Insights into Nonalcoholic Fatty-Liver Disease Heterogeneity

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Abstract

Keywords
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The acronym nonalcoholic fatty-liver disease (NAFLD) groups a heterogeneous patient population. Although in many patients the primary driver is metabolic dysfunction, a complex and dynamic interaction of different factors (i.e., sex, presence of one or more genetic variants, coexistence of different comorbidities, diverse microbiota composition, and various degrees of alcohol consumption among others) takes place to determine disease subphenotypes with distinct natural history and prognosis and, eventually, different response to therapy. This review aims to address this topic through the analysis of existing data on the differential contribution of known factors to the pathogenesis and clinical expression of NAFLD, thus determining the different clinical subphenotypes observed in practice. To improve our understanding of NAFLD heterogeneity and the dominant drivers of disease in patient subgroups would predictably impact on the development of more precision-targeted therapies for NAFLD.

Nonalcoholic fatty-liver disease (NAFLD) is currently considered the commonest liver disease worldwide with an estimated global prevalence of 25%.[1,2] The acronym refers to a range of hepatic histological alterations including isolated steatosis (also referred as NAFL), non-alcoholic streatohepatitis (NASH which is hallmarked by the presence of hepatocellular injury accompanied by inflammation and variable degrees of fibrosis), and cirrhosis.[3] Importantly, NAFLD is a risk factor for hepatocellular carcinoma (HCC) development even in the absence of cirrhosis.[4] NAFLD relevance is underscored by data pointing to NAFLD as the most fast-growing cause of cirrhosis

and the need liver transplantation in the United States.[5] Moreover, epidemiological figures are worrisome as, according to epidemiological modeling, NAFLD burden is expected to grow in the coming decades burdening health care systems and causing substantial economic costs and compromising individual health, determining significant morbidity and mortality.[6]

To date, dietary caloric restriction and exercise are the cornerstone of NAFLD therapy, and although these measures have been proven to be efficacious, therapeutic goals are difficult to achieve or sustain.[7–9] Unfortunately, no Food and Drug Administration (FDA)-approved therapies for NAFLD

are currently available. Clinical trials conducted so far with a myriad of investigational agents have shown relatively low rates of response compared with placebo.[10] The latter may be related to several factors[11] with disease heterogeneity being a relevant aspect. In fact, inclusion criteria for most of clinical studies is usually based mainly on histological diagnosis of NAFLD which result in mixed population of patients.[12] Thus, more granular data on the effectiveness of new therapies in NAFLD subgroups is needed to create the proper grounds for personalized or precision medicine approaches in the near future.[13–15]

As mentioned above, the NAFLD acronym groups a heterogeneous patient population. NAFLD clinical expression results from a complex and multilayered dynamic interaction of different factors including sex,[16] presence of several genetic variants,[17] coexistence of different comorbidities,[18] diverse microbiota composition,[19–21] and various degrees of alcohol consumption[22] among others. This complex interaction results in several disease subphenotypes with distinct natural history and prognosis and, eventually, different response to therapy (►Fig. 1). Of note, NAFLD heterogeneity

was one of the reasons argued by an expert panel that suggested to replace NAFLD by a new acronym derived from the initial letters of metabolic (dysfunction)-associated fatty-liver disease (i.e., MAFLD). NAFLD renaming was proposed aiming to better capture the main driver of the disease in the majority of patients.[23,24] However, this suggestion had not been universally accepted and a debate on the matter is ongoing.[25,26] In the present review, we aim to address the issue of NAFLD heterogeneity through the analysis of existing data on the differential contribution of known factors to the clinical expression of NAFLD. The impact of better deciphering NAFLD heterogeneity and the dominant drivers of disease in patient subgroups on the development of more targeted therapies for NAFLD is also highlighted.

