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Impact of PNPLA3 I148M on Drug-Induced Hepatocellular Liver Injury: A Systematic Review and Meta-Analysis

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ABSTRACT

Background and Aims: The role of the *patatin-like phospholipase domain-containing protein 3* (PNPLA3)-I148M variant in drug-induced liver injury (DILI) remains unclear. This systematic review with meta-analysis investigated whether carriage of PNPLA3 I148M may contribute to the development of hepatocellular DILI.

Methods: Scientific databases were searched up to January 31st, 2025 for studies investigating serum transaminase elevations in response to drug treatment. Studies were included if serum transaminases were elevated in at least one genetic subgroup (II/IM/MM), and the possible presence of relevant hepatocellular DILI was estimated based on Hy's law. The meta-analysis reported on any increase in transaminases compared to baseline levels.

Results: Eight articles covering thirteen studies with a total of 8235 patients were included in the systematic review, of which 5 studies comprising 3480 individuals were eligible for the meta-analysis. Patients were treated with small molecules ($n = 602$), biologicals ($n = 3221$), or chemotherapeutic treatments ($n = 4412$). None of the studies reported conclusively more occurrence of relevant hepatocellular DILI based on PNPLA3 I148M carriage. The meta-analysis suggested amplified drug-induced transaminase elevations in response to adomeglivant and basal insulin peglispro in metabolically impaired individuals with PNPLA3-IM/MM compared to PNPLA3-II (odds ratio = 1.53, 95% CI = 1.22–1.92). None of the studies were primarily designed to detect the outcome of interest.

Abbreviations: ALL, acute lymphoblastic leukaemia; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CFB, change from baseline; CI, confidence interval; CTCAE, common terminology criteria for adverse events; DILI, drug-induced liver injury; EASL, European Association for the Study of the Liver; FDR, false discovery rate; IFNβ-1α, interferon beta 1 alpha; LFC, liver fat content; MASLD, metabolic dysfunction-associated steatotic liver disease; NOS, Newcastle-Ottawa quality assessment scale; OR, odds ratio; PNPLA3, patatin-like phospholipase domain-containing protein 3; PRISMA, Preferred Reporting Items for Systematic reviews and Meta-Analyses; RCT, randomised controlled trial; RoB 2, revised Cochrane Risk-of-Bias tool for randomised trials; ROBINS-I, Risk Of Bias In Non-randomised Studies—of Interventions assessment tool; SMD, standardised mean difference; T2DM, type 2 diabetes mellitus; ULN, upper limit of normal; VLDL, very low density lipoprotein.

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Tamara Vanhaecke and Joost Boeckmans are last authors.

Conclusions: Low to no evidence suggests that carriage of PNPLA3 I148M confers susceptibility to develop clinically relevant hepatocellular DILI; although it may potentiate drug-induced transaminase elevations in response to adomeglivant and basal insulin peglispro in metabolically impaired individuals.

1 | Introduction

Drug-induced liver injury (DILI) can result in acute liver failure, making it a relevant cause of liver-related death. The global incidence of DILI is unclear but prospective studies from France and Iceland reported annual incidences in the general population of 13.9 and 19.1 per 100 000 inhabitants [1, 2]. DILI is a challenging clinical condition responsible for roughly half of the cases of acute liver failure reported in the USA and Western Europe [3, 4], and accounts for 8% of liver transplantations in Europe [5].

Further, DILI is amongst the leading reasons for late-stage discontinuation of agents during clinical trials or post-marketing regulatory actions. Specifically, hepatotoxicity accounted for one in every four terminations during clinical development and one third of market withdrawals between 1975 and 2007 in the USA [6]. The lack of specific, validated biomarkers to predict DILI complicates its management during clinical trials, where monitoring of alanine and aspartate aminotransferases (ALT and AST) in serum remains the most accessible and recommended approach to evaluate potential hepatic injury in study participants. According to the guidelines of the European Association for the Study of the Liver (EASL), DILI should be suspected in a clinical trial when ALT and/or AST levels were normal at baseline and exceed 3-fold the upper limit of normal (ULN) during treatment [7]. Apart from transaminase elevations > 3-fold the ULN, Hy's law, which serves as an indicator that a drug is likely to cause severe hepatocellular DILI, additionally considers elevations of total serum bilirubin > 2-fold, and the elevations in AST/ALT should have occurred in the absence of elevated serum alkaline phosphatase or other alternative explanations [8].

The underlying mechanisms culminating in DILI are often of multifactorial nature and involve an interplay between both characteristics of the DILI-causing agent and patient-related factors including age, sex, environment and genetics [9]. In this context, a single nucleotide polymorphism in *patatin-like phospholipase domain-containing 3* (PNPLA3), PNPLA3 I148M, has been robustly associated with metabolic dysfunction-associated steatotic liver disease (MASLD) susceptibility and progression [10, 11], and also with advanced liver disease due to other aetiologies [12]. PNPLA3 I148M has also been linked to increased serum ALT and AST levels, and in vitro studies suggest that it may also impact the drug metabolising capacity of hepatocytes, possibly altering their sensitivity to hepatotoxicity [10, 13, 14]. While a recent systematic review showed that carriage of PNPLA3 I148M may influence the response to treatment for hepatic steatosis, its potential impact on the susceptibility to develop DILI remains elusive [15].

This systematic review with meta-analysis investigated whether hetero- or homozygous carriage of the PNPLA3 I148M variant may potentiate drug-induced hepatocellular liver injury.

2 | Methods

The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) checklist is available as a supplement.

