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Metabolic-associated fatty liver disease: from simple steatosis towards liver cirrhosis and potential complications. Proceedings of the *Third Translational Hepatology Meeting*, endorsed by the Spanish Association for the Study of the Liver (AEEH)

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Abbreviations (in order of appearance):

AEEH: Spanish Association for the Study of the Liver; MAFLD: Metabolic-associated fatty liver disease; MASH: Metabolic-associated steatohepatitis; HCC: Hepatocellular carcinoma; T2DM: Type 2 Diabetes mellitus; SM: Metabolic syndrome; HVPG: Hepatic venous pressure gradient; PH: Portal hypertension; LSECs: Liver sinusoidal endothelial cells; HSCs: Hepatic stellate cells; HFD: High fat diet; ECM: Extracellular matrix; FXR: farnesoid X receptor;

TGR5: Takeda G protein-coupled receptor 5; BMI: Body mass index; DAMPS: Damage-associated molecular patterns; PAMPS: Pathogen-associated molecular patterns; MCJ: methylation-controlled J protein; KCs: Kupffer cells; TCA: Tricarboxylic acid; ROS: Reactive oxygen species; RNS: Reactive nitrosative species; $O_2^{\bullet-}$: Superoxide radicals; $^{\bullet}OH$: hydroxyl radicals; H_2O_2: Hydrogen peroxide radicals; SOD: Superoxide dismutase; CAT: catalase; GPX: Glutathione peroxidase; GST: Glutathione-S-transferase; SCFA: Short chain fatty acids; TLR: Toll-like receptor (TLR); PMBCs: Peripheral mononuclear blood cells; MAIT: mucosal-associated invariant T (MAIT); ihTh17: Inflammatory hepatic T helper 17 EVs: Extracellular vesicles; DILI: drug-induced liver injury; CCl_4: Carbon tetrachloride.

Abstract

This is a meeting report of the 3rd Translational Hepatology Meeting held in Alicante, Spain, in October 2021. The meeting, which was organized by the Spanish Association for the Study of the Liver (AEEH), provided an update on the recent advances in the field of basic and translational Hepatology, with a particular focus on the molecular and cellular mechanisms and therapeutic targets involved in metabolic-associated fatty liver disease (MAFLD), metabolic-associated steatohepatitis (MASH), cirrhosis and end-stage hepatocellular carcinoma (HCC).

1- Definition, comorbidities and pathophysiology

Introduction

Metabolic-associated fatty liver disease (MAFLD), formerly known as non-alcoholic fatty liver disease (NAFLD)[i], is one of the leading causes of liver cirrhosis and hepatocellular carcinoma (HCC) worldwide. This disease has surfaced as a growing public health issue, since MAFLD is becoming increasingly prevalent in parallel with the pandemics of obesity and diabetes. Prevalence of MAFLD in Spain is predicted to increase up to 27.6% and metabolic-associated steatohepatitis (MASH), the inflammatory advanced stage of this disease, will reach about 6% of the Spanish population in 2030, whereas mortality and advanced liver disease will double in 2030[ii].

MAFLD and MASH are strongly associated to risk factors such as obesity, type 2 diabetes mellitus (T2DM), dyslipidaemias and metabolic syndrome (SM) [iii] and reciprocally, patients with significant fibrosis in the context of MAFLD have been proven to be at risk of developing both T2DM and arterial hypertension[iv]. Besides, mounting evidence from both preclinical and human studies has highlighted the relationship of this disease with inflammatory phenotypes, especially related to immune-mediated inflammatory diseases, such as Crohn's disease[v], coeliac disease[vi] or hidradenitis suppurativa[vii].

MAFLD pathophysiology

MAFLD is a complex entity involving numerous genetic, epigenetic and environmental factors. This disease begins with the accumulation of triglycerides and other lipids in the main hepatic cell type, the hepatocyte, and this can progress from simple steatosis to steatohepatitis, cirrhosis or even liver cancer[viii].

Initially, obesity induces insulin resistance and chronic inflammation that promotes lipolysis of adipose tissue[ix]. Excess blood fatty acids begin to accumulate in the hepatocytes, causing the onset of the so-called fatty liver. These lipids come from the systemic circulation,

although they are also synthesized *de novo* within the hepatocyte which, in addition, decreases both their degradation and export. Storage of lipids in the form of triglycerides is not particularly harmful, but other lipids such as diacylglycerols, cholesterol, phosphatidylcholines and certain saturated fatty acids are particularly toxic.

At the same time, insulin resistance and inflammation in adipose tissue lead to increased secretion of adipokines and inflammatory cytokines that generate a state of chronic inflammation[x]. This inflammation, in the liver, is coupled with lipotoxicity due to lipid accumulation promoting the activation of stress kinases and hepatocyte cell death-related pathways[xi]. Then, the repair mechanisms including proliferation and fibrosis are activated, which will be decisive for the disease to develop into liver cirrhosis or HCC.

Main unresolved clinical issues in MAFLD

Epidemiological modelling studies have shown that MAFLD incidence and prevalence is rapidly increasing and advanced-MAFLD will be one of the main causes of liver-related complications, liver transplantations and liver-related deaths in the near future[xii]. The main challenges that clinicians will face in this context are: (i) Identification of patients that will progress to advanced-NAFLD and rapid-progressors: the main strategy to overcome this challenge would be to understand deeply the intrahepatic and extrahepatic mechanisms of disease progression and discover new non-invasive test with pathophysiological profile[xiii, xiv]; (ii) Determination of hepatic venous pressure gradient (HVPG) as the main predictor for clinical decompensation in patients with MAFLD, and validation of the current cut-offs of HVPG defined in other aetiologies for MAFLD patients: Previous data from retrospective studies showed that HVPG in MAFLD is not as accurate as in other aetiologies[xv, xvi], therefore the thorough characterization of advanced MAFLD patients with longitudinal assessment would help to identify the best cut-offs and to assess their prognostic value. (iii) Evaluation of the usefulness of non-invasive tests for the follow-up in MAFLD patients in

order to assess disease progression, clinically significant portal hypertension (PH) or signs of oesophageal varices: Recent Baveno Consensus proposed the use of non-invasive tests to avoid invasive assessment in patients with PH signs, but data coming from MAFLD patients are still scarce[xvii]. The prospective longitudinal assessment of patients with MAFLD will generate data regarding non-invasive tests and transient elastography and their role in follow-up to predict clinical outcomes. (iv) Multidisciplinary management of advanced-MAFLD patients: Management of comorbidities in MAFLD patients is key in order to avoid disease progression. Among them, the incorporation of combined treatments and monitorization for potential side-effects, the identification of the best time-frame and the best clinical approach (surgical *vs.* endoscopic) and the best candidates for obesity in MAFLD patients are crucial for MAFLD management[xviii, xix, xx].

Role of sinusoidal cells in MAFLD

Non-parenchymal liver cells, mainly liver sinusoidal endothelial cells (LSECs), hepatic stellate cells (HSCs) and resident macrophages, play essential roles maintaining liver homeostasis and their de-regulation represent a key underlying mechanism of all hepatopathies. Indeed, the complexity of the hepatic sinusoidal milieu is defined by specific functions of each cell type together with intense paracrine communication between them[xxi]. In the context of fatty liver disease, all sinusoidal cells become dysfunctional. Different studies have described the rapid de-differentiation of LSECs upon administration of high fat diet (HFD) in animal models, which become vasoconstrictor and pro-inflammatory. Subsequently, the dysfunctional endothelium activates and recruits local and systemic myeloid cells, thus promoting an amplification of the damage, and paracrinally affects HSCs which start to become activated[xxii]. As MAFLD progresses and together with hepatocyte dysfunction and death, sinusoidal dysfunctionality is further aggravated resulting in disease-modifying consequences including the development of hepatic microcirculatory dysfunction

and PH, exacerbation of hepatic and systemic inflammation, and synthesis and release of large amounts of extracellular matrix (ECM) components resulting in hepatic fibrosis. Interestingly, recent studies propose a change in the paradigm demonstrating that the biomechanical properties of the chronically injured liver (i.e. high stiffness and high vascular tone) are not only consequences of the disease but active players in the aggravation and perpetuation of sinusoidal cells dysfunctionality and liver disease[xxiii], and therefore potential targets for therapy.

Role of fat in MAFLD

In 2020, an international expert consensus panel proposed a new definition of fatty liver, MAFLD, which is based on a set of positive diagnostic criteria for fatty liver disease associated with metabolic dysfunction[i]. The guiding sign is the hepatic steatosis, which can be evidenced by biopsy, imaging or blood biomarker. To date, there are no data supporting an association between the degree of steatosis and the risk of MAFLD progression or clinical outcomes. However, experimental studies showed that PH may begin to develop in the absence of fibrosis[xxiv]. Indeed, preliminary data in MAFLD patients suggest that the degree of steatosis may be associated with PH, but the clinical relevance of this subclinical PH (6-9.5 mmHg) is unknown until date[xxv]. On the other hand, the liver fat content could be relevant for treatment monitoring. Emerging data support the use of magnetic resonance imaging derived proton density fat fraction (MRI-PDFF), a non-invasive and quantitative measure of liver fat content, for treatment response assessment in NASH trials[xxvi, xxvii]. Finally, visceral fat constitutes a relevant type of fat in MAFLD pathogenesis, since the expansion of this fat is associated with MAFLD progression and development of cardiovascular disease, the main cause of mortality among MAFLD patients.