Role of Genetic Determinants

NAFLD has a strong heritability and during the last year's genome-wide association studies (GWAS) have allowed the discovery of the main common genetic determinants of this condition and of the susceptibility to hepatic fat
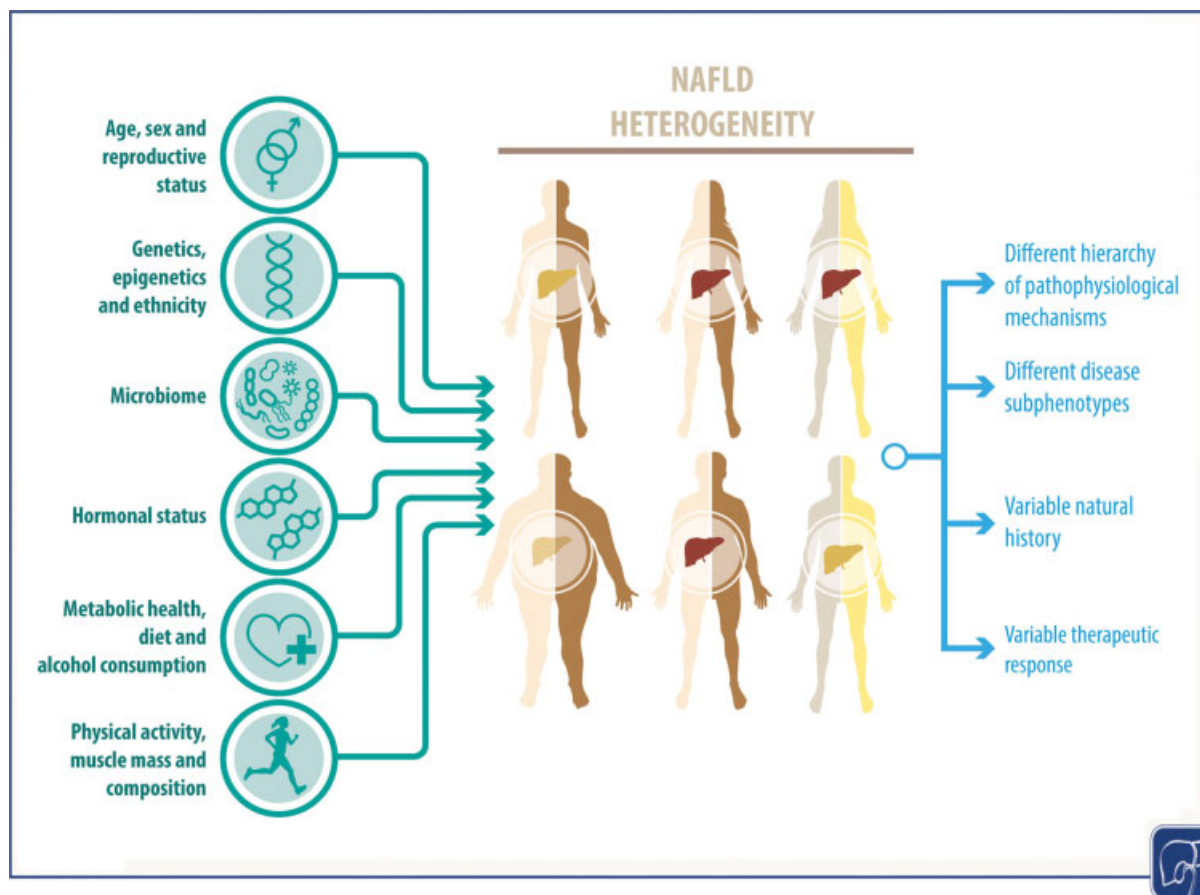
Fig. 1 The acronym nonalcoholic fatty-liver disease (NAFLD) groups a heterogeneous patient population. The different phenotypes observed in clinical practice stem from a complex and dynamic interaction of different factors (age, sex, reproductive status, presence of one or more genetic variants and epigenetic factors, a diverse microbiota composition, coexistence of different comorbidities, the degree of alcohol consumption, and muscle mass and physical activity among other). These variables critically influence NAFLD development and progression. Disease heterogeneity imply that pathophysiological mechanisms underlying NAFLD may have different hierarchy or trajectory in different patients defining different subphenotypes which may be relevant to molecular pathway-based therapies. NAFLD heterogeneity may also influence natural history and prognosis and, eventually, response to therapy.

accumulation.[17] Their common pathogenic mechanism turned out to be the alteration of hepatocellular lipid handling, favoring on one hand retention in lipid droplets over secretion within very-low density lipoproteins and on the other hand synthesis over catabolism.[27] Increased quantity and altered composition of lipids determines lipotoxicity and potentially progressive liver disease.[27]

Identification of patatin-like phospholipase domain-containing 3 (PNPLA3) p.I148M and transmembrane 6 superfamily member 2 (TM6SF2) p.E167K, as the main risk variants, allowed soon to pinpoint a subset of patients carrying multiple variants who are at higher risk of progression to the hepatic rather than to the cardiometabolic complications of NAFLD.[14,17,28,29] Due to the increased power of studies conducted in large population-based cohorts, the number of NAFLD risk variants is now increasing by the day.[30–33] We are therefore presently moving to a model where, across the continuum of the distribution of the inherited predisposition to NAFLD in the population, the development of polygenic risk scores (PRS) can stratify the risk of this condition and of liver-related complications.[30,31,34–36] For example, "high" PRS can predict the evolution to cirrhosis and HCC independently of classical risk factors in individuals with dysmetabolism and/or NAFLD, potentially being able to aid in the clinical management and in the design of referral pathways.[35]

On the other hand, it has become clear that the main common risk variant and PRS cannot discriminate accurately between "metabolic" and "genetic" NAFLD.[28] Vice versa, very robust evidence has accumulated that increased adiposity, and dysmetabolism is the main trigger for the phenotypic expression of this genetic predisposition; the higher the body mass index, the higher the risk of liver disease in carriers of NAFLD risk variants.[30,35,37,38] In addition, genetic predisposition to NAFLD contributes to insulin resistance via inducing liver injury.[34] Finally, the main genetic risk variants in PNPLA3 and TM6SF2 are similarly enriched in "overweight/obese" versus "lean" NAFLD.[39,40] Indeed, central adipose tissue distribution, genetic causes of insulin resistance,[37] and other factors predisposing to hepatic fat accumulation can vicariate the role of excess adiposity among the small fraction of lean individuals who develop NAFLD.

Excess intake of alcohol is another key determinant of the phenotypic expression of NAFLD risk variants[17,30] which are also the main genetic risk factors of alcoholic liver disease.[41] Fructose intake, chronic hepatitis C, and iron accumulation are additional triggers of liver disease.[42–44] This new evidence suggests that several conditions that were previously considered as separated diseases share major pathogenic mechanism of liver damage centered on fat accumulation and lipotoxicity which are initiated by specific but often concomitant insults. The most frequent is the combination of obesity, excessive alcohol, and genetic predisposition. Environmental and toxic insults may further push this deleterious mix toward liver disease. As such, genetics has clearly highlighted that clinical heterogeneity should perhaps be the best considered in the overall context of fatty-liver diseases than in NAFLD per se.

