Received: 15 July 2024 | Revised: 12 August 2024 | Accepted: 19 August 2024

DOI: 10.1111/liv.16088

Liver INTERNATIONAL WILEY

MINI REVIEW

Sex-specific effects of PNPLA3 I148M

Alessandro Cherubini[1] | Chiara Rosso[2] | Sara Della Torre[3]

[1]Department of Transfusion Medicine, Precision Medicine—Biological Resource Center, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy

[2]Department of Medical Sciences, University of Turin, Turin, Italy

[3]Department of Pharmaceutical Sciences, Università degli Studi di Milano, Milan, Italy

Correspondence
Alessandro Cherubini, Department of Transfusion Medicine, Precision Medicine—Biological Resource Center, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Via Francesco Sforza 35, Milan 20122, Italy.
Email: alessandro.cherubini@policlinico.mi.it

Handling Editor: Luca Valenti.

Abstract

Metabolic dysfunction–associated steatotic liver disease (MASLD, previously termed NAFLD, nonalcoholic fatty liver disease) is a complex multifactorial disease showing generally higher prevalence and severity in men than in women. With respect to women, men are also more prone to develop metabolic dysfunction–associated steatohepatitis, fibrosis and liver-related complications. Several genetic, hormonal, environmental and lifestyle factors may contribute to sex differences in MASLD development, progression and outcomes. However, after menopause, the sex-specific prevalence of MASLD shows an opposite trend between men and women, pointing to the relevance of oestrogen signalling in the sexual dimorphism of MASLD. The patatin-like phospholipase domain-containing protein 3 (*PNPLA3*) gene, that encodes a triacylglycerol lipase that plays a crucial role in lipid metabolism, has emerged as a key player in the pathogenesis of MASLD, with the I148M variant being strongly associated with increased liver fat content and disease severity. Recent advances indicate that carrying the *PNPLA3* I148M variant can be a risk factor for MASLD especially for women. To elucidate the molecular mechanisms underlying the sex-specific role of *PNPLA3* I148M in the development of MASLD, several in vitro, ex vivo and in vivo models have been developed.

KEYWORDS
hepatocellular carcinoma (HCC), human liver organoids, metabolic dysfunction–associated steatotic liver disease (MASLD), sexual dimorphism

1 | INTRODUCTION

Metabolic dysfunction–associated steatotic liver disease (MASLD), defined as an excess of the amount of fat stored in the liver (≥5% of weight), represents a spectrum of liver disease ranging from hepatic fat accumulation without inflammation to steatohepatitis (metabolic dysfunction–associated steatohepatitis, MASH), fibrosis, cirrhosis and potentially hepatocellular carcinoma (HCC) in the absence of excessive alcohol consumption.[1] MASLD is characterized by substantial inter-individual variability in terms of severity and rate of progression.[1] However, the early identification of individuals at high risk of progression remains an important unmet

Abbreviations: ERα, oestrogen receptor alpha; ERE, oestrogen receptor response element; HCC, hepatocellular carcinoma; HFD, high-fat diet; KI, knock-in; MASH, metabolic dysfunction–associated steatohepatitis; MASLD, metabolic dysfunction–associated steatotic liver; PNPLA3, patatin-like phospholipase domain-containing protein 3; SNP, single nucleotide polymorphism; SREBP, steroid regulator element binding protein.

Alessandro Cherubini, Chiara Rosso, and Sara Della Torre equally contributed to this study.

In this review, we provide an overview of the current state of knowledge regarding the sex-specific effects of *PNPLA3* I148M in the pathophysiology of MASLD.

need in the clinical context. Genetics plays an important role influencing both, the onset of steatosis and the worsening of liver disease.[2] Moreover, the liver is characterized by sexual dimorphism, and the pathogenic mechanisms involved in the onset and progression of MASLD differ, at least in part, according to sex and sex-related factors.[3] This observation has been further corroborated by a recent large-scale analysis of hepatic transcriptomic profiles that emphasized the sexually dimorphic nature of MASLD and its association with the progression of liver disease suggesting the importance to consider sex as one of the main tools for patient risk stratification.[4]

