PNPLA3 I148M and response to treatment for hepatic steatosis: A systematic review

Boeckmans, Joost; Gatzios, Alexandra; Schattenberg, Jörn M; Koek, Ger H; Rodrigues, Robim; Vanhaecke, Tamara

Published in:
Liver International : Official Journal of the International Association for the Study of the Liver

DOI:
10.1111/liv.15533

Publication date:
2023

Received: 11 January 2023 | Revised: 25 January 2023 | Accepted: 28 January 2023

DOI: 10.1111/liv.15533

Liver INTERNATIONAL WILEY

ORIGINAL ARTICLE

PNPLA3 I148M and response to treatment for hepatic steatosis: A systematic review

Joost Boeckmans[1] | Alexandra Gatzios[1] | Jörn M. Schattenberg[2] | Ger H. Koek[3,4] | Robim M. Rodrigues[1] | Tamara Vanhaecke[1]

[1]Department of In Vitro Toxicology and Dermato-Cosmetology, Faculty of Medicine and Pharmacy, Vrije Universiteit Brussel, Brussels, Belgium

[2]Metabolic Liver Research Center, Department of Medicine, University Medical Center Mainz, Mainz, Germany

[3]Division of Gastroenterology and Hepatology, Department of Internal Medicine, Maastricht University Medical Centre+, Maastricht, The Netherlands

[4]School of Nutrition and Translational Research in Metabolism, Maastricht University, Maastricht, The Netherlands

Correspondence
Joost Boeckmans, Department of In Vitro Toxicology and Dermato-Cosmetology, Faculty of Medicine and Pharmacy, Vrije Universiteit Brussel, Brussels, Belgium.
Email: joost.boeckmans@vub.be

Funding information
Chair Mireille Aerens for the Development of Alternative Methods; Colgate-Palmolive - Society of Toxicology; Fonds Wetenschappelijk Onderzoek; Onderzoeksraad Vrije Universiteit Brussel

Handling Editor: Luca Valenti

Abstract

Background: It is unclear whether the *patatin-like phospholipase domain-containing protein 3* (*PNPLA3*) rs738409 C-to-G single nucleotide polymorphism, resulting in the substitution of isoleucine to methionine at position 148 (I148M), impedes regression of hepatic steatosis when treating non-alcoholic fatty liver disease (NAFLD).

Objectives: Investigate if carriage of the *PNPLA3* 148M allele affects the anti-steatotic efficacy of all possible anti-NAFLD interventions, identify gaps in current knowledge and provide guidance for individual treatment.

Methods: Research available in public databases was searched up to 13 November 2022. Studies were included if a treatment in NAFLD patients decreased hepatic steatosis in the pooled patient group or a *PNPLA3* I148M polymorphism subgroup (II/IM/MM). The risk of bias was assessed using the Cochrane Risk-Of-Bias 2 Tool and the Newcastle–Ottawa Scale.

Results: Moderate evidence indicates that NAFLD patients homozygous for the *PNPLA3* 148M allele benefit less or not at all from omega-3 carboxylic acids to decrease liver fat, while the *PNPLA3* 148I allele shows moderate benefit. Low evidence suggests that interventions employing lifestyle changes are more effective to reduce liver fat in NAFLD patients homozygous for the *PNPLA3* 148M allele compared to patients with wild-type *PNPLA3*.

Conclusions: NAFLD patients homozygous for the *PNPLA3* 148M allele might not benefit from omega-3 carboxylic acids to reduce hepatic steatosis in contrast to patients with wild-type *PNPLA3*. Instead, patients with two *PNPLA3* 148M alleles should be especially advised to adopt lifestyle changes. Genotyping for *PNPLA3* I148M should be encouraged in therapeutic studies for NAFLD.

Registration Number (Prospero): CRD42022375028.

Abbreviations: ^{1}H-MRS, proton magnetic resonance spectroscopy; BMI, body mass index; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; GCKR, glucokinase regulator; MAFLD, metabolic-associated fatty liver disease; MBOAT7, membrane-bound O-acyltransferase 7; MRI-PDFF, magnetic resonance imaging proton density fat fraction; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; PNPLA3, patatin-like phospholipase domain-containing protein 3; PRISMA, Preferred Reporting Items for Systematic reviews and Meta-Analyses; PRS-HFC, polygenic risk score for hepatic fat content; RoB2, Risk-Of-Bias 2; SNP, single nucleotide polymorphism; SREBP-1c, sterol regulatory element-binding protein-1c; TM6SF2, transmembrane 6 superfamily member 2.

Joost Boeckmans and Alexandra Gatzios share first authorship.

KEYWORDS
genetics, hepatic steatosis, non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), patatin-like phospholipase domain-containing protein 3 (PNPLA3)

1 | INTRODUCTION

1.1 | Rationale

Non-alcoholic fatty liver disease (NAFLD) is estimated to affect 32.4% of the world population[1] and represents an umbrella term for successive conditions.[2] Steatosis, which is characterized by intrahepatic lipid accumulation, is the first stage in NAFLD and does not pose immediate health risks. However, 5% to 10% of the patients suffering from liver steatosis advance to non-alcoholic steatohepatitis (NASH), which is accompanied by hepatic inflammation and is considered the tipping point to life-threatening conditions including liver cirrhosis and hepatocellular carcinoma.[3] Overall, the socioeconomic burden of NAFLD is high.[4]

In the past decade, multiple single nucleotide polymorphisms (SNPs) have been discovered that contribute to the earlier onset and faster progression of NAFLD, including variants of *patatin-like phospholipase domain-containing protein 3* (*PNPLA3*), *transmembrane 6 superfamily member 2* (*TM6SF2*), *membrane-bound O-acyltransferase 7* (*MBOAT7*) and *glucokinase regulator* (*GCKR*). In addition, a polygenic risk score for hepatic fat content (PRS-HFC) based on the aforementioned genes has been established based on the effect size of each variant.[5,6] The *PNPLA3* rs738409 variant with C-to-G loss-of-function mutation, by which isoleucine is substituted by methionine at position 148 (I148M), is considered a robust genetic factor associated with susceptibility to NAFLD and the progression of the disease.[7–10] One of the mechanisms behind the effect of this SNP on NAFLD is related to the impairment of PNPLA3-mediated triglyceride hydrolysis in lipid droplets of hepatocytes.[11,12] In addition, PNPLA3 mediates retinol release in hepatic stellate cells, which is protective against liver fibrosis. The PNPLA3 I148M variant shows, however, impaired retinyl-palmitate lipase activity and could therefore predispose to liver fibrosis.[13,14]

Although the presence of isolated steatosis is not a major health issue, intrahepatic lipids are involved in lipid-induced stress and lipotoxicity resulting in the activation of inflammation and progression of NAFLD.[15] Therefore, reducing intrahepatic lipids is a valuable strategy for anti-NASH drug development to halt and reverse the disease before it progresses to end-stage liver conditions. In addition, several potential anti-NASH drugs that target de novo lipogenesis are in clinical phases II and III, including aramchol (a stearoyl-CoA desaturase 1 inhibitor)[16] and TVB-2640 (a fatty acid synthase inhibitor),[17] in which reductions of intrahepatic lipids are shown along with improvements in biomarkers of inflammation and fibrosis.