2.1 | Eligibility Criteria

Study eligibility criteria are given in Table S1. Clinical studies were included from full-text research papers or conference proceedings, written in English. Studies were eligible if serum aminotransferase test disturbances were detected during drug treatment for any non-hepatic disease in at least one subgroup of PNPLA3 I148M (II/IM/MM). Studies were excluded when no genotyping for PNPLA3 I148M was done, no serum aminotransferase tests were performed after treatment, or when the agent could be classified as a dietary supplement according to European (Directive 2002/46/EC) or USA law (Dietary Supplement Health and Education Act of 1994).

2.2 | Information Sources

MEDLINE, Cochrane Library and Web of Science were searched for publications up to January 31st, 2025.

2.3 | Search Strategy

MEDLINE: (((PNPLA3) OR (patatin-like phospholipase domain–containing 3) OR (adiponutrin)) AND (rs738409)) AND ((DILI) OR (drug induced liver injury) OR (liver toxicity) OR (hepatotoxicity)).

Cochrane Library: 'PNPLA3' AND 'rs738409' AND ('Drug induced liver injury' OR 'DILI' OR 'liver toxicity' OR 'aminotransferases') + filter Trials.

Web of Science: AB = (PNPLA3 OR patatin-like phospholipase domain-containing protein 3 OR adiponutrin) AND AB = (DILI OR drug-induced liver injury OR liver toxicity OR hepatotoxicity) + filter Review article (exclude).

Additionally, citation searching was performed to identify any other reports that could meet the predefined eligibility criteria.

2.4 | Selection Process

Duplicates were manually removed from the search results, and pre-screening of titles, abstracts and keywords was done to identify potentially relevant records based on study type and the mentioning of the terms 'PNPLA3' and 'DILI', or synonyms thereof. Remaining full-text reports were subsequently assessed for all eligibility criteria listed in Table S1. The search

Summary

- Carriage of PNPLA3 I148M is unlikely to increase the risk of clinically relevant hepatocellular drug-induced liver injury.
- In metabolically impaired patients, carriage of the PNPLA3 I148M variant may confer susceptibility to develop more pronounced drug-induced transaminase elevations in response to adomeglivant and basal insulin peglispro, which both influence lipid metabolism.
- (Exacerbation of) underlying MASLD could have mediated the increased propensity to develop higher levels of transaminases in PNPLA3 I148M carriers in response to certain drugs.
- Studies investigating the effect of PNPLA3 I148M on drug-induced hepatotoxicity are scarce.

was conducted independently by two researchers; inconsistencies were discussed to reach a consensus. A third researcher was available for discussion in case consensus could not be reached by the two primary researchers.

2.5 | Data Collection Process

A premade extraction sheet in Microsoft Excel was used for data collection by two independent researchers. The collected data were compared, and inconsistencies were discussed to reach a consensus. A third researcher was available for discussion in case consensus could not be reached by the two primary researchers.

2.6 | Data Items

2.6.1 | Outcomes

The primary outcome was the effect of PNPLA3 I148M IM/MM vs II genotypes on treatment-related increase of ALT and/or AST levels. Secondary outcomes were the occurrence of hepatocellular DILI based on thresholds defined by Hy's law [8]: ALT and/or AST $\geq$ 3-fold ULN (or 3-fold the pre-existing level) and total bilirubin $>$ 2-fold ULN without alkaline phosphatase (ALP) elevation or any alternative explanation; and the risks of serum transaminase elevation and hepatocellular DILI based on treatment type by PNPLA3 genotype (IM/MM vs II).

Data were collected on the outcome of serum aminotransferases (enzyme(s) tested, numerical outcome/change from baseline, *p*-value, odds ratio). If available, bilirubin and alkaline phosphatase levels were also noted. If applicable, specific criteria used for classification of the observed hepatotoxicity were also noted.

2.6.2 | Other Variables

Report details (first author, year of publication), study details (design, duration, participating countries, registration number), patient characteristics (disease, age, sex, body mass index (BMI), PNPLA3 I148M genotype) and intervention details (intervention, treatment arms, dosage, frequency, duration, number of patients per group) were collected.

2.7 | Study Risk of Bias Assessment

Risk of bias was assessed independently by two researchers using the revised Cochrane Risk-of-Bias tool for randomised trials (RoB 2) [16], the Risk of Bias in Non-randomised Studies—of Interventions (ROBINS-I) assessment tool (version for cohort-type studies) [17] and the Newcastle-Ottawa quality assessment scale (NOS) for case-control studies [18]. Inconsistencies were discussed to reach a consensus, and a third researcher was available for discussion in case consensus could not be reached by the two primary researchers.

2.8 | Effect Measures

A meta-analysis was performed for the composite outcome of increased serum ALT and/or AST level after treatment with a small molecule, biological or chemotherapy protocol in PNPLA3 IM/MM carriers compared to PNPLA3 II carriers. The effect measure used in the meta-analysis was the corresponding odds ratio (OR) and its 95% confidence interval (CI).

2.9 | Synthesis Methods

All studies that met the eligibility criteria were included in the narrative synthesis of results. When studies reported on ALT and/or AST elevations $\geq$ 3-fold ULN, but without additional information to apply Hy's law, the impact of PNPLA3 I148M was graded as 'possibly contributing to relevant hepatocellular DILI'. When no cutoff data for ALT and/or AST was available and only minor increases in their levels were observed without any other notice of severe hepatocellular liver injury, the effect of PNPLA3 I148M was considered as 'unlikely contributing to relevant hepatocellular DILI'.

For the meta-analysis, only studies that either reported an OR and its 95% CI from a dominant (IM/MM vs. II) logistic regression model regarding ALT and/or AST, or reported sufficient data allowing calculation of the standardised mean difference (SMD) as Cohen's *d* between PNPLA3 IM/MM and II groups, were eligible for inclusion [19]. Studies were excluded from the meta-analysis if baseline ALT and/or AST levels were not accounted for.

