the interindividual variability in the predisposition to NAFLD and ALD are largely shared. This concept was first demonstrated for the *PNPLA3* I148M rs738409 variant, the major genetic determinant of hepatic fat content (Romeo et al., 2008) that increases susceptibility to the whole spectrum of NAFLD, from steatosis to inflammation and NASH, finishing with fibrosis, cirrhosis, and hepatocellular carcinoma (HCC) (Dongiovanni et al., 2013; Pirazzi et al., 2012; Sookoian and Pirola, 2011; Valenti et al., 2010). The I148M variant is also associated with an increased risk of steatosis and fibrosis in other chronic and acute liver diseases (Buch et al., 2015; Stättermayer et al., 2015; Valenti et al., 2012; Valenti et al., 2011; Viganò et al., 2013), with a larger effect observed in ALD (Dongiovanni et al., 2013) but also an evident effect in chronic hepatitis C (CHC) (Valenti et al., 2011). Therefore, this genetic variant may be considered a general modulator of liver disease progression. Notably, carriage of the I148M variant was the strongest genetic determinant of severe steatohepatitis due to alcohol intoxication (Atkinson et al., 2017).

Along this line, the *TM6SF2* E167K rs58542926 variant predisposes patients to hepatic fat accumulation (Kozlitina et al., 2014) by altering very-low-density lipoprotein export, resulting in an increased risk of progressive NAFLD/ALD and CHC (Buch et al., 2015; Dongiovanni et al., 2015a; Liu et al., 2017b). Remarkably, the first genome-wide association study examining the risk of alcoholic cirrhosis identified the variants in *PNPLA3* and *TM6SF2* and at the *MBOAT7* locus (rs641738) as the major common determinants. We showed that the rs641738 variant is similarly associated with hepatic fat content and fibrosis (Mancina et al., 2016). Furthermore, the *MBOAT7* variation also confers an increased risk of progression of NAFLD/ALD to HCC (Donati et al., 2017; Liu et al., 2014; Trépo et al., 2014). Genetic variations in *HSD17B13* have been linked to protection against the development of cirrhosis due to ALD and NAFLD and against fibrosis in individuals with CHC (About et al., 2018; Abul-Husn et al., 2018; Ma et al., 2019). Finally, different predominant triggers, including CHC and other metabolic or toxic causes of liver damage (Krawczyk et al., 2013; Stättermayer et al., 2015; Valenti et al., 2012), result in subtle differences in the histological features and small changes in the natural history.

In conclusion, the overlap in the genetic architecture suggests that ALD and NAFLD share common pathogenetic mechanisms, which indicates that NAFLD and ALD are spectra of the same condition, namely, FLD. This concept would not be surprising considering that there is no difference in the pathophysiology of progressive liver disease associated with hepatic fat accumulation. A practical implication of these observations is that therapies aimed at FLD resolution targeting a specific pathway leading to liver damage may have a preventive effect against FLD progression irrespective of whether the disease is metabolic in nature or related to excessive alcohol intake.

Same Fatty Liver with Diverse Clinical Presentations

The most common phenotype associated with NAFLD is excess body weight (Chalasani et al., 2018). However, NAFLD has been reported in individuals with normal BMI in both Western and Asian countries (Das et al., 2010). Individuals with normal BMI and NAFLD usually have central fat accumulation and sarcopenia. The severity of insulin resistance also varies across these populations, although in general, lean individuals with NAFLD are less insulin resistant and have milder histologic severity of disease (Fracanzani et al., 2011), although this has not been universally reported (Denkmayr et al., 2018). The distribution of potential etiologies of NAFLD has not been systematically studied across these populations beyond the description of varying prevalence of the *PNPLA3* I148M variant (Fracanzani et al., 2017). Based on epidemiological considerations, it is expected that conditions such as celiac disease and hypoalphalipoproteinemia/hypobetalipoproteinemia are more likely to be present in lean individuals with NAFLD in Western populations (Pelusi et al., 2019a). In Asian countries, an increase in caloric intake often occurs in adulthood and is associated with hypertrophy of adipocytes, whereas in other countries with a high prevalence of childhood obesity, there is both hyperplasia and hypertrophy of adipocytes, which can affect biology and the ability to contribute to the systemic inflammatory state associated with NAFLD (Skurk et al., 2007). It is noteworthy that even in apparently lean individuals, increasing adiposity, especially in the visceral compartments, and insulin resistance are key risk factors for the development of NAFLD (Wong et al., 2015).