However, there is initial evidence from the first next generation sequencing studies that rare genetic variants with a large impact on hepatic fat can predispose to NAFLD irrespective of dysmetabolism.[14,45] For example, carriage of apolipoprotein B (APOB) variants predispose to severe NAFLD, cirrhosis, and HCC in nonobese individuals.[45,46] This subset of patients is at low risk of cardiovascular disease due to reduced circulating lipoproteins and may also possibly benefit from vitamin E supplementation due to malabsorption of lipophilic vitamins.[45–47]

There is additional evidence that the genetic setup of NAFLD can influence the profile of extrahepatic organ damage and the response to therapy. Thanks to the recent discoveries in NAFLD genetics,[14,17,28,29] the frontier of disease phenotyping can move away from the "metabolic vs. genetic" simplistic dichotomy, toward a subclassification integrated with the clinical risk factors and based on combined environmental-genetic pathways underlying disease predisposition. The challenge ahead will be to develop reliable clusters of genetic variants that predict disease subtypes. Based on the current knowledge at least three groups can be identified. The first is defined by the presence of high genetic risk of obesity and insulin resistance[37,48] in the absence of specific risk determinants of liver injury ("insulin-resistant fatty-liver disease"). This roughly corresponds to the previous classification of "metabolic NAFLD," at high risk of cardiometabolic complications, but lower risk of liver-related events. Among the specific determinants of liver involvement in individuals with dysmetabolism, a group of genetic variants including those in glucokinase regulator (GCKR) that facilitate fat accumulation by diverting carbon substrates from glucose metabolism to lipid synthesis (de novo lipogenesis [DNL]) can define "enhanced lipogenesis fatty-liver disease," at high risk of dyslipidemia.[17,49] Insulin-resistant patients carrying these variants may possibly benefit from approaches reducing lipogenesis, such as statins and omega-3 fatty acids.[14,50] A pathophysiological distinct subset ("impaired lipid remodeling fatty liver disease") is represented by those with a high burden of genetic variants, including those in PNPLA3, TM6SF2, membrane bound O-acyl transferase (MBOAT7), and APOB, impairing lipid remodeling and secretion and favoring lipotoxicity. These patients are at a particularly high risk of liver-related complications, including hepatocellular carcinoma before cirrhosis development.[14] They develop type-2 diabetes,[34,48] but are relatively protected from cardiovascular disease.[14,35] From a therapeutic point of view, they respond well to weight loss, but when they carry the PNPLA3 variant do not benefit from statins and are at increased risk of liver injury due to enhanced hepatic lipid accumulation during insulin therapy (reviewed by Cespiati et al[14]).

Overall, the newest research developments have suggested new possibilities to integrate, rather than oppose the genetic markers with clinical information to improve fatty-liver disease classification and management. The efficacy and cost effectiveness of this approach remains to be proven before wide implementation can be advised.

Differential Contribution of Pathogenetic Pathways

NAFLD pathogenesis is associated with a wide spectrum of aberrant cellular and molecular mechanisms.[51–53] As described above, genetic[54] and metabolic[55] alterations are key drivers of the development of NAFLD resulting in an environment that favors hepatic fat accumulation and toxic lipid metabolites via an increase in circulating free fatty acids released from a dysfunctional inflamed adipose tissue[56,57] and an increased DNL in hepatocytes.[58,59] These changes may lead to a state of "lipotoxicity" that triggers hepatocellular stress and consequently may result in liver injury and cell death, activating a sterile liver inflammatory response[51,53,60] and ultimately liver fibrogenesis contributing to disease progression. The mechanisms thought to be at play in NAFLD pathogenesis may act in a sequential or parallel fashion and with different hierarchies within the spectrum of disease and over time.[57,60] Thus, it is likely that the observed heterogeneity of NAFLD in the clinic, where some patients develop isolated steatosis, whereas others develop NASH and advanced chronic liver disease stems from a differential contribution of pathogenetic pathways,[60,61] as well as from the activation or deactivation of redundant mechanisms. Also, it is likely that, besides genes, many factors including diet, exercise, sex, age, and coexistence of obesity and type 2 diabetes mellitus (T2DM) among others, critically influence disease pathogenesis in a given patient.[15] However, to dissect the precise contribution of each pathway to NAFLD development and progression is difficult due to limitations of available animal models[62,63] or lack of robust human data. Remarks on potentially relevant pathogenic differences among NAFLD patients are provided below.

One important mechanism underlying NAFLD development is an upregulated DNL which has been reported to be three-fold increased compared with control patients.[58] Interestingly, DNL rates are influenced by age and sex, and this pathway may be pathogenically more relevant in older and male patients than others.[64,65]

A previously overlooked player in NAFLD pathogenesis is the skeletal muscle. Several studies have underscored that sarcopenia (i.e., decreased muscle mass) or adverse muscle composition (i.e., low muscle volume and muscle fat infiltration or myosteatosis) may influence both NAFLD development and progression.[66–68] Although important methodological differences exist across published studies, not all patients with NAFLD exhibit muscle alterations and it is likely that this pathogenic factor be more relevant in particular populations, such as nonobese or lean NAFLD, as well as older patients in which sarcopenia is more frequent.[69]

Activation of the innate immune system has been implicated in NAFLD progression.[70,71] After liver damage, a physiological immune response is key for resolution of the injurious process, liver regeneration and restauration of a healthy liver state, while an exuberant and persistent innate immune response may lead to chronic liver inflammation. In the context of NAFLD, an imbalance of the cross-talk of the gut microbiome and the liver, known as the gut-liver axis, as well as occurrence of hepatocyte cell injury and death are two key upstream triggers of a persistent pathogenic innate immune response.[57,71] Intestinal dysbiosis, defined as the imbalance between resident microbial communities that disturb the symbiotic relationship between gut microbes and the host, and an increased intestinal permeability may lead to a pathological translocation of microbial products to the liver via the portal vein.[19,20] In this setting, pathogen-associated molecular patterns (PAMPs) are recognized by selective pattern recognition receptors, mainly toll-like receptors, in the liver and cause a chronic innate immune response. In addition, microbiota-derived metabolites such as modified bile acids, choline, and ethanol alter hepatic metabolism and trigger an inflammatory response.[72] The above-mentioned mechanisms may be at play in only a fraction of individuals with NAFLD. In fact, clinical studies have uncovered that only a subset of NAFLD patients present with dysbiosis and/or a dysfunction in the gut barrier.[19,72] These findings point that in addition to the genetic and metabolic modifiers, assessment of microbiome profile, and or intestinal permeability may allow for a more precise classification and risk stratification of NAFLD patients to define an adequate therapeutic intervention.[72–74]