Overall, the prevalence of MASH and hepatic fibrosis is higher in men than in women. Similarly, the incidence of advanced hepatic fibrosis and cirrhosis complications such as the MASH-related HCC is 2–4 fold higher in men than women.[5] However, the molecular mechanisms underlying sex differences are not yet fully elucidated. What is known is that sex hormones, mainly oestrogens, exert a protective effect on the liver as demonstrated by several studies showing that the prevalence of MASLD and MASH in postmenopausal women with a mean age of 51 years is comparable to that observed in men.[6,7] Overall, menopause in women is associated with advanced fibrosis ($F \geq 2$),[8] while conflicting data are reported on the impact of oestrogens in the progression of liver disease in fertile and pre-menopausal women.[9,10] Further studies are necessary to understand the effective contribute of such hormones species to the progression of MASLD (Table 2). However, oestrogen-based therapies may help reduce MASLD risk, especially in postmenopausal women. On the other hand, the role of progesterone in MASLD is less clear. Progesterone can decrease insulin sensitivity and has complex effects on inflammation. Its impact on liver fat accumulation and MASLD risk is less pronounced compared to oestrogens. So, further research is needed to understand progesterone's role in liver metabolism and MASLD (Table 2).

The patatin-like phospholipase domain-containing protein 3 (*PNPLA3*) gene, that encodes a triacylglycerol lipase that plays a crucial role in lipid metabolism, has emerged as a key player in the pathogenesis of MASLD, with the I148M variant being strongly associated with increased liver fat content and disease severity.[11] Specifically, individuals carrying the GG risk homozygosis for the rs738409 single nucleotide polymorphism (SNP) have a higher risk of developing hepatic fibrosis and MASLD/MASH-related HCC compared to those with the wild-type allele.[12] Recently, the interaction between the I148M variant in the *PNPLA3* gene and the oestrogen receptor alpha (ERα) has been described and seems to explain, at least in part, the sexual dimorphism in the context of MASLD.[13] Unfortunately, clinical longitudinal studies are scanty, and few data on the impact of genetics on the development of clinical events over time are available at present.

In this review, we provide an overview of the current state of knowledge regarding the sex-specific effects of *PNPLA3* I148M variant in the pathophysiology of MASLD from clinical to in vitro and in vivo studies (Figure 1; Table 1).

Key points

- Metabolic dysfunction–associated steatotic liver disease (MASLD) prevalence and incidence are higher in men then in pre-menopausal women, while tend to become more common in women after menopause.
- Women carrying patatin-like phospholipase domain-containing protein 3 (*PNPLA3*) I148M variant affected by MASLD show a larger impact in all liver damage outcomes than men.
- Female mice show higher hepatic *Pnpla3* expression during the follicular phase of the cycle characterized by high oestradiol levels than during the luteal phase and then in males.
- Hepatic in vitro model carrying *PNPLA3* I148M variant shows lipid droplet accumulation and collagen deposition when exposed to oestrogen receptor α agonists.

2 | UNDERSTANDING THE SEX-SPECIFIC ROLE OF *PNPLA3* IN MASLD: AN UPDATE FROM CLINICAL STUDIES

From a clinical point of view, the strong association between the *PNPLA3* I148M polymorphism and sexual dimorphism dates back over 10 years, when a meta-analysis showed a negative association between the genetic variant on hepatic steatosis and male sex, suggesting its possible effect on MASLD susceptibility.[14] Specifically, the meta-analysis analysed 16 studies selected for the common aim to assess the strength of the effect of the rs738409 SNP on MASLD across different populations, as well as on intermediate phenotypes such as insulin resistance and overweight/obesity.[14] Just a year later, Li and colleagues observed a strong association between the rs738409 variant and elevated ALT levels in men from the Cameron County Hispanic Cohort (a Mexican American cohort including 1532 individuals with a high prevalence of obesity, diabetes and MASLD), confirming previous results.[15] In addition, the study reported a significant association between the rs738409 genetic variant and ALT levels only in women aged ≤50 compared to those older than 50 years. Considering the differences in oestrogen levels between the two groups, the authors hypothesized the existence of an interaction between the I148M variant and oestrogen levels that, in turn, led to the onset and worsening of liver disease.[15] Sexual dimorphism of the rs738409 SNP was further observed in a study on 121 participants with primary sclerosing cholangitis (PSC). Specifically, in men with severe liver disease, the carriage of the G risk allele was a risk factor for reduced survival.[16] Recently, a longitudinal study performed in a large European cohort of biopsied-proven MASLD patients identified a group of individuals consisting of non-obese women with 50 years or older carrying the I148M GG risk genotype who had a higher risk of developing liver-related events during follow-up compared to the counterpart.[17]

