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Dysfunctional VLDL metabolism in MASLD

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Lipidomics has unveiled the intricate human lipidome, emphasizing the extensive diversity within lipid classes in mammalian tissues critical for cellular functions. This diversity poses a challenge in maintaining a delicate balance between adaptability to recurring physiological changes and overall stability. Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD), linked to factors such as obesity and diabetes, stems from a compromise in the structural and functional stability of the liver within the complexities of lipid metabolism. This compromise inaccurately senses an increase in energy status, such as during fasting-feeding cycles or an upsurge in lipogenesis. Serum lipidomic studies have delineated three distinct metabolic phenotypes, or “metabotypes” in MASLD. MASLD-A is characterized by lower very low-density lipoprotein (VLDL) secretion and triglyceride (TG) levels, associated with a reduced risk of cardiovascular disease (CVD). In contrast, MASLD-C exhibits increased VLDL secretion and TG levels, correlating with elevated CVD risk. An intermediate subtype, with a blend of features, is designated as the MASLD-B metabotype. In this perspective, we examine into recent findings that show the multifaceted regulation of VLDL secretion by S-adenosylmethionine, the primary cellular methyl donor. Furthermore, we explore the differential CVD and hepatic cancer risk across MASLD metabotypes and discuss the context and potential paths forward to gear the findings from genetic studies towards a better understanding of the observed heterogeneity in MASLD.

Lipid diversity in humans

Fatty acids (FA) synthesis from not lipid sources including glucose, known as *de novo* lipogenesis (DNL), begins with the conversion of glucose to pyruvate through glycolysis in the cytoplasm. Pyruvate is subsequently converted into acetyl-CoA, a 2-carbon (2C) molecule, in the mitochondria, serving as the precursor for FA synthesis. Acetyl-CoA is then transported from the mitochondria to the cytoplasm, where it undergoes a series of enzymatic reactions catalyzed by FA synthase (FAS), a multifunctional cytosolic enzyme with seven distinct catalytic activities which is virtually identical in all biological systems. This enzyme sequentially adds 2C units derived from acetyl-CoA to a growing FA chain, resulting in the formation of the saturated 16C FA commonly known as palmitate (C16:0)¹. Contrariwise, throughout evolution, multiple FA elongases and desaturases related to FAS have emerged, producing a wide array of FA².

In the liver, palmitate serves as the precursor for the synthesis of a diverse assembly of saturated FA, including long-chain (18C) and very-long-chain (≥ 20 C) molecular species like C18:0 (stearic acid), and C20:0 (arachidic acid). Additionally, the liver engages in the synthesis of mono-unsaturated and polyunsaturated FA (PUFA) from palmitate, spanning various types, including n-10, n-9, and n-7, as well as n-6 and n-3 types (where “n” indicates the position of the double bond from the methyl end of

the FA), with the later derived from dietary precursors. This intricate synthesis process of FA relies on the coordinated action of different enzymes, specifically elongases and desaturases².

Several transcription factors, including PPAR α , LXR, SREBP-1, ChREBP, and MLX, intricately regulate diverse aspects of FA synthesis, encompassing the early stages of the process as well as the expression of elongases and desaturases. PPAR α and LXR regulate FA by controlling the expression of transcription factors, such as SREBP-1 and ChREBP. SREBP-1 promotes the expression of genes involved in DNL, including FAS and acetyl-CoA carboxylase, thereby stimulating the early stages of FA synthesis, while ChREBP, activated by high carbohydrate intake, also enhances DNL. Furthermore, ChREBP and MLX form complexes that regulate the expression of elongases and desaturases^{3,4}.

The process of FA synthesis and dietary FA uptake serves as a foundational pathway in lipid metabolism, generating precursors for the synthesis of an extensive array of essential lipid molecules (glycerolipids, phospholipids, and sphingolipids, among others) each fulfilling distinct roles crucial for cellular structure and function. This diversity underscores the pivotal role of FA in sustaining cellular structural integrity, the functionality of cellular membranes, signaling pathways, and energy storage mechanisms essential for the proper functioning of living organisms.

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A query into the Human Metabolome Database (<https://hmdb.ca/>) returned 13908 results for triglycerides (TG), 5478 for phosphatidylcholines (PC), 5110 results for phosphatidylethanolamines (PE), 950 for sphingomyelins (SM), 422 for ceramides, and 148 for sterols⁵. This complex molecular landscape highlights the diversity of lipid composition in the human circulatory system^{6–8}. Of note is the liver's predominant role in synthesizing the majority of circulating lipids, underscoring its central position in orchestrating the complex and dynamic processes of lipid metabolism^{8,9}.