Where ORs could not be directly extracted from the publications or Appendix S1, the SMD (Cohen's *d*) was calculated using the least squares mean values reported for the change from baseline (CFB) after treatment in PNPLA3 IM/MM and PNPLA3 II individuals. From the SMD, the lnOR and its 95% CI were subsequently calculated and transformed to OR and its 95% CI [19]. The input data used for the meta-analysis is summarised in Table S2.

Meta-analysis was performed in R (version 4.4.2) using the meta package (version 8.0–1) [20]. A random effects model

was fitted, and heterogeneity between the studies was explored by subgroup analysis based on treatment type and by using Cochran's Q, Higgins & Thompson's I^2 index and tau-squared (τ^2) metrics. Univariate meta-regression was performed for the mean difference between the PNPLA3 IM/MM and PNPLA3 II groups in baseline age, proportion of males, BMI, %Hb1Ac, duration of T2DM, serum triglycerides and transaminase levels. In the subset of studies for which liver fat content (LFC) measurements and CFB in LFC, triglycerides and %Hb1Ac were available in both PNPLA3 IM/MM and II genetic subgroups, meta-regression was also performed using the mean difference of these factors as covariates. The Benjamini-Hochberg method for multiple comparisons was applied to control the false discovery rate (FDR) and adjusted p was considered significant when <0.05. Publication bias was assessed through a trim-and-fill contour-enhanced funnel plot. The results from the meta-analysis were presented in a forest plot.

2.10 | Reporting Bias Assessment

Pre-registration of the study protocol in a publicly accessible registry and pre-specification of genetic subgroup analysis for PNPLA3 I148M was evaluated for all included studies as a measure for reporting bias.

2.11 | Certainty Assessment

All included studies were discussed in the context of their risk of bias assessments, meaning that outcomes from studies with intermediate or high risk of bias were nuanced using that perspective during the narrative synthesis of results. For the meta-analysis, every study was weighted according to its 95% CI; based thereon, a proportional symbol size was given in the summarising forest plot.

3 | Results

3.1 | Study Selection

Thirty-five unique records were retrieved based on the predefined search criteria, and 27 records were excluded after prescreening for keywords. The full texts of the remaining eight reports were assessed for the in- and exclusion criteria (Table S1), after which two records were excluded. Five additional records were identified through citation searching and retrieved for full-text screening, of which two were retained. Overall, eight full-text reports covering thirteen unique studies were included in the systematic review (Figure S1), of which five accounted for baseline transaminase levels, which were included in the meta-analysis.

3.2 | Study Characteristics

Study characteristics are summarised in Table 1. From the thirteen unique studies, Liu et al. [27] combined the randomised TOTXV and TOTXVI studies into one discovery cohort; therefore, these studies are discussed as one cohort. Liu et al. also reported on a replication cohort that is the randomised controlled trial (RCT) AALL0232. The report by Guzman et al. [22] covered a randomised crossover study (GLDI) and a RCT (GLDJ), and the report by Pillai et al. [25] included three RCTs, namely IMAGINE-2, IMAGINE-4 and IMAGINE-5. Further, Yang et al. [28] included two studies, the RCTs AALL0232 and AALL0434, and the remaining reports yielded one study each. Two of these studies, by Feagins et al. [24] and Sundbaum et al. [21], were case–control studies and another two studies, by Capone et al. [23] and Gutierrez-Camino et al. [26], were retrospective cohort studies. The duration of treatment covered by the studies varied from 4 weeks to 12 months, with some treatments running for several years.

The included studies covered a total of 8235 patients, of which 602 received treatment with a small molecule, 3221 with a biological agent, and 4412 were treated with a chemotherapy protocol consisting of multiple drugs. Patients were treated for inflammatory diseases (rheumatoid arthritis, Crohn's disease, psoriatic arthritis, ankylosing spondylitis), multiple sclerosis, type 2 diabetes mellitus (T2DM), or acute lymphoblastic leukaemia (ALL). Details of all treatments are provided in Table S3. Overall, the allelic frequencies were 0.712 and 0.288 for PNPLA3 148I and 148M, respectively, and II/IM/MM genotype frequencies were 0.527/0.370/0.103. Both the allelic and genotype frequencies were similar to those reported for the general population in the 1000 Genomes Project Phase 3 [29]. Five studies were conducted in paediatric patients. Study participant characteristics are summarised in Table S4.

Studies used various outcome measures (Table 1) such as ALT/AST numerically expressed as fold of the ULN or as CFB, or ALT/AST categorically expressed as a grade of hepatotoxicity according to various criteria (e.g., common terminology criteria for adverse events (CTCAE) versions 3.0 and 4.0). None of the included studies were primarily powered to detect a difference in this outcome based on PNPLA3 I148M genotype.

3.3 | Risk of Bias in Studies

Results of the bias analyses are summarised in Tables S5–S7. None of the studies prespecified our outcome of interest in their study protocol, resulting in some concerns of bias in the domain 'selection of the reported result' for all studies.

Some studies were assessed for risk of bias as a cohort study despite being originally randomised trials, due to their specific *post hoc* analyses resembling a cohort study design. This was the case for TOTXV + TOTXVI and AALL0232 (Liu et al. [27]), and AALL0232 and AALL0434 (Yang et al. [28]). The study by Guttierez-Camino et al. [26] was determined to have a serious risk of confounding (Domain 1), as the report did not mention any evaluation or correction for potential confounding domains; nor did it mention the measurement or gradation of ALT levels before treatment initiation. Similarly, baseline ALT levels were unavailable for the AALL0232 study reported by Liu et al. [27] and only gradations using the CTCAE 4.0 criteria were available; thus, the analysis was performed using cases (grade >2) and controls (grade ≤ 2). Therefore, this study was considered to have a moderate risk of bias due to missing data (Domain 5).