Variants Regulating Lipid Biology Increase the Risk of FLD

A common mechanism that could explain the damage induced by alcohol and excess nutrients is related to the accumulation of enlarged lipid droplets. Indeed, both ethanol and metabolic substrates are converted into lipids once in the hepatocyte and are then stored within these intracellular organelles (Stickel and Hampe, 2012). The striking observation when examining human molecular genetics of NAFLD is that all the main genetic variations contributing to NAFLD susceptibility impact the activity of proteins involved in lipid droplet biology. The PNPLA3 protein is an enzyme located on the surface of lipid droplets of hepatocytes and hepatic stellate cells. PNPLA3 displays hydrolase activity toward triglycerides and retinyl esters and has potential transacetylase activity to incorporate polyunsaturated fatty acids into phospholipids (Mitsche et al., 2018). The I148M mutation induces a loss of function in lipase activity and accumulates at the surface of lipid droplets where CGI-58, an essential cofactor for the adipose triglyceride lipase (ATGL), is sequestered (Wang et al., 2019). The tridimensional structure of protein domains and whether the mutant induces a change in the protein topology are not yet known. Irrespective of the mechanism, genetic variation reducing PNPLA3 expression seems to have a beneficial effect against NAFLD in carriers of the I148M mutant (Donati et al., 2016), and pharmacological downregulation of the protein induces amelioration of hepatic fat accumulation in both wild-type and mutant mice and inflammation and fibrosis in mice carrying the mutant protein fed with a NAFLD-inducing diet (BasuRay et al., 2019; Kumashiro et al., 2013; Lindén et al., 2019).

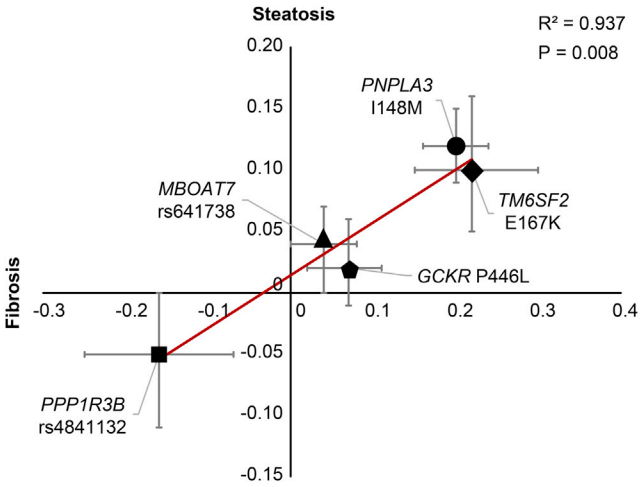


Figure 1. Hepatic Fat Accumulation Is Causally Linked with Fibrosis
Comparison of the impact of naturally occurring risk variants in *PNPLA3* I148M (rs738409), *TM6SF2* E167K (rs58542926), *GCKR* P446L (rs1260326), *MBOAT7* rs641738, and *PPP1R3B* (rs4841132) on hepatic histological steatosis versus fibrosis in a cross-sectional liver biopsy cohort (n = 1388) (Dongiovanni et al., 2018a; Dongiovanni et al., 2018b). Normalized correlation coefficients were estimated by generalized linear regression models adjusted for age, sex, body mass index, and presence of type 2 diabetes. Modified from Valenti and Baselli, (2019).

TM6SF2 is another membrane protein located in the Golgi whose loss of function is responsible for lipid retention in the hepatocyte via reduction in lipidation and number of lipoprotein particles (Kim et al., 2017; Prill et al., 2019; Smagris et al., 2016). While the E167K mutation increases the risk of NAFLD by determining the compartmentalization of lipids within intracellular droplets, it reduces the risk of cardiovascular disease by reducing circulating lipoproteins (Dongiovanni et al., 2015a; Kozlitina et al., 2014). MBOAT7 is a six-transmembrane protein anchored to lipid endomembranes, specifically lipid droplets, mitochondrial-associated membranes, and the endoplasmic reticulum (Caddeo et al., 2019; Mancina et al., 2016). Interestingly, the enzymatic activity of this protein, although it has yet to be studied in detail, seems to be related to the remodeling of phospholipids with polyunsaturated fatty acids in the Lands cycle.