Balancing adaptability and stability in liver lipid metabolism

Conceptually, this large diversity of lipid species can be likened to the strands of a “frayed rope” gradually separating from the main body. This analogy captures a fundamental trade-off: while the fraying may weaken the structure integrity of the rope, it simultaneously enhances its versatility and adaptability. Similarly, the diverse array of lipids in the liver (FA, TG, PC, PE, sphingolipids, sterols, etc.) enables it to respond dynamically to shifting physiological demands, including energy requirements, biosynthesis of membranes, the production of lipid signaling molecules, and lipid storage and transport. However, this heightened adaptability also carries inherent risks and potential compromises, symbolized by the fraying of the rope. In the context of liver lipid metabolism, these compromises may manifest as issues such as the accumulation of excessive TG (liver steatosis), the potential harmful effects of TG accumulation (lipotoxicity), and an imbalance between reactive oxidative substances (ROS) generated during FA β -oxidation and antioxidants defenses (oxidative stress).

In essence, the liver faces the challenge of maintaining a delicate balance between adapting to recurrent metabolic and physiological changes (such as fasting-feeding cycles and rest-activity variations) and preserving its structural and functional stability throughout the complexities of lipid metabolism. Achieving and sustaining this equilibrium lacks a one-size-fits-all solution, as it hinges on a combination of genetic, nutritional, and environmental factors. Metabolic dysfunction-associated steatotic liver disease (MASLD), a name recently adopted by consensus for the disease previously known as non-alcoholic fatty liver disease (NAFLD)¹⁰, arises from multiple interconnected causes that are challenging to disentangle. MASLD is linked with and may be caused by factors contributing to obesity, diabetes, cholesterol issues, chronic kidney disease (CKD), and cardiovascular disease (CVD)¹¹. Whether an individual will eventually develop

MASLD and if it does, whether will go on to develop cirrhosis, liver failure and eventually hepatocellular carcinoma (HCC) or CVD, is not known.

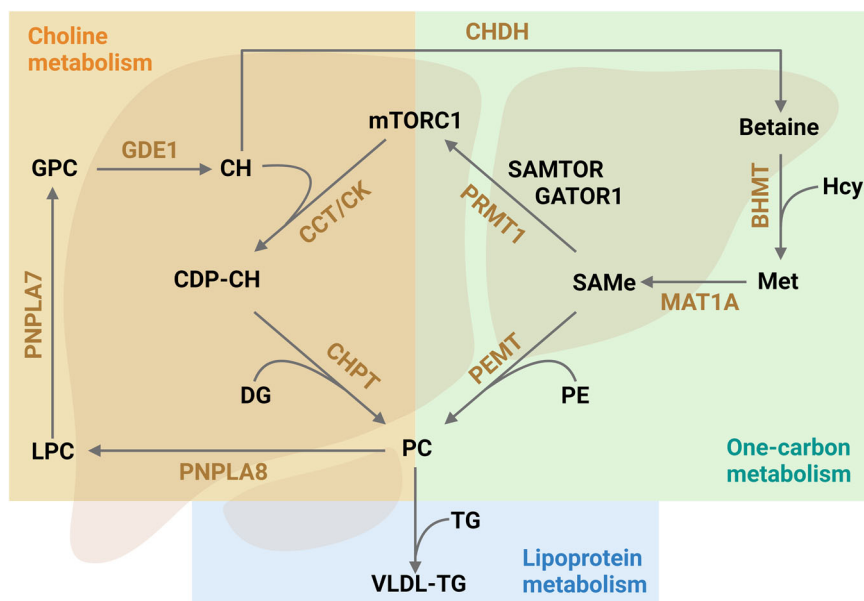
This is an important question since MASLD has been on the rise in Western countries, closely associated with the increasing rates of obesity, sedentary lifestyles, and poor dietary choices^{11–13}. In the United States and many European countries, MASLD has become a prevalent liver disease. The Western diet, characterized by high consumption of processed foods, sugars, and unhealthy fats, as well as other factors like the lack of exercise, are considered contributing factors. The impact of MASLD is not confined to Western countries. In India, for example, there has been a noticeable increase in the prevalence of MASLD, and it is becoming a significant public health concern. While traditionally India has had a lower prevalence compared to Western countries, the rising incidence of obesity, insulin resistance, and lifestyle changes are contributing to the increased prevalence of MASLD. Genetic factors and a transition to a more Westernized diet and sedentary lifestyle are influencing the disease's emergence in the Indian population^{12–14}.