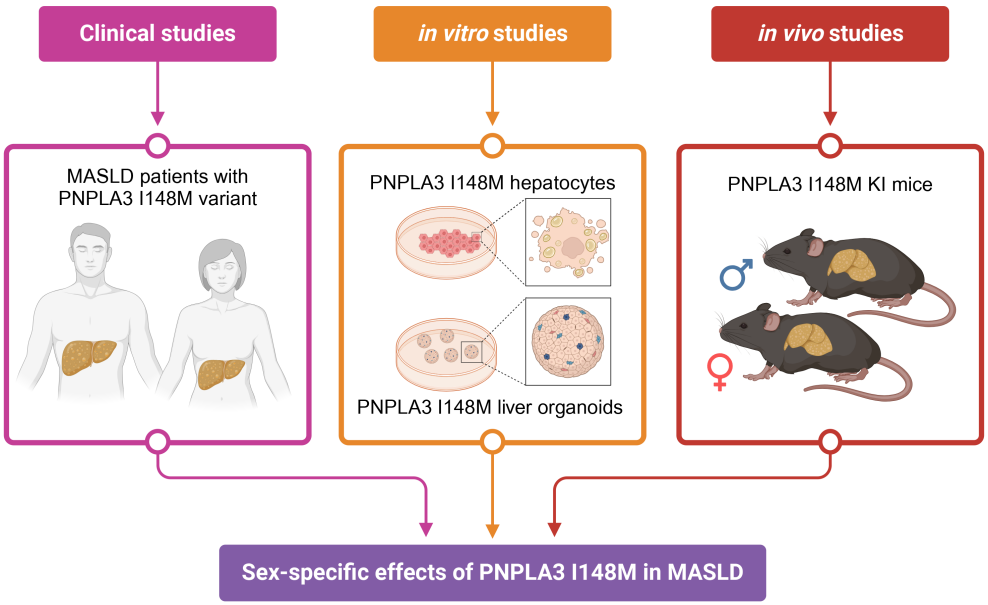

FIGURE 1 Recent advances indicate that carrying the PNPLA3 I148M variant can be a risk factor for MASLD and accounts for sex differences in MASLD susceptibility, progression and outcomes. To elucidate the molecular mechanisms underlying the sex-specific role of PNPLA3 I148M in the pathophysiology of MASLD, further clinical and pre-clinical studies with in vitro and in vivo models are needed. MASLD, metabolic dysfunction–associated steatotic liver; PNPLA3, patatin-like phospholipase domain-containing protein 3.

TABLE 1 Sex differences in PNPLA3. Evidence from clinical and experimental studies.

Experimental model	Main findings	Author, year
Human studies	• Meta-analysis of the influence of I148M variant of patatin-like phospholipase domain-containing 3 gene (PNPLA3) on the susceptibility and histological severity of nonalcoholic fatty liver disease	Sookoian and Pirola, 2011[14]
	• PNPLA3 polymorphisms and liver aminotransferase levels in a Mexican American population	Li et al., 2012[15]
	• A frequent PNPLA3 variant is a sex-specific disease modifier in PSC patients with bile duct stenosis	Friedrich et al., 2013[16]
	• Impact of PNPLA3 rs738409 polymorphism on the development of liver-related events in patients with MASLD	Rosso et al., 2023[17]
In vitro studies	• Selective oestrogen receptor modulators stimulate the activity of SREBP pathway regulating in turn the expression of PNPLA3, resulting in the lipid metabolism changes and then the onset of liver steatosis	Huang et al., 2010[18] Fernández-Suárez et al., 2021[19]
	• ER-α agonists regulate the PNPLA3 mRNA expression, protein synthesis and accumulation on intracellular lipid droplets, leading to the intracellular lipid droplet accumulation	Cherubini et al., 2023[13]
In vivo studies	• No comparative studies between male and female mice carrying the PNPLA3 I148M variant • In control mice, the endogenous *Pnpla3* mRNA is differently regulated in the liver of males and females. *Pnpla3* mRNA is induced by high oestrogen levels in the liver of females	Cherubini et al., 2023[13]

Abbreviations: MASLD, metabolic dysfunction–associated steatotic liver; PNPLA3, patatin-like phospholipase domain-containing protein 3; SREBP, steroid regulator element binding protein.