Immune and systemic inflammatory disorders in MAFLD

Evolution has selected those species best endowed to survive and reproduce in a given environment, both from a metabolic (nutrition) and an immune/inflammatory (defense against pathogens) point of view. This would explain the close relationship between metabolism, immunity and inflammation along the entire evolutionary chain up to humans[xxviii]. Moreover, humans are adapted to the lack of nutrients after millions of years of being hunter-gatherers. The excess of nutrients is a consequence of the emergence of agriculture, livestock and the industrial revolution, very recent phenomena leading to a new situation to which we have not yet adapted. This would be the evolutionary explanation for the current epidemic of obesity and MAFLD [xxviii]. Systemic inflammation in obesity is triggered by ischemia of hypertrophic adipose tissue, whose capillaries are not sufficient to oxygenate it properly. However, in later stages, various organs including the liver contribute to the systemic inflammation that in turn affect the rest of the body [xxviii,xxix]. This explains the frequent coincidence of MAFLD with extrahepatic diseases such as psoriasis, cardiovascular events (a major cause of mortality in patients with MAFLD) and cognitive impairment [xxviii, xxx].

Systemic inflammation in MAFLD has a variable intensity in a given patient due to the fluctuating nature of the injuries causing the disease[xxxi]. As a consequence, determining the parameters of systemic inflammation is not yet useful in the diagnosis, prognosis or treatment of patients in clinical practice[xxxii], although it may be in the future [xxix].

Metabolic alterations of bile acids during MAFLD progression

Bile acids are key components of bile that perform essential functions beside facilitate the absorption of dietary lipids. Thus, they participate in the homeostasis of hepatic lipid and glucose metabolism and energy expenditure acting as signaling molecules through nuclear and membrane receptors such as the nuclear farnesoid X receptor (FXR) and the membrane Takeda G protein-coupled receptor 5 (TGR5)[xxxiii].

Several studies have described alterations in serum/plasma bile acid levels and/or in the proportion of molecular species in MAFLD patients[xxxiv], which overall indicated that bile acids may play a role in the pathophysiology and progression of MAFLD. However, the results are not consistent, mainly because there is a strong association of this metabolic condition with obesity, insulin resistance, and T2DM, where alterations in bile acid metabolism already occur, but also because some studies do not have appropriate patient matching, with control groups presenting lower body mass index (BMI) or fasting glycemia. The comparison of fasting plasma bile acids in obese subjects with and without steatohepatitis matched for BMI and insulin resistance found no differences in total bile acid levels or in the proportion of molecular species[xxxv], but a more complete study concluded that plasma bile acid concentrations were elevated in steatohepatitis patients with severe insulin resistance[xxxvi]. Future studies may clarify the role of bile acids in the development of MAFLD and their potential therapeutic utility.

Advanced MASH - Pathophysiology of decompensated cirrhosis

Decompensated cirrhosis is associated to poor prognosis specially when acute-on-chronic liver failure occurs. Even though the mechanisms associated to this condition are partially understood, several interacting key factors have been identified.

(i) Portal hypertension: Portal pressure is determined by the interaction between vascular resistance and portal blood flow. Increased vascular resistance, the initial factor in PH development, has two components. The first is structural, associated to the architectural disturbance characteristics of cirrhosis (fibrosis, parenchymal extinction, sinusoidal capillarization etc.)[xxxvii]. The second is the dynamic component caused by the dysregulation of liver vascular tone and by the activation of contractile cells such as myofibroblasts and hepatic stellate cells. PH is the most important factor in early stages of cirrhosis and its worsening is strongly related to disease progression[xxxviii]. (ii) Systemic hemodynamics: PH

promotes marked splanchnic vasodilation leading to effective hypovolemia and hyperdynamic circulation, activation of homeostatic compensating systems (sympathetic nervous system, non-osmotic secretion of antidiuretic hormone, and activation of renin-angiotensin system), sodium and water retention, and renal failure[xxxix]. In advanced stages, cardiac systolic and diastolic function may be also affected, contributing to circulatory derangements. (iii) Systemic inflammation: There is growing evidence indicating the existence of marked systemic inflammation in cirrhosis. Initially triggered by damage-associated molecular patterns (DAMPS) and pathogen-associated molecular patterns (PAMPS) overproduction, the continuous stimulation of innate immune system cells induces the overproduction of inflammatory mediators that extended damage to different organs. Interestingly, the intensity of inflammatory response is associated with acute-on-chronic liver failure (ACLF) development and prognosis[xl]. Additionally, albumin dysfunction, caused not only by a decrease in its synthesis but also due to several posttranscriptional changes, increases the severity of inflammation and worsens prognosis[xli]. (iv) Metabolic alterations and mitochondrial dysfunction: The exacerbated systemic inflammation promotes a catabolic state, similar to the observed in sepsis and severe trauma, with mitochondrial dysfunction and shifting of ATP production from oxidative phosphorylation to a less efficient aerobic glycolysis. Additionally, the increase in energetic needs derived from systemic inflammation lead to peripheral organs to hypometabolism, dysfunction and failure[xlii].

Carcinogenesis in MAFLD

Among other complications, individuals with MAFLD are at higher risk than healthy individuals of malignancies, predominantly HCC, but also other extra-hepatic cancers[xliii]. Indeed, although the incidence of HCC in MAFLD patients is lower than that of other liver diseases, the ongoing global epidemic of MAFLD is causing a worldwide increase in HCC incidence[xliv]. Unlike other aetiologies, a high percentage of MAFLD patients develop HCC

without cirrhosis [xliv]. MAFLD-related HCC molecular features are not yet well defined, however as far as it is known to date, they do not differ much compared to another HCC aetiologies. Notably, these tumors display higher rates of *ACVR2A* mutations, and are enriched in bile and fatty acid signaling, oxidative stress and inflammation, and present a higher fraction of Wnt/TGF-β proliferation subclass tumours[xlv]. Even more pronounced than HCCs of other aetiologies, the majority of MAFLD-associated HCCs are diagnosed at advanced or very advanced stage[xlvi]. Importantly, recent evidence shows that although immunotherapy improved survival of advanced HCCs, it was not superior in patients with non-viral HCC, particularly NASH–HCC, probably owing to NASH-related aberrant T cell activation[xlvii]. Future research is still needed to better understand MAFLD-related HCC and to develop specific biomarkers and therapeutic options.

2- *Update on the mechanisms of liver injury in MAFLD*

Adipose tissue and liver crosstalk

Currently, it is well-accepted that there is a close crosstalk between the adipose tissue and the liver[xlviii]. The excess of fat is commonly correlated with a generalized proinflammatory state and the elevated production of adipokines is likely to play a role in the pathogenesis of MAFLD, which can progress to advanced stages such as fibrosis. However, different cells in the liver display a divergent response to the excess of fat. While hepatocytes store more fatty acids (namely steatosis), HSCs (the primary fibrogenic cells) get activated and lose their intracellular lipids normally observed when they are in a quiescent stage[xlix]. The mechanism mediating the reduction of fatty acids in activated HSCs remain largely unknown but there seems to be related with a decrease in the expression of adipogenic genes[l]. Identifying the causal pathways mediating this crucial event for the activation of these fibrogenic cells might open new avenues to find potential targets for the treatment of fibrosis.

Mitochondrial (dys)function in MAFLD

Besides, mitochondria play a plethora of functions in the liver which include regulation of cellular signaling, energetics and redox balance. In patients with fatty liver, fatty acids are preferably oxidized through fatty acid oxidation into acetyl-CoA and further metabolized through the tricarboxylic acid (TCA) cycle[li] and oxidative phosphorylation[lii] rather than safely disposed through ketogenesis[liii]. Under these circumstances, excessive oxidative burst in the mitochondria results in exacerbated production of reactive oxygen species and oxidative stress. Increased oxidative stress markers with lowering of the hepatic antioxidant machinery and decreased mitochondria biogenesis cause mitochondrial damage. In fact, mitochondria structural and functional impairment with reduced respiratory capacity and decreased activity of the respiratory complexes are hallmarks of NASH [lii]. In the last years, even though therapies targeting mitochondria have been proposed for the treatment of MAFLD, these are still rather experimental. In fact, it has been recently shown that the inhibition of Glutaminase 1[liv], a mitochondrial enzyme, as well as silencing an endogenous inhibitor of the complex I of the electron transport chain, the methylation-controlled J protein (MCJ)[lv], ameliorates liver steatosis in mouse models of diet-induced MAFLD by different underlying mechanisms.

Oxidative stress and cell death

As introduced above, oxidative stress is the result of an imbalance between the production of reactive oxygen (ROS) and nitrosative (RNS) species and the antioxidant capacity. Oxidative stress includes superoxide ($O_2^{\bullet-}$), hydroxyl ($^{\bullet}OH$), and hydrogen peroxide (H_2O_2) radicals. Apart from mitochondria, other subcellular structures or organelles, including the plasma membrane, endoplasmic reticulum, and peroxisomes contribute to the production of oxidative stress. The antioxidant system may rely on enzymatic and non-enzymatic reactions. The enzymatic system comprises superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), and glutathione-S-transferase (GST)[lvi]. Excessive oxidative stress can

result in lipid peroxidation and cause damage to proteins and DNA. Peroxidation of membrane lipids leads to both functional and structural damage, which finally results in cell death.