GCKR rs1260326 encodes a loss-of-function variant (P446L) that increases the activity of intracellular glucokinase and results in a net increase in intracellular glucose phosphate, leading to *de novo* lipogenesis via the induction of glycolysis and the stimulation of carbohydrate-responsive element-binding protein (ChREBP) (Raimondo et al., 2015). This process has a net effect on carriers of the variant reflected by higher liver fat and circulating triglycerides but lower insulin resistance and reduced predisposition to diabetes. Similarly, the rs4841132 variation in *PPP1R3B* shifts energy storage from glycogen to lipid accumulation in the liver, resulting in an increased risk of NAFLD in at-risk individuals (Dongiovanni et al., 2018a).

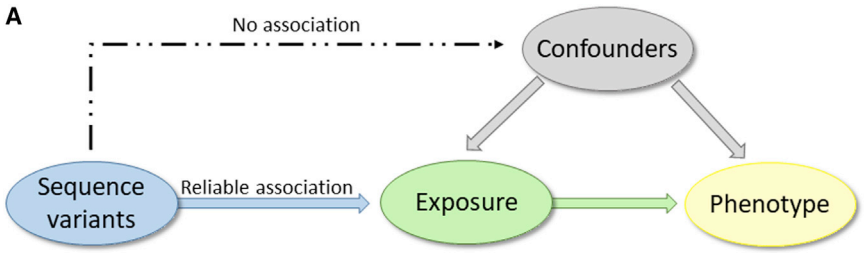
Inactivating variants in the *HSD17B13* gene, which encodes the hepatic lipid droplet protein hydroxysteroid 17-β dehydrogenase 13, have recently been linked with a reduced risk of alcoholic and non-alcoholic chronic liver disease without affecting liver fat content (Abul-Husn et al., 2018; Grimaudo et al., 2019b; Kozlitina et al., 2018; Ma et al., 2019). Among these, the rs72613567 T to TA insertion variant adjacent to the donor splice site downstream of exon 6 of *HSD17B13* may affect mRNA splicing and lead to the production of a truncated protein (Abul-Husn et al., 2018). Interestingly, the rs72613567 interacted with *PNPLA3* I148M, such that additional *HSD17B13* TA alleles reduced the risk of liver disease conferred by *PNPLA3* I148M (Abul-Husn et al., 2018). The enzymatic activity of HSD17B13 is still subject to debate. Abul-Husn et al. show that the protein has enzymatic activity against several bioactive lipid species (e.g., leukotriene B) that potentially cause the inflammation (Abul-Husn et al., 2018).

In Figure 1, we showed the correlation, which was derived from the normalized estimates of the impact of genetic variation in the same cohort (Dongiovanni et al., 2018a; Dongiovanni et al., 2018b; Valenti and Baselli, 2019) between the effect size of these variants on liver fat and fibrosis. Remarkably, there was an almost complete and robust linear relationship between the impact of genetic variants on fat and fibrosis, the major predictors of liver-related events in patients with NAFLD. This evidence points to a deleterious effect of liver fat accumulation. Indeed, a formal Mendelian randomization (MR) analysis (Figure 2) indicated that the epidemiological association between hepatic fat and fibrosis was fully consistent with a causal role of hepatic fat on liver disease independent of the severity of dysmetabolism (Dongiovanni et al., 2018b). In other words, MR suggests that liver steatosis should not be considered merely "benign," but rather as a form of FLD that has yet to develop into steatohepatitis (Pelusi et al., 2019b; Pelusi and Valenti, 2019).

One should bear in mind that the quality of lipid species may also contribute to the onset of steatohepatitis. Another caveat is that the genetic predisposition to obesity and diabetes is well established (Ingelsson and McCarthy, 2018; McCarthy, 2017; Goodarzi, 2018) and therefore, in studies investigating the genetic susceptibility of FLD, it would be important to account for the obesity and diabetes genetic predisposition irrespective of the adjustment in the analyses for metabolic diseases.