In certain settings, the immune system may be activated by other stimuli and contribute to NAFLD development and progression. This may be the case for the association of NAFLD with some immune-mediated inflammatory disorders, such as psoriasis[75,76] and suppurative hidradenitis,[77] which are associated to a low-grade systemic inflammatory state. Of note, Psoriasis patients exhibit significantly increased serum levels of proinflammatory cytokines such as tumor necrosis factor-a (TNF-a) interleukin (IL)-6 and reduced levels of the anti-inflammatory adipokine, adiponectin than matched controls.[78] Also, psoriasis patients with NAFLD have higher serum levels of C-reactive protein (CRP) and have an increased prevalence of advanced liver fibrosis.[79] In the same line, patients with suppurative hidradenitis exhibit a high prevalence of NAFLD independently of classic metabolic risk factors.[80] However, it remains difficult to dissect whether the underlying systemic inflammation observed in psoriasis and suppurative hidradenitis is a major contributor to NAFLD development and progression given the frequent coexistence of obesity in these patient populations.[76,81] Interestingly, an imbalance in T-cell subtypes, like Th17/Treg, leading to an IL-17–mediated inflammation may be a common pathogenic link in this regard.[76,82]

Hepatocyte cell death as a result of lipotoxicity is another key driver of the progression of NAFLD. Cellular release of damage-associated molecular patterns (DAMPs) by damaged[71] or stressed hepatocytes are recognized by the innate immune system and may trigger chronic liver inflammation.[60,83] Recently pyroptosis has been identified as a novel form of programmed cell death in NAFLD and NASH. Pyroptosis is characterized by the activation and assembly of multiprotein complexes, called inflammasomes. The most studied one is the nucleotide-binding domain, leucine-rich–repeat (NLR) family, pyrin domain containing 3 (NLRP3) inflammasome. Once activated, the NLRP3 inflammasome

cleaves procaspase-1 to its mature form caspase-1 that subsequently cleaves IL-1β, IL-18, and gasdermin D. IL-1b and IL-18 are highly proinflammatory cytokines that are released into the extracellular space partly through gasdermin D formed transmembrane pores.[60,84] NLRP3 was shown to promote NASH and liver fibrosis in various mouse models. Genetic constitutive overactivation of the NLRP3 inflammasome causes pyroptosis, inflammation, and fibrosis in the liver, while global NLRP3 knock out mice show less inflammation and liver fibrosis in models of diet induced NASH.[85,86] Human data on the contribution of NLRP3 to NAFLD development and progression is scarce; thus, future translational studies to examine the human relevance of these pathways to NAFLD heterogeneity are warranted.

As described above, PAMPs and DAMPs activate and sustain a pattern recognition receptor-mediated innate immune response characteristic for NAFLD. Once activated, a complex intercellular cross-talk between the different innate immune cells, mainly macrophages, neutrophils, natural killer cells, and T-cells, as well as hepatocytes and hepatic stellate cells (HSCs), defines the progression to steatohepatitis and fibrosis. Macrophages are known to play a crucial role in orchestrating immune response and the recruitment of further immune cells.[87] Kupffer's cells, the resident liver macrophages, are dominant over monocytes and monocyte-derived macrophages (MoMFs) in a healthy liver. However, after liver injury, both monocytes and subsequently MoMFs are rapidly recruited to the liver.[88] The functional plasticity of macrophages was historically subclassified in the two polarizations, proinflammatory-type M1 and anti-inflammatory-type M2. This classification was shown to lack accuracy of the macrophage activation program. Instead, during liver injury, macrophages display wide gene expression profiles that led to the identification of different subpopulations of macrophages. The new technology of single-cell RNA sequencing (scRNA-seq) revealed an accumulation of MoMF in murine livers in a diet-induced NASH model.[89] The population of MoMF was further subclassified in the three clusters MoMF I to III with each cluster characterized by particular marker genes. Interestingly, similar subpopulations of bone marrow monocytes were also detected. These findings indicate a NAFLD gene signature in myeloid leukocytes in both liver and bone marrow.[88] Another study using scRNA-seq identified further subpopulations of macrophages in NASH characterized by the expression of Trem2, termed NASH-associated macrophages. In human samples, Trem2 expression correlated with the severity of NAFLD activity score and the extent of fibrosis. Therefore, markers of NASH-associated macrophages may function as potential novel key classifiers of NAFLD/NASH phenotypes.[90] The specific role of Kupffer's cells during the progression of NAFLD to NASH has continued to evolve. Recent evidence has uncovered a reduction of mature Kupffer's cell population during this process due to increased Kupffer's cell death. Monocyte-derived macrophages are then able to enter the stage of differentiation into Kupffer's cells. While Kupffer's cell acts mainly by facilitating hepatocyte triglyceride accumulation, MoMFs are more proinflammatory.[91,92] A better

understanding and profiling of the immune cell niche during NAFLD and NASH may provide a precision medicine approach to the current pathological scoring of inflammation and allow for risk stratification and identify patients more likely to have a progressive disease versus those who will have a nonprogressive liver phenotype.