TABLE 2 Potential findings and implications of experimental research approaches aimed to fulfil the gap of knowledge on the sex-specific effects of PNPLA3 I148M variant in MASLD.

Study	Objective	Design	Potential findings and implications
Sex-Specific GWAS (Genome-Wide Association Studies)	To identify genetic variants associated with MASLD that may have different effects in men and women	Perform separate GWAS analyses for male and female cohorts to uncover sex-specific genetic predispositions	This approach can help identify sex-specific genetic variants involved in MASLD
Cellular and molecular mechanism studies	To elucidate the cellular and molecular pathways through which PNPLA3 I148M variant and sex hormones influence liver metabolism	Use hepatocyte cultures from male and female donors to study the direct effects of PNPLA3 I148M variant and sex hormones on liver cells Perform transcriptomic and proteomic analyses to identify hormone-responsive genes and proteins involved in lipid metabolism, inflammation and fibrosis	This approach can help to shed light on the molecular mechanisms underlying sex differences in MASLD according to PNPLA3 I148M variant and hormone interactions Moreover, this approach can help identify new therapeutic targets and biomarkers for sex-specific treatments
Organoid models	To study the impact of sex and sex hormones on PNPLA3-related pathways in human liver	Use liver organoids from male and female MASLD patients with/without PNPLA3 I148M variant Perform time and dose-dependent analysis with sex hormones (i.e. oestrogens and testosterone) incubation	This approach can provides a human-relevant model to study sex differences in MASLD Moreover, organoids can be useful in personalized medicine and drug testing
Innovative approaches	To study the direct effects of PNPLA3 I148M variant and their interaction with sex hormones	Employ CRISPR/Cas9 technology to create precise genetic modifications in organoids, hepatic cells and animal models	This approach can help to uncover sex- and cell-specific responses to PNPLA3 I148M variant and to profile changes in liver metabolism associated with hormonal influences
Sex-specific animal studies	To unravel the sex-specific role of PNPLA3 in vivo and its modulation by sex hormones To understand the differential impact of sex and sex hormones on MASLD development and progression	Use genetically engineered mouse models with human PNPLA3 I148M variant to study its role in liver metabolism Include both male and female mice in MASLD models; administer specific hormone treatments (oestrogens, testosterone) to observe differential effects	Comparative studies between male and female mice can help to elucidate sex-specific effects of PNPLA3 I148M and sex hormones on liver fat accumulation
Hormone replacement studies in animal models	To investigate the effects of hormone replacement therapy on MASLD	Use orchiectomized (ORX) and ovariectomized (OVX) rodents to mimic hormone deficiencies. Administer controlled doses of oestrogens, progesterone and testosterone, both individually and in combination, to assess their individual and combined effects on liver fat accumulation, inflammation and fibrosis	This approach can help evaluate sex-specific response to hormone replacement therapy
Longitudinal human cohort studies	To track the development of MASLD in relation to PNPLA3 variants and hormonal changes over time	Enrol a large, diverse cohort of men and women and follow them over several years. Collect data on hormone levels, genetic background, lifestyle factors and liver health through imaging and biopsy when applicable	This approach can help establish causal relationships between PNPLA3 I148M variant, hormonal changes and MASLD progression Moreover, this approach can help to define timing and type of interventions

TABLE 2 (Continued)

Study	Objective	Design	Potential findings and implications
Interventional trials	To evaluate the therapeutic potential of PNPLA3 I148M variant targeting and/or hormone modulation in MASLD patients	Conduct randomized controlled trials (RCTs) with pharmacological agents, gene-editing techniques or administering hormone replacement therapy (e.g. testosterone for men and oestrogens/progesterone for postmenopausal women) to MASLD patients Measure liver fat content, liver enzyme levels, insulin sensitivity and inflammatory markers before and after the intervention	This approach can help to evaluate sex-specific personalized approaches for MASLD treatment Moreover, this approach can support the inclusion of hormonal status in therapeutic trials
Gene–environment interactions	To explore how lifestyle factors interact with PNPLA3 I148M variant in a sex-specific fashion	Cohort studies assessing diet, exercise, PNPLA3 genotype, hormone levels and liver health	This approach can help to unravel the extent to which lifestyle factors can differently modulate the impact of PNPLA3 I148M on MASLD in men and women Moreover, this approach can help to identify personalized lifestyle interventions alongside genetic and hormonal factors in MASLD management

Abbreviations: MASLD, metabolic dysfunction–associated steatotic liver; PNPLA3, patatin-like phospholipase domain-containing protein 3.