The liver is a central hub in FA metabolism that orchestrates complex processes for energy production and lipid storage. Mitochondrial β -oxidation of FA generates energy, while simultaneously, TG are synthesized and stored within lipid droplets (LD)¹⁵. The continuous influx of FA is ensured by DNL and FA uptake from circulation¹⁶. Additionally, the liver releases very low-density lipoproteins (VLDL), crucial for transporting lipids to peripheral tissues^{17–19}. Liver steatosis and dysregulation of energy metabolism occur when the acquisition of lipids, through FA uptake and DNL, exceeds their disposal through FA β -oxidation and VLDL secretion.

Regulation of VLDL synthesis and secretion by S-adenosylmethionine

The synthesis and secretion of VLDL particles represents a dynamic anabolic response, particularly pronounced during fasting-feeding cycles. These anabolic processes involve intricate mechanisms that synthesize complex molecules from simpler ones, typically demanding an input of energy. Within hepatocytes, TG and other lipids undergo assembly to form VLDL particles through anabolic processes. These particles consist of a core made of TG and cholesterol esters, coated by a hydrophilic monolayer of phospholipids (predominantly PC), free cholesterol, and apolipoproteins (mainly APOB)¹⁹. The significance of PC and PE in regulating lipoprotein metabolism and energy utilization has been well-established. Dysregulated PC/PE ratios, either low or high, adversely affect VLDL secretion, impair LD

Fig. 1 | Multifaceted regulation of VLDL synthesis and export by S-adenosylmethionine. See text for a description. BHMT betaine-homocysteine methyltransferase, CCT, CTP phosphocholine cytidyltransferase, CDP-CH CDP-choline, CH choline, CHDH choline dehydrogenase, CHPT choline phosphate transferase, CK choline kinase, DG diglycerides, GATOR1 GTPase-activating protein activity toward Rag 2, GDE1 glycerophosphocholine phosphodiesterase 1, GPC glycerophosphocholine, Hcy homocysteine, LPC lysophosphatidylcholine, MAT1A methionine adenosyltransferase 1A, Met methionine, mTORC1 mechanistic target of rapamycin kinase 1, PC phosphatidylcholine, PE phosphatidylethanolamine, PEMT phosphatidylethanolamine N-methyltransferase, PNPLA7 lysophosphatidylcholine phospholipase, PNPLA8 phosphatidylcholine phospholipase, PRMT1 protein methyltransferase 1, SAME S-adenosylmethionine, SAMTOR SAME sensor upstream of mTORC1, TG triglycerides, VLDL very low-density lipoproteins.



size and function, and affect mitochondrial energy production, thereby promoting the onset of liver steatosis^{20,21}.

In the liver, PC is synthesized by two complementary pathways: the PE *N*-methyltransferase (PEMT) pathway and the CDP-choline (CDP-CH) pathway^{21,22}. In the PEMT pathway, PC is synthesized from methionine through its conversion to *S*-adenosylmethionine (SAME), followed by the methylation of PE to form PC (Fig. 1). On the other hand, the CDP-CH pathway, also known as the Kennedy pathway, utilizes choline (CH) as the initial substrate and is catalyzed by three enzymes: choline kinase, CTP: phosphocholine cytidyltransferase (CCT), and choline phosphate transferase, with CCT, the enzyme that synthesizes CDP-CH, acting as the rate-limiting step (Fig. 1). The choline moiety of PC, through sequential deacylation by the phospholipases PNPLA8 and PNPLA7, and conversion to betaine, can be utilized to remethylate homocysteine, through a reaction catalyzed by betaine-homocysteine methyltransferase, and regenerate methionine, ultimately replenishing hepatic SAME levels (Fig. 1)²³.