Liver fibrosis is the critical pathological feature that predicts liver-related outcome in NASH.[93,94] Various cytokines activate HSCs and their transformation toward collagen secreting myofibroblasts (MFB) which are key for triggering and progression of liver fibrosis as in other hepatic diseases.[95] However, considering the disturbed metabolic milieu present in NAFLD, it is likely, although not certain that a myriad of disease-specific mechanisms may be at play (recently reviewed by Schwabe et al).[96] Interestingly, a recent study investigating the population of activated HSCs in liver fibrosis revealed various clusters of MFBs, suggesting the existence of heterogeneity within the fibrogenic cell population. While all clusters showed a high expression of collagen, the ability to secret chemokines to modulate inflammation was limited to certain clusters.[97] Another study uncovered specific liver zonation of HSCs, classifying them into portal vein-associated HSCs (PaHSCs) and central vein-associated HSCs (CaHSCs). Notably, CaHSCs were identified as the dominant pathogenic collagen-producing cells. The lysophosphatidic acid receptor 1 (LPAR1) is upregulated in NASH patients and selectively expressed in CaHSCs but not PaHSCs and NPCs. Pharmacological inhibition of LPAR1 in a murine NASH model has shown to ameliorate liver fibrosis.[98] These findings underline the complexity and diversity of HSCs in the context of the pathogenesis of liver fibrosis in general and in NAFLD in particular.[96] Further studies are warranted to investigate whether certain subpopulations of HSCs favor remodeling after liver injury or triggering liver fibrosis. These studies may allow to identify patients with fibrotic NASH that are at particular risk of worsening of fibrosis versus those that are more likely to show a robust wound healing response with potential for spontaneous improvement of fibrosis. In summary, these novel studies dissecting the distinct immune cell and HSC niche may allow to provide two additional groups to the ones described in the previous section including an "Inflammatory fatty liver disease" and a "profibrotic fatty-liver disease." This classification may be central to identify patients with NAFLD/NASH that are at particular risk for disease progression or "rapid progressors," a subphenotype that represents the central target for therapeutic intervention.

Heterogeneity of Clinical Disease

In the clinical arena, NAFLD patients present with diverse features. Sources of heterogeneity that can be considered in the clinic at present time are multiple and include age, sex, cardiometabolic comorbidities, ethnicity, endocrine status, as well as alcohol and tobacco consumption.[23]

Considerations on the influence of age and sex in NAFLD clinical presentation and prognosis has been reviewed elsewhere,[99–101] underscoring that while pediatric patients may

have a different natural history and unique susceptibilities, as well as more severe long-term consequences,[101] older patients exhibit an increased prevalence and severity of NAFLD.[102] With regard to sex, NAFLD is considered a sexually dimorphic disease with clear differences between male and females with the latter being protected of severe fibrotic disease during premenopausal stage.[103]

Being a multisystem disease, NAFLD is commonly associated with metabolic features, with a prevalence of 80% of obesity, 72% dyslipidemia, and 44% of T2DM.[1,104] Although the combination of these features acts in an additive manner to promote NAFLD progression, T2DM is the feature most strongly and consistently associated with a more severe disease and progression.[105–107] Considering the ominous effect of T2DM on liver disease progression and extrahepatic complications (i.e., cardiovascular disease, cancer risk, and chronic kidney disease), most guidelines recommend patients that NAFLD need to be screened and treated for metabolic comorbidities.[3,108,109] In addition, while obesity is also considered a risk factor for disease progression,[107,110] lean patients with NAFLD have been increasingly recognized as being at risk of progression to advanced liver disease, as well as of developing metabolic comorbidities, and cardiovascular disease with impact in overall and liver-related mortality.[111,112] Lean patients account for 20 to 30% of NAFLD patients.[113] The same as obese NAFLD, its presence is associated with presence of metabolic comorbidities, increased visceral adiposity and unfavorable genetic polymorphisms.[114] Of note, lean NAFLD is not a benign condition, since it is independently associated with increased overall and cardiovascular mortality.[115]

Ethnicity is an important aspect in NAFLD heterogeneity. Several studies describe Hispanic Latino population to be at the highest risk for development of NAFLD, whereas non-Hispanic Black and non-Hispanic Asian population are at lowest risk.[116] In the United States, most of this risk is explained specifically by Mexican American population (50% NAFLD prevalence, compared with only 30% of prevalence in other Hispanic populations). The cause for these ethnicity differences is probably explained by genetic and sociocultural factors including diet, exercise, alcohol consumption, education, family income, and quality of life. Interestingly, novel data suggest that advanced fibrosis assessed by noninvasive serum test is more prevalent in non-Hispanic black (28.5%) and non-Hispanic white populations (25.8%) compared with Mexican American (10.8%) and Asian non-Hispanic population (2.65%).[117] Indeed, further studies are needed to validate and explore these findings.

Sex differences is another important factor to be considered in NAFLD heterogeneity. It has been repeatedly shown that female patients at reproductive age have a significantly lower NAFLD prevalence and severity[118] which may be explained by a myriad of sex differences in the pathobiology of NAFLD (e.g., different storing of surplus calories, differential activation of hepatic DNL, and sex-specific in mitochondrial function and immune responses among others).[99] After menopause, sex differences are reduced in NAFLD and, in

fact, women have a higher risk of advanced fibrosis than men, especially after age 50 years,[119] suggesting that estrogen could be a protector factor for NAFLD.[99,118] Again, sociocultural characteristics are likely to be involved in sex differences in NAFLD risk.

Obstructive sleep apnea (OSA) is a common complication of obesity, inducing repetitive cycles of hypoxia and reoxygenation. Presence of OSA is associated with increased liver steatosis, inflammation, and fibrosis.[120,121] Unfortunately, trials evaluating OSA therapy with continuous positive airway pressure (CPAP) have shown nonconclusive results in reducing liver fibrosis of NAFLD patients.[120,122]

Alcohol consumption has classically been considered an exclusion criterion for NAFLD. However, it has been increasingly recognized that a group of patients may present a dual etiology (i.e., alcoholic and nonalcoholic) of their fatty-liver disease.[22] Recent population based longitudinal studies demonstrate that even mild consumption of alcohol can have a synergistic effect when combined with obesity, insulin resistance, and metabolic dysfunction to increase risk of cirrhosis and hepatocellular carcinoma.[22] In this context, the renaming of NAFLD into MAFLD proposes a set of positive criteria for diagnosis and does not consider the exclusion of alcohol consumption as a prerequisite criterion for diagnosis.[23,24]