Driven by insufficient drug response rates and failures of late-stage NASH clinical trials,[18] it has recently become clear that NAFLD is a heterogenous disease in which genetic, metabolic and environmental disease drivers are present in different proportions among patients. In that context, it has been stipulated that patients should be stratified in clinical studies according to their predominant disease driver in order to advance anti-NASH drug development.[19] In line with the genetic drivers of NAFLD, carriage of two *PNPLA3* 148M alleles has been linked to poor anti-NASH treatment response, although conflicting data exist.[20] Since a poor response to anti-NASH interventions in homozygous carriers of *PNPLA3* 148M may be explained by the prominent role of PNPLA3 in triglyceride hydrolysis,[11,12] it is hypothesized that the *PNPLA3* I148M variant affects treatment response by compromising the reduction of intrahepatic lipids compared to non-carriers. To underline the critical role of PNPLA3, genetic strategies to target PNPLA3 in patients with NASH are underway.[21]

Key points

- Patients with NAFLD homozygous for the *PNPLA3* 148M allele might benefit less or not at all from omega-3 carboxylic acids to reduce hepatic steatosis compared to patients with two 148I alleles.
- Homozygosity for *PNPLA3* 148M might enhance the effect of lifestyle changes, including calorie restriction and physical exercise to reduce liver fat.
- Pharmacological studies for NAFLD investigating the effect of *PNPLA3* I148M on treatment response for hepatic steatosis are scarce.
- Genotyping for *PNPLA3* I148M should be encouraged in therapeutic studies for NAFLD and NASH.

1.2 | Objectives

The objective of this study is to investigate whether patients with hetero- or homozygous carriage of the *PNPLA3* 148M allele respond differently to anti-NAFLD therapies than patients with homozygous carriage of the 148I allele.

2 | METHODS

2.1 | Protocol and registration

This study has been prospectively registered in the PROSPERO database with registration number CRD42022375028. The systematic review was performed according to the guidelines of the Preferred

Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) (Table S1).[22]

2.2 | Eligibility criteria

Eligible studies were full-text papers, conference proceedings and posters written in English in which patients suffering from NAFLD/NASH received or underwent treatment that reduced the hepatic lipid load compared to placebo or baseline in the pooled patient group or patient subgroups based on PNPLA3 I148M polymorphism. Studies enrolling either heterozygous or homozygous carriers of the *PNPLA3* 148M allele were eligible for inclusion. Elaborated eligibility criteria are given in Table S2.

2.3 | Information sources

Studies published until 13 November 2022 were searched using Medline, Web of Science and the Cochrane Library (covering PubMed and Embase).

2.4 | Search

Medline (PubMed): (((((‘Non-alcoholic Fatty Liver Disease’[Mesh]) OR (NAFLD)) OR (non-alcoholic fatty liver disease)) OR (NASH)) OR (non-alcoholic steatohepatitis) OR (MAFLD) OR (metabolic-associated fatty liver disease)) AND (((PNPLA3) OR (Patatin-like phospholipase domain-containing protein 3)) OR (adiponutrin)) + filter ‘Clinical Trial’.

Cochrane Library (PubMed + Embase): (((((‘Non-alcoholic Fatty Liver Disease’[Mesh]) OR (NAFLD)) OR (non-alcoholic fatty liver disease)) OR (NASH)) OR (non-alcoholic steatohepatitis) OR (MAFLD) OR (metabolic-associated fatty liver disease)) AND (((PNPLA3) OR (Patatin-like phospholipase domain-containing protein 3)) OR (adiponutrin)).

Web of Science: AB = (NAFLD OR non-alcoholic fatty liver disease OR NASH OR non-alcoholic steatohepatitis OR MAFLD OR metabolic dysfunction-associated fatty liver disease) AND AB = (PNPLA3 OR patatin-like phospholipase domain-containing protein 3 OR adiponutrin) + Filter ‘Trial’.

2.5 | Study selection

Search results were extracted and duplicates were manually removed. Titles and abstracts were pre-screened based on the reporting of the term ‘PNPLA3’ in the pre-specified article types (Table S2) to identify potentially relevant records. Then, the full-text papers were assessed for all eligibility criteria. Two researchers conducted the search independently. Inconsistencies between the two authors were discussed to reach a consensus in one round by reading the respective articles together.

2.6 | Data collection process

Data were collected independently by two researchers using a predefined extraction sheet in Microsoft Excel. The collected data were compared upon completion of the collection process. Disagreements were discussed to reach a consensus in one round by reading the respective articles together.

2.7 | Data items

Data were collected on the study details (first author, country, publication year, study type and total number of patients), patient characteristics (genotype composition, ethnicity, age, sex, body mass index (BMI) and NAFLD stage), intervention (intervention, dosage and frequency), outcome (numerical outcome and *p*-value) and method used for assessing hepatic steatosis.

2.8 | Risk of bias in individual studies

The validity of the included studies was assessed using the Cochrane Risk-Of-Bias 2 (RoB2) Tool (for assessing randomized controlled clinical trials)[23] and the Newcastle–Ottawa Scale (for assessing case–control and cohort studies).[24] Bias assessments were performed independently by two researchers. Inconsistencies between the two authors were discussed to reach a consensus in one round by reading the respective articles together.

2.9 | Summary measures

Preliminary searches showed heterogenous study outcome measures of the effect of the determinant. Therefore, the summary measure was of qualitative nature, being treatment response ‘in favor of PNPLA3 148I’, ‘in favor of PNPLA3 148M’ or ‘no effect of PNPLA3 I148M’.

2.10 | Synthesis of results

Data pooling was not possible because of different outcome measures, including relative and absolute reductions from baseline and correlation coefficients. Results were narratively synthesized in which studies of poor and intermediate quality were discussed from that perspective.

2.11 | Risk of bias across studies

As a measure of publication bias and selective reporting, it was evaluated whether the study protocols of the included studies were pre-registered in a publicly available database and whether subgroup analysis for the PNPLA3 I148M variant was pre-defined. The

assessments of the risk of bias across studies were performed independently by two researchers. Inconsistencies between the two authors were solved in one round by doing the search together.

3 | RESULTS

3.1 | Study selection

The initial search yielded 36 unique records based on the predefined search criteria. Of those 36 records, 26 were removed in the pre-screen based on the title and the abstract in which no indication was given that the selection parameters were present. After assessment of the full-text versions of the 10 records that were left, 3 studies were excluded since there was no genetic analysis of the treatment group of interest. One record was manually added, resulting in seven full-text articles and one poster for inclusion in the systematic review (Figure 1).

3.2 | Study characteristics

3.2.1 | Studies

The eight records selected for inclusion consisted of 10 studies since the study from Eriksson et al.[25] made use of a parallel-group design in which three therapies were evaluated. Three studies were prospective cohort studies, six were randomized-controlled studies and one was a case–control study. Study durations ranged from 6 days to 24 months and are provided for each study in Table 1. For one study, only a meeting abstract with a poster was available from an interim analysis. The outcome measures were heterogenous and most often calculated relative to baseline values.

3.2.2 | Participants and interventions

The included studies covered a total of 659 enrolled NAFLD patients, of which one control group of 21 patients was used in triplicate (Table 2). Forty-two patients were part of a pharmacological treatment study, 208 of lifestyle intervention studies with focus on calorie restriction, 204 of food supplement studies, 204 of lifestyle intervention studies with focus on physical exercise and dietary habits and 43 of a combination treatment study (Table 3). The *PNPLA3* genotypes II, IM and MM occurred in a ratio of 1.0/1.0/0.5, respectively, indicating that patients with two *PNPLA3* 148M alleles were the least represented. The number of patients per group with respective genotypes in each study is specified in Table 2. The ethnicity of the study participants was mentioned in three studies. Two studies included paediatric patients. All patients were assessed for hepatic steatosis at baseline using non-invasive imaging techniques (Table 2).

3.2.3 | Outcome

The primary outcome was the effect of PNPLA3 I148M on treatment response based on reducing intrahepatic lipids. All patients were evaluated for end-of-study liver fat using the same techniques as used for the baseline measurements within each study. Only the study of Krawczyk et al.[27] was primarily powered to show a difference in treatment response between patients with different *PNPLA3* I148M genotypes, although a dropout of 35% resulted in a loss of power.