Human Genetics to Identify Drug Targets: Lessons from Cardiovascular Disease

Studies conducted in the field of cardiovascular disease may help in understanding the significance of the MR analysis cited above and the clinical implications for patients with NAFLD. Indeed, epidemiological studies describe the association of various traits with diseases and clinical outcomes. These studies are the foundation of descriptive medicine, but even in well-conducted prospective studies, causality between the variables under study cannot be inferred due to the roles of unmeasured confounders. The best way to assess causality in human studies is performing interventions that specifically modify an exposure potentially causing the clinical outcome with randomization of participants. However, a valid alternative is using MR. Indeed, the combination of alleles in a single individual is a result of a randomization performed by nature during meiotic recombination. Therefore, a genetic variation with a primary effect on a trait can be used if a series of assumptions are fulfilled, as recently reviewed (Holmes et al., 2017; Neeland and Kozlitina, 2017), to establish whether changes in this biological trait cause modifications in the outcome.



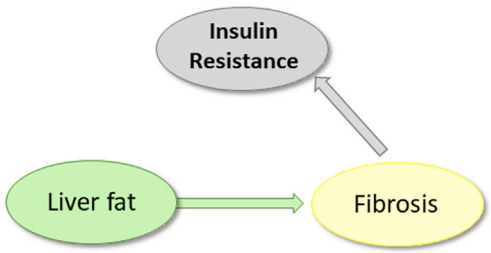


Figure 2. Mendelian Randomization as a Tool to Understand Causality

(A) Mendelian randomization consists of an instrumental variable analysis meeting the following assumptions: (1) single or multiple variants (instrumental variable) in blue must be associated with the exposure in green; (2) must not associate with confounders in gray; and (3) must not affect the phenotype in yellow except through the risk factor. (B) A Mendelian randomization study on fatty liver disease by Dongiovanni et al. (2018b) showed that excess in liver fat causes fibrosis, which in turn causes insulin resistance.

Examples from the field of atherosclerosis can elucidate how this approach has been successfully used to understand disease mechanisms and identify drug targets. High circulating low-density lipoprotein (LDL-C) levels are associated with accelerated atherosclerosis, while high levels of high-density lipoprotein (HDL-C) have been shown to be protective against this disease in epidemiological studies. Early epidemiological studies coupled with studies in mice have prompted the idea that while LDL-C accumulates in the atherosclerotic plaque, HDL-C is responsible for the opposite event, namely, clearing the lipids from the plaque. In the last 35 years, several intervention studies have been conducted to lower LDL-C or increase HDL-C to prevent cardiovascular disease. Interestingly, all the studies reducing LDL-C, with different drugs acting on the same (rosuvastatin, atorvastatin, and simvastatin) or different mechanisms (ezetimibe or PCSK9 inhibitors), have succeeded in reducing atherosclerosis, while all the drugs raising HDL-C (e.g., torcetrapib) have had a neutral or detrimental effect (Neeland and Kozlitina, 2017). Importantly, the reduction in atherosclerosis was proportional to the size of the reduction in LDL-C in all trials. All of these results showed that LDL-C plays a causal role, while HDL-C is only a marker of a disease process. Learning from this lesson, the novel clinical trials of drugs for reducing atherosclerosis are coming from MR studies and providing successful results (for example, inhibitors of Lp(a), ANGPTL3, and APOC3) (Gaudet et al., 2017; Graham et al., 2017; Tsimikas et al., 2015).

A recent work by Sun et al. (2019) shows elegantly how oxidized phospholipids and key inflammatory and atherogenic factors arising from oxidative stress accumulate in human and mouse NASH. Oxidized phospholipids promote reactive oxygen species, resulting in hepatocyte mitochondrial dysfunction, and their neutralization improved the entire spectrum of FLD. This work further strengthens the parallel between atherosclerosis and FLD.

Using the Lessons from Human Genetics to Discover Drugs for FLD

Coming back to NAFLD, an MR study using, as instrumental variables, four variants in *PNPLA3*, *TM6SF2*, *MBOAT7*, and *GCKR*, demonstrated a causal role of liver fat in inducing liver inflammation, ballooning, and fibrosis (Dongiovanni et al., 2018b). Interestingly, this study suggests that insulin resistance that is often associated with NAFLD is due to the degree of fibrosis and not the presence of fat in the liver. This finding suggests that the most obvious target to counteract liver disease progression should be represented by reduction in the hepatic fat within intracellular lipid droplets, which is the common feature linking genetic risk variants with disease predisposition (Figure 3). Therefore, hepatic fat (including the accumulation of triglycerides, cholesterol, and possibly specific lipotoxic species) is analogous to LDL-C accumulation in the plaque as a driver and therapeutic target for atherosclerosis (Figure 4).