Among other relevant factors that may modulate disease development and severity are presence of skin inflammatory disorders, such as psoriasis and suppurative hidradenitis, hypothyroidism, polycystic ovarian syndrome, and periodontitis, all of which have been associated with increased prevalence of NAFLD.[123]

Besides systemic comorbidities, patients with NAFLD represent a wide spectrum of liver disease severity. In the previous decade, emphasis was made in a dichotomic differentiation between those patients with isolated steatosis and those with NASH. More recently, emphasis is made on patients with NASH and liver fibrosis as the latter histological feature has been shown to be a major prognostic determinant in NAFLD patients.[94] Natural history studies, as well as studies examining patients on the placebo arms of drug clinical trials, using paired biopsies, showed that evolution of NAFLD/NASH overtime is highly dynamic and that, although NASH has a higher risk of fibrosis progression and development of cirrhosis than non-NASH patients, some individuals will have spontaneous improvement while some patients with isolated steatosis could also progress to NASH and cirrhosis.[124–131] Current data support the idea that approximately 20 to 25% of patients with isolated steatosis will progress to NASH, and from those, approximately 20 to 40% will progress to advanced fibrosis and cirrhosis. Additionally, 5 to 7% of NASH-cirrhotic patients will develop HCC.[56] Thus, identifying the group of patients who most likely will progress to advanced fibrosis is a primary goal of NAFLD/NASH-related research. In this regard, those at higher risk are patients with metabolic abnormalities, such as T2DM, metabolic syndrome, and obesity.[2,132]

Most natural history studies of NAFLD have assessed liver-related morbidity and mortality in relation to a single time-point evaluation of histology to identify risk factors.[124,131,133–135] In spite of their intrinsic selection bias, paired-biopsy studies are informative regarding identification of factors associated with disease progression.[125,126,128–130] In these studies, the main risk factors for presence of NAFLD are metabolic abnormalities such as T2DM, metabolic syndrome, and obesity.[136–138] Regarding predictors of fibrosis progression, studies have shown that the best predictors are histological parameters including hepatocellular ballooning degeneration and inflammation associated with age in patients with NASH.[130] As mentioned above, the presence of fibrosis on initial biopsy or progression of fibrosis on serial biopsies appears to be the most prominent histologic predictor of overall mortality and liver-related outcomes.[139–141] During the last decade, efforts have been made to identify noninvasive surrogates of liver biopsy that can be followed over time. Serum aminotransferase levels are good predictors of improvement of NASH in clinical trials.[10] Likewise, markers of cell death including soluble cytokeratin-18 (CK-18) forms (total and fragmented) have been shown to be good diagnostic markers of NASH and predict response to therapeutic interventions but currently remain a research tool and are not clinically available.[142,143] Other circulating markers such as proinflammatory markers, including circulating levels of cytokines (onlinTNF-a, IL-6, IL-8, and CRP), or other molecules, such as adiponectin, have shown inconsistent results in part due to the challenges with the sensitivity of the assays used to detect changes in blood.[143–146] To identify patients who already are progressing and patients at early stages of fibrosis, noninvasive scores have been developed. Among them, the NAFLD Fibrosis Score, Fibrosis-4 (FIB-4), and Hepamet are nonexpensive, noncommercial, and easily available.[147–152] The enhanced liver fibrosis (ELF) test is a commercial panel that combines various constituents of collagen matrix deposition and turnover.[153] Additionally, imaging assessment of steatosis and fibrosis has been developed as a noninvasive method and has focused mainly on transient elastography (TE) with controlled attenuation parameter (CAP) and magnetic resonance imaging (MRI)-based technologies.[153–158] Details on modern concepts about noninvasive assessment of hepatic fibrosis can be found in recent in-depth reviews on the topic.[159,160]

Implications for Clinical Practice and Research

The differential contribution of genetic/epigenetic, environmental, and metabolic factors deters a significant interpatient variation regarding the major driver of disease (►Fig. 2). Proper consideration of NAFLD heterogeneity is relevant for both clinical practice and research. In the clinical arena, a more precise patient phenotyping could allow to a more granular grouping of patients to better stratify them into those at higher risk of adverse outcomes. Also, a better phenotyping would allow the implementation of tailored treatment approaches in line with concepts of precision medicine or individualized care.[13,15,161] Since the degree

of liver fibrosis has been shown to be closely related to liver-related mortality,[93,94] assessment of this variable is now considered crucial in patient stratification and should be performed with the available noninvasive tools.[159] However, most of these tools are not able to distinguish those patients that show a strong wound healing response and thus more likely to have a resolutive phenotype with spontaneous regression of fibrosis versus those that will have a strong fibrogenic response and will more likely show progression of fibrosis. The challenge for the future will be to develop deep phenotyping approaches that also takes into consideration these processes in a given patient, and incorporates associated comorbidities, race, genetic and epigenetic influences, and environmental factors.[10,162] Thus, clinical variables known to be associated with rapid disease progression, such as T2DM, arterial hypertension, severe obesity, and worsening metabolic health (i.e., presence of disglycemia and having one or more components of metabolic syndrome), should be factor in association with molecular and cellular profiling of liver phenotypes and if present, may confer the label of "high-risk" NAFLD/NASH[163,164] which may imply higher risk of all-cause mortality, as well as of fibrosis, cirrhosis, and HCC.[165] In addition, consideration on differences linked to age, sex, and hormonal status may be proven relevant given that these factors may influence NAFLD natural history, as well as the performance of several diagnostic tools.[15] With regard to genetic factors, routine genotyping of genetic variants known to influence NAFLD has not yet been proven to be cost effective but likely will contribute to individualized management when used integrated with all pertinent clinical information[14,166] (see section Role of Genetic Determinants). Finally, detailed phenotyping of behavioral (i.e., physical activity and diet), alcohol use, and socioeconomic factors, as well as of muscle mass and composition (i.e., using MRI techniques), could aid in better defining NAFLD patient cohorts.