SAME not only regulates hepatic PC synthesis through the PEMT pathway but also exerts control over the Kennedy pathway by modulating the activity of mTORC1 (mechanistic target of rapamycin kinase 1), a key sensor of nutrient and energy status²⁴. This regulation occurs via the methylation of NRPL2, the catalytic subunit of GATOR1 (GTPase-activating protein activity toward Rag 2), by protein methyltransferase 1 (PRMT1)²⁵. Methylation by PRMT1 inhibits the GTPase-activating protein activity of NRPL2, resulting in mTORC1 activation²⁵. Active mTORC1, in turn, stimulates PC synthesis through the CDP-CH pathway by activation of CCT, thereby stimulating the synthesis and secretion of VLDL particles (Fig. 1)^{26,27}. Additionally, SAME facilitates the methylation of NRPL2 by directly interacting with SAMTOR, prompting its dissociation from GATOR1²⁸. However, SAME binds SAMTOR with a dissociation constant of about 7 μM, which is significantly lower than the physiological concentration of SAME in the liver, approximately 35 μM^{29,30}. This raises the question about the functionality of this mechanism in the liver.

SAME is the main biological methyl donor found across all mammalian cells, being most abundant in the adult liver. The synthesis of SAME is carried out by the enzyme methionine adenosyltransferase (MAT). In mammals, MAT is encoded by two genes: *MAT1A*, predominantly expressed in normal liver tissue, and *MAT2A*, which is expressed in all extrahepatic tissues. Mice deficient in *Mat1a* (*Mat1a*-KO mice)³¹, exhibit characteristic metabolic disturbances. These include markedly reduced hepatic SAME levels along with decreased PC levels and reduced PC to PE molar ratio, resulting in impaired VLDL secretion^{31–34}. These mice develop liver steatosis spontaneously, which can progress to steatohepatitis, fibrosis, and even HCC^{31–34}. Notably, patients with MASLD often exhibit diminished expression of *MAT1A* and low levels of hepatic SAME^{35–38}.

Diversity in MASLD: unveiling subtypes and their lipidomic profiles

The serum lipidome encompasses a broad spectrum of lipids circulating in the bloodstream, influenced by various factors. Conditions like liver disease or metabolic disorders significantly impact hepatic lipid metabolism, consequently altering the serum lipidome. Numerous studies have sought differences in FA, TG, phospholipids, and sphingolipids to identify non-invasive serum biomarkers that differentiate between MASLD and metabolic dysfunction-associated steatohepatitis (MASH), the progressive stages previously known as non-alcoholic fatty liver (NAFL) and non-alcoholic steatohepatitis (NASH), respectively¹⁰. For example, Oresic et al.³⁹ identified a serum lipid signature comprised of three lipids (1TG and 2 PC) for the diagnosis of MASLD in a large cohort (*n* = 679) using proton magnetic resonance spectroscopy to determine liver fat content. This “lipid triplet” had area under the receiver operating characteristic curve (AUROC) values of 0.74 in the discovery cohort and of 0.71 in the validation cohort³⁹. Another study aiming to develop and validate a lipidomic panel to identify individuals with MASH and significant fibrosis (fibrosis stage 2 or higher)⁴⁰, a condition referred to as at-risk MASH due to its association with elevated morbidity and mortality risk^{41,42}, the authors included discovery (*n* = 790) and validation (*n* = 565) cohorts of liver biopsied individuals recruited internationally. In this study, Nouredin et al.⁴⁰ identified 12 lipids, including 2 TG, 5 PC, 3 SM, and 1 ceramide. This panel of lipids in combination with the body mass index (BMI), ALT, and AST, showed an AUROC of 0.76 for the discovery cohort and of 0.79 for the validation cohort. Overall, these and other studies have demonstrated alterations in the levels of circulating FA, TG, phospholipids, sphingolipids, and bile acids in MASLD patients compared to subjects with normal liver. However, most studies identifying panels of potential biomarkers for differentiating between MASLD and normal liver, or between MASLD and MASH, have involved small cohorts, with few being validated or showing an AUROC ≥ 0.80 [reviewed in ref. 8].

The failure of these and other studies to identify lipidomic signatures accurately differentiating between individuals with MASLD and those without, as well as differentiating between the disease’s various stages (reviewed in ref. 8), suggests MASLD may be a heterogenous condition exhibiting different subtypes or metabotypes.