It would be predicted that drugs targeting liver fat content (and the accumulation of lipotoxic species) will most likely be successful in impeding FLD progression. This may be achieved by different approaches: (1) by reducing the delivery of lipotoxic loads and excess carbohydrates to the liver (e.g., by administering drugs targeting GLP-1, which directly modulates food intake; SGLT2, which induces a negative caloric balance; and PPAR-γ, which modulates adipose tissue insulin sensitivity and adiponectin release); (2) by promoting the oxidation of lipids within hepatocytes (e.g., via ω-3 fatty acids, PPARα and δ agonists, metabolism, and TRH receptor β agonists); (3) by inhibiting lipogenesis (e.g., via ACC and SCD1 inhibitors); and (4) by exerting a more complex modulation of lipid metabolism with diversion of lipids from the liver, partly via the modulation of the farnesoid X receptor (FXR) and fibroblast growth factor-19 (FGF19) axis. Although these pathways also have several pleiotropic effects, they showed promising results in terms of the reduction in fibrosis or biomarkers, as recently reviewed (Pelusi and Valenti, 2019).

The path of reducing *de novo* lipogenesis is not free from negative effects. The *GCKR* P446L variant leads to a reduction in hepatic glucose metabolism, most likely through ChREBP, resulting in lower concentrations of malonyl-CoA, a substrate for *de novo* lipogenesis (van de Bunt and Gloyn, 2010). Although carriers of this variant have less liver disease than noncarriers,

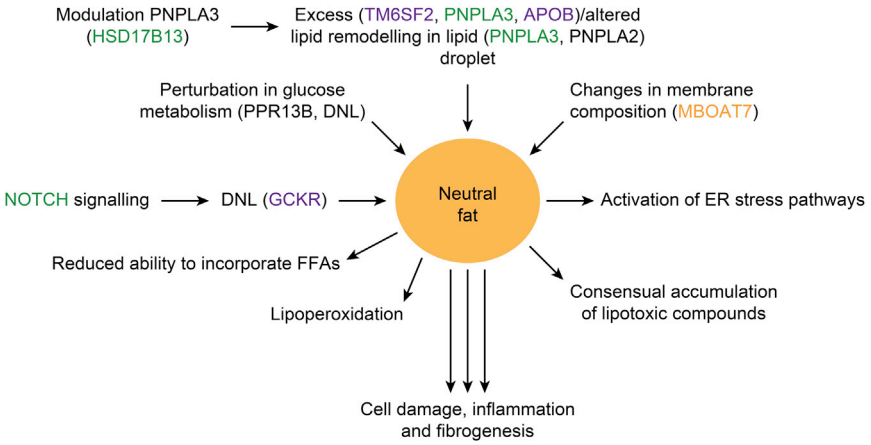


Figure 3. Mechanism by Which Genetic Risk Variants Involved in the Susceptibility to Hepatic Fat Accumulation Lead to Liver Damage and Therapeutic Opportunities
Green indicates a druggable target. Purple indicates druggable targets with possible side effects as suggested by human genetics. Yellow indicates undruggable targets.

they have a higher risk of diabetes. Overall, one could hypothesize that by reducing *de novo* lipogenesis, liver disease would also be reduced, but insulin resistance and hyperglycemia may be worsened. If this will be the case, the question is whether the effect on liver disease reduction will outweigh the effect on increasing cardiovascular disease risk.

Because liver fat content can be reduced by increasing triglyceride secretion from the liver, an obvious question is whether this could be used as a therapeutic strategy. The *TM6SF2* E167K variant results in an increase in liver fat content by reducing liver fat secretion through quantitative (reduced lipidation) and qualitative (lower number of particles) changes in lipoprotein particle secretion (Kim et al., 2017; Prill et al., 2019; Smagris et al., 2016). The upregulation of this gene could technically free the liver from fat, but the prediction will be, given that carriers of the genetic variant are protected from cardiovascular events, that by increasing TM6SF2 function we will cause not only a reduction in liver fat but also an increased risk of cardiovascular disease mediated by the increase in circulating lipoproteins. Overall, the data suggest that the modulation of secreted lipoprotein particles will change the risk of both liver disease and cardiovascular disease, unfortunately in the opposite direction.