From a therapeutic standpoint, NAFLD heterogeneity should be considered when structuring a therapeutic plan for disease management. As previously outlined in several clinical guidelines or position papers released and endorsed by several scientific societies or expert panels,[3,108,167] lifestyle modifications remain the cornerstone of NAFLD management and are indicated in all patients.[7] Of note, sex differences in the response to lifestyle changes have been observed in several studies. Interestingly in the most-cited study assessing lifestyle interventions in NAFLD,[168] men showed a greater histological improvement than women after weight loss. In this study, male sex was one of the factors predicting beneficial histological response following a relatively modest weight loss (between 7 and 10%), while in women, a more substantial weight loss (> 10%) was required to achieve a significant histological improvement.[168,169] Thus, based in these observations, women may require additional support to achieve the goals to be achieved after introduction of lifestyle changes. In contrast, patients carrying the p.I148M variant of the *PNAPL3* gene exhibit a better response to lifestyle modification and bariatric surgery than patients not carrying the variant.[170–172] This information may be of
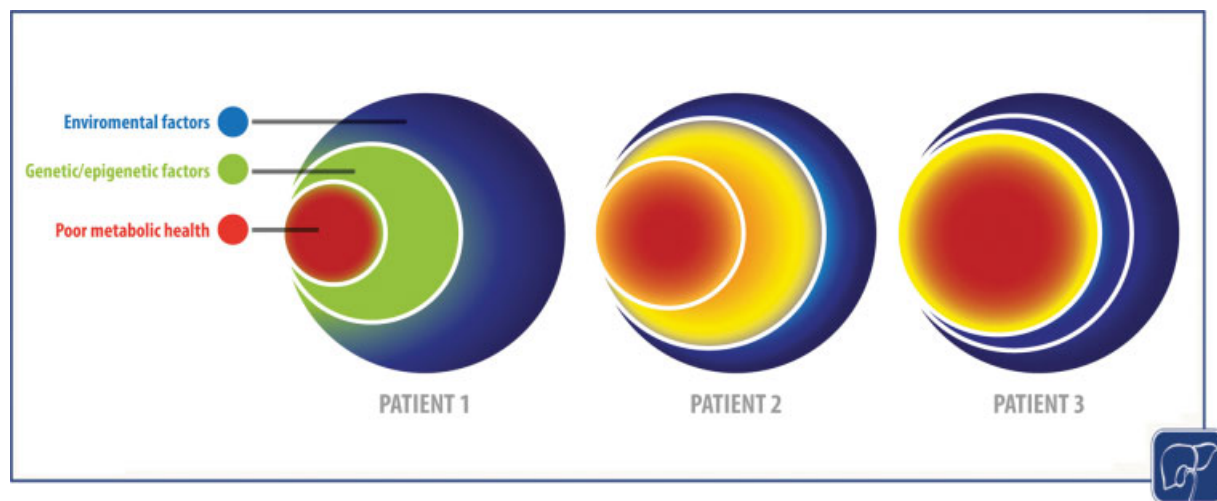
Fig. 2 The differential contribution of genetic/epigenetic, environmental and metabolic factors deters a significant interpatient variation regarding the major driver of disease. Circles depict three hypothetical patients that exhibit a different predominance of the three above-mentioned factors. While patient 1 has environmental factors (e.g., poor diet and/or physical activity) as main driver of disease, patients 2 and 3 has a mixed predominance of genetic factors and metabolic derangement as determinants of their phenotypes. Better distinction of main drivers in each patient may help to implementation of individualized or precision medicine approaches in nonalcoholic fatty-liver disease (NAFLD) management (adapted from Eslam et al).[23]

aid when considering obesity surgery in NAFLD patients. The same applies to the possibility of applying tailored cardiovascular risk management in patient subgroups as patients with the p.I148M variant of PNAPL3, as well as those with the *TM6SF2 E167K* gene variant, exhibit a lower risk of cardiovascular disease compared with noncarriers.[17] Finally, tailored HCC screening may be envisioned for some patients using genetic information as use of PRS, as shown by Bianco et al in a recent paper in which PNPLA3–TM6SF2–GCKR–MBOAT7 were combined, may improve HCC risk stratification in NAFLD.[173]

With regard to interpatient variability to therapeutic agents, available information is scarce. It is likely that better and more detailed assessment of certain specific pathways could be of help in selecting therapeutic agents. This could be made with the use of metabolomics[174,175] or other system biology tools. Thus, if DNL is shown to be overactive,[65] specific use of agents targeting this pathway, such as acetyl-CoA carboxylase (ACC) inhibitors, could be indicated.[174,175] Also, if appropriate tools to assess intestinal dysbiosis[19] are available, gut microbiome-targeted therapies could be also used only in those with altered microbial composition. Finally, drugs that improve dyslipidemia or decrease cardiovascular disease may be more effective for those patients with a more profound derangement of metabolic health such as patients with concurrent T2DM and NAFLD. In the latter population, one should also consider the potential benefits of antidiabetic drugs, such as pioglitazone and sodium-glucose cotransporter-2 (SGLT2) inhibitors,[176,177] or the preferential use of the glucagon-like peptide 1 (GLP-1) receptor agonists, such as liraglutide or semaglutide, with potentially beneficial impact on liver histology and

cardiovascular outcomes in this particular patient population.[178–181]

Finally, consideration of psychosocial factors in the evaluation of any given patient is also relevant for NAFLD management and should be part of a comprehensive phenotyping approach. These factors might influence motivation to both adopt and sustain lifestyle changes. In this regard, necessary support to patients' efforts through health coaching and/or motivational interventions is key to successful adherence to any program aimed at naturalizing physical activity in daily life.