Lipidomics has emerged as potent tool for assessing the heterogeneity of MASLD and uncovering underlying mechanisms. Comparison of the serum lipidomic profile of *Mat1a*-KO mice with that of a large cohort (*n* = 535) of liver-biopsied MASLD subjects identified a subset of MASLD patients whose serum lipidome resembled that of *Mat1a*-KO⁹. This finding was further validated using an independent international cohort of 1154 liver biopsied MASLD patients⁴³. Additionally, this study identified a panel

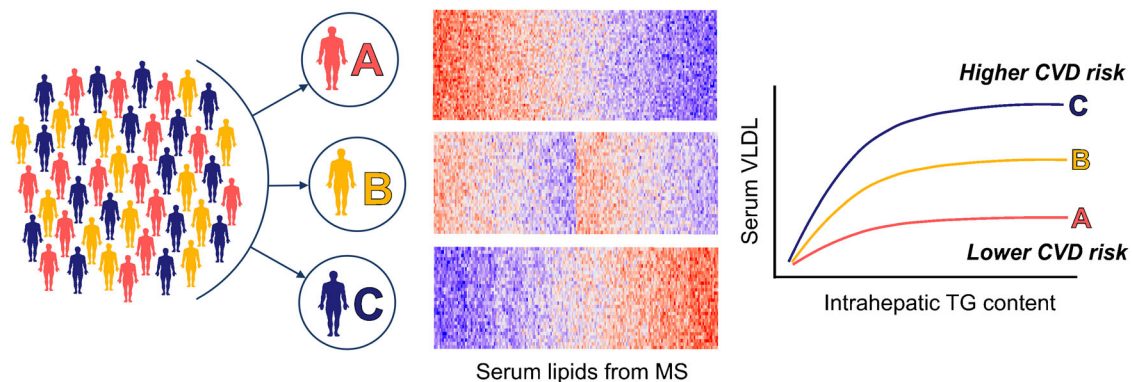


Fig. 2 | Diversity in MASLD: unveiling subtypes. Unsupervised statistical analysis classified MASLD patients into distinct subtypes or “metabotypes” based on their serum lipidomic profiles, obtained using mass spectrometry (MS), with two primary classifications: MASLD-A metabotype, characterized by lower serum VLDL levels

and associated with reduced cardiovascular disease (CVD) risk; and MASLD-C metabotype, featuring increased VLDL levels and linked to elevated CVD risk. An intermediate MASLD-B metabotype was identified, showcasing a combination of features.

of 11 lipids (7 PC, 2 TG, and 2 SM) that categorized MASLD into three distinct subtypes or metabotypes, each characterized by unique alterations in serum lipid homeostasis (Fig. 2). Specifically, two primary metabotypes were identified: MASLD-A metabotype, featuring lower VLDL secretion and serum TG levels, associated with a reduced CVD risk—the primary cause of mortality in MASLD⁴⁴—; and the MASLD-C metabotype, characterized by increased VLDL secretion and serum TG levels, associated with elevated CVD risk⁴³. An intermediate subtype, exhibiting a blend of features, was classified as MASLD-B metabotype. The percent of patients with MASH and fibrosis was comparable among metabotypes, although metabotypes B and C exhibited higher liver enzymes. Furthermore, the classification of MASLD into metabotypes was independent of age, sex, BMI, diabetes, or lipid lowering medication⁴³.

The primary distinction between MASLD-A and MASLD-C metabotypes lies in the correlation between serum VLDL-TG concentration and intrahepatic TG content (Fig. 2)⁴³. MASLD-C patients exhibit a curvilinear correlation, with serum VLDL-TG concentration saturating at steatosis grade 2 (34–66% of hepatocytes exhibiting fat accumulation). Conversely, MASLD-A patients show a serum VLDL-TG concentration independent of steatosis grade, significantly much lower than that observed in MASLD-C patients (Fig. 2). As a result, individuals with MASLD-A metabotype have reduced circulating levels of APOB containing lipoproteins, including VLDL, intermediate-density lipoproteins, and low-density lipoproteins (LDL), explaining the lower CVD risk observed in MASLD-A patients⁴³. Essentially, these findings suggest that MASLD-A patients compromise the structural and functional stability of the liver by inaccurately sensing increases in its energy status, such as during an upsurge in lipogenesis, accelerating the rate of VLDL secretion.

Differential hepatic cancer risk across MASLD subtypes

Numerous studies have highlighted, that genetic defects impairing hepatic VLDL secretion (APOB, APOC3, MTTP, TM6SF2) can lead to MASLD even in the absence of obesity or insulin resistance^{45–47}. Notably, the serum metabolomic profile of *Mttp*-KO and *Tm6sf2*-KO mice closely mirrors that of MASLD-A patients⁴³. These murine models of MASLD, like *Mat1a*-KO³⁴, spontaneously develop MASLD ultimately progressing to HCC^{48,49}. In contrast, the serum metabolomic profile of *Ldl*-receptor-KO mice, fed a high-fat/high-cholesterol diet—a mouse model of MASLD exhibiting increased VLDL secretion, liver steatohepatitis, and fibrosis without progressing to liver cancer⁵⁰—resembles that of MASLD-C patients⁴³. These findings prompt the question: can hepatic steatosis itself drive hepatic tumorigenesis in the context of impaired VLDL secretion? This is an important question, given that the prevalence of HCC in non-cirrhotic MASLD patients is estimated to be 38%, compared to 14% among non-cirrhotics with other etiologies⁵¹.