TABLE 1 | Included reports and studies.

	Country	Study type	Duration of study or follow-up	Total number of patients	Liver enzymes outcome measure
Small molecules					
Sundbaum et al. 2021 [21]	Sweden	Case–control study	6 months	198	New ALT elevation, defined as > 1.5-fold the upper limit of normal (ULN)
Guzman et al. 2018 (GLDI) [22]	USA, Czech Republic, Mexico, Poland and Puerto Rico	Multicentre randomised crossover study, triple masked	2 × 6 weeks with a 4-week washout period (preceded by a 2-week open-label placebo lead-in)	246	ALT elevation at end of treatment, expressed as change from baseline (CFB) in IU/L Patients with ALT > 2.5-fold ULN were excluded from the study
Guzman et al. 2018 (GLDJ) [22]	USA, Greece, Puerto Rico and Taiwan	Multicentre randomised, double-blind, placebo- and active comparator-controlled study	12 months (6 months interim)	158	ALT elevation at end of treatment, expressed as CFB in IU/L Patients with ALT > 2-fold ULN were excluded from the study
Biologicals					
Capone et al. 2020 [23]	Italy	Retrospective multicentre cohort study	Median duration of therapy 4.4 (2.1–7.8) years.	113	New ALT and/or AST elevation exceeding the ULN, graded using Common Toxicity Criteria for Adverse Events (CTCAE) version 4.0 Patients with known liver disease, other disease modifying drug treatment, or ALT/AST > ULN were excluded from the study
Feagins et al. 2015 [24]	The United States of America (USA)	Case–control study	Variable	32	New ALT and/or AST elevation exceeding the ULN All cases have biopsy-proven MASLD
Pillai et al. 2018 (IMAGINE-2) [25]	USA, Argentina, Australia, Brazil, Canada, Finland, Germany, Greece, Hungary, Israel, Italy, Lithuania, Mexico, New Zealand, Poland, Puerto Rico, Romania, Russia, Slovakia, South Africa, Spain, Turkey and the United Kingdom (UK)	Multicentre randomised, double-blind, active comparator-controlled study	12 months (6 months interim)	1364	ALT and/or AST elevation at end of treatment, expressed as CFB in IU/L (least squares mean) Patients with ALT > 2.5-fold ULN were excluded from the study

(Continues)

TABLE 1 | (Continued)

	Country	Study type	Duration of study or follow-up	Total number of patients	Liver enzymes outcome measure
Pillai et al. 2018 (IMAGINE-4) [25]	USA, Australia, Austria, Brazil, Croatia, Czechia, Denmark, Germany, Greece, Hungary, Israel, Italy, Japan, Lithuania, Mexico, the Netherlands, Poland, Puerto Rico, Romania, Russia, Slovakia, Spain, Taiwan, Turkey and UK	Multicentre randomised, quadruple-blind, active comparator-controlled study	6 months	1284	ALT and/or AST elevation at end of treatment, expressed as CFB in IU/L (least squares mean) Patients with ALT > 2.5-fold ULN were excluded from the study
Pillai et al. 2018 (IMAGINE-5) [25]	USA, Czechia, Germany, Greece, Israel, Puerto Rico, Romania, Russia and Spain	Multicentre randomised, open label, active comparator-controlled study	12 months (6 months interim)	428	ALT and/or AST elevation at end of treatment, expressed as CFB in IU/L (least squares mean) Patients with ALT > 2.5-fold ULN were excluded from the study
Combinatory chemotherapy protocols					
Gutierrez-Camino et al. 2017 [26]	Spain	Retrospective cohort study	±4 weeks (induction phase)	138	ALT and/or AST elevation at end of induction, graded by the Spanish Society of Paediatric Haematology and Oncology standards No ALT/AST-based exclusion criteria
Liu et al. 2017 (TOTXV + TOTXVI) [27]	USA	Cohort consisting of two randomised, open-label studies	±6 weeks (induction phase)	715	ALT elevation at end of induction, graded using CTCAE version 3.0 No ALT/AST-based exclusion criteria
Liu et al. 2017 (AALL0232, induction only) [27]	USA, Canada, New Zealand, Switzerland and Australia	Randomised, open-label, active comparator-controlled study	±6 weeks (induction phase)	2285	ALT elevation at end of induction, graded using CTCAE version 4.0 No ALT/AST-based exclusion criteria
Yang et al. 2022 (AALL0232) [28]	USA, Canada, New Zealand, Switzerland and Australia	Randomised, open-label, active comparator-controlled study	Treatment duration ranged from 2.5 to 3.5 years	2283	ALT and/or AST elevation throughout treatment protocol, graded using CTCAE version 4.0 No ALT/AST-based exclusion criteria
Yang et al. 2022 (AALL0434) [28]	USA, Canada, New Zealand, Switzerland and Australia	Randomised, open-label, active comparator-controlled study	Treatment duration ranged from 2.5 to 3.5 years	1274	ALT and/or AST elevation throughout treatment protocol, graded using CTCAE version 4.0 No ALT/AST-based exclusion criteria

Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase; CFB, change from baseline; CTCAE, common toxicity criteria for adverse events; UK, United Kingdom; ULN, upper limit of normal; USA, the United States of America.

As outcome assessors could have been aware of the treatment received by the patients in all evaluated studies, the risk of bias in Domain 6 was considered moderate. Baseline transaminase levels were also not available for both the AALL0232 and AALL0434 studies as reported by Yang et al. [28], resulting in a moderate risk of bias due to missing data (Domain 5). The remaining non-randomised study, TOTXV + TOTXVI as reported by Liu et al. [27], had a low overall risk of bias.