The impact of NAFLD heterogeneity in therapeutic clinical trials has been also emphasized recently.[12,23] Precise stratification of patient cohorts, included in a given study, is a key when assessing therapeutic response in a complex disease such as NAFLD. The suboptimal responses observed in several clinical trials of different drugs may be in part due to the fact that disease heterogeneity was not taken into consideration.[11,182] In their recent review on the matter Ampuero and Romero-Gomez concluded that clinical trial reporting for NAFLD has been suboptimal with no detailed mention of dysmetabolic comorbidities or proper recording of daily dietary and exercise habits. These authors suggest that future trials should carefully consider the presence of comorbidities expected to impact on the treatment response (trying to keep similar proportion of patients in the different arms of a given trial), as well as proper recording of alcohol intake and exercise both during the trial and not only at entry, which will improve capturing of the effects of these variables in treatment responses. Also, adoption of innovative trials designs to study novel NAFLD treatments, such as the adaptative, umbrella, or basket strategies, could be useful to improve trial efficiency.[12,23]

Conclusion and Outlook

NAFLD groups a heterogeneous patient population. The heterogeneity of NAFLD is reflected by the fact that some patients develop only steatosis, whereas others develop hepatocyte injury, steatohepatitis, and progressive liver fibrosis and, ultimately, cirrhosis. The interaction of different factors (e.g., sex, presence of one or more genetic variants, coexistence of different comorbidities such as obesity and T2DM, diverse microbiota composition, and various degrees of alcohol consumption) critically influence different disease pathways to determine disease subphenotypes with distinct natural history and prognosis and potentially different response to treatment. The recent proposal of NAFLD, renaming into MAFLD that sets positive criteria for diagnosis,[24] has been generally welcomed by the scientific community, although debate is ongoing.[25] The use of MAFLD may be a step forward in addressing disease heterogeneity by grouping a more homogenous patient population with more severe metabolic and liver disease (i.e., presence of significant fibrosis).[183–185] In addition, differentiation of three subgroups (i.e., obese MAFLD, lean MAFLD, and diabetic MAFLD) that may have different prognosis and could be appraised differently, may be also relevant. However, further discussion is necessary and consensus needs to be reached on this matter.[26,184]

Taking into account, disease heterogeneity is relevant both clinically, to apply more individualized approaches in NAFLD diagnosis and treatment, and to drug development due to the need of including more homogeneous patient populations in clinical trials. In addition to clinical factors, availability of modern tools to define activation of DNL with metabolomics, development of accurate biomarkers of inflammatory, cell death or liver fibrosis pathways activation, better detection of presence of dysbiosis assisted by the use of microbiota signatures, or evaluation of genetic risk of cirrhosis or HCC with PRS would surely impact our ability to discriminate patients' subgroups and stratify them according to their risks of poor outcomes. This also would help to select the most appropriate treatment for a given patient following the precision medicine principles.[13,15] Thus, in the future, it can be envisaged that precise phenotyping of pathogenic pathways would lead to select a specific drug[10] or that a genetic treatment[27,186] could be chosen based on global ethnic-specific genetic information. Also, it is likely that principles of precision nutrition[187] and precision exercise[188] would apply to patient management to increase the therapeutic efficacy of lifestyles changes in NAFLD management.

Indeed, further research is needed to improve our understanding of NAFLD heterogeneity. Building of large biobanks from well-characterized patient populations integrating clinical, genetic, biomarkers, and OMICs information is key to this end. The use of artificial intelligence approaches offers the opportunity to combine and decode this information to develop useful algorithms for patient stratification and management. A better definition of the dominant drivers of disease in patient subgroups would predictably impact on the development of more precision-targeted therapies for NAFLD.

Main Concepts and Learning Points

- Nonalcoholic fatty-liver disease (NAFLD) is an umbrella term that groups a heterogeneous patient population.
- NAFLD heterogeneity is influenced by multiple variables including age, sex, presence of one or more genetic variants, coexistence of different comorbidities, diverse microbiota composition, and various degrees of alcohol consumption among other factors.
- The heterogeneous nature of NAFLD results from a different hierarchy or trajectory of pathophysiological mechanisms that determine several disease subphenotypes with distinct natural history and prognosis and, eventually, different response to therapy.
- Proper consideration of NAFLD heterogeneity is relevant for both clinical practice to implement individualized medicine approaches, as well as for clinical research, to improve clinical trials efficiency.
- The recent renaming of NAFLD into MAFLD (i.e., metabolic [dysfunction]-associated fatty-liver disease) is a step forward in taking into account NAFLD heterogeneity by setting specific diagnostic criteria and grouping patients with disturbed metabolic health. However, more research is needed to refine discrimination of subtypes of the disease.

Abbreviations
ACC, acetyl-CoA carboxylase; APOB, apolipoprotein B; CaHSCs, central vein-associated HSCs; CRP, C-reactive protein; DAMPs, damage-associated molecular patterns; DNL, de novo lipogenesis; GCKR, glucokinase regulator; GWAS, genome-wide association studies; HCC, hepatocellular carcinoma; HSC, hepatic stellate cells; IL, interleukin; MBOAT7, membrane bound O-acyl transferase; MoMFs, monocyte-derived macrophages; NAFLD, nonalcoholic fatty-liver disease; NAFL, nonalcoholic fatty-liver; NASH, nonalcoholic steatohepatitis; NLRP3, NLR family pyrin domain containing 3; PaHSCs, portal vein-associated HSCs; PAMPs, pathogen-associated molecular patterns; PNPLA3, patatin-like phospholipase domain-containing 3; PRS, polygenic risk scores; scRNA-seq, single cell RNA sequencing; TDM2, type 2 diabetes mellitus; TM6SF2: transmembrane 6 superfamily member 2; TNF-a, tumor necrosis factor-a.

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Conflict of Interest

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