The mode of cell death is not only pivotal in directing the severity but also the outcome of liver injury. Apoptosis, necrosis, necroptosis, autophagy, pyroptosis and ferroptosis[lvii] overlap and even crosstalk in a variety of liver diseases including MAFLD/MASH, where apoptotic effectors CASP3/6/7/8 are predominantly involved in intrinsic (via lipotoxicity and organelle stress) as well as in extrinsic (via cell surface receptors) apoptosis thus driving inflammation, whilst the role of necroptosis via the involvement of RIPK1/3/MLKL might lead to fibrosis and metabolic changes[lviii].

Inflammation and fibrogenesis

As described above, hepatocyte and endothelial injury triggers multiple proinflammatory and profibrogenic pathways and promotes the release of extracellular vesicles, activating other liver cell populations and contributing to MAFLD progression. Monocyte recruitment to the damaged liver and its polarization in inflammatory macrophages is promoted by chemokine release by Kupffer cells (KCs) and other activated non-parenchymal cells including HSCs. The diversity of liver macrophage subsets and their plasticity adapting to changes in the microenvironment explain their different functional responses in MAFLD, in which they regulate inflammation, fibrosis, and tumor progression, as well as tissue repair[lix]. Activated HSC are the main source of ECM and drive the fibrotic response to damage and the development of cirrhosis. Besides some well-known proliferative and profibrogenic cytokines, novel routes are emerging as important regulators of HSC activation, such as Hippo-Yap and Notch pathways[lx, lxi].

Recent studies are unveiling the enormous complexity of liver biology. In this line, two different populations of liver-resident macrophages with inflammatory and

immunoregulatory functions have been described, and several subclasses of HSCs have been also defined in murine models of steatohepatitis, including a subclass of activated HSCs with properties similar to inflammatory fibroblasts associated with cancer. Although further research is needed, these transcriptomic analyzes provide new insights into cell heterogeneity and its role in liver disease, and offer hope that specific cell subtypes may be targeted by precision drugs to reduce inflammation or fibrosis[lxii, lxiii].

3- How to approach MAFLD

Genetics, epigenetics and other risk factors

A really critical unanswered question is why there are certain patients that progress to severe symptomatic states, whereas another important group does not. The reasons for this interindividual variability are not completely understood but can be at least partially attributed to differences in genetic background, epigenetic modifications and also in new recently described events known as epitranscriptomics. Different variants of genes mainly implicated in the cellular metabolism of lipids in the liver define the genetic risk factors for MAFLD. As such, the most relevant loci affecting MAFLD are PNPLA3 (rs738409 C>G), TM6SF2 (rs58542926 C>T), GCKR (rs1260326), MBOAT7 (rs641738 C>T) or HSD17B13 (rs6834314) among others[lxiv]. Epigenetics is the second branch able to explain the variability, participating in the development and progression of fatty liver to MASH. Methylation of DNA, chemical modification of histone tails and non-coding RNA-mediated regulation are the principal epigenetic events, many of them involved in hepatic lipid metabolism, insulin resistance, mitochondrial disfunction and oxidative stress thus participating in the development and progression of fatty liver to MASH[lxv]. Additionally, epitranscriptomics describe chemical RNA modifications, also dynamic and reversible, that controls its structure and function without affecting its sequence. To date, more than 100 different chemical RNA modifications have been identified, being N6 -methyladenosine (m6 A) the most

characterized m6 A modification that plays an important role in glucose and lipid homeostasis, while some m6 A regulators are involved in the progression of MAFLD[lxvi].

A deep knowledge and integrative analysis of the genetic, epigenetic and epitranscriptomics modifiers and events can help enormously for individual risk stratification and constitute the basis for further developing prevention and treatment strategies.

Nutritional geometry in MAFLD

The understanding of the effect of nutrients on MAFLD, in order to define nutritional interventions for treatment and prevention, is unclear[lxvii]. Nutritional geometry is a novel approach that analyzes how nutrients and foods can be combined in a system that allows us to know the interaction of foods to regulate the properties of diets that affect health. It consists of the graphical representation of n-dimensions of nutrients in a diet and their comparison by response surfaces of different physiological or health/disease parameters. Using this geometric approach, it was shown that in humans energy intake increases as protein intake decreases, a phenomenon known as "protein leverage"[lxviii]. A study with mice showed that macronutrient composition of diets determines the probability of having fatty liver disease, such that diets low in proteins and high in fats are the strongest drivers of MAFLD[lxix]. This may indicate, that for MAFLD treatment, we should consider weight loss and the proportion of macronutrients, their quality and the overall intake of energy. Further studies are warranted to evaluate whether MAFLD patients have an adequate protein intake, with a higher energy intake coming from the consumption of high-fat foods.

Role of the microbiome in immunological and inflammatory alterations in MAFLD

Intestinal microbiota dysbiosis has a deep impact in the hepatic immune response in MAFLD. Though healthy gut microbial phylum ratio is higher in Bacteroides *vs.* Firmicutes, MAFLD and further advanced stages show a disturbance in this ratio towards Firmicutes and Proteobacteria phylum, with increasing bacterial abundance and decreasing bacterial

diversity[lxx]. The unbalance of short chain fatty acids (SCFA) production, bile acids pool and dysbiotic microbial products induce a potent liver immune response via TLR activation of hepatocytes, KCs and HSC[lxxi]. In a multicenter study, MAFLD patients with BMI above 30 kg/m^2 showed a higher number of different antigens in serum and increased toll-like receptor (TLR) expression in peripheral mononuclear blood cells (PMBCs) both at RNA and protein levels[lxxii]. Independently of BMI, when bacterial antigen was present in serum, there was a significant increase of TNF-α and IL-6.

Though antigen-specific response of resident hepatic immune cells is essential, mucosal-associated invariant T (MAIT) cells responding to bacterial riboflavin metabolite are emerging potential contributors. They produce IL-17 and IL-22 favoring the intestinal barrier in steady state and access the liver to promote regulatory macrophage activation as shown in MCD murine model[lxxiii]. Interestingly, new potential contribution in fueling MAFLD has been proposed by described inflammatory hepatic Th17 (ihTh17) in obesity[lxxiv]. This CXCR3+ subset shows increased glycolytic capacity and produces IL-17, IFN-γ and TNF-α, driving to NAFLD worsening.

4- Methods and technologies for the study of MAFLD

Omics & Exosomes

Extracellular vesicles (EVs) constitute a novel biological entity that has awaked great interest to identify biomarkers, and as active players in the development of liver diseases. Omics technologies have been widely applied to characterize the content and function of EVs secreted by liver cells in different pathological scenarios including drug-induced liver injury (DILI), NAFLD and MS[lxxv]. Thus, transcriptomics and proteomics of these EVs have provided several low invasive candidate biomarkers for cirrhosis in serum[lxxvi] and urine[lxxvii]. The transcriptomic analysis of EVs and the cells that secrete those EVs made possible the identification of a sorting RNA signal that can incorporate the RNAs into the EVs to be

exported out of the cells[lxxviii]. Another important contribution of the omics technologies to the hepatic EVs, in this case done by metabolomics has been the demonstration that hepatic EVs carry several active enzymes that are able to modify the serum metabolic composition that could have important implications for endothelial functioning[lxxix]. The integration of several "omics" technologies combined in different experimental settings including the analysis of the cells, the EVs secreted by those cells, and the cells exposed to those EVs allow to dissect the EVs-mediated mechanisms underlying the development and progression of liver diseases and it provides novel therapeutics targets.

Application of a wide range of "omics" to MAFLD has provided a huge amount of information valuable from a clinical perspective [lxxxii]. First, "omics" have greatly enhanced our knowledge of the pathophysiology and mechanisms of the disease, revealing relevant risk factors such as polymorphisms of PNPLA3 or other genes[lxxx] and potential epigenomic[lxxxi] and metabolomic[lxxxii] pathways amenable to diagnostic or therapeutic exploitation. Second, information from "omics" may help to predict the response to specific therapeutic interventions[**lxxxiii**]. Finally, "omics" have a great potential for identifying non-invasive biomarkers for the diagnosis, staging and monitoring of MAFLD. In this sense, specific miRNAs, metabolomic/lipidomic factors, and the combination of different "omics" have shown considerable ability to detect and differentiate the stages of disease [lxxxii]. Despite their potential, no "omics"-based tools have demonstrated to be superior to current and more simple tools outside of the trials in which they were described, and they are not recommended for routine medical practice in the most recent MAFLD European and American guidelines[lxxxiv], [lxxxv]. Further insights into the natural history of MAFLD, development of specific therapies, better validation studies, and further technological improvements may all be needed for "omics" to fully integrate into clinical practice.