TABLE 3 Open questions and knowledge gaps on the interaction between sex and PNPLA3 on MASLD pathophysiology.

Main question	Open questions and knowledge gaps
Mechanisms of PNPLA3 action	• How does the I148M variant of PNPLA3 contribute to lipid accumulation in hepatocytes? • What are the downstream molecular pathways influenced by PNPLA3 in liver cells?
Interaction with sex hormones	• How do sex hormones influence PNPLA3 expression and activity? • Are there sex-specific effects of the PNPLA3 I148M variant on MASLD progression?
Gene–environment interactions	• How do lifestyle factors (diet, exercise) interact with PNPLA3 I148M variant to influence MASLD risk? • What is the role of other genetic and epigenetic factors in modulating the impact of PNPLA3 I148M on MASLD?
Therapeutic targeting	• Can PNPLA3 be effectively targeted for therapeutic intervention in MASLD? • What are the potential side effects of modulating PNPLA3 activity in humans?

Abbreviations: MASLD, metabolic dysfunction–associated steatotic liver; PNPLA3, patatin-like phospholipase domain-containing protein 3.

All the evidences suggest that a careful follow-up should be important in specific subgroup of individuals and that *PNPLA3* genotyping and sex should be considered in the clinical setting for patient risk stratification. However, additional studies are required to validate previous results in external and independent cohorts.

3 | UNDERSTANDING THE SEX-SPECIFIC ROLE OF PNPLA3 IN MASLD: INSIGHTS FROM IN VITRO STUDIES

Although sex hormones, such as oestrogens and androgens, are known to influence adiposity and insulin resistance,[18] results of

in vitro studies are generally not contextualized and interpreted in a unisex way. Several in vitro studies reported that *PNPLA3* I148M, with a critical role in homeostasis of lipid metabolism, plays a key role in the development of MASLD. However, mechanisms explaining sex biological specificities in liver disease susceptibility are largely unknown.

Some evidence shows that the *PNPLA3* gene is under nutritional control, being in strong relationship with the steroid regulator element binding protein (SREBP)-1c through liver X receptor, two important factors involved in the regulation of fatty acid synthesis.[19] Contextually, sex hormones, like oestrogens, modulate lipogenic genes such as SREBP family, changing lipid metabolism and then contributing to the onset of liver steatosis.[20]

Recently, Cherubini and colleagues reported existence of an interaction between female hormones and *PNPLA3* I148M variant in determining MASLD progression.[13] The authors identified the presence of an oestrogen receptor response element (ERE), that is highly conserved in mammals, in *PNPLA3* promoter. In this study, immortalized cell line and tissue-derived human liver organoids of both sexes were treated with ERα agonists, observing *PNPLA3* transcription induction. Next, they exposed cells to excess fatty acids (either oleic acid or palmitic acid) to model liver disease, showing an accumulation of PNPLA3 I148M protein on lipid droplets, intracellular lipid accumulation and collagen deposition by stellate cells in response to ERα agonists. To corroborate these findings, the authors removed the *PNPLA3*-ERE by CRISR/Cas9 showing a reduction of ERα agonists effects on lipid accumulation and collagen deposition. All in all, this evidence demonstrates for the first time an axis between female hormones, genetic variant and altered lipid metabolism. However, further in vitro studies that consider sex-specific approaches are needed to better understand sex differences in MASLD susceptibility and progression (Table 2).

4 | UNDERSTANDING THE SEXUAL DIMORPHISM IN MASLD: INSIGHTS FROM MOUSE MODELS

Several diet-induced and genetic mouse models for MASLD have been developed to investigate the molecular drivers accounting for MASLD susceptibility, development and progression.[21] Recently, a wide-ranging retrospective review tried to rank diet-induced mouse models for MASLD according to metabolic phenotype, liver histopathology and transcriptome benchmarked based on their proximity to human MASLD.[22]

Besides mouse models can recapitulate most of the features of human MASLD, including MASH and fibrosis, the majority of the studies has been limited to the male sex,[22] with female mice being excluded from analysis for the potential confounding effects ascribable to fluctuating levels of oestrogens typical of oestrous cycle.[23]