All randomised trials had a low overall risk of bias, except for the IMAGINE-5 trial by Pillai et al. [25], which was determined to have a high risk of bias in Domain 2 due to its open-label study design. Further, outcome assessors were also aware of the treatment received by the patients, raising some concerns of bias in Domain 4 as well.

Both case–control studies were determined to have some concerns of bias. In the study by Feagins et al. [24], this was mainly due to the fact that all but one of the participants were male. In the study of Sundbaum et al. [21], concerns arose from the likelihood that the study was underpowered to detect an effect of PNPLA3 I148M.

3.4 | Results of Individual Studies

3.4.1 | Small Molecules

Sundbaum et al. [21] investigated the effect of PNPLA3 I148M on ALT elevation after 6 months of methotrexate treatment for rheumatoid arthritis, and did not find a significant association between either an increase of ALT > 1.5-fold the ULN (OR = 0.3147 [0.0845–1.132], $p = 0.0768$) or peak ALT (beta estimate = −0.08791 [−0.2650–0.08913], $p = 0.3317$) (Table 2), although a trend towards a detrimental effect for the II group was seen. It should be noted that in this study, 50% of patients included as cases had a history of ALT elevations compared to 12.5% of the controls ($p = 0.001$) which might have biased the results. Furthermore, the study was powered at 80% to detect an OR of ±7.5 at a genome-wide level for a marker with a minor allele frequency of 0.4. Considering the reported minor allele frequencies of 0.083 in cases and 0.206 in controls, this study was underpowered to detect more subtle effects of this specific variant [29]. Therefore, no general conclusion can be drawn regarding a possible effect of the PNPLA3 I148M variant on methotrexate-induced ALT/AST elevations.

The report by Guzman et al. [22] covered two separate studies concerning treatment with the glucagon receptor antagonist adomeglivant (LY2409021) in patients with T2DM. The first study was a 6-week randomised cross-over study in which 246 patients received either placebo or adomeglivant, alongside diet and exercise alone or in combination with a stable dose of metformin. While no difference in ALT CFB was found based on PNPLA3 I148M genotype during the placebo treatment, the CFB in the treatment arm was significantly higher in patients carrying the PNPLA3 I148M variant ($p = 0.005$). PNPLA3 I148M explained 4.22% of variance in ALT CFB in the adomeglivant arm compared to 0.2% in the placebo arm. While no numerical values were reported, the mean CFB interpreted from Figure 1A in the article was around 7.4, 14.7 and 14 IU/L for II, IM and MM genotypes, respectively [22]. The second study was a randomised placebo and active comparator-controlled trial in which 158 T2DM patients on a stable dose of metformin and a sulfonylurea received either placebo, active comparator (sitagliptin) or adomeglivant. Similar to the first study, no difference in CFB based on PNPLA3 I148M was found for the placebo and sitagliptin group, whereas in the adomeglivant group, ALT CFB was increased in carriers of the PNPLA3 I148M variant ($p = 0.059$, $p < 0.1$ was considered significant). In this study, PNPLA3 I148M explained 7.29% of variance in the adomeglivant group compared to 5.79% and 0.44% in the sitagliptin and placebo groups, respectively. The mean CFB interpreted from Figure 1B was around 4.3, 13.2 and 31.8 IU/L for II, IM and MM genotypes, respectively [22] (Table 2). Taken together, these two studies support the hypothesis that the PNPLA3 I148M variant potentiates ALT elevations upon treatment with adomeglivant.

3.4.2 | Biological Agents

The five studies employing biological agents covered varying diseases and therapeutic classes. Feagins et al. [24] conducted a case–control study in patients with inflammatory diseases receiving treatment with tumour necrosis factor alpha inhibitors (Table S3). They reported that the PNPLA3 I148M minor allele was more frequent in patients who developed transaminase elevations exceeding the ULN compared to matched controls (75% of cases compared to 38% of inflammatory controls, $p = 0.31$). However, the study included only eight cases, all male and the magnitudes of transaminase elevations were not ascertained, questioning the relevance of these results.

Capone et al. [23] investigated the effect of PNPLA3 I148M on transaminase elevations exceeding the ULN upon treatment with interferon beta 1 alpha (IFNβ-1α) for multiple sclerosis. In their retrospective cohort study covering 113 patients, they found that carriage of the PNPLA3 I148M variant (IM/MM) increased the risk of such elevations (OR = 2.82 [1.08–7.83], $p < 0.05$).

In the report by Pillai et al. [25], the association between PNPLA3 I148M and CFB in ALT and/or AST after treatment with basal insulin peglispro (LY2605541) was investigated in three randomised active comparator-controlled studies, IMAGINE-2, IMAGINE-4 and IMAGINE-5, and a pooled analysis was also conducted. In IMAGINE-2, 1364 insulin naïve T2DM patients who were on at least two stable-dose oral antihyperglycaemic medications received treatment with either peglispro or active comparator insulin glargine. While no statistically significant effect of PNPLA3 I148M was found at the interim analysis after 26 weeks, both ALT and AST mean CFB were significantly higher in carriers of the variant after 52 weeks of treatment with peglispro (II/IM/MM, ALT: 2.46, 6.31, 4.74 IU/L, $p < 0.001$; AST: 2.50, 4.33, 3.14 IU/L, $p = 0.023$). A similar effect was found in the active comparator group although in that case, specifically homozygous carriage of PNPLA3 I148M predisposed patients to increased ALT and AST CFB (II/IM/MM, ALT: −2.76, −2.89, 2.76 IU/L, $p = 0.021$; AST: −0.70, −0.95, 3.55 IU/L, $p = 0.008$). In the IMAGINE-4 trial, 1284 T2DM patients on insulin lispro were treated with either peglispro or active comparator insulin glargine for 26 weeks. Significantly increased ALT and AST mean CFB values were found in carriers of the PNPLA3 I148M

TABLE 2 | Results of individual studies.