Beyond fat accumulation, human genetics has revealed another, until recently, unsuspected major player in liver damage progression in FLD; that is, the derangement of hepatic retinol metabolism. Retinol is released from hepatic stellate cells after liver damage (Friedman et al., 1985; Friedman, 2008), transdifferentiating in the fibrogenic phenotype. Retinol is converted into its active form, retinoic acid, a potent nuclear transcription factor that increases the secretion of metalloproteases and metalloproteases involved in fibrosis remodeling and repair (Pingitore et al., 2016). Therefore, it may be speculated that the *PNPLA3* I148M variant has direct fibrogenic activity in hepatic stellate cells depending on the reduction in retinol availability (Pingitore et al., 2016; Pingitore and Romeo, 2019; Pirazzi et al., 2014). This reduction may lead to a failure to repair collagen deposition resulting in fibrosis. Modulation of retinol metabolism (PNPLA3) represents another possible therapeutic approach, but a better understanding of the mechanism by which retinoids regulate hepatic inflammation and fibrosis is required.

Regarding the concept of translating the MR approach into drug discovery for the validation of potential targets identified in pathophysiological studies, as is currently the practice for cardiovascular disease, we already have some evidence that this concept will also work for FLD. Indeed, initial data suggest that the rs35724 variant in the *N1RH4* gene (encoding FXR), linked with increased gene expression, was associated with protection against fibrosis and fat but not inflammation, but this variant was associated with increases in LDL-C levels (Grimaudo et al., 2019a). This pattern recapitulates the results of randomized controlled trials evaluating obeticholic acid, a potent FXR agonist, for the treatment of severe NASH (Neuschwander-Tetri et al., 2015). Although this approach needs further validation, it suggests that the genetic variation affecting the expression and activity of candidate pharmacological targets may be used as a proxy to forecast the likely impact of pharmacological manipulation in clinical trials.

A Precision Medicine Approach to NAFLD Based on Human Genetics

In medicine, there is yet no precision treatment for metabolic diseases tackling a specific inborn genetic variant. The human genetics of FLD offers the opportunity to implement precision medicine with the *PNPLA3* I148M variant. As described before, this variant is the strongest genetic determinant increasing susceptibility to FLD. A recent study in mice with *PNPLA3* variant knock in attempted to prevent FLD progression in mice by downregulating the PNPLA3 mutant protein by means of antisense oligonucleotides (Lindén et al., 2019). Although the mechanisms are not entirely clear, there is a general consensus that the mutant protein has a deleterious effect by inhibiting lipid droplet remodeling in hepatocytes and hepatic stellate cells (Pingitore and Romeo, 2019). Downregulation of the mutant protein resulted in a reduction in fatty liver in these mice and, importantly, a specific effect in ameliorating inflammation and fibrosis specifically in mice homozygous for the Pnpla3 I148M mutant protein (Lindén et al., 2019). If these results are translated into humans, downregulation of the mutant protein may be the first example of human genetics-based precision medicine. The novelty will be that with this drug, we will treat the cause of the disease and not merely the phenotype.

Using Human Genetics in Clinical Trials

The accumulating evidence on the role of genetics in FLD can already be useful for the design and management of clinical trials in the field. For example, as screening failure represents one of the major limitations in NASH trials, scores encompassing the determination of the *PNPLA3* variant may help to noninvasively