Recent studies show that liver deletion of *Mttp* (*Mttp*-LKO) increases susceptibility to diethylnitrosamine (DEN)-induced hepatic carcinogenesis in mice⁵². Intriguingly, an acceleration in liver carcinogenesis was observed in mice with simultaneous deletions of both *Mttp* and FA acid-binding protein 1 (FABP1) (*Fabp1*/*Mttp* DKO)⁵². This observation is noteworthy, especially considering that deletion of *Fabp1* (*Fabp1*-KO), a FA binding protein primarily involved in binding and transporting FA within hepatocytes⁵³, has been shown to provide protection against diet-induced steatosis in mice⁵⁴. DEN-treated *Mttp*-KO and *Fabp1*/*Mttp* DKO mice exhibit alterations in lipid metabolism (mainly TG, phospholipids, and ceramides) and energy substrate utilization when compared to control animals and show that these alterations are greater in the double KO⁵¹. Additionally, the double KO (*Fabp1*/*Mttp* DKO) exhibited reduced steatosis and fibrosis compared to the *Mttp*-KO mice⁵⁵.

The transition from MASH to HCC is often associated with a reduction of steatosis⁵⁶. This reduction could be due to a remodeling of hepatic metabolism, as tumor cells often require a different metabolic environment, favoring the utilization of FA as a carbon source and energy supply for tumor growth. Therefore, the mechanism by which impaired VLDL

secretion promotes liver tumorigenesis might be analogous to mechanisms observed in liver metastatic tumors^{57,58}. These studies have revealed that fatty liver conditions create a metastatic-friendly environment by enhancing the mobilization of LD stores, accelerating TG lipolysis, and transferring resulting FA as an energy source to neighboring tumor cells⁵⁷. Furthermore, these studies have associated liver metastasis with heightened production of extracellular vesicles, creating an immunosuppressive tumor environment⁵⁸. Alternatively, the reduction in FA uptake in *Fabp1*/*Mttp* DKO may result in decreased mitochondrial FA oxidation, leading to a decrease in the production of NADPH. This reduction in NADPH availability could compromise the cell's ability to neutralize the elevated generation of ROS induced by DEN, a phenomenon associated with the development of HCC⁵⁹. Additionally, it has also been shown, that *MAT1A* loss promotes liver cancer metastasis and sensitizes to colorectal liver metastasis⁶⁰.

In summary, these compelling findings collectively prompt the question: Could the reduced CVD risk observed in MASLD-A metabotype patients, attributed to impaired VLDL secretion, potentially come at the expense of an elevated susceptibility to HCC? If so, to what extent are genetic factors contributing to the formation of this metabotype?