Although very few, the comparative studies between the two sexes have highlighted the sexually dimorphic nature of MASLD, with male mice generally developing more severe pathogenic features of MASLD, including steatosis, insulin resistance and inflammation compared to females.[7,10,18] When exposed to an excess of dietary lipids (high-fat diet, HFD), female mice, contrary to males, counteract liver lipid deposition thank to the regulatory activity of oestrogens, acting mainly through ERα at the hepatic level.[10] The lack of a proper oestrogen signalling, indeed, impairs liver metabolic homeostasis, leading to hepatic lipid deposition and inflammation in ovariectomized as well as in liver-specific ERα knockout females.[10,24]

Such a female-specific and ERα-dependent ability to counteract MASLD may be likely the consequence of the strict interplay between metabolism and reproduction gained during evolution by the female liver[23] and aimed to modulate the hepatic metabolism according to nutrient availability and to the energy requirements characterizing each reproductive stage.[25] The peculiar role exerted by ERα in the female liver contributes to sex differences in the regulation of hepatic metabolism under several nutritional and fasting conditions.[10,26]

Given the nutritional regulation of PNPLA3 and given the interaction between ERα and PNPLA3 I148M variant in women with MASLD,[13,19] studies with mice of both sexes carrying this genetic variant may represent a useful research tool for investigating the sex-specific role of PNPLA3 in MASLD pathogenesis.

Several animal models, including PNPLA3 I148M knock-in (KI) mice, which carry the human PNPLA3 I148M variant, have been developed to elucidate the role of PNPLA3 I148M variant in hepatic triglyceride (TG) metabolism and its association with MASLD.

PNPLA3 I148M KI mice had normal levels of hepatic fat on a chow diet, but under dietary challenges (i.e. high-sucrose or HFD) develop hepatic steatosis, inflammation and fibrosis, recapitulating key features of human MASLD/MASH.[27] The increased liver fat in PNPLA3 I148M KI mice is associated with the accumulation of catalytically inactive PNPLA3 on the surfaces of lipid droplets (LDs).[27] Studies using these mice have revealed that the I148M variant disrupts ubiquitylation and proteasomal degradation of PNPLA3, leading to accumulation of PNPLA3 I148M and impaired mobilization of TG from LDs resulting in aberrant lipid accumulation in the liver.[28]

The I148M variant acts as a loss-of-function mutation, disrupting the mobilization of polyunsaturated fatty acids (PUFAs) from intracellular TG pools, thus impairing the hepatic secretion of very-large low-density lipoproteins.[29] These last findings derive from a study where several mouse models (PNPLA3 I148M KI mice; mice overexpressing PNPLA3 I148M; liver KO *Pnpla3* mice; ASO-treated mice) have been used, without performing sex-based comparative analysis.

Although the majority of the studies on PNPLA3 I148M KI mice has been limited to males, research indicates that there are sex-specific differences in the impact of PNPLA3 I148M variant on liver metabolism and disease progression, pointing to the relevance of this variant in accounting for sexual dimorphism in MASLD development and progression.[18] As occurs for women, female mice carrying the

PNPLA3 I148M variant tend to have higher liver fat content compared to PNPLA3 I148M KI males after 4 weeks on a high-sucrose diet.[27] In another report, PNPLA3 I148M KI mice of both sexes were studied to investigate their susceptibility to MASLD; however, the analysis of differences between the two sexes is not suitable since PNPLA3 I148M KI females were fed with a high-sucrose diet (70%) for 15 weeks, while PNPLA3 I148M KI males were fed with a MASH-inducing diet for 26 weeks.[30]

Several other metabolic parameters such as glucose metabolism, insulin sensitivity, lipid profiles and gene expression profiles related to lipid metabolism, inflammation and fibrosis may vary between male and female PNPLA3 I148M KI mice, potentially influencing the development and progression of MASLD in a sex-specific manner.

Further research is needed to uncover the intricate molecular mechanisms by which the PNPLA3 I148M variant contributes to MASLD pathogenesis (Table 2). Studies with mice of both sexes carrying the PNPLA3 I148M variant may represent a valuable tool to elucidate the sex-specific role of PNPLA3 I148M in MASLD and to disentangle the contribution of genetic and hormonal drivers accounting for the sexually dimorphic nature of MASLD. Additionally, exploring sex-specific potential therapeutic strategies targeting PNPLA3 and its downstream effectors in the context of MASLD holds promise for the development of therapeutic strategies useful to hamper MASLD in both sexes, even also according to hormonal status.