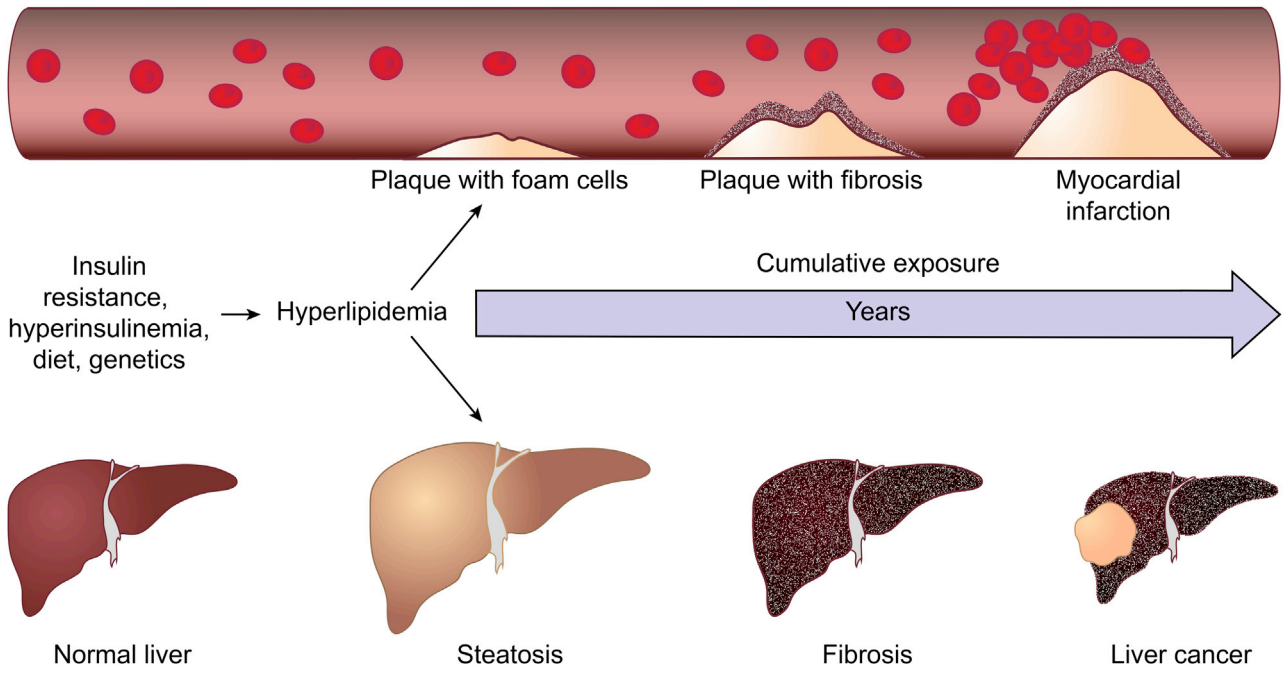


Figure 4. A Parallel between the Development and Progression of Atherosclerosis and NAFLD
In both diseases, there is an initial trigger due to the accumulation of lipids, mainly cholesterol during atherosclerosis and triglycerides, cholesterol, and lipids in the subintima layer of the arterial walls and the liver, respectively. This process causes fatty streaks with intima-media thickening and simple steatosis, respectively. The subsequent lipid oxidation and accumulation of lipotoxic compounds causes inflammation and the activation of fibrogenesis, leading to the development of plaques and fibrosing NASH, respectively. When buffering mechanisms are exhausted, especially in the presence of facilitating factors, these processes lead to plaque rupture and thrombosis or to liver failure and the development of liver cancer.

predict those patients with a higher likelihood of being affected by NASH with severe histological activity (Hyysalo et al., 2014). Incorporating a larger set of genetic risk variants (e.g., using a weighted genetic risk score with variants in *PNPLA3*, *TM6SF2*, *GCKR*, and *MBOAT7*) would lead to further improvement of the diagnostic accuracy (Dongiovanni et al., 2018b). Furthermore, carriage of the *PNPLA3* variant can help identify patients at risk of progressive liver disease in the absence of histological NASH at a definite time point, which may be worth targeting in future studies (Pelusi et al., 2019b). As the shift of late phase studies is moving from histological changes to hard clinical events, the inclusion of a proportion of patients at high genetic risk may allow for an increase in the power of the studies. Indeed, the genetic risk profile correlates with the risk of developing hepatocellular carcinoma (Donati et al., 2017; Pelusi et al., 2019a) and liver-related events (Grimaudo et al., 2019b). Furthermore, stratification of genetic risk would certainly help to balance arms of clinical intervention studies and help achieve success, highlighting at the same time potential sources of variability related to disease heterogeneity.