	Intervention	Outcome measure(s) used	Numerical outcome [brackets indicate 95% CI] PNPLA3 I148M IM/MM vs II unless other specified	*p*
Small molecules				
Sundbaum et al. 2021 [21]	Methotrexate	Primary: ALT > 1.5-fold upper limit of normal (ULN) Secondary: peak ALT within 6 months	Primary: odds ratio (OR) = 0.3147 [0.08745–1.132] Secondary: beta estimate = −0.08791 [−0.2650–0.08913]	$p = 0.0768$ $p = 0.33170$
Guzman et al. 2018 (GLDI) [22]	Adomeglivant (LY2409021)	ALT elevation, expressed as change from baseline (CFB) in IU/L	No CFB in placebo group for any PNPLA3 I148M genotype. CFB in treatment group higher in PNPLA3 I148M carriers than non-carriers (linear regression model). PNPLA3 I148M explains 4.22% (R^2%) of variance in ALT CFB in the treatment group compared to 0.2% in the placebo group. Mean CFB (SE) interpreted from Figure 1A: II ≈7.4 (2.1), IM ≈14.7 (2.3), MM ≈14 (2.6) IU/L	$p = 0.83$ $p = 0.005$
Guzman et al. 2018 (GLDJ) [22]	Adomeglivant (LY2409021) Active comparator (AC): sitagliptin	ALT elevation, expressed as CFB in IU/L	ALT CFB: No difference in CFB between PNPLA3 I148M genotypes in placebo and active comparator groups. CFB in treatment group higher in PNPLA3 I148M carriers than non-carriers (linear regression model) PNPLA3 I148M explains 7.29% (R^2%) of variance in ALT CFB in the LY2409021 group compared to 5.79% and 0.44% in the placebo and active comparator groups. Mean CFB (SE) interpreted from Figure 1B: II ≈4.3 (2.7), IM ≈13.2 (3.5), MM ≈31.8 (10) IU/L Liver fat content (LFC) % (magnetic resonance imaging), CFB [95% CI], II/IM/MM: Treatment = 3.2 [1.2–5.1]/4.6 [2.2–7.1]/8.6 [1.5–15.7] AC = −1.2 [−4.2–1.8]/1.7 [−1.2–4.6]/0.4 [−4.7–5.4]	$p = 0.45$ and 0.61, resp. $p = 0.059$* $p = 0.27$ $p = 0.40$
Biologicals				
Capone et al. 2020 [23]	Interferon beta 1 alpha	ALT and/or AST > ULN, graded using the common terminology criteria for adverse events (CTCAE) version 4.0	OR (dominant model) = 2.82 [1.08–7.83]	$p < 0.05$

(Continues)

TABLE 2 | (Continued)

	Intervention	Outcome measure(s) used	Numerical outcome [brackets indicate 95% CI] PNPLA3 I148M IM/MM vs II unless other specified	*p*
Feagins et al. 2015 [24]	Tumour necrosis factor alpha inhibitors	ALT > ULN	Mean peak ALT cases = 111 IU/L (range 69–180) Mean peak AST cases = 63 IU/L (range 38–88) 75% of cases had PNPLA3 I148M mutations compared to 38% of inflammatory controls. Mean peak bilirubin cases = 0.98 mg/dL (range 0.6–1.6) Mean peak alkaline phosphatase cases = 110 IU/L (range 76–172)	$p = 0.31$
Pillai et al. 2018 (IMAGINE-2) [25]	Basal insulin peglispro (LY2605541) AC: insulin glargine	ALT and/or AST elevation, expressed as CFB in IU/L	At 26 weeks, ALT CFB (SE), II/IM/MM: Treatment = 1.92 (0.6)/2.77 (0.72)/1.37 (1.40) AC = −3.12 (0.82)/−3.47 (0.98)/−0.15 (1.86) At 26 weeks, AST CFB (SE), II/IM/MM: Treatment = 1.62 (0.42)/1.83 (0.51)/0.98 (0.97) AC = −0.84 (0.57)/−1.43 (0.69)/1.46 (1.30) At 52 weeks, ALT CFB (SE), II/IM/MM: Treatment = 2.46 (0.61)/6.31 (0.73)/4.74 (1.43) AC = −2.76 (0.84)/−2.89 (1.01)/2.76 (1.89) At 52 weeks, AST CFB (SE), II/IM/MM: Treatment = 2.50 (0.42)/4.33 (0.51)/3.14 (1.00) AC = −0.70 (0.59)/−0.95 (0.71)/3.55 (1.33)	$p = 0.555$ $p = 0.277$ $p = 0.740$ $p = 0.145$ $p = 0.0002$ $p = 0.021$ $p = 0.023$ $p = 0.008$
Pillai et al. 2018 (IMAGINE-4) [25]	Basal insulin peglispro (LY2605541) AC: insulin glargine	ALT and/or AST elevation, expressed as CFB in IU/L	ALT CFB (SE), II/IM/MM: Treatment = 7.08 (0.79)/5.46 (0.94)/13.78 (1.87) AC = −0.61 (0.78)/−0.52 (0.93)/−4.19 (1.92) AST CFB (SE), II/IM/MM: Treatment = 3.65 (0.62)/3.43 (0.73)/11.02 (1.46) AC = 0.11 (0.61)/−0.31 (0.72)/−1.82 (1.49)	$p = 0.0004$ $p = 0.197$ $p = 7.64 \times 10^{-6}$ $p = 0.482$
Pillai et al. 2018 (IMAGINE-5) [25]	Basal insulin peglispro (LY2605541) AC: insulin glargine	ALT and/or AST elevation, expressed as CFB in IU/L	At 26 weeks, ALT CFB (SE), II/IM/MM: Treatment = 7.55 (1.14)/13.90 (1.47)/15.36 (3.45) AC = 0.96 (1.64)/0.56 (2.07)/−1.27 (3.90) At 26 weeks, AST CFB (SE), II/IM/MM: Treatment = 3.49 (0.79)/8.46 (1.02)/10.18 (2.42) AC = 0.40 (1.14)/0.29 (1.44)/−1.85 (2.70) At 52 weeks, ALT CFB (SE), II/IM/MM: Treatment = 6.78 (1.10)/12.33 (1.41)/8.67 (3.29) AC = −0.05 (1.57)/0.18 (2.02)/−1.29 (3.67) At 52 weeks, AST CFB (SE), II/IM/MM: Treatment = 3.32 (0.79)/7.15 (1.01)/5.45 (2.36) AC = 0.07 (1.13)/−0.95 (0.71)/3.55 (1.33)	$p = 0.001$ $p = 0.872$ $p = 0.00019$ $p = 0.956$ $p = 0.009$ $p = 0.939$ $p = 0.012$ $p = 0.655$