5 | CONCLUSION

MASLD is a progressive wide spectrum pathology that involves several factors; among them, gene variants and sex hormones play a critical role. Here, we tried to summarize the sex differences reported in the role of *PNPLA3* I148M variant on disease progression, reviewing recent literature from the perspective of clinical, in vitro and in vivo studies. Particularly, results obtained so far pave the way for future precision medicine approaches targeted to women or in individual with higher oestrogen levels carrying *PNPLA3* I148M variant. However, despite advances in understanding the role of *PNPLA3* variants in MASLD, several open questions and gaps remain (Table 3). So, to elucidate the underlying mechanisms and extend them to other genetic variants, further studies will need to be conducted in which males and females are included as separate groups and compared with each other.

AUTHOR CONTRIBUTIONS

All authors were involved in writing the article. The final draft article was approved by all the authors.

ACKNOWLEDGEMENTS

Open access funding provided by BIBLIOSAN.

FUNDING INFORMATION

This work was supported by the Italian Ministry of Health (Ministero della Salute), Fondazione IRCCS Ca′ Granda Ospedale Maggiore Policlinico, Ricerca Corrente; Italian Ministry of University and Research (Italy), PNRR—M4—C2 'National Center for Gene Therapy and Drugs based on RNA Technology' CN3 Spoke 4, group 'ASSET: A sex-specific approach to NAFLD targeting'.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflicts of interest relevant to the present study.

ORCID

Alessandro Cherubini https://orcid.org/0000-0003-4756-2046
Chiara Rosso https://orcid.org/0000-0001-6518-7089
Sara Della Torre https://orcid.org/0000-0001-9498-5266

REFERENCES

1. Anstee QM, Targher G, Day CP. Progression of NAFLD to diabetes mellitus, cardiovascular disease or cirrhosis. *Nat Rev Gastroenterol Hepatol*. 2013;10:330-344.
2. Trépo E, Valenti L. Update on NAFLD genetics: from new variants to the clinic. *J Hepatol*. 2020;72:1196-1209.
3. Lefebvre P, Staels B. Hepatic sexual dimorphism—implications for non-alcoholic fatty liver disease. *Nat Rev Endocrinol*. 2021;17:662-670.
4. Vandel J, Dubois-Chevalier J, Gheeraert C, et al. Hepatic molecular signatures highlight the sexual dimorphism of nonalcoholic Steatohepatitis (NASH). *Hepatology*. 2021;73:920-936.
5. Petrick JL, Florio AA, Znaor A, et al. International trends in hepatocellular carcinoma incidence, 1978-2012. *Int J Cancer*. 2020;147:317-330.
6. Yang JD, Abdelmalek MF, Pang H, et al. Gender and menopause impact severity of fibrosis among patients with nonalcoholic steatohepatitis. *Hepatology*. 2014;59:1406-1414.
7. Della TS. Beyond the X factor: relevance of sex hormones in NAFLD pathophysiology. *Cells*. 2021;10:2502.
8. Turola E, Petta S, Vanni E, et al. Ovarian senescence increases liver fibrosis in humans and zebrafish with steatosis. *Dis Model Mech*. 2015;8:1037-1046.
9. Klair JS, Yang JD, Abdelmalek MF, et al. A longer duration of estrogen deficiency increases fibrosis risk among postmenopausal women with nonalcoholic fatty liver disease. *Hepatology*. 2016;64:85-91.
10. Meda C, Barone M, Mitro N, et al. Hepatic ERα accounts for sex differences in the ability to cope with an excess of dietary lipids. *Molecular Metabolism*. 2020;32:97-108.
11. Romeo S, Kozlitina J, Xing C, et al. Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. *Nat Genet*. 2008;40:1461-1465.
12. Valenti L, Al-Serri A, Daly AK, et al. Homozygosity for the patatin-like phospholipase-3/adiponutrin I148M polymorphism influences liver fibrosis in patients with nonalcoholic fatty liver disease. *Hepatology*. 2010;51:1209-1217.
13. Cherubini A, Ostadreza M, Jamialahmadi O, et al. Interaction between estrogen receptor-α and PNPLA3 p.I148M variant drives fatty liver disease susceptibility in women. *Nat Med*. 2023;29:2643-2655.
14. Sookoian S, Pirola CJ. Meta-analysis of the influence of I148M variant of patatin-like phospholipase domain containing 3 gene (PNPLA3) on the susceptibility and histological severity of nonalcoholic fatty liver disease. *Hepatology*. 2011;53:1883-1894.
15. Li Q, Qu H-Q, Rentfro AR, et al. PNPLA3 polymorphisms and liver aminotransferase levels in a Mexican American population. *Clin Invest Med*. 2012;35:E237-E245.