Technological advances in the study of MAFLD

Over the past century, novel technological advances have driven discoveries related to both hepatocyte organization and function. This includes hepatocyte separation techniques, novel immunohistochemistry and microscopy approaches, cell sorting and single cell RNA sequencing, among others[lxxxvi]. Despite its high prevalence, this disease is still lacking from pharmacological therapies to prevent and treat the MAFLD outbreak, therefore, preclinical research is crucial to identify and test new therapeutic agents. The absence of models able to reflect the unique cellular structure recapitulating the liver microenvironment constitutes a significant limiting factor in MAFLD. Thus, novel 3D models have been established from different cells sources, including spheroids, derived from different hepatic cell types, and hepatic organoids, produced by stem cell differentiation in parenchymal and non-parenchymal liver cells[lxxxvii]. Organoids are 3D physiological *in vitro* structures that recapitulate morphological and functional features of *in vivo* tissues and offer significant advantages over traditional cell culture methods[lxxxviii]. Besides, livers-on-a chip are designed to mimic the physiological microenvironment of the hepatic lobule, even reproducing blood circulation[lxxxix]. More recently, it has been successfully developed a microfluidic NASH-on-a-chip platform that recapitulates the main NASH histologic endpoints in a single chip and that can emerge as a human-relevant, *in vitro* platform to study disease pathogenesis and develop novel anti-NASH drugs[xc]. Finally, precision cut liver slices from rat or human origin retain the structure and cellular composition of the native liver and represent an improved system to study liver fibrosis compared to two-dimensional mono- or co-cultures[xci].

Animal models of MAFLD

Given the epidemic of "Diabesity" (obesity and T2DM) and concomitant meteoric rise in MAFLD there is an urgent need in preclinical animal models. The theoretical "ideal" MAFLD model should: (i) fully recapitulate the liver phenotype (macrovesicular steatosis, inflammation, hepatocellular ballooning and fibrosis) plus features of the associated

metabolic syndrome (dyslipidemia, adiposity, insulin resistance), (ii) have the ability to further progress to advanced fibrosis, cirrhosis and ultimately HCC, (iii) be stable, reliable and reproducible, (iv) have high success rate and low mortality, (v) be simple and feasible[xcii]. All existing models of MAFLD can be broadly classified into:

1. Dietary models including: a) Overnutrition-diets with high fat and/or rich in saturated fatty acids, fructose and cholesterol (HFD, Western diet, ALIOS)[xciii]; b) Deficient diets lacking methionine and/or choline (MCD, CDAA).

2. Genetic models with different genetic alterations leading to hepatic lipid accumulation (ob/ob, db/db, PTEN knock-out, DIAMOND mice).

3. "Hybrid" or intensified models presenting the combination of dietary or genetic factors with other hepatotoxins (carbon tetrachloride- CCl_4, alcohol, DUAL model, streptozotocin)[xciv, xcv]

No single animal model has encompassed the whole spectrum of human MAFLD progression but could simulate particular characteristics of the disease. Therefore, the appropriate selection strongly depends on the specific research questions being addressed [xcii].

The future in animal models

Currently there exists a poor rate of translation from the bench to the bedside. One possible explanation could be the failure of preclinical animal models to predict clinical efficacy and safety. Thus, it is important to improve the validity of animal models. One problem that undermine their use is the species differences between animals (mouse) and humans[xcvi]. Nevertheless, an increased methodological rigor in the way animal research is planned, conducted, reported, analyzed and interpreted is important to overcome the quality of preclinical studies. Preclinical studies should be conducted, reported and analyzed like clinical trials. Recently, it has been developed a tool to validate the clinical translatability of animal model[xcvii]. This may help to select the most relevant model. Molecular and cellular

pathways responsible for MAFLD progression are not well understood. This complicates the look for an ideal MAFLD preclinical model. A MAFLD mouse model may ideally exhibit weight gain, adipose inflammation, insulin resistance, glucose intolerance and the complete pathological spectrum from MAFLD to MASH, including fibrosis[xcviii]. New technologies and research approaches, the coordination with clinicians and the development of animal models' platforms to model their heterogeneity will help us to develop relevant animal models for MAFLD.

5- *Conclusions*

MAFLD is the term for a range of conditions caused by a build-up of fat in the liver, and it is usually seen in people who are overweight or obese. This disease encompasses a spectrum of histological liver changes ranging from simple steatosis to the concomitant presence of inflammation and ballooning, which define metabolic-associated steatohepatitis (MASH). Diverse pathologic events, occurring in different cell types, contribute to MAFLD development and progression, and therefore represent potential targets for therapeutic strategies. Future translational research in the field should combine multidisciplinary expertise, the use of conventional and new methods, together with proof-of-concept studies using human-based advanced models.

REFERENCES

[i] Eslam M, Newsome PN, Sarin SK, Anstee QM, Targher G, Romero-Gomez M, Zelber-Sagi S, Wai-Sun Wong V, Dufour JF, Schattenberg JM, Kawaguchi T, Arrese M, Valenti L, Shiha G, Tiribelli C, Yki-Järvinen H, Fan JG, Grønbæk H, Yilmaz Y, Cortez-Pinto H, Oliveira CP, Bedossa P, Adams LA, Zheng MH, Fouad Y, Chan WK, Mendez-Sanchez N, Ahn SH, Castera L, Bugianesi E, Ratziu V, George J. A new definition for metabolic dysfunction-associated fatty liver disease: An international expert consensus statement. J Hepatol. 2020 Jul;73(1):202-209. doi: 10.1016/j.jhep.2020.03.039.

[ii] Estes C, Anstee QM, Arias-Loste MT, Bantel H, Bellentani S, Caballeria J, Colombo M, Craxi A, Crespo J, Day CP, Eguchi Y, Geier A, Kondili LA, Kroy DC, Lazarus JV, Loomba R, Manns MP, Marchesini G, Nakajima A, Negro F, Petta S, Ratziu V, Romero-Gomez M, Sanyal A, Schattenberg JM, Tacke F, Tanaka J, Trautwein C, Wei L, Zeuzem S, Razavi H. Modeling NAFLD disease burden in China, France, Germany, Italy, Japan, Spain, United Kingdom, and United States for the period 2016-2030. J Hepatol. 2018 Oct;69(4):896-904. doi: 10.1016/j.jhep.2018.05.036.

[iii] Sheka AC, Adeyi O, Thompson J, Hameed B, Crawford PA, Ikramuddin S. Nonalcoholic Steatohepatitis: A Review. JAMA. 2020 Mar 24;323(12):1175-1183. doi: 10.1001/jama.2020.2298.

[iv] Ampuero J, Aller R, Gallego-Durán R, Crespo J, Calleja JL, García-Monzón C, Gómez-Camarero J, Caballería J, Lo Iacono O, Ibañez L, García-Samaniego J, Albillos A, Francés R, Fernández-Rodríguez C, Diago M, Soriano G, Andrade RJ, Latorre R, Jorquera F, Morillas RM, Escudero D, Estévez P, Guerra MH, Augustín S, Banales JM, Aspichueta P, Benlloch S, Rosales JM, Salmerón J, Turnes J, Romero Gómez M; HEPAmet Registry. Significant fibrosis predicts new-onset diabetes mellitus and arterial hypertension in patients with NASH. J Hepatol. 2020 Jul;73(1):17-25. doi: 10.1016/j.jhep.2020.02.028.

[v] McHenry S, Sharma Y, Tirath A, Tsai R, Mintz A, Fraum TJ, Salter A, Browning JD, Flores AG, Davidson NO, Fowler KJ, Ciorba MA, Deepak P. Crohn's Disease Is Associated With an Increased Prevalence of Nonalcoholic Fatty Liver Disease: A Cross-Sectional Study Using Magnetic Resonance Proton Density Fat Fraction Mapping. Clin Gastroenterol Hepatol. 2019 Dec;17(13):2816-2818. doi: 10.1016/j.cgh.2019.02.045.

[vi] Tovoli F, Negrini G, Farì R, Guidetti E, Faggiano C, Napoli L, Bolondi L, Granito A. Increased risk of nonalcoholic fatty liver disease in patients with coeliac disease on a gluten-free diet: beyond traditional metabolic factors. Aliment Pharmacol Ther. 2018 Sep;48(5):538-546. doi: 10.1111/apt.14910.

[vii] Durán-Vian C, Arias-Loste MT, Hernández JL, Fernández V, González M, Iruzubieta P, Rasines L, González-Vela C, Vaqué JP, Blanco R, Crespo J, González-López MA. High prevalence of non-alcoholic fatty liver disease among hidradenitis suppurativa patients independent of classic metabolic risk factors. J Eur Acad Dermatol Venereol. 2019 Nov;33(11):2131-2136. doi: 10.1111/jdv.15764.

[viii] Heeren J, Scheja L. Metabolic-associated fatty liver disease and lipoprotein metabolism. Mol Metab. 2021 Aug;50:101238. doi: 10.1016/j.molmet.2021.101238.

[ix] Nikolic I, Leiva M, Sabio G. The role of stress kinases in metabolic disease. Nat Rev Endocrinol. 2020 Dec;16(12):697-716. doi: 10.1038/s41574-020-00418-5.