(Continues)

TABLE 2 | (Continued)

	Intervention	Outcome measure(s) used	Numerical outcome [brackets indicate 95% CI] PNPLA3 I148M IM/MM vs II unless other specified	*p*
Pillai et al. 2018 (pooled) [25]	Basal insulin peglispro (LY2605541) AC: insulin glargine	ALT and/or AST elevation, expressed as CFB in IU/L	ALT CFB (SE), II/IM/MM: Treatment = 5.27 (0.46)/6.05 (0.56)/8.60 (1.11) AC = −0.88 (0.56)/−1.07 (0.66)/−1.96 (1.30) AST CFB (SE), II/IM/MM: Treatment = 2.89 (0.34)/3.72 (0.41)/6.36 (0.82) AC = 0.11 (0.41)/−0.48 (0.49)/−0.48 (0.95) LFC CFB (SE), II/IM/MM: Treatment = 1.26 (0.47)/2.6 (0.54)/4.24 (1.27) AC = −2.82 (0.65)/−1.19 (0.82)/−5.2 (1.74)	$p = 0.019$ $p = 0.742$ $p = 0.0004$ $p = 0.599$ $p = 0.009$ $p = 0.974$
Combinatory chemotherapy protocols				
Gutierrez-Camino et al. 2017 [26]	LAL/SHOP94/99/2005 protocols (induction)	Grade ≥ 2 elevation in ALT and/or AST, graded by the Spanish Society of Paediatric Haematology and Oncology standards	Hepatotoxicity (grade ≥ 2) OR = 2.06 [0.99–4.29] (dominant model) High hepatotoxicity (grade ≥ 3) OR = 2.69 [1.09–6.64] (dominant model)	$p = 0.051$ $p = 0.029$
Liu et al. 2017 (TOTXV + TOTXVI) [27]	TOTXV or TOTXVI protocols (induction)	ALT elevation, graded using CTCAE version 3.0	Median increase of 10 IU/L per minor allele (TOTXV) Median increase of 15 IU/L per minor allele (TOTXVI) Median increase of 12 IU/L per minor allele (TOTXV + TOTXVI) Elevation grade ≥ 2, exponent beta coefficient = 1.29 [1.18–1.40] (linear regression) PNPLA3 I148M explains 3.8% (R^2%) of variance in ALT elevation PNPLA3 I148M remains significant risk factor for postinduction ALT elevation after including diagnostic ALT as covariate	$p = 5.5 \times 10^{-6}$ $p = 2.7 \times 10^{-4}$ $p = 0.032$ $p = 1.2 \times 10^{-8}$ $p = 4.4 \times 10^{-6}$
Liu et al. 2017 (AALL0232) [27]	COG AALL0232 protocols (induction)	ALT elevation, graded using CTCAE version 4.0	Association between PNPLA3 I148M and frequency of grade > 2 elevations	$p = 0.024$
Yang et al. 2022 (AALL0232) [28]	AALL0232 protocols (all treatment phases)	Grade ≥ 3 ALT and/or AST elevation, graded using CTCAE version 4.0 Grade ≥ 3 hyperbilirubinemia, graded using CTCAE version 4.0	Association between PNPLA3 I148M and grade ≥ 3 elevations ORs: Transaminases: AALL0232 + AALL0434 = 1.27 [1.15–1.40] AALL0232 = 1.26 [1.12–1.41] Hyperbilirubinemia: AALL0232 = 1.43 (95% CI not defined)	$p < 0.002$ $p = 0.0001$ $p = 4.28 \times 10^{-4}$
Yang et al. 2022 (AALL0434) [28]	AALL0434 protocols (all treatment phases)		Association between PNPLA3 I148M and grade ≥ 3 elevations ORs: Transaminases: AALL0434 = 1.29 [1.07–1.55] Hyperbilirubinemia: AALL0434 = 1.05 (95% CI not defined)	$p = 0.007$ $p = 0.76$

Abbreviations: AC, active comparator; ALT, alanine transaminase; AST, aspartate transaminase; CFB, change from baseline; CTCAE, common terminology criteria for adverse events; LFC, liver fat content; OR, odds ratio; SE, standard error; ULN, upper limit of normal.
*Adjusted $p < 0.10$ was considered significant.