Diversity in MASLD: genetic variants

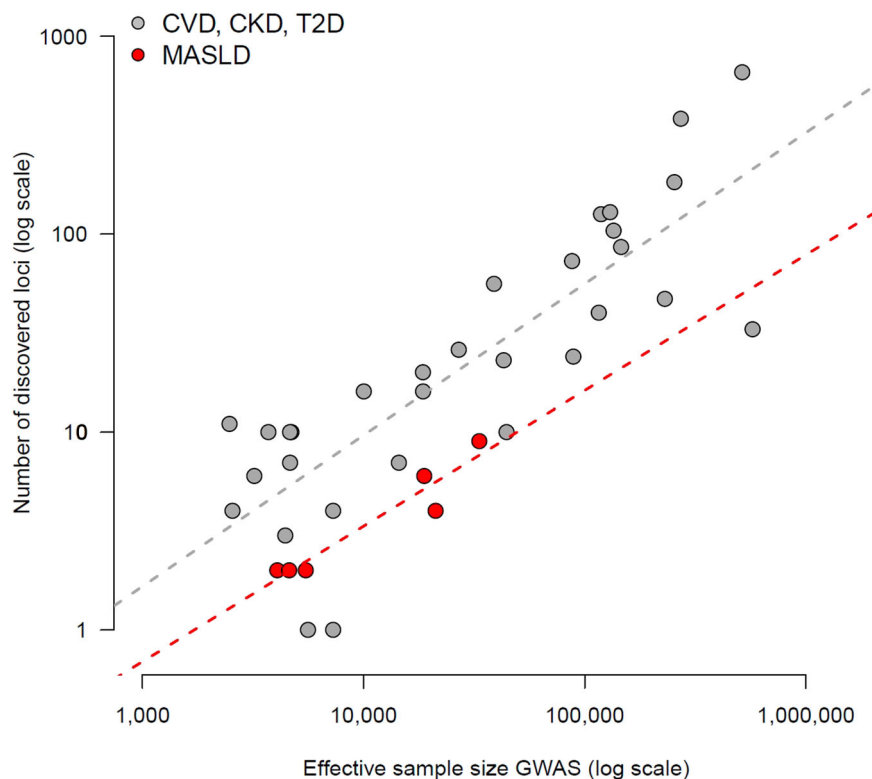
The year 2008 marked a significant breakthrough with the discovery of the first gene variant, *PNPLA3*, strongly associated to intrahepatic TG accumulation, utilizing proton magnetic resonance spectroscopy, in a genome-wide association study (GWAS) conducted in a multiethnic population⁶¹. Notably, this study revealed no evidence of an association between the *PNPLA3* allele and BMI or insulin resistance, indicating a specific association between *PNPLA3* and hepatic TG content distinct from obesity or diabetes⁶¹. Subsequent publications not only validated this seminal study but also broadened the spectrum of genetic variants associated with this trait, confirming that MASLD is genetically influenced⁶².

These studies have uncovered a robust and reproducible association between variants in the genes *PNPLA3*, *TM6SF2*, *MBOAT7*, *HSD17B13*, and *GCKR*, and MASLD^{63,64}. Furthermore, variants in *TRIB1*, *MTTP*, *GPAM*, *MARCI*, and *APOE* genes are also associated to MASLD^{63,64}. *PNPLA3*, located on LD, plays a crucial role in TG and phospholipid metabolism, with its variant allele associated to altered lipid remodeling characterized by the accumulation of PUFA in diglycerides and TG, along with decreased incorporation into PC, although it does not affect VLDL secretion⁶⁵. Interestingly, this *PNPLA3* variant is more prevalent in MASLD patients with metabotypes B and C⁴³. Similarly, *MBOAT7*, involved in phosphatidylinositol remodeling⁶⁶, and *HSD17B13*, located on LD, also impact lipid remodeling⁶⁷. *GPAM*, a mitochondrial glycerol 3-phosphate acyltransferase, promotes lipid accumulation by preferring saturated FA as substrates for TG and phospholipids synthesis⁶⁸. The E167K variant of *TM6SF2* enhances hepatic lipid accumulation by decreasing VLDL secretion, while concurrently protecting against CVD by lowering circulating lipids⁶⁹. Other variants, such as those in *TRIB1* and *MTTP*, influence VLDL metabolism, lowering circulating TG levels^{70,71}. The P446L variant of *GCKR* enhances glucokinase activity, increasing glycolytic flux and TG synthesis⁷², and *MARCI* encodes a mitochondrial enzyme that reduces trimethylamine N-oxide, a CVD risk factor⁷³. Finally, *APOE* maintains lipid equilibrium in the liver by regulating the influx and efflux of lipids, with its dysregulation leading to TG accumulation⁶⁸. It is inferred from these variants that lipid remodeling, VLDL synthesis, and export are crucial for the liver to adapt its functionality in response to changing physiological demands.

The role of genetic heterogeneity in risk for MASLD

The collective experience from the GWAS era, including metabolic diseases, suggests that these studies have only identified a small fraction of the total number of genetic variants associated with disease susceptibility⁷⁴. An overview of the situation with T2D illustrates this point well. As early as 2012, 63 independent signals had been associated with the disease⁷⁵. This figure rapidly surged to the hundreds, reaching 243 loci discovered by 2018,

Fig. 3 | Association between sample size and GWAS discoveries in metabolic diseases. X-axis: effective sample size of GWAS studies based on the number of cases and controls used in each study. Y-axis: number of independent associations discovered in each study. Both axes are in logarithmic scale. We used the GWAS Catalog to identify studies for four metabolic diseases, namely MASLD, T2DM, CVD, and CKD. As a quality control, we selected only GWAS fulfilling four criteria, namely: i) including at least 1000 cases and 1000 controls, ii) studying individuals of a single genetic ancestry (e.g. East Asians), iii) performing association tests focused exclusively on additive effects, and iv) testing markers spread genome-wide (e.g. exome studies excluded). In total, 37 studies passed these filters: six studies for MASLD and 31 for the other three diseases (red and gray points, respectively). The dashed lines indicate the regression line for each subgroup.