[x] Leiva M, Matesanz N, Pulgarín-Alfaro M, Nikolic I, Sabio G. Uncovering the Role of p38 Family Members in Adipose Tissue Physiology. Front Endocrinol (Lausanne). 2020 Dec 23;11:572089. doi: 10.3389/fendo.2020.572089.

[xi] Cicuéndez B, Ruiz-Garrido I, Mora A, Sabio G. Stress kinases in the development of liver steatosis and hepatocellular carcinoma. Mol Metab. 2021 Aug;50:101190. doi: 10.1016/j.molmet.2021.101190.

[xii] Estes C, Razavi H, Loomba R, Younossi Z, Sanyal AJ. Modeling the epidemic of nonalcoholic fatty liver disease demonstrates an exponential increase in burden of disease. Hepatology. 2018 Jan;67(1):123-133. doi: 10.1002/hep.29466.

[xiii] Govaere O, Cockell S, Tiniakos D, Queen R, Younes R, Vacca M, Alexander L, Ravaioli F, Palmer J, Petta S, Boursier J, Rosso C, Johnson K, Wonders K, Day CP, Ekstedt M, Orešič M, Darlay R, Cordell HJ, Marra F, Vidal-Puig A, Bedossa P, Schattenberg JM, Clément K, Allison M, Bugianesi E, Ratziu V, Daly AK, Anstee QM. Transcriptomic profiling across the nonalcoholic fatty liver disease spectrum reveals gene signatures for steatohepatitis and fibrosis. Sci Transl Med. 2020 Dec 2;12(572):eaba4448. doi: 10.1126/scitranslmed.aba4448.

[xiv] Caussy C, Tripathi A, Humphrey G, Bassirian S, Singh S, Faulkner C, Bettencourt R, Rizo E, Richards L, Xu ZZ, Downes MR, Evans RM, Brenner DA, Sirlin CB, Knight R, Loomba R. A gut microbiome signature for cirrhosis due to nonalcoholic fatty liver disease. Nat Commun. 2019 Mar 29;10(1):1406. doi: 10.1038/s41467-019-09455-9.

[xv] Bassegoda O, Olivas P, Turco L, Mandorfer M, Serra-Burriel M, Tellez L, Kwanten W, Laroyenne A, Farcau O, Alvarado E, Moga L, Vuille-Lessard E, Fortea JI, Ibañez L, Tosetti G, Vanwolleghem T, Larrue H, Burgos-Santamaría D, Stefanescu H, Paternostro R, Cippitelli A, Lens S, Augustin S, Llop E, Laleman W, Trebicka J, Chang J, Masnou H, Zipprich A, Miceli F, Semmler G, Forns X, Primignani M, Bañares R, Puente A, Berzigotti A, Rautou PE, Villanueva C, Ginès P, Garcia-Pagan JC, Procopet B, Bureau C, Albillos A, Francque S, Reiberger T, Schepis F, Graupera I, Hernandez-Gea V. Decompensation in Advanced Nonalcoholic Fatty Liver Disease May Occur at Lower Hepatic Venous Pressure Gradient

Levels Than in Patients With Viral Disease. Clin Gastroenterol Hepatol. 2021 Oct 21:S1542-3565(21)01136-8. doi: 10.1016/j.cgh.2021.10.023.

[xvi] Ferrusquía-Acosta J, Bassegoda O, Turco L, Reverter E, Pellone M, Bianchini M, Pérez-Campuzano V, Ripoll E, García-Criado Á, Graupera I, García-Pagán JC, Schepis F, Senzolo M, Hernández-Gea V. Agreement between wedged hepatic venous pressure and portal pressure in non-alcoholic steatohepatitis-related cirrhosis. J Hepatol. 2021 Apr;74(4):811-818. doi: 10.1016/j.jhep.2020.10.003.

[xvii] Franchis, R. de et al. BAVENO VII - RENEWING CONSENSUS IN PORTAL HYPERTENSION: Report of the Baveno VII Consensus Workshop: personalized care in portal hypertension. J. Hepatol. 0, (2021).

[xviii] Klebanoff MJ, Corey KE, Samur S, Choi JG, Kaplan LM, Chhatwal J, Hur C. Cost-effectiveness Analysis of Bariatric Surgery for Patients With Nonalcoholic Steatohepatitis Cirrhosis. JAMA Netw Open. 2019 Feb 1;2(2):e190047. doi: 10.1001/jamanetworkopen.2019.0047.

[xix] Romero-Gómez M, Zelber-Sagi S, Trenell M. Treatment of NAFLD with diet, physical activity and exercise. J Hepatol. 2017 Oct;67(4):829-846. doi: 10.1016/j.jhep.2017.05.016.

[xx] Abu Dayyeh BK, Bazerbachi F, Graupera I, Cardenas A MD, MMSc, PhD. Endoscopic bariatric and metabolic therapies for non-alcoholic fatty liver disease. J Hepatol. 2019 Dec;71(6):1246-1248. doi: 10.1016/j.jhep.2019.07.026.

[xxi] Marrone G, Shah VH, Gracia-Sancho J. Sinusoidal communication in liver fibrosis and regeneration. J Hepatol. 2016 Sep;65(3):608-17. doi: 10.1016/j.jhep.2016.04.018.

[xxii] Gracia-Sancho J, Caparrós E, Fernández-Iglesias A, Francés R. Role of liver sinusoidal endothelial cells in liver diseases. Nat Rev Gastroenterol Hepatol. 2021 Jun;18(6):411-431. doi: 10.1038/s41575-020-00411-3.

[xxiii] Guixé-Muntet S, Ortega-Ribera M, Wang C, Selicean S, Andreu I, Kechagia JZ, Fondevila C, Roca-Cusachs P, Dufour JF, Bosch J, Berzigotti A, Gracia-Sancho J. Nuclear deformation mediates liver cell mechanosensing in cirrhosis. JHEP Rep. 2020 Jul 17;2(5):100145. doi: 10.1016/j.jhepr.2020.100145.

[xxiv] Baffy G. Origins of Portal Hypertension in Nonalcoholic Fatty Liver Disease. Dig Dis Sci. 2018 Mar;63(3):563-576. doi: 10.1007/s10620-017-4903-5.

[xxv] Baffy G, Bosch J. Overlooked subclinical portal hypertension in non-cirrhotic NAFLD: Is it real and how to measure it? J Hepatol. 2021 Oct 2:S0168-8278(21)02090-0. doi: 10.1016/j.jhep.2021.09.029.

[xxvi] Ajmera V, Park CC, Caussy C, Singh S, Hernandez C, Bettencourt R, Hooker J, Sy E, Behling C, Xu R, Middleton MS, Valasek MA, Faulkner C, Rizo E, Richards L, Sirlin CB, Loomba R. Magnetic Resonance Imaging Proton Density Fat Fraction Associates With Progression of Fibrosis in Patients With Nonalcoholic Fatty Liver Disease. Gastroenterology. 2018 Aug;155(2):307-310.e2. doi: 10.1053/j.gastro.2018.04.014.

[xxvii] Caussy C, Reeder SB, Sirlin CB, Loomba R. Noninvasive, Quantitative Assessment of Liver Fat by MRI-PDFF as an Endpoint in NASH Trials. Hepatology. 2018 Aug;68(2):763-772. doi: 10.1002/hep.29797.

[xxviii] Hotamisligil GS. Inflammation, metaflammation and immunometabolic disorders. Nature. 2017 Feb 8;542(7640):177-185. doi: 10.1038/nature21363.

[xxix] Lefere S, Tacke F. Macrophages in obesity and non-alcoholic fatty liver disease: Crosstalk with metabolism. JHEP Rep. 2019 Feb 23;1(1):30-43. doi: 10.1016/j.jhepr.2019.02.004.

[xxx] Kjærgaard K, Mikkelsen ACD, Wernberg CW, Grønkjær LL, Eriksen PL, Damholdt MF, Mookerjee RP, Vilstrup H, Lauridsen MM, Thomsen KL. Cognitive Dysfunction in Non-Alcoholic Fatty Liver Disease-Current Knowledge, Mechanisms and Perspectives. J Clin Med. 2021 Feb 9;10(4):673. doi: 10.3390/jcm10040673.

[xxxi] Schuppan D, Surabattula R, Wang XY. Determinants of fibrosis progression and regression in NASH. J Hepatol. 2018 Feb;68(2):238-250. doi: 10.1016/j.jhep.2017.11.012.

[xxxii] Zimmermann E, Anty R, Tordjman J, Verrijken A, Gual P, Tran A, Iannelli A, Gugenheim J, Bedossa P, Francque S, Le Marchand-Brustel Y, Clement K, Van Gaal L, Sørensen TIA, Jess T. C-reactive protein levels in relation to various features of non-alcoholic fatty liver disease among obese patients. J Hepatol. 2011 Sep;55(3):660-665. doi: 10.1016/j.jhep.2010.12.017.

[xxxiii] Marin JJ, Macias RI, Briz O, Banales JM, Monte MJ. Bile Acids in Physiology, Pathology and Pharmacology. Curr Drug Metab. 2015;17(1):4-29. doi: 10.2174/1389200216666151103115454.