3.3 | Risk of bias within studies

The four included randomized controlled trials were rated as having some concerns since the outcome of interest was not

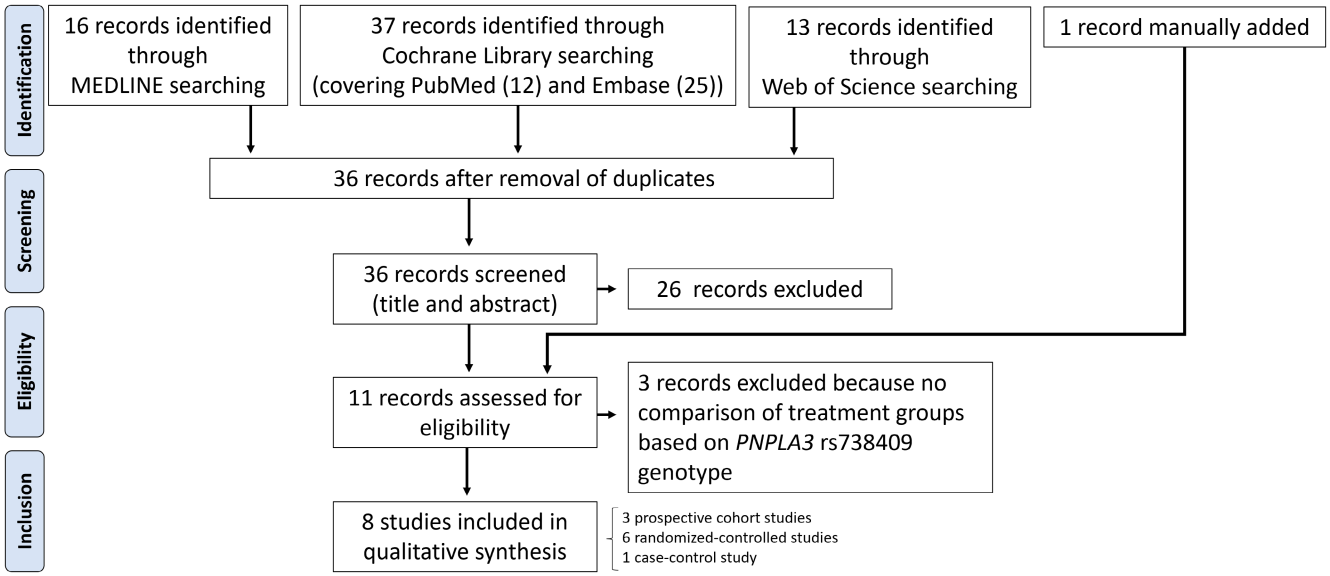

FIGURE 1 PRISMA flow diagram of studies included in the systematic review. Eight articles, covering 10 studies, were included in the systematic review.

TABLE 1 Characteristics of included studies.

	Country	Study type	Study duration	Total number of patients	Outcome measure
Pharmacological					
Eriksson et al. 2018[25]	Sweden	Multi-centre randomized placebo-controlled double-blind parallel-group study	12 weeks	42	Relative reduction of hepatic steatosis from baseline
Lifestyle intervention with focus on calorie restriction					
Teufelhart et al. 2017[26]	Austria	Prospective cohort study (interim analysis)	2 weeks +6 weeks	47 patients at 2 weeks and 36 at 8 weeks	Difference in controlled attenuation parameter from baseline
Krawczyk et al. 2016[27]	Poland	Prospective cohort study	4 months	143	Absolute difference in Hamaguchi score from baseline
Sevastianova et al. 2011[28]	Finland	Case–control study	6 days	18	Relative reduction of hepatic steatosis from baseline
Food supplements					
Eriksson et al. 2018[25]	Sweden	Multi-centre randomized placebo-controlled double-blind parallel-group study	12 weeks	41	Relative reduction of hepatic steatosis from baseline
Scorletti et al. 2015[29]	United Kingdom	Multi-centre randomized placebo-controlled double-blind study	15 to 18 months	103	B coefficient for end-of-study liver fat percentage
Nobili et al. 2013[30]	Italy	Randomized placebo-controlled study	24 months	60	Probability of more severe liver steatosis
Lifestyle intervention with focus on physical exercise and dietary habits					
Koot et al. 2015[31]	The Netherlands	Prospective cohort study	6 months	51	B coefficient for end-of-study liver fat percentage
Shen et al. 2015[32]	China	Randomized single-blind controlled study	12 months	154	Spearman's correlation coefficient
Combinations					
Eriksson et al. 2018[25]	Sweden	Multi-centre randomized placebo-controlled double-blind parallel-group study	12 weeks	43	Relative reduction of hepatic steatosis from baseline

pre-specified in the study protocol (Table S3). The study by Nobili et al.[30] holds some additional concerns since the primary outcome was based on the subjective estimation of the degree of liver steatosis using ultrasonography although its overall bias risk is the same as for the other randomized controlled trials. One cohort study[26] was rated as being of poor quality since the study was an interim analysis of a limited number of enrolled patients, and sparse information was present regarding the selection of the patients. The cohort study by Krawczyk et al.[27] was rated as having some concerns because it scored moderately for each domain. The cohort study by Koot et al.[31] and the case–control study by Sevastianova et al.[28] were the only studies considered to have a low risk of bias (Table S4), although the latter study consisted only of 18 patients.

3.4 | Results of individual studies and synthesis of results

3.4.1 | Pharmacological intervention

The relative reduction of hepatic fat from baseline in response to 12 weeks of dapagliflozin (10 mg, once daily) measured using magnetic resonance imaging proton density fat fraction (MRI-PDFF) in 20 genotyped patients was found to be larger in *PNPLA3* 148II patients (−22.0% [−26.8% to −19.2%]) compared to a pooled patient group with one and two *PNPLA3* 148M alleles (7.0% [−2.2% to 11.3%]), suggesting that dapagliflozin is a less effective treatment to reduce hepatic steatosis in patients with at least one *PNPLA3* 148M allele as most of the patients with the *PNPLA3* 148M allele

TABLE 2 Baseline details of participants from the included studies.