277 by 2022 and as many as 611 by 2024 through the recent multi-ethnic analysis conducted by the DIAMANTE consortium^{76–78}. This approximately threefold increase in discovered loci every five years has been achieved through similarly large expansions in the number of cases included, which reached as many as 428,452 in the latest scan⁷⁸. Quantitative genetic modeling indicates that the genetic architecture of most complex diseases is likely influenced by 10^4 to 10^5 variants spread across the genome^{79,80}. Hence, an ambitious strategy focused on larger sample sizes will be needed to uncover the myriad of genes and pathways that underlie the polygenic component of MASLD.

Nonetheless, a comparison of the yield from GWAS suggests that the genetic architecture of MASLD presents important challenging aspects that will not be offset with brute force gained through larger sample sizes. In particular, the figure of 17 loci associated in the recent scan by Chen et al.⁶⁴ pales clearly with the total number of genes discovered for other metabolic diseases, which in some cases such as T2DM is in the hundreds⁷⁸. To evaluate this question, we examined the statistical delivery of GWAS for MASLD with the results for three other diseases, namely T2DM, CVD and CKD. As expected, the ability to discover new loci correlates positively with the sample size of the study (Fig. 3). However, the yield from the six selected studies for MASLD is clearly in the low range of the overall cloud of points.

Even if a qualitative approximation, this pattern suggests that the payoff of genetic studies for MASLD is notably lower. Besides the above-mentioned challenges associated with phenotyping methodologies, this observation hints at a role for biological heterogeneity within disease, which is known to play an important role in determining the statistical power of genetic studies^{81–83}. Although human disease genomics has focused on large-scale comparisons of cases and controls in the last two decades, increasing attention will likely pivot towards this research question. The “frayed rope” analogy discussed above also provides a useful framework to examine heterogeneity at the level of patients. Briefly, the genetic makeup of each individual does not constitute a single liability, but a range of molecular pathway-specific situations that in combination determine the risk profile and disease presentation in each person⁸⁴. Although genetic studies have identified the backbone components of MASLD risk, new approximations

are needed to characterize the hundreds of low-risk variants that underlie the diversities in molecular profiles that are observed across MASLD patients. Notably, this new mindset has already delivered useful insights into the heterogeneity involved in MASLD susceptibility. For instance, decomposition of pleiotropic effects using the phenome-wide association study (PheWAS) framework uncovered that genetic risk for MASLD clusters into 7 mechanistic subgroups of effects⁶⁴. In this regard, the successful characterization of the three metabolotypes that emerge from liver metabolism variability demonstrates the potential of research programs focused on dissection of heterogeneity to identify subtypes within MASLD.

Taken together, the diverse array of genes and metabolomic pathways identified through GWAS underscores the intricate and multifaceted nature of the pathogenic mechanisms underlying MASLD. This complexity supports the concept that the development and progression of MASLD diverge significantly based on an individual’s unique genetic makeup. This individual variability is further manifested in the distinct lipidomic profile exhibited by each person, highlighting the intricate interplay between genetic factors and lipid metabolism pathways. For instance, variants in genes such as PNPLA3, TM6SF2, and MTTP have been associated with low VLDL secretion and the retention of TG and other lipids, leading to increased liver lipid accumulation^{85,86}. On the other hand, genes like GSKR and TRIB1 contribute to MASLD by increasing glycolysis to synthesize TG via DNL^{64,87}, resulting in different phenotypic expressions of MASLD. This highlights the need for a nuanced understanding of the heterogeneity within MASLD, recognizing that different combinations of genetic variants may lead to diverse metabolic outcomes and clinical presentations.

The intricate interplay between genetic factors and lipid metabolism pathways emphasizes the necessity of a personalized approach in comprehending and treating MASLD. This tailored approach allows for the development of more precise and effective therapeutic interventions that consider the unique genetic and metabolic characteristics of each individual. Therefore, the comprehensive integration of omics data—including genomics, metabolomics, and proteomics—with clinical, lifestyle, and environmental data is essential for unraveling the complexities of MASLD and advancing towards precision medicine in the field.

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Author contributions

U.M.M. and J.M.M. wrote the manuscript and O.M. and S.C.L. reviewed the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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