[xxxiv] Chávez-Talavera O, Haas J, Grzych G, Tailleux A, Staels B. Bile acid alterations in nonalcoholic fatty liver disease, obesity, insulin resistance and type 2 diabetes: what do the human studies tell? Curr Opin Lipidol. 2019 Jun;30(3):244-254. doi: 10.1097/MOL.0000000000000597.

[xxxv] Legry V, Francque S, Haas JT, Verrijken A, Caron S, Chávez-Talavera O, Vallez E, Vonghia L, Dirinck E, Verhaegen A, Kouach M, Lestavel S, Lefebvre P, Van Gaal L, Tailleux A, Paumelle R, Staels B. Bile Acid Alterations Are Associated With Insulin Resistance, but Not With NASH, in Obese Subjects. J Clin Endocrinol Metab. 2017 Oct 1;102(10):3783-3794. doi: 10.1210/jc.2017-01397.

[xxxvi] Grzych G, Chávez-Talavera O, Descat A, Thuillier D, Verrijken A, Kouach M, Legry V, Verkindt H, Raverdy V, Legendre B, Caiazzo R, Van Gaal L, Goossens JF, Paumelle R, Francque S, Pattou F, Haas JT, Tailleux A, Staels B. NASH-related increases in plasma bile acid levels depend on insulin resistance. JHEP Rep. 2020 Dec 16;3(2):100222. doi: 10.1016/j.jhepr.2020.100222.

[xxxvii] Gracia-Sancho J, Marrone G, Fernández-Iglesias A. Hepatic microcirculation and mechanisms of portal hypertension. Nat Rev Gastroenterol Hepatol. 2019 Apr;16(4):221-234. doi: 10.1038/s41575-018-0097-3.

[xxxviii] Garcia-Tsao G, Abraldes JG, Berzigotti A, Bosch J. Portal hypertensive bleeding in cirrhosis: Risk stratification, diagnosis, and management: 2016 practice guidance by the American Association for the study of liver diseases. Hepatology. 2017 Jan;65(1):310-335. doi: 10.1002/hep.28906.

[xxxix] Angeli P, Garcia-Tsao G, Nadim MK, Parikh CR. News in pathophysiology, definition and classification of hepatorenal syndrome: A step beyond the International Club of Ascites (ICA) consensus document. J Hepatol. 2019 Oct;71(4):811-822. doi: 10.1016/j.jhep.2019.07.002.

[xl] Arroyo V, Angeli P, Moreau R, Jalan R, Clària J, Trebicka J, Fernández J, Gustot T, Caraceni P, Bernardi M; investigators from the EASL-CLIF Consortium, Grifols Chair and European Foundation for the Study of Chronic Liver Failure (EF-Clif). The systemic inflammation hypothesis: Towards a new paradigm of acute decompensation and multiorgan failure in cirrhosis. J Hepatol. 2021 Mar;74(3):670-685. doi: 10.1016/j.jhep.2020.11.048.

[xli] Bernardi M, Angeli P, Claria J, Moreau R, Gines P, Jalan R, Caraceni P, Fernandez J, Gerbes AL, O'Brien AJ, Trebicka J, Thevenot T, Arroyo V. Albumin in decompensated cirrhosis: new concepts and perspectives. Gut. 2020 Jun;69(6):1127-1138. doi: 10.1136/gutjnl-2019-318843.

[xlii] Moreau R, Clària J, Aguilar F, Fenaille F, Lozano JJ, Junot C, Colsch B, Caraceni P, Trebicka J, Pavesi M, Alessandria C, Nevens F, Saliba F, Welzel TM, Albillos A, Gustot T, Fernández J, Moreno C, Baldassarre M, Zaccherini G, Piano S, Montagnese S, Vargas V, Genescà J, Solà E, Bernal W, Butin N, Hautbergue T, Cholet S, Castelli F, Jansen C, Steib C, Campion D, Mookerjee R, Rodríguez-Gandía M, Soriano G, Durand F, Benten D, Bañares R, Stauber RE, Gronbaek H, Coenraad MJ, Ginès P, Gerbes A, Jalan R, Bernardi M, Arroyo V, Angeli P; CANONIC Study Investigators of the EASL Clif Consortium; Grifols Chair; European Foundation for the Study of Chronic Liver Failure (EF Clif). Blood metabolomics uncovers inflammation-associated mitochondrial dysfunction as a potential mechanism underlying ACLF. J Hepatol. 2020 Apr;72(4):688-701. doi: 10.1016/j.jhep.2019.11.009.

[xliii] Mantovani A, Scorletti E, Mosca A, Alisi A, Byrne CD, Targher G. Complications, morbidity and mortality of nonalcoholic fatty liver disease. Metabolism. 2020 Oct;111S:154170. doi: 10.1016/j.metabol.2020.154170.

[xliv] Huang DQ, El-Serag HB, Loomba R. Global epidemiology of NAFLD-related HCC: trends, predictions, risk factors and prevention. Nat Rev Gastroenterol Hepatol. 2021 Apr;18(4):223-238. doi: 10.1038/s41575-020-00381-6.

[xlv] Pinyol R, Torrecilla S, Wang H, Montironi C, Piqué-Gili M, Torres-Martin M, Wei-Qiang L, Willoughby CE, Ramadori P, Andreu-Oller C, Taik P, Lee YA, Moeini A, Peix J, Faure-Dupuy S, Riedl T, Schuehle S, Oliveira CP, Alves VA, Boffetta P, Lachenmayer A, Roessler S, Minguez B, Schirmacher P, Dufour JF, Thung SN, Reeves HL, Carrilho FJ, Chang C, Uzilov AV, Heikenwalder M, Sanyal A, Friedman SL, Sia D, Llovet JM. Molecular characterisation of hepatocellular carcinoma in patients with non-alcoholic steatohepatitis. J Hepatol. 2021 Oct;75(4):865-878. doi: 10.1016/j.jhep.2021.04.049.

[xlvi] Anstee QM, Reeves HL, Kotsiliti E, Govaere O, Heikenwalder M. From NASH to HCC: current concepts and future challenges. Nat Rev Gastroenterol Hepatol. 2019 Jul;16(7):411-428. doi: 10.1038/s41575-019-0145-7.

[xlvii] Pfister D, Núñez NG, Pinyol R, Govaere O, Pinter M, Szydlowska M, Gupta R, Qiu M, Deczkowska A, Weiner A, Müller F, Sinha A, Friebel E, Engleitner T, Lenggenhager D, Moncsek A, Heide D, Stirm K, Kosla J, Kotsiliti E, Leone V, Dudek M, Yousuf S, Inverso D, Singh I, Teijeiro A, Castet F, Montironi C, Haber PK, Tiniakos D, Bedossa P, Cockell S, Younes R, Vacca M, Marra F, Schattenberg JM, Allison M, Bugianesi E, Ratziu V, Pressiani T, D'Alessio A, Personeni N, Rimassa L, Daly AK, Scheiner B, Pomej K, Kirstein MM, Vogel A, Peck-Radosavljevic M, Hucke F, Finkelmeier F, Waidmann O, Trojan J, Schulze K, Wege H, Koch S, Weinmann A, Bueter M, Rössler F, Siebenhüner A, De Dosso S, Mallm JP, Umansky V, Jugold M, Luedde T, Schietinger A, Schirmacher P, Emu B, Augustin HG, Billeter A, Müller-Stich B, Kikuchi H, Duda DG, Kütting F, Waldschmidt DT, Ebert MP, Rahbari N, Mei HE, Schulz AR, Ringelhan M, Malek N, Spahn S, Bitzer M, Ruiz de Galarreta M, Lujambio A, Dufour JF, Marron TU, Kaseb A, Kudo M, Huang YH, Djouder N, Wolter K, Zender L, Marche PN, Decaens T, Pinato DJ, Rad R, Mertens JC, Weber A, Unger K, Meissner F, Roth S, Jilkova ZM, Claassen M, Anstee QM, Amit I, Knolle P, Becher B, Llovet JM, Heikenwalder M. NASH limits anti-tumour surveillance in immunotherapy-treated HCC. Nature. 2021 Apr;592(7854):450-456. doi: 10.1038/s41586-021-03362-0.

[xlviii] Rosso C, Kazankov K, Younes R, Esmaili S, Marietti M, Sacco M, Carli F, Gaggini M, Salomone F, Møller HJ, Abate ML, Vilstrup H, Gastaldelli A, George J, Grønbæk H, Bugianesi E. Crosstalk between adipose tissue insulin resistance and liver macrophages in non-alcoholic fatty liver disease. J Hepatol. 2019 Nov;71(5):1012-1021. doi: 10.1016/j.jhep.2019.06.031.

[xlix] Tsuchida T, Friedman SL. Mechanisms of hepatic stellate cell activation. Nat Rev Gastroenterol Hepatol. 2017 Jul;14(7):397-411. doi: 10.1038/nrgastro.2017.38.

[l] Trivedi P, Wang S, Friedman SL. The Power of Plasticity-Metabolic Regulation of Hepatic Stellate Cells. Cell Metab. 2021 Feb 2;33(2):242-257. doi: 10.1016/j.cmet.2020.10.026.