variant after treatment with peglispro (II/IM/MM, ALT: 7.08, 5.46, 13.78 IU/L, $p < 0.001$; AST: 3.65, 3.43, 11.02, $p < 0.001$) but not in the active comparator group. This effect seemed most pronounced in homozygous carriers of the variant allele. In both aforementioned studies, PNPLA3 I148M carriage was also associated with increased ALT and AST at baseline (IMAGINE-2, $p = 0.007$ for both and IMAGINE-4, $p < 0.001$ and $p = 3.75 \times 10^{-3}$ respectively). In the IMAGINE-5 trial, 428 T2DM patients who were already on insulin, or with up to three stable-dose oral antihyperglycaemic medications, were switched to either peglispro or glargine for 52 weeks. At the interim analysis after 26 weeks, mean ALT and AST CFB were significantly increased in the peglispro group (II/IM/MM, ALT: 7.55, 13.90, 15.36 IU/L, $p = 0.001$; AST: 3.49, 8.46, 10.18 IU/L, $p < 0.001$) but not the glargine group. After 52 weeks, these effects persisted (II/IM/MM ALT: 6.78, 12.33, 8.67 IU/L, $p = 0.009$; AST: 3.32, 7.15, 5.45 IU/L, $p = 0.019$). The pooled analysis of all three aforementioned studies further emphasised the association between PNPLA3 I148M and increases in CFB of both ALT and AST after 26 weeks of treatment (II/IM/MM, ALT: 5.27, 6.05, 8.60 IU/L, $p = 0.019$, AST: 2.89, 3.72, 6.36 IU/L, $p < 0.001$) (Table 2). Taken together, these studies provide some evidence for a link between PNPLA3 I148M and moderate serum transaminase elevations after treatment with basal insulin peglispro in T2DM patients.

3.4.3 | Combinatory Chemotherapy Protocols

Five studies investigating chemotherapeutic treatment protocols for children with ALL (Table S3) were included. A retrospective study by Gutierrez-Camino et al. [26] reported an association between carriage of PNPLA3 I148M and the occurrence of ALT and/or AST elevations > 5.1-fold the ULN during the induction phase of treatment for B-cell lineage ALL with LAL/SHOP94/99/2005 protocols in a Spanish cohort of 138 children (OR = 2.69 [1.09–6.64], $p = 0.029$). However, this study appeared to be bias-prone (Table S5).

The report by Liu et al. [27] included 715 patients in a discovery cohort and 2285 patients in a replication cohort. The authors investigated the impact of PNPLA3 I148M on ALT levels during the induction phase of treatment (Table S3). Median increases in ALT per PNPLA3 I148M minor allele of 10 and 15 IU/L were reported for TOTXV and TOTXVI, respectively ($p < 0.001$ for both), resulting in a pooled median increase of 12 IU/L per allele ($p = 0.032$). Linear regression analysis further showed an association between PNPLA3 I148M and ALT increases during the induction phase (exponent beta = 1.29 [1.18–1.40], $p < 0.001$). In this study, PNPLA3 I148M explained 3.8% of variance in postinduction ALT elevations, and PNPLA3 I148M remained a significant risk factor for postinduction ALT elevations when baseline ALT was included as a covariate ($p < 0.001$). Analysis in the replication cohort confirmed these findings, showing an association between PNPLA3 I148M and the occurrence of ALT elevations > 5-fold the ULN ($p = 0.024$), yet no baseline ALT levels were reported, and therefore this result might be biased (Table 2).

The report by Yang et al. [28] included similar studies, but in this case, the entire duration of treatment was considered. The first study, AALL0232, involved 2283 children receiving treatment for B-cell lineage ALL, while the second study, AALL0434, included 1274 children being treated for T-cell lineage ALL (Table S3). An association between PNPLA3 I148M and ALT and/or AST elevations > 5-fold the ULN was observed in both the AALL0232 study (OR = 1.26 [1.12–1.41], $p < 0.001$) and AALL0434 study (OR = 1.29 [1.07–1.55], $p = 0.007$), and in a pooled analysis (OR = 1.27 [1.15–1.40], $p < 0.002$). No interaction with treatment phase was found for this effect ($p = 0.78$) (Table 2). However, it should be noted that baseline ALT and AST levels were not available in these studies.

Altogether, these studies provide moderate evidence that the PNPLA3 I148M variant may enhance transaminase elevations in paediatric patients upon treatment with chemotherapeutics in the context of ALL.

3.5 | Results of Syntheses

3.5.1 | Narrative Synthesis

Results of the narrative synthesis are visually summarised in Figure 1. Moderate evidence suggests that the PNPLA3 I148M variant potentiates elevations in serum aminotransferases following treatment with biological agents for inflammatory and metabolic diseases, chemotherapeutic protocols in the context of ALL, and treatment with the small molecule adomeglivant for T2DM. There is low to no evidence that individuals who are homozygous for the PNPLA3 major allele (II) may be at increased risk of ALT elevations after treatment with methotrexate.

Some paediatric studies reported on a higher hepatocellular DILI risk in children with PNPLA3 I148M following ALL treatment (ALT and/or AST ≥ 3-fold ULN), but none of the included studies could show a definite effect of PNPLA3 I148M on clinically relevant hepatocellular DILI because of lacking baseline transaminase levels and individual-level total bilirubin and ALP levels.

3.5.2 | Meta-Analysis

All studies included in the meta-analysis encompassed patients with metabolic impairment, as the study population in all studies consisted of patients with T2DM. Further, all patients were on treatment with diet and exercise or at least one anti-diabetic medication at a stable dose (i.e., metformin, sulfonylurea and/or insulin) prior to study initiation (Table S3). For three of these studies, LFC measurements by magnetic resonance imaging within each PNPLA3 I148M genetic subgroup were available; these data suggest that overall these patient populations exhibited hepatic steatosis at baseline (LFC around 10%) without significant differences between the IM/MM and II groups (13.7% vs. 12.3%, Table S4).

A random effects model suggested that individuals carrying PNPLA3 IM/MM genotypes may develop higher serum aminotransferase levels after treatment with the small molecule adomeglivant and the biological basal insulin peglispro, both agents that are known to influence lipid metabolism, compared