	Genotype composition	Ethnicity	Age	Sex (male: female)	BMI	Method used for assessing hepatic steatosis	NAFLD stage	Additional information
Pharmacological								
Eriksson et al.[25]	Placebo: 21 Intervention: 21 N.B.: 20 genotyped patients per group, number of patients per genotype not specified	Ethnicity not specified. A majority of Northern European descent can be assumed.	Mean: Placebo: 65.6 Intervention: 65.0	Placebo: 17/4 Intervention: 16/5	Mean: Placebo: 30.3 Intervention: 30.5	MRI-PDFF	At least hepatic steatosis	Concurrent treatment with metformin (82%) and/or sulfonylurea (18%). Drug naivety in 14% of patients. Patients with *PNPLA3* 148M were mostly heterozygous ($n = 47$ II, $n = 30$ IM and $n = 3$MM, genotyping for $n = 20$ per group)
Lifestyle intervention with focus on calorie restriction								
Teufelhart et al.[26]	2 weeks: II: 20 IM: 20 MM: 7 2 + 6 weeks: II: 19 IM: 11 MM: 6	Ethnicity not specified. A majority of Western European descent can be assumed.	Mean: 46.0	28/19	II: 32.2 IM + MM: 31.3	Transient elastography with controlled attenuation parameter	At least hepatic steatosis	Interim analysis of a limited number of patients (tentative enrolment: 27 patients per genotype)
Krawczyk et al.[27]	II: 71 IM: 53 MM: 19	Eastern European descent	Median: II: 46 IM: 49 MM: 48	II: 40/31 IM: 35/18 MM: 13/6	Median: II: 32.2 IM: 33.1 MM: 31.1	Ultrasonography	At least hepatic steatosis	
Sevastianova et al.[28]	II: 10 MM: 8	Northern European descent.	Mean: II: 52 MM: 48	II: 4/6 MM: 2/6	Mean: II: 30.7 MM: genotype: 29.6	^{1}H-MRS	At least hepatic steatosis	Serum fasting insulin and C-reactive protein were lower in the *PNPLA3* 148MM group at baseline
Food supplements								
Eriksson et al.[25]	Placebo: 21 Intervention: 20 N.B.: 20 genotyped patients per group, number of patients per genotype not specified	Ethnicity not specified. A majority of Northern European descent can be assumed.	Mean: Placebo: 65.6 Intervention: 66.2	Placebo: 17/4 Intervention: 15/7	Mean: Placebo: 30.3 Intervention: 33.0	MRI-PDFF	At least hepatic steatosis	Concurrent treatment with metformin (82%) and/or sulfonylurea (18%). Drug naivety in 14% of patients. Patients with *PNPLA3* 148M were mostly heterozygous ($n = 47$ II, $n = 30$ IM and $n = 3$MM, genotyping for $n = 20$ per group)
Scorletti et al.[29]	Placebo: 52 Treatment: 51 II: 42 IM: 43 MM: 13	Ethnicity not specified. A majority of Northern European descent can be assumed.	Mean: Placebo: 54.0 Treatment:48.6	Placebo: 35/17 Treatment:25/26	Mean: Placebo: 32.4 Treatment: 34.0 II: 34.6 IM: 33.2 MM: 31.7	^{1}H-MRS	At least hepatic steatosis	

TABLE 2 (Continued)

	Genotype composition	Ethnicity	Age	Sex (male: female)	BMI	Method used for assessing hepatic steatosis	NAFLD stage	Additional information
Nobili et al.[30]	Placebo: 20 Treatment low dose: 20 Treatment high dose: 20 II: 6 IM: 34 MM: 20	Ethnicity not specified. A majority of Southern European descent can be assumed.	Median: Placebo: 13 Treatment low dose: 11 Treatment high dose: 11 II: 10 IM: 11 MM: 12	Placebo: 8/12 Treatment low dose: 8/12 Treatment high dose: 9/11 II: 5:1 IM: 20:14 MM: 10:10	Median: Placebo: 26.1 Treatment low dose: 26.6 Treatment high dose: 24.4 II: 25.5 IM: 26.0 MM: 26.7	Ultrasonography	At least hepatic steatosis	Paediatric NAFLD
Lifestyle intervention with focus on physical exercise and dietary habits								
Koot et al.[31]	Usual care: 18 II: 7 IM: 5 MM: 6 Ambulatory treatment: 21 II: 6 IM: 9 MM: 6 Inpatient treatment: 23 II: 12 IM: 3 MM: 8	Mainly Caucasian and Middle-Eastern descent	Mean: Usual care: 14.7 Ambulatory treatment: 14.4 Inpatient treatment: 14.9	Usual care: 10/8 Ambulatory treatment: 13/8 Inpatient treatment: 10/13	BMI z score: Usual care: 3.3 Ambulatory treatment: 3.5 Inpatient treatment: 3.6	^{1}H-MRS	At least hepatic steatosis	Paediatric NAFLD Randomization was done for inpatient treatment vs. usual care arms but not for the ambulatory treatment arm After 6 months, 12 patients moved from the usual care group to the inpatient treatment group.
Shen et al.[32]	Control: 77 II: 22 IM: 38 MM: 17 Intervention: 77 II: 22 IM: 38 MM: 17	Ethnicity not specified. A majority of Eastern Asian descent can be assumed.	Mean: Control: 51 II: 51 IM: 51 MM: 50 Intervention: 51 II: 50 IM: 53 MM: 49	Control: 31/46 II: 12/10 IM: 12/26 MM: 8/9 Intervention: 41/36 II: 10/12 IM: 19/19 MM: 10/7	Control: 25.3 II: 26.3 IM: 25.3 MM: 23.9 Intervention: 25.5 II: 26.1 IM: 25.0 MM: 25.8	^{1}H-MRS	At least hepatic steatosis	
Combinations								
Eriksson et al.[25]	Placebo: 21 Intervention: 22 N.B.: 20 genotyped patients per group and number of patients per genotype not specified	Ethnicity not specified. A majority of Northern European descent can be assumed.	Mean: Placebo: 65.6 Intervention: 65.0	Placebo: 17/4 Intervention: 15/7	Mean: Placebo: 30.3 Intervention: 31.1	MRI-PDFF	At least hepatic steatosis	Concurrent treatment with metformin (82%) and/or sulfonylurea (18%). Drug naivety in 14% of patients. Patients with *PNPLA3* 148M were mostly heterozygous ($n = 47$ II, $n = 30$ IM and $n = 3$ MM, genotyping for $n = 20$ per group)

Note: II = homozygous 148I; IM = heterozygous 148M; MM = homozygous 148M.

Abbreviations: ^{1}H-MRS, proton magnetic resonance spectroscopy; MRI-PDFF, magnetic resonance imaging proton density fat fraction.

TABLE 3 Details of the interventions and results of individual studies.

	Intervention	Dosage	Frequency	Numerical outcome [brackets indicate 95% CI]	*p*-value
Pharmacological					
Eriksson et al.[25]	Dapagliflozin	10 mg	Once daily	Relative reduction of hepatic fat from baseline: II: −22.0% [−26.8% to −19.2%] IM + MM: 7.0% [−2.2% to 11.3%]	<0.01
Lifestyle intervention with focus on calorie restriction					
Teufelhart et al.[26]	Low-calorie diet (max. 1000 kcal/day) with Hepafast® (protein shake enriched with liver protective substances) followed by 6 weeks low glycaemic and insulinaemic diet	NA	NA	Controlled attenuation parameter from baseline (2 weeks): II: 310.8 ± 29.3 (n = 20) to 268.7 ± 40.1 (n = 20) (difference 42.1 ± 34.5) IM + MM: 322.9 ± 28.0 (n = 25) to 272.0 ± 28.5 (n = 25) (difference 50.9 ± 30.7) Controlled attenuation parameter from baseline (8 weeks): II: 310.8 ± 29.3 (n = 20) to 238.2 ± 33.1 (n = 19): (difference 74.0 ± 30.1) IM + MM: 322.9 ± 28 (n = 25) to 252.6 ± 29.0 (n = 17) (difference 77.3 ± 27.6)	$p = 0.59$ $p = 0.91$
Krawczyk et al.[27]	Restriction of daily caloric intake without changes in physical activity Overweight/obese patients: 500 kcal restriction Normal weight patients: energy consistent with physiological needs	NA	NA	Mean difference in Hamaguchi score (at entry–after diet): II: 1 IM: 1 MM: 0.7	n.s. (no *p*-value given)
Sevastianova et al.[28]	Hypocaloric (1000 kcal deficit) low-carbohydrate (<20 g/day) diet	NA	NA	Relative and absolute reductions of hepatic fat from baseline: II: −18.4 ± 7.8% (from −11.9 ± 2.1% to 9.9 ± 2.0%) MM −44.6 ± 7.0% (from −10.2 ± 1.8% to 6.5 ± 1.7%)	$p = 0.027$ (relative) $p = 0.042$ (absolute)
Food supplements					
Eriksson et al.[25]	Omega-3 carboxylic acids (EPA + DHA + DPA)	2000–2400 mg + 600–800 mg + 40–320 mg	Once daily	Relative reduction of hepatic fat from baseline: II: −18.6% [−20.2 to −15.6%] IM + MM: −12.6% [−15.9 to −4.3%]	n.s (no *p*-value given)
Scorletti et al.[29]	Omega-3 carboxylic acids (EPA + DHA)	1840 mg + 1520 mg	Once daily	Unstandardized B coefficient for end-of-study liver fat percentage: MM: 9.5 [2.53–16.39]	$p = 0.008$
Nobili et al.[30]	Omega-3 carboxylic acids (DHA) + advice for following a low-calorie diet and doing aerobic exercise	250 mg or 500 mg	Once daily	Probability of more severe liver steatosis: MM vs. IM: 37% [26% to 48%] II vs. IM: −12% [−21% to −3%]	$p < 0.001$ $p < 0.05$