[li] Sunny NE, Parks EJ, Browning JD, Burgess SC. Excessive hepatic mitochondrial TCA cycle and gluconeogenesis in humans with nonalcoholic fatty liver disease. Cell Metab. 2011 Dec 7;14(6):804-10. doi: 10.1016/j.cmet.2011.11.004.

[lii] Koliaki C, Szendroedi J, Kaul K, Jelenik T, Nowotny P, Jankowiak F, Herder C, Carstensen M, Krausch M, Knoefel WT, Schlensak M, Roden M. Adaptation of hepatic mitochondrial function in humans with non-alcoholic fatty liver is lost in steatohepatitis. Cell Metab. 2015 May 5;21(5):739-46. doi: 10.1016/j.cmet.2015.04.004.

[liii] Fletcher JA, Deja S, Satapati S, Fu X, Burgess SC, Browning JD. Impaired ketogenesis and increased acetyl-CoA oxidation promote hyperglycemia in human fatty liver. JCI Insight. 2019 Apr 23;5(11):e127737. doi: 10.1172/jci.insight.127737.

[liv] Simon J, Nuñez-García M, Fernández-Tussy P, Barbier-Torres L, Fernández-Ramos D, Gómez-Santos B, Buqué X, Lopitz-Otsoa F, Goikoetxea-Usandizaga N, Serrano-Macia M, Rodriguez-Agudo R, Bizkarguenaga M, Zubiete-Franco I, Gutiérrez-de Juan V, Cabrera D, Alonso C, Iruzubieta P, Romero-Gomez M, van Liempd S, Castro A, Nogueiras R, Varela-Rey M, Falcón-Pérez JM, Villa E, Crespo J, Lu SC, Mato JM, Aspichueta P, Delgado TC, Martínez-Chantar ML. Targeting Hepatic Glutaminase 1 Ameliorates Non-alcoholic Steatohepatitis by Restoring Very-Low-Density Lipoprotein Triglyceride Assembly. Cell Metab. 2020 Mar 3;31(3):605-622.e10. doi: 10.1016/j.cmet.2020.01.013.

[lv] Barbier-Torres L, Fortner KA, Iruzubieta P, Delgado TC, Giddings E, Chen Y, Champagne D, Fernández-Ramos D, Mestre D, Gomez-Santos B, Varela-Rey M, de Juan VG, Fernández-Tussy P, Zubiete-Franco I, García-Monzón C, González-Rodríguez Á, Oza D, Valença-Pereira F, Fang Q, Crespo J, Aspichueta P, Tremblay F, Christensen BC, Anguita J, Martínez-Chantar ML, Rincón M. Silencing hepatic MCJ attenuates non-alcoholic fatty liver disease (NAFLD) by increasing mitochondrial fatty acid oxidation. Nat Commun. 2020 Jul 3;11(1):3360. doi: 10.1038/s41467-020-16991-2.

[lvi]Tang D, Kang R, Berghe TV, Vandenabeele P, Kroemer G. The molecular machinery of regulated cell death. Cell Res. 2019 May;29(5):347-364. doi: 10.1038/s41422-019-0164-5.

[lvii] Galluzzi, L.; Vitale, I.; Aaronson, S.A.; Abrams, J.M.; Adam, D.; Agostinis, P.; Alnemri, E.S.; Altucci, L.; Amelio, I.; Andrews, D.W.*, et al.* Molecular mechanisms of cell death: Recommendations of the nomenclature committee on cell death 2018. *Cell Death Differ* **2018**, *25*, 486-541.

[lviii] Shojaie L, Iorga A, Dara L. Cell Death in Liver Diseases: A Review. Int J Mol Sci. 2020 Dec 18;21(24):9682. doi: 10.3390/ijms21249682.

[lix] Wen Y, Lambrecht J, Ju C, Tacke F. Hepatic macrophages in liver homeostasis and diseases-diversity, plasticity and therapeutic opportunities. Cell Mol Immunol. 2021 Jan;18(1):45-56. doi: 10.1038/s41423-020-00558-8.

[lx] Lee YA, Wallace MC, Friedman SL. Pathobiology of liver fibrosis: a translational success story. Gut. 2015 May;64(5):830-41. doi: 10.1136/gutjnl-2014-306842.

[lxi] Yu HX, Yao Y, Bu FT, Chen Y, Wu YT, Yang Y, Chen X, Zhu Y, Wang Q, Pan XY, Meng XM, Huang C, Li J. Blockade of YAP alleviates hepatic fibrosis through accelerating apoptosis and reversion of activated hepatic stellate cells. Mol Immunol. 2019 Mar;107:29-40. doi: 10.1016/j.molimm.2019.01.004.

[lxii] MacParland SA, Liu JC, Ma XZ, Innes BT, Bartczak AM, Gage BK, Manuel J, Khuu N, Echeverri J, Linares I, Gupta R, Cheng ML, Liu LY, Camat D, Chung SW, Seliga RK, Shao Z, Lee E, Ogawa S, Ogawa M, Wilson MD, Fish JE, Selzner M, Ghanekar A, Grant D, Greig P, Sapisochin G, Selzner N, Winegarden N, Adeyi O, Keller G, Bader GD, McGilvray ID. Single cell RNA sequencing of human liver reveals distinct intrahepatic macrophage populations. Nat Commun. 2018 Oct 22;9(1):4383. doi: 10.1038/s41467-018-06318-7.

[lxiii] Rosenthal SB, Liu X, Ganguly S, Dhar D, Pasillas MP, Ricciardelli E, Li RZ, Troutman TD, Kisseleva T, Glass CK, Brenner DA. Heterogeneity of HSCs in a Mouse Model of NASH. Hepatology. 2021 Aug;74(2):667-685. doi: 10.1002/hep.31743.

[lxiv] Jonas W, Schürmann A. Genetic and epigenetic factors determining NAFLD risk. Mol Metab. 2021 Aug;50:101111. doi: 10.1016/j.molmet.2020.101111.

[lxv] Eslam M, Valenti L, Romeo S. Genetics and epigenetics of NAFLD and NASH: Clinical impact. J Hepatol. 2018 Feb;68(2):268-279. doi: 10.1016/j.jhep.2017.09.003.

[lxvi] Zhao Z, Meng J, Su R, Zhang J, Chen J, Ma X, Xia Q. Epitranscriptomics in liver disease: Basic concepts and therapeutic potential. J Hepatol. 2020 Sep;73(3):664-679. doi: 10.1016/j.jhep.2020.04.009.

[lxvii]Berná G, Romero-Gomez M. The role of nutrition in non-alcoholic fatty liver disease: Pathophysiology and management. Liver Int. 2020. 40:102-108. doi: 10.1111/liv.14360.

[lxviii] Raubenheimer D, Simpson SJ. Nutritional Ecology and Human Health. Annu Rev Nutr. 2016. 36:603-26. doi: 10.1146/annurev-nutr-071715-051118.

[lxix] Simpson SJ, Raubenheimer D, Cogger VC, Macia L, Solon-Biet SM, Le Couteur DG, George J. The nutritional geometry of liver disease including non-alcoholic fatty liver disease. J Hepatol. 2018. 68:316-325. doi: 10.1016/j.jhep.2017.10.005.

[lxx] Behary J, Amorim N, Jiang XT, Raposo A, Gong L, McGovern E, Ibrahim R, Chu F, Stephens C, Jebeili H, Fragomeli V, Koay YC, Jackson M, O'Sullivan J, Weltman M, McCaughan G, El-Omar E, Zekry A. Gut microbiota impact on the peripheral immune response in non-alcoholic fatty liver disease related hepatocellular carcinoma. Nat Commun. 2021 Jan 8;12(1):187. doi: 10.1038/s41467-020-20422-7.

[lxxi] Kolodziejczyk AA, Zheng D, Shibolet O, Elinav E. The role of the microbiome in NAFLD and NASH. EMBO Mol Med. 2019 Feb;11(2):e9302. doi: 10.15252/emmm.201809302.

[lxxii] Gómez-Hurtado I, Gallego-Durán R, Zapater P, Ampuero J, Aller R, Crespo J, Arias-Loste M, García-Monzón C, Bellot P, González-Rodríguez Á, Juanola O, Romero-Gómez M, Francés R. Bacterial antigen translocation and age as BMI-independent contributing factors on systemic inflammation in NAFLD patients. Liver Int. 2020 Sep;40(9):2182-2193. doi: 10.1111/liv.14571.

[lxxiii] Li Y, Huang B, Jiang X, Chen W, Zhang J, Wei Y, Chen Y, Lian M, Bian Z, Miao Q, Peng Y, Fang J, Wang Q, Tang R, Gershwin ME, Ma X. Mucosal-Associated Invariant T Cells Improve Nonalcoholic Fatty Liver Disease Through Regulating Macrophage Polarization. Front Immunol. 2018 Sep 4;9:1994. doi: 10.3389/fimmu.2018.01994.

[lxxiv] Moreno-Fernandez ME, Giles DA, Oates JR, Chan CC, Damen MSMA, Doll JR, Stankiewicz TE, Chen X, Chetal K, Karns R, Weirauch MT, Romick-Rosendale L, Xanthakos SA, Sheridan R, Szabo S, Shah AS, Helmrath MA, Inge TH, Deshmukh H, Salomonis N, Divanovic S. PKM2-dependent metabolic skewing of hepatic Th17 cells regulates pathogenesis of non-alcoholic fatty liver disease. Cell Metab. 2021 Jun 1;33(6):1187-1204.e9. doi: 10.1016/j.cmet.2021.04.018.