Indeed, several studies have shown genetic background results in specific changes in liver physiology. For example, *PNPLA3* I148M carriers have lower *de novo* lipogenesis and a higher content of polyunsaturated fatty acids than noncarriers (Mancina et al., 2015). *GCKR* and *TM6SF2* carriers have higher *de novo* lipogenesis (Prill et al., 2019). Based on these data, one may speculate that carriers of *GCKR* and *TM6SF2* would respond better to inhibitors of DNL, while carriers of *PNPLA3* variant would not. Moreover, *PNPLA3* variant carriers also respond differently to the administration of specific drugs. As an example, they do not experience the beneficial effect of statins (Dongiovanni et al., 2015b), have higher transaminase levels after administration of hormones (Guzman et al., 2018; Pillai et al., 2018), and have higher prevalence of liver-related side effects of chemotherapy (Dold et al., 2017; Liu et al., 2017a). These findings suggest that it would be a clever step to genotype patients for these common variants in clinical trials and perform subanalyses to test whether specific genetically defined subgroups have a higher response rate and fewer side effects to drug administration. This process may lead to the demonstration of efficacy and approval of treatment, which may have no or only modest benefits in the overall population.

Future Perspectives

As discussed, FLD is a complex pathology with multiple facets and histological subtypes. While human genetics clearly show that excess liver fat content and lipotoxicity cause liver fibrosis, a question remains for the other features of FLD; namely, inflammation, ballooning, and direct activation of hepatic stellate cells and the role played by retinoids in the progression of liver disease remain to be elucidated. To understand the implication of having higher liver inflammation or ballooning, per se, the identification and characterization of genetic variants specifically altering the susceptibility to hepatocellular damage and inflammation are needed.

Notwithstanding, current genetic knowledge already allows for the identification of a subset of patients with an increased risk of liver-related complications of FLD, which is associated

with a distinct pathophysiological profile and altered response to some therapeutic approaches. Genetic scores may therefore be able to help stratify the risk of progressive liver disease and hepatocellular carcinoma independently of fibrosis staging to allow for the indication of specific treatments and surveillance for liver-related complications. Moreover, modulation of the expression of the main protein associated with FLD susceptibility, namely PNPLA3, holds the promise to become the first precision-based approached for the treatment of a chronic disorder.

Currently, the Food and Drug Administration requires that clinical trials for the treatment of FLD show efficacy in the reduction of hepatic fibrosis and inflammation (i.e., NASH) in order to approve any new drug therapies in this space. This requirement effectively focuses on secondary prevention in FLD. Drugs targeting defects in lipid biology are likely to reach these clinical endpoints, because abolishing the cause of inflammation and fibrosis will allow the liver to take full advantage of its high regenerative potential as shown for drugs targeting hepatitis C virus (Belli et al., 2016). But the current enrollment requirements in such clinical trials leads to an important question: should simple steatosis be treated as a primary prevention to halt FLD progression to NASH? Results from the MR studies suggest that, yes, simple steatosis should be treated (Dongiovanni et al., 2018b) in the same way that treating elevated LDL-C is recommended to prevent atherosclerosis. If these results are confirmed by longitudinal studies, the next step will be to define the threshold of liver fat required to start treatment as a primary prevention strategy.

DECLARATION OF INTERESTS

S.R. served as a consultant for GSK, Pfizer, AMGEN, SANOFI, AKCEA therapeutics, Novonordisk, IONIS, CELGENE, CAMP4, AstraZeneca, MedaCorp, and ForsiteLabs, and received research grants from AMGEN, SANOFI and AstraZeneca.

L.V. has been consulting for Gilead, Pfizer, Astra Zeneca, Novo Nordisk, Diatech Pharmacogenetics, and Intercept, and received research grants from Gilead. He has served as a speaker for MSD, Gilead, AlfaSigma, and AbbVie.

A.S. is president of Sanyal Biotechnology and has stock options in Genfit, Akarna, Tiziana, Indalo, Durect, and Galmed. He has served as a consultant to Astra Zeneca, Nitto Denko, Enyo, Ardelyx, Conatus, Nimbus, Amarin, Salix, Tobira, Takeda, Jannsen, Gilead, Terns, Birdrock, Merck, Valeant, Boehringer-Ingelheim, Lilly, Hemoshear, Zafgen, Novartis, Novo Nordisk, Pfizer, Exhalenz, and Genfit. He has been an unpaid consultant to Intercept, Echosens, Immuron, Galectin, Fractyl, Syntlogic, Affimune, Chemomab, Zydus, Nordic Bioscience, Albireo, Prosciento, Surrozen, and Bristol Myers Squibb. His institution has received grant support from Gilead, Salix, Tobira, Bristol Myers, Shire, Intercept, Merck, Astra Zeneca, Malinckrodt, Cumberland, and Novartis. He receives royalties from Elsevier and UptoDate.

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