TABLE 3 (Continued)

	Intervention	Dosage	Frequency	Numerical outcome [brackets indicate 95% CI]	*p*-value
Lifestyle intervention with focus on physical exercise and dietary habits					
Koot et al.[31]	Lifestyle modification in different settings: inpatient care, ambulatory care and usual care	NA	• Long inpatient setting: 6 months inpatient treatment on working days + monthly follow-up of 2 days for 6 months • Short inpatient setting: 2 months of inpatient treatment on working days + biweekly return of 2 days during 4 months + monthly follow-up of 2 days for 6 months • Ambulatory setting: 16 ambulatory visits during 6 months with homework assignments + follow-up after 6 weeks and 3, 6, 9 and 12 months • Usual care: continuation of local care	B coefficient from univariable regression for improvement in degree of liver steatosis: II: reference IM: −3.8 (−10 to 2.7) MM: −1.4 (−7.9 to 5.2)	IM vs. II $p = 0.63$ MM vs. II $p = 0.67$
Shen et al.[32]	Dietitian-led lifestyle modification (reducing calorie intake and increasing energy expenditure)	NA	• First 4 months: weekly dietary consultations • Last 8 months: monthly dietary consultations	Change in intrahepatic lipids (%) (with SD): Control: −2.1 (6.4) II: −2.0 (6.4) IM: −0.8 (5.7) MM: −5.2 (7.2) Spearman's correlation coefficient for the control group for 148M: 0.10 Intervention: −6.7 (6.1) II: −3.7 (5.2) IM: −6.5 (3.6) MM: −11.3 (8.8) Spearman's correlation coefficient for the intervention group for 148M: 0.34 B coefficient from multivariable regression (II = 1, IM = 2 and MM = 3): Control group: / Intervention group: B = 2.97 [95% CI: 1.19 to 4.76]	 $p = 0.384$ $p = 0.002$ n.s. (no *p*-value given) $p = 0.001$
Combinations					
Eriksson et al.[25]	Omega-3 carboxylic acids (EPA + DHA + DPA) Dapagliflozin	2000–2400 mg + 600–800 mg + 40–320 mg / 10 mg	Once daily	Relative reduction of hepatic fat from baseline: II: −16.1% [−20.5% to −11.6%] IM + MM: −25.4% [−27.3% to −19.0%]	n.s. (no *p*-value given)

Note: Brackets indicate 95% confidence intervals. II = homozygous 148I, IM = heterozygous 148M, MM = homozygous 148M.

Abbreviations: DHA: docosahexaenoic acid; DPA: docosapentaenoic acid; EPA: eicosapentaenoic acid.

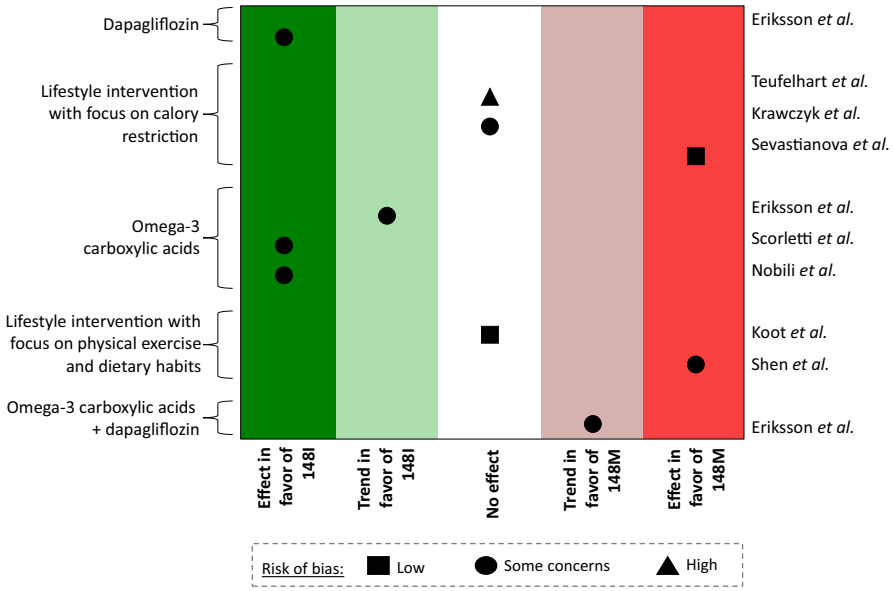

FIGURE 2 Data summary. NAFLD patients carrying a *PNPLA3* 148M allele are less sensitive to omega-3 carboxylic acids to decrease their hepatic fat but seem to benefit more from lifestyle interventions including calorie restriction and physical exercise. NAFLD, non-alcoholic fatty liver disease; PNPLA3, patatin-like phospholipase domain-containing protein 3.

were heterozygous (Table 3).[25] It must be noted, however, that only one study investigated the effect of PNPLA3 I148M on a pharmacologically mediated reduction of hepatic steatosis. No general conclusion can, therefore, be drawn for the lower susceptibility to pharmacological treatments of patients carrying the *PNPLA3* 148M allele (Figure 2).

3.4.2 | Lifestyle intervention with focus on calorie restriction

Three studies investigated the impact of PNPLA3 I148M on response to diet and calorie restriction (Table 3). Teufelhart et al. investigated whether calorie restriction (1000 kcal/day) and a protein shake enriched with liver protective substances for 2 weeks, followed by a low glycaemic and insulinaemic diet for 6 weeks could reduce intrahepatic lipids differently between *PNPLA3* I148M genotypes. Hepatic steatosis was measured using transient elastography with controlled attenuation parameters at 2 and 8 weeks, but no differences were found between the *PNPLA3* 148II and pooled homo- and heterozygous *PNPLA3* 148M patient groups. It must be noted, however, that this study was a bias-prone conference abstract and poster of an interim analysis of a limited number of patients of a prospective cohort study, likely resulting in an underpowered analysis.[26] A second study included 143 genotyped NAFLD patients and lasted 4 months. Obese and overweight patients followed a diet with 500 kcal restriction while normal-weight patients received a diet according to their physiological needs. Although this study was powered on detecting differences in treatment response between *PNPLA3* I148M genotypes, no differences were found in the decrease in the ultrasound-based Hamaguchi score between the genetic subgroups.[27] Nonetheless, dropout occurred for 35% of patients, and the power analysis was based on an odds ratio of 2.1, which might have been too substantive. The third study was a small case–control study (18 patients; 8 with two *PNPLA3* 148M alleles and 10 with two 148I alleles) using a hypocaloric (1000 kcal deficit) low-carbohydrate (20 g) diet for 6 days.[28] After 6 days only, the relative and absolute reductions in hepatic lipids, measured using proton magnetic resonance spectroscopy (^{1}H-MRS), was already significantly larger in *PNPLA3* 148MM patients compared to the patients with *PNPLA3* 148II (*PNPLA3* 148II: −18.4 ± 7.8% (from 11.9% ± 2.1% to 9.9 ± 2.0%) vs. *PNPLA3* 148MM −44.6 ± 7.0% (from 10.1% ± 1.8% to 6.5 ± 1.7%)), indicating that this type of diet is more effective in patients homozygous for the *PNPLA3* I148M variant to reduce hepatic steatosis.

3.4.3 | Food supplement intervention

In the studies in which food supplements were evaluated, all consisting of omega-3 carboxylic acids, two[29,30] of three studies reported that the decrease in liver steatosis was in favour of *PNPLA3* 148II, while a trend in the same direction was observed for the third study (Table 3).[25] In the study performed by Scorletti et al., using a mixture of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (1840 mg EPA + 1520 mg DHA) with liver fat measurements using ^{1}H-MRS, an unstandardized B-value of 9.5 [95% CI 2.53 to 16.39] was found for the homozygous carriage of the *PNPLA3* 148M allele after adjusting for baseline liver fat percentage and most important confounders, indicating that this genotype is independently associated with end-of-study liver fat. In

line with this finding, Nobili et al. reported on the probability of having more severe liver steatosis (graded on a scale from 0 to 3) in obese children with NAFLD after 24 months of treatment with DHA (250 or 500 mg, once daily) with concurrent advice for following a low-calorie diet and doing aerobic exercise. Children in the control group were also advised to adopt the aforementioned lifestyle changes. Nevertheless, DHA supplementation appeared to significantly reduce liver fat, indicating that the decrease in liver steatosis was at least partially caused by the omega-3 carboxylic acids.[33] Within the treatment groups, they found that the probability of having more severe liver steatosis after 24 months in children with two *PNPLA3* 148M alleles was 37% [95% CI 26% to 48%] higher compared with children having one *PNPLA3* 148M allele. Furthermore, the probability of having more severe liver steatosis was significantly lower in homozygous *PNPLA3* 148I children compared to children with one *PNPLA3* 148M allele (−12% [95% CI −21% to −3%]). Assessment of liver steatosis was, however, based on subjective criteria using ultrasonography. In the third study by Eriksson et al.,[25] in which a similar mixture of omega-3 carboxylic acids was used as in the study by Scorletti et al., with the addition of a minor amount of docosapentaenoic acid (DPA), a larger but non-significant reduction in liver steatosis could be measured using MRI-PDFF in the patient group homozygous for the *PNPLA3* 148I allele compared to the pooled patient group with one or two *PNPLA3* 148M alleles after 12 weeks. A decrease in liver fat from baseline of −18.6% [95% CI −20.2% to −15.6%] in the *PNPLA3* 148II group compared to −12.6% [95% CI −15.9% to −4.3%] in the patient group with one or two *PNPLA3* 148M alleles was observed. Nonetheless, only 20 patients were included in the intervention group resulting in low power. Although these three studies have some bias concerns regarding the selection of the reported result and the study by Nobili et al.[30] also regarding the outcome measurement (Table S3), it is likely that patients with two *PNPLA3* 148M alleles benefit less from food supplements consisting of omega-3 carboxylic acids compared to patients homozygous for *PNPLA3* 148I (Figure 2).

3.4.4 | Lifestyle intervention with focus on physical exercise and dietary habits

Lifestyle interventions that employed physical exercise were investigated in two studies. The first study was a prospective cohort study of children with NAFLD and severe obesity.[31] The children followed intensive lifestyle treatments in different settings, being inpatient, ambulatory or usual care with hepatic fat measurement at baseline and after 6 months. Hepatic fat, measured using ^{1}H-MRS, decreased in all settings without significant differences between the groups. Then, the effect of PNPLA3 I148M on the treatment response related to reducing hepatic steatosis was assessed using linear regression analysis. When using data of patients homozygous for the *PNPLA3* 148I allele as a reference, B coefficients of −3.8 [95% CI −10 to 2.7] for one 148M allele and −1.4 [95% CI −7.9 to 5.2] for two 148M alleles were found, indicating that differences in *PNPLA3* genotype did not predict the treatment response (a positive B value here indicates a larger decrease in the histological per cent of steatosis since the ^{1}H-MRS scanner was validated using histology). The second study was a post hoc analysis of a 12-month community-based lifestyle intervention programme with 154 genotyped NAFLD patients. Here, it was explored whether a dietitian-led lifestyle modification approach to reduce calorie intake and increase energy expenditure[34] could result in a different response depending on PNPLA3 I148M polymorphism. It was observed that the presence of the 148M allele correlated with a better response to the lifestyle modifications compared to patients with two *PNPLA3* 148I alleles to reduce hepatic fat, which was measured using ^{1}H-MRS. The Spearman's correlation coefficient for the 148M allele in the intervention group was 0.34 ($p = 0.002$), while this was not significant for the control group (Spearman's correlation coefficient 0.10 with $p = 0.384$). On multivariable analyses, the *PNPLA3* polymorphism was associated with changes in hepatic steatosis in the intervention group only (B = 2.97 [95% CI: 1.19 to 4.76] with $p < 0.001$), indicating that NAFLD patients with *PNPLA3* 148M alleles respond better to lifestyle changes than patients homozygous for *PNPLA3* 148I.[32]

3.4.5 | Combinatory intervention

Lastly, one study examined a combination treatment with dapagliflozin (10 mg, once daily) and a mixture of omega-3 carboxylic acids (2000–2400 mg EPA + 600–800 mg DHA + 40–320 mg DPA, once daily) during 12 weeks in patients with NAFLD.[25] The relative reduction in hepatic fat, measured using MRI-PDFF, was numerically larger in the pooled hetero- and homozygous *PNPLA3* 148M group (−25.4% [95% CI −27.3% to −19.0%]) compared to patients with two *PNPLA3* 148I alleles (−16.1% [95% CI −20.5% to −11.6%]), but this was not significantly different (Table 3). The sample size was possibly too small to achieve enough power since it consisted of only 20 genotyped patients. In addition, it was not mentioned how many patients were hetero- or homozygous for the *PNPLA3* 148M allele, which makes interpretation and reproduction difficult.

3.5 | Risk of bias across studies

For two of the eight included articles, no study protocol registration was mentioned in the published article or poster, and one study protocol was untraceable despite registration being mentioned in the manuscript. None of the registered studies prospectively mentioned a genetic subgroup analysis (Table S5). These factors might have resulted in publication bias and selected reporting.

4 | DISCUSSION

4.1 | Summary of evidence

Moderate evidence indicates that NAFLD patients with two *PNPLA3* 148I alleles benefit more from the intake of omega-3 carboxylic acids compared to patients with two 148M alleles to reduce liver fat. Low evidence exists for the findings that homozygous *PNPLA3* 148M carriers are more sensitive to lifestyle interventions and that NAFLD patients carrying at least one *PNPLA3* 148M allele do not benefit from dapagliflozin to reduce their liver fat.

4.2 | Mechanistic underpinnings of study results

The exact mechanistic basis and cellular consequences of PNPLA3 I148M are not yet completely understood. Yet, Luukkonen et al. reported that the *PNPLA3* 148M variant is a loss-of-function mutation resulting in impaired transacylation and hydrolysis of polyunsaturated fatty acids from diacylglycerols. Very low-density lipoproteins in patients carrying two *PNPLA3* 148M alleles were depleted from polyunsaturated fatty acids compared to wild-type *PNPLA3* 148I carriers. As a consequence, more triglycerides consisting of polyunsaturated fatty acids are retained in the livers of patients with the *PNPLA3* 148M variant.[35] This study could explain why especially NAFLD patients with two *PNPLA3* 148M alleles benefit less or not at all from the intake of omega-3 carboxylic acids to reduce their hepatic lipid load. An open-label study with a limited number of participants including 10 homozygous *PNPLA3* 148I and 10 homozygous 148M NAFLD patients showed, however, that 4-week daily supplementation with 1840 mg EPA and 1520 mg DHA does not alter hepatic steatosis in any genetic subgroup.[36] The included studies in this systematic review in which omega-3 carboxylic acids supplementation was studied and that showed a significant effect of PNPLA3 I148M lasted from 15 to 18 months[29] and 24 months[30] respectively. Therefore, the effect of the PNPLA3 I148M variant on omega-3 carboxylic acids supplementation could require long-term intake to occur.