[lxxv] Azparren-Angulo M, Royo F, Gonzalez E, Liebana M, Brotons B, Berganza J, Goñi-de-Cerio F, Manicardi N, Abad-Jordà L, Gracia-Sancho J, Falcon-Perez JM. Extracellular vesicles in hepatology: Physiological role, involvement in pathogenesis, and therapeutic opportunities. Pharmacol Ther. 2021 Feb;218:107683. doi: 10.1016/j.pharmthera.2020.107683.

[lxxvi] Garcia Garcia de Paredes A, Manicardi N, Tellez L, Ibañez L, Royo F, Bermejo J, Blanco C, Fondevila C, Fernandez Lanza V, Garcia-Bermejo L, Falcon-Perez JM, Bañares R, Gracia-Sancho J, Albillos A. Molecular Profiling of Decompensated Cirrhosis by a Novel MicroRNA Signature. Hepatol Commun. 2020 Dec 2;5(2):309-322. doi: 10.1002/hep4.1642.

[lxxvii] Gonzalez E, Azkargorta M, Garcia-Vallicrosa C, Prieto-Elordui J, Elortza F, Blanco-Sampascual S, Falcon-Perez JM. Could protein content of Urinary Extracellular Vesicles be useful to detect Cirrhosis in Alcoholic Liver Disease? Int J Biol Sci. 2021 May 5;17(8):1864-1877. doi: 10.7150/ijbs.59725.

[lxxviii] Szostak N, Royo F, Rybarczyk A, Szachniuk M, Blazewicz J, del Sol A, Falcon-Perez JM. Sorting signal targeting mRNA into hepatic extracellular vesicles. RNA Biol. 2014;11(7):836-44. doi: 10.4161/rna.29305.

[lxxix] Royo F, Moreno L, Mleczko J, Palomo L, Gonzalez E, Cabrera D, Cogolludo A, Vizcaino FP, van-Liempd S, Falcon-Perez JM. Hepatocyte-secreted extracellular vesicles modify blood metabolome and endothelial function by an arginase-dependent mechanism. Sci Rep. 2017 Feb 17;7:42798. doi: 10.1038/srep42798.

[lxxx] Romeo S, Kozlitina J, Xing C, Pertsemlidis A, Cox D, Pennacchio LA, Boerwinkle E, Cohen JC, Hobbs HH. Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. Nat Genet. 2008 Dec;40(12):1461-5. doi: 10.1038/ng.257.

[lxxxi] Lee J, Kim Y, Friso S, Choi SW. Epigenetics in non-alcoholic fatty liver disease. Mol Aspects Med. 2017 Apr;54:78-88. doi: 10.1016/j.mam.2016.11.008.

[lxxxii] Perakakis N, Stefanakis K, Mantzoros CS. The role of omics in the pathophysiology, diagnosis and treatment of non-alcoholic fatty liver disease. Metabolism. 2020 Oct;111S:154320. doi: 10.1016/j.metabol.2020.154320.

[lxxxiii] Romeo S, Sanyal A, Valenti L. Leveraging Human Genetics to Identify Potential New Treatments for Fatty Liver Disease. Cell Metab. 2020 Jan 7;31(1):35-45. doi: 10.1016/j.cmet.2019.12.002.

[lxxxiv] Chalasani N, Younossi Z, Lavine JE, Charlton M, Cusi K, Rinella M, Harrison SA, Brunt EM, Sanyal AJ. The diagnosis and management of nonalcoholic fatty liver disease: Practice guidance from the American Association for the Study of Liver Diseases. Hepatology. 2018 Jan;67(1):328-357. doi: 10.1002/hep.29367.

[lxxxv] European Association for the Study of the Liver (EASL); European Association for the Study of Diabetes (EASD); European Association for the Study of Obesity (EASO). EASL-EASD-EASO Clinical Practice Guidelines for the Management of Non-Alcoholic Fatty Liver Disease. Obes Facts. 2016;9(2):65-90. doi: 10.1159/000443344.

[lxxxvi] Cunningham RP, Porat-Shliom N. Liver Zonation - Revisiting Old Questions With New Technologies. Front Physiol. 2021 Sep 9;12:732929. doi: 10.3389/fphys.2021.732929.

[lxxxvii] Xu Q. (2021). Human Three-Dimensional Hepatic Models: Cell Type Variety and Corresponding Applications. *Frontiers in bioengineering and biotechnology, 9,* 730008. https://doi.org/10.3389/fbioe.2021.730008.

[lxxxviii] Nuciforo S, Heim MH. Organoids to model liver disease. JHEP Rep. 2020 Oct 22;3(1):100198. doi: 10.1016/j.jhepr.2020.100198.

[lxxxix] Soret PA, Magusto J, Housset C, Gautheron J. In Vitro and In Vivo Models of Non-Alcoholic Fatty Liver Disease: A Critical Appraisal. J Clin Med. 2020 Dec 24;10(1):36. doi: 10.3390/jcm10010036.

[xc] Freag MS, Namgung B, Reyna Fernandez ME, Gherardi E, Sengupta S, Jang HL. Human Nonalcoholic Steatohepatitis on a Chip. Hepatol Commun. 2020 Nov 29;5(2):217-233. doi: 10.1002/hep4.1647.

[xci] Paish HL, Reed LH, Brown H, Bryan MC, Govaere O, Leslie J, Barksby BS, Garcia Macia M, Watson A, Xu X, Zaki MYW, Greaves L, Whitehall J, French J, White SA, Manas DM, Robinson SM, Spoletini G, Griffiths C, Mann DA, Borthwick LA, Drinnan MJ, Mann J, Oakley F. A Bioreactor Technology for Modeling Fibrosis in Human and Rodent Precision-Cut Liver Slices. Hepatology. 2019 Oct;70(4):1377-1391. doi: 10.1002/hep.30651

[xcii] Nevzorova YA, Boyer-Diaz Z, Cubero FJ, Gracia-Sancho J. Animal models for liver disease - A practical approach for translational research. J Hepatol. 2020 Aug;73(2):423-440. doi: 10.1016/j.jhep.2020.04.011.

[xciii] Estévez-Vázquez O, Benedé-Ubieto R, Guo F, Gómez-Santos B, Aspichueta P, Reissing J, Bruns T, Sanz-García C, Sydor S, Bechmann LP, Maranillo E, Sañudo JR, Vázquez MT, Lamas-Paz A, Morán L, Mazariegos MS, Ciudin A, Pericàs JM, Peligros MI, Vaquero J, Martínez-Naves E, Liedtke C, Regueiro JR, Trautwein C, Bañares R, Cubero FJ, Nevzorova YA. Fat: Quality, or Quantity? What Matters Most for the Progression of Metabolic Associated Fatty Liver Disease (MAFLD). Biomedicines. 2021 Sep 22;9(10):1289. doi: 10.3390/biomedicines9101289.

[xciv] Benedé-Ubieto R, Estévez-Vázquez O, Guo F, Chen C, Singh Y, Nakaya HI, Gómez Del Moral M, Lamas-Paz A, Morán L, López-Alcántara N, Reissing J, Bruns T, Avila MA, Santamaría E, Mazariegos MS, Woitok MM, Haas U, Zheng K, Juárez I, Martín-Villa JM, Asensio I, Vaquero J, Peligros MI, Argemi J, Bataller R, Ampuero J, Romero Gómez M, Trautwein C, Liedtke C, Bañares R, Cubero FJ, Nevzorova YA. An Experimental DUAL Model of Advanced Liver Damage. Hepatol Commun. 2021 Mar 11;5(6):1051-1068. doi: 10.1002/hep4.1698.

[xcv] Maeso-Díaz R, Boyer-Diaz Z, Lozano JJ, Ortega-Ribera M, Peralta C, Bosch J, Gracia-Sancho J. New Rat Model of Advanced NASH Mimicking Pathophysiological Features and Transcriptomic Signature of The Human Disease. Cells. 2019 Sep 10;8(9):1062. doi: 10.3390/cells8091062.

[xcvi] Pound P, Ritskes-Hoitinga M. Is it possible to overcome issues of external validity in preclinical animal research? Why most animal models are bound to fail. J Transl Med. 2018 Nov 7;16(1):304. doi: 10.1186/s12967-018-1678-1.

[xcvii] Ferreira GS, Veening-Griffioen DH, Boon WPC, Moors EHM, Gispen-de Wied CC, Schellekens H, van Meer PJK. A standardised framework to identify optimal animal models for efficacy assessment in drug development. PLoS One. 2019 Jun 13;14(6):e0218014. doi: 10.1371/journal.pone.0218014.

[xcviii] Farrell G, Schattenberg JM, Leclercq I, Yeh MM, Goldin R, Teoh N, Schuppan D. Mouse Models of Nonalcoholic Steatohepatitis: Toward Optimization of Their Relevance to Human Nonalcoholic Steatohepatitis. Hepatology. 2019 May;69(5):2241-2257. doi: 10.1002/hep.30333.