16. Friedrich K, Rupp C, Hov JR, et al. A frequent PNPLA3 variant is a sex specific disease modifier in PSC patients with bile duct stenosis. *PLoS One*. 2013;8:e58734.
17. Rosso C, Caviglia GP, Birolo G, et al. Impact of PNPLA3 rs738409 polymorphism on the development of liver-related events in patients with nonalcoholic fatty liver disease. *Clin Gastroenterol Hepatol*. 2023;S1542-3565(23):00324-5. doi:10.1016/j.cgh.2023.04.024
18. Cherubini A, Della Torre S, Pelusi S, Valenti L. Sexual dimorphism of metabolic dysfunction-associated steatotic liver disease. *Trends Mol Med*. 2024;S1471-4914(24):00135-7. doi:10.1016/j.molmed.2024.05.013
19. Huang Y, He S, Li JZ, et al. A feed-forward loop amplifies nutritional regulation of PNPLA3. *Proc Natl Acad Sci USA*. 2010;107:7892-7897.
20. Fernández-Suárez ME, Daimiel L, Villa-Turégano G, et al. Selective estrogen receptor modulators (SERMs) affect cholesterol homeostasis through the master regulators SREBP and LXR. *Biomed Pharmacother*. 2021;141:111871.
21. Flessa C-M, Nasiri-Ansari N, Kyrou I, et al. Genetic and diet-induced animal models for non-alcoholic fatty liver disease (NAFLD) research. *Int J Mol Sci*. 2022;23:15791.
22. Vacca M, Kamzolas I, Harder LM, et al. An unbiased ranking of murine dietary models based on their proximity to human metabolic dysfunction-associated steatotic liver disease (MASLD). *Nat Metab*. 2024;6:1178-1196.
23. Della Torre S, Maggi A. Sex differences: a resultant of an evolutionary pressure? *Cell Metab*. 2017;25:499-505.
24. Della Torre S, Benedusi V, Pepe G, et al. Dietary essential amino acids restore liver metabolism in ovariectomized mice via hepatic estrogen receptor α. *Nat Commun*. 2021;12:6883.
25. Della Torre S, Rando G, Meda C, et al. Amino acid-dependent activation of liver estrogen receptor alpha integrates metabolic and reproductive functions via IGF-1. *Cell Metab*. 2011;13:205-214.
26. Della Torre S, Mitro N, Meda C, et al. Short-term fasting reveals amino acid metabolism as a major sex-discriminating factor in the liver. *Cell Metab*. 2018;28:256-267.e5.
27. Smagris E, BasuRay S, Li J, et al. Pnpla3I148M knockin mice accumulate PNPLA3 on lipid droplets and develop hepatic steatosis. *Hepatology*. 2015;61:108-118.
28. BasuRay S, Smagris E, Cohen JC, Hobbs HH. The PNPLA3 variant associated with fatty liver disease (I148M) accumulates on lipid droplets by evading ubiquitylation. *Hepatology*. 2017;66:1111-1124.
29. Johnson SM, Bao H, McMahon CE, et al. PNPLA3 is a triglyceride lipase that mobilizes polyunsaturated fatty acids to facilitate hepatic secretion of large-sized very low-density lipoprotein. *Nat Commun*. 2024;15:4847.
30. Lindén D, Ahnmark A, Pingitore P, et al. Pnpla3 silencing with antisense oligonucleotides ameliorates nonalcoholic steatohepatitis and fibrosis in Pnpla3 I148M knock-in mice. *Mol Metab*. 2019;22:49-61.

How to cite this article: Cherubini A, Rosso C, Della Torre S. Sex-specific effects of PNPLA3 I148M. *Liver Int*. 2024;00:1-8. doi:10.1111/liv.16088