The effects of lifestyle interventions including calorie restriction and physical exercise seem to be in favour of NAFLD patients with *PNPLA3* 148M alleles, although this effect was not reproduced in all included studies. Marzuillo et al. investigated the effect of waist/hip ratio and BMI standard deviation score (SDS) in response to weight loss in obese children without confirmed NAFLD according to the *PNPLA3* I148M status. In their cohort study, children with two *PNPLA3* 148M alleles showed indeed a larger decrease in steatosis and alanine aminotransferase (ALT) levels compared to children hetero- and homozygous for the *PNPLA3* 148I allele. By using regression analysis, they found that the decrease in ALT levels in homozygous *PNPLA3* 148M children correlated significantly with the waist/hip ratio compared to the other genotypes while there was no significant association with BMI-SDS. Therefore, abdominal fat changes could dictate the effect of PNPLA3 I148M variation in response to diet and lifestyle changes.[37] Although the regression analysis was not performed for liver steatosis, the effect of weight loss on ALT levels in children with two *PNPLA3* 148M alleles supports the findings of this systematic review. Another hypothesis for the lifestyle-mediated effects on liver fat in favour of NAFLD patients with two *PNPLA3* 148M alleles is the fact that PNPLA3 is regulated by sterol regulatory element-binding protein-1c (SREBP-1c) that in its turn is activated by insulin signalling. Therefore, lower insulin levels in response to calorie restriction and physical exercise might lessen the role of PNPLA3 in the liver and perhaps also result in less hepatic trapping of polyunsaturated fatty acids. This hypothesis is supported by the fact that insulin resistance in non-diabetic individuals intensifies the risk for hepatic steatosis based on the presence of *PNPLA3* 148M.[38] The role of insulin sensitivity in the effect of the *PNPLA3* genotype on liver steatosis is, however, in conflict with the finding that the patient group with two *PNPLA3* 148I alleles responded better to dapagliflozin compared to the pooled homo- and heterozygous 148M patient group[25] since dapagliflozin inhibits the renal sodium–glucose cotransporter 2 to improve glycaemic control in diabetes type 2 patients.[39] Dapagliflozin was, however, earlier found to not improve tissue insulin sensitivity upon reducing liver fat.[40] Nonetheless, the group sizes were small and the number of patients for each *PNPLA3* genotype was not specified, making thorough interpretation delicate.

4.3 | Strengths and limitations

The main strength of this systematic review is the narrow research question. Limitations of this study are the heterogenous outcome measures making data pooling impossible and that most studies were not primarily powered to detect differences between *PNPLA3* I148M subgroups or were post hoc analyses of the treatment arm of randomized controlled studies. A limitation of the bias assessments is the fact that the Newcastle–Ottawa scale for assessing bias of non-randomized studies does not capture analysis pre-specification. Therefore, we assessed whether all included studies were prospectively registered and whether genetic analysis was pre-specified in the study protocol as a measure of publication bias and selective reporting.

4.4 | Conclusion and future directions

In summary, NAFLD patients with two *PNPLA3* 148M alleles may benefit less or not at all from the long-term intake of omega-3 carboxylic acids to reduce their hepatic lipid load compared to *PNPLA3* wild-type patients. Albeit the low level of evidence that patients with NAFLD homozygous for the *PNPLA3* 148M allele benefit more from lifestyle changes, including calorie restriction and physical exercise, these therapies remain effective interventions to reduce liver fat and should therefore be especially encouraged to these patients. Pharmacological studies investigating the effect of PNPLA3 I148M on changes in liver fat are largely lacking.

Since the ancestral and thus geographical distribution of PNPLA3 I148M varies widely,[41,42] clinical trial results may vary depending on study site and specific population. Therefore, future research should focus on prospective cohort studies primarily powered to detect differences in treatment response between the different *PNPLA3* I148M genotypes, while genotyping patients enrolled in randomized controlled trials should be encouraged to allow for post hoc analysis.

FUNDING INFORMATION

This work was funded by Research Foundation Flanders (1S91923N), Colgate-Palmolive – Society of Toxicology, the Chair Mireille Aerens for the Development of Alternative Methods and Onderzoeksraad Vrije Universiteit Brussel.

CONFLICT OF INTEREST STATEMENT

Independent of this research study, JMS has acted as a consultant to Apollo Endosurgery, Albireo Pharma Inc, Bayer, Boehringer Ingelheim, Gilead Sciences, GSK, Intercept Pharmaceuticals, Ipsen, Inventiva Pharma, Madrigal Pharmaceuticals, MSD, Northsea Therapeutics, Novartis, Novo Nordisk, Pfizer, Roche, Sanofi and Siemens Healthineers; has received research funding from Gilead Sciences, Boehringer Ingelheim, Siemens Healthcare GmbH and Speaker Honorarium from Boehringer Ingelheim, Echosens, MedPublico GmbH, Novo Nordisk and Madrigal Pharmaceuticals. All other authors declare to have no relevant conflict of interest.

ORCID

Joost Boeckmans https://orcid.org/0000-0001-8399-5872
Alexandra Gatzios https://orcid.org/0000-0001-7478-3405
Jörn M. Schattenberg https://orcid.org/0000-0002-4224-4703
Robim M. Rodrigues https://orcid.org/0000-0003-2927-6791
Tamara Vanhaecke https://orcid.org/0000-0002-6685-7299

REFERENCES

1. Riazi K, Azhari H, Charette JH, et al. The prevalence and incidence of NAFLD worldwide: a systematic review and meta-analysis. *Lancet Gastroenterol Hepatol*. 2022;7:851-861.
2. Boeckmans J, Natale A, Buyl K, et al. Human-based systems: mechanistic NASH modelling just around the corner? *Pharmacol Res*. 2018;134:257-267.
3. Loomba R, Sanyal AJ. The global NAFLD epidemic. *Nat Rev Gastroenterol Hepatol*. 2013;10:686-690.
4. Schattenberg JM, Lazarus J, Newsome PN, et al. Disease burden and economic impact of diagnosed non-alcoholic steatohepatitis in five European countries in 2018: a cost-of-illness analysis. *Liver Int*. 2021;41:1227-1242.
5. Bianco C, Tavaglione F, Romeo S, Valenti L. Genetic risk scores and personalization of care in fatty liver disease. *Curr Opin Pharmacol*. 2021;61:6-11.
6. Dongiovanni P, Stender S, Pietrelli A, et al. Causal relationship of hepatic fat with liver damage and insulin resistance in nonalcoholic fatty liver. *J Intern Med*. 2018;283:356-370.
7. Dongiovanni P, Anstee QM, Valenti L. Genetic predisposition in NAFLD and NASH: impact on severity of liver disease and response to treatment. *Curr Pharm des*. 2013;19:5219-5238.
8. 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.
9. Dongiovanni P, Donati B, Fares R, et al. PNPLA3 I148M polymorphism and progressive liver disease. *World J Gastroenterol*. 2013;19:6969.
10. Anstee QM, Darlay R, Cockell S, et al. Genome-wide association study of non-alcoholic fatty liver and steatohepatitis in a histologically characterised cohort☆. *J Hepatol*. 2020;73:505-515.
11. He S, Mcphaul C, Li JZ, et al. A sequence variation (I148M) in PNPLA3 associated with nonalcoholic fatty liver disease disrupts triglyceride hydrolysis. *J Biol Chem*. 2010;285:6706-6715.
12. Trépo E, Romeo S, Zucman-Rossi J, Nahon P. PNPLA3 gene in liver diseases. *J Hepatol*. 2016;65:399-412.
13. Pirazzi C, Valenti L, Motta BM, et al. PNPLA3 has retinyl-palmitate lipase activity in human hepatic stellate cells. *Hum Mol Genet*. 2014;23:4077-4085.
14. Pingitore P, Dongiovanni P, Motta BM, et al. PNPLA3 overexpression results in reduction of proteins predisposing to fibrosis. *Hum Mol Genet*. 2016;25:5212-5222.
15. Parthasarathy G, Revelo X, Malhi H. Pathogenesis of nonalcoholic steatohepatitis: an overview. *Hepatol Commun*. 2020;4:478-492.
16. Ratziu V, de Guevara L, Safadi R, et al. Aramchol in patients with nonalcoholic steatohepatitis: a randomized, double-blind, placebo-controlled phase 2b trial. *Nat Med*. 2021;27:1825-1835.
17. Loomba R, Mohseni R, Lucas KJ, et al. TVB-2640 (FASN inhibitor) for the treatment of nonalcoholic steatohepatitis: FASCINATE-1, a randomized, placebo-controlled phase 2a trial. *Gastroenterology*. 2021;161:1475-1486.
18. Drenth JPH, Schattenberg JM. The nonalcoholic steatohepatitis (NASH) drug development graveyard: established hurdles and planning for future success. *Expert Opin Investig Drugs*. 2020;29:1365-1375.
19. Eslam M, Sanyal AJ, George J, et al. MAFLD: a consensus-driven proposed nomenclature for metabolic associated fatty liver disease. *Gastroenterology*. 2020;158:1999-2014.e1.
20. Wang JZ, Cao HX, Chen JN, Pan Q. PNPLA3 rs738409 underlies treatment response in nonalcoholic fatty liver disease. *World J Clin Cases*. 2018;6:167-175.
21. Cherubini A, Casirati E, Tomasi M, Valenti L. PNPLA3 as a therapeutic target for fatty liver disease: the evidence to date. *Expert Opin Ther Targets*. 2021;25:1033-1043.
22. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *BMJ*. 2009;339:332-336.
23. Sterne JAC, Savović J, Page MJ, Elbers RG, et al. RoB 2: a revised tool for assessing risk of bias in randomised trials. *BMJ*. 2019;366:l4898.
24. Wells GA, Shea B, O'Connell D, Peterson J, Welch V, Losos M. The Newcastle-Ottawa Scale (NOS) for Assessing the Quality of Nonrandomised Studies in Meta-Analyses. Accessed November 2022. http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp
25. Eriksson JW, Lundkvist P, Jansson PA, et al. Effects of dapagliflozin and n-3 carboxylic acids on non-alcoholic fatty liver disease in people with type 2 diabetes: a double-blind randomised placebo-controlled study. *Diabetologia*. 2018;61:1923-1934.
26. Teufelhart M, Hofer H, Haslacher H, et al. A prospective study to evaluate the efficacy of a standardized low calorie diet (HEPAFAST®) according to PNPLA3 genotype in patients with Non Alcoholic Fatty Liver Disease (NAFLD) – 2 months data (interim analysis). Poster at the International Liver Congress 2017 (European Association for the Study of the Liver (EASL)).
27. Krawczyk M, Stachowska E, Milkiewicz P, Lammert F, Milkiewicz M. Reduction of caloric intake might override the prosteatotic effects

of the PNPLA3 p.I148M and TM6SF2 p.E167K variants in patients with fatty liver: ultrasound-based prospective study. *Digestion*. 2016;93:139-148.
28. Sevastianova K, Kotronen A, Gastaldelli A, et al. Genetic variation in PNPLA3 (adiponutrin) confers sensitivity to weight loss-induced decrease in liver fat in humans. *Am J Clin Nutr*. 2011;94:104-111.
29. Scorletti E, West AL, Bhatia L, et al. Treating liver fat and serum triglyceride levels in NAFLD, effects of PNPLA3 and TM6SF2 genotypes: results from the WELCOME trial. *J Hepatol*. 2015;63:1476-1483.
30. Nobili V, Bedogni G, Donati B, Alisi A, Valenti L. The I148M variant of PNPLA3 reduces the response to docosahexaenoic acid in children with non-alcoholic fatty liver disease. *J Med Food*. 2013;16:957-960.
31. Koot BGP, van der Baan-Slootweg OH, Vinke S, et al. Intensive lifestyle treatment for non-alcoholic fatty liver disease in children with severe obesity: inpatient versus ambulatory treatment. *Int J Obes (Lond)*. 2016;40:51-57.
32. Shen J, Wong GLH, Chan HLY, et al. PNPLA3 gene polymorphism and response to lifestyle modification in patients with nonalcoholic fatty liver disease. *J Gastroenterol Hepatol*. 2015;30:139-146.
33. Nobili V, Alisi A, Della Corte C, et al. Docosahexaenoic acid for the treatment of fatty liver: randomised controlled trial in children. *Nutr Metab Cardiovasc Dis*. 2013;23:1066-1070.
34. Wong VWS, Chan RSM, Wong GLH, et al. Community-based lifestyle modification programme for non-alcoholic fatty liver disease: a randomized controlled trial. *J Hepatol*. 2013;59:536-542.
35. Luukkonen PK, Nick A, Hölttä-Vuori M, et al. Human PNPLA3-I148M variant increases hepatic retention of polyunsaturated fatty acids. *JCI Insight*. 2019;4:1-13.
36. Kuttner CS, Mancina R, Wagenpfeil G, Lammert F, Stokes CS. Four-week Omega-3 supplementation in carriers of the prosteatotic PNPLA3 p.I148M genetic variant: an open-label study. *Lifestyle Genom*. 2019;12:10-17.
37. Marzuillo P, Grandone A, Perrone L, Miraglia Del Giudice E. Weight loss allows the dissection of the interaction between abdominal fat and PNPLA3 (adiponutrin) in the liver damage of obese children. *J Hepatol*. 2013;59:1143-1144.
38. Barata L, Feitosa MF, Bielak LF, et al. Insulin resistance exacerbates genetic predisposition to nonalcoholic fatty liver disease in individuals without diabetes. *Hepatol Commun*. 2019;3:894.
39. Op Den Kamp YJM, de Ligt M, Dautzenberg B, et al. Effects of the SGLT2 inhibitor dapagliflozin on energy metabolism in patients with type 2 diabetes: a randomized, double-blind crossover trial. *Diabetes Care*. 2021;44:1334-1343.
40. Latva-Rasku A, Honka MJ, Kullberg J, et al. The SGLT2 inhibitor dapagliflozin reduces liver fat but does not affect tissue insulin sensitivity: a randomized, double-blind, placebo-controlled study with 8-week treatment in type 2 diabetes patients. *Diabetes Care*. 2019;42:931-937.
41. Kubiliun MJ, Cohen JC, Hobbs HH, Kozlitina J. Contribution of a genetic risk score to ethnic differences in fatty liver disease. *Liver Int*. 2022;42:2227-2236.
42. Lazo M, Xie J, Alvarez CS, et al. Frequency of the PNPLA3 rs738409 polymorphism and other genetic loci for liver disease in a Guatemalan adult population. *Liver Int*. 2022;42:1470-1474.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Boeckmans J, Gatzios A, Schattenberg JM, Koek GH, Rodrigues RM, Vanhaecke T. PNPLA3 I148M and response to treatment for hepatic steatosis: A systematic review. *Liver Int*. 2023;43:975-988. doi:10.1111/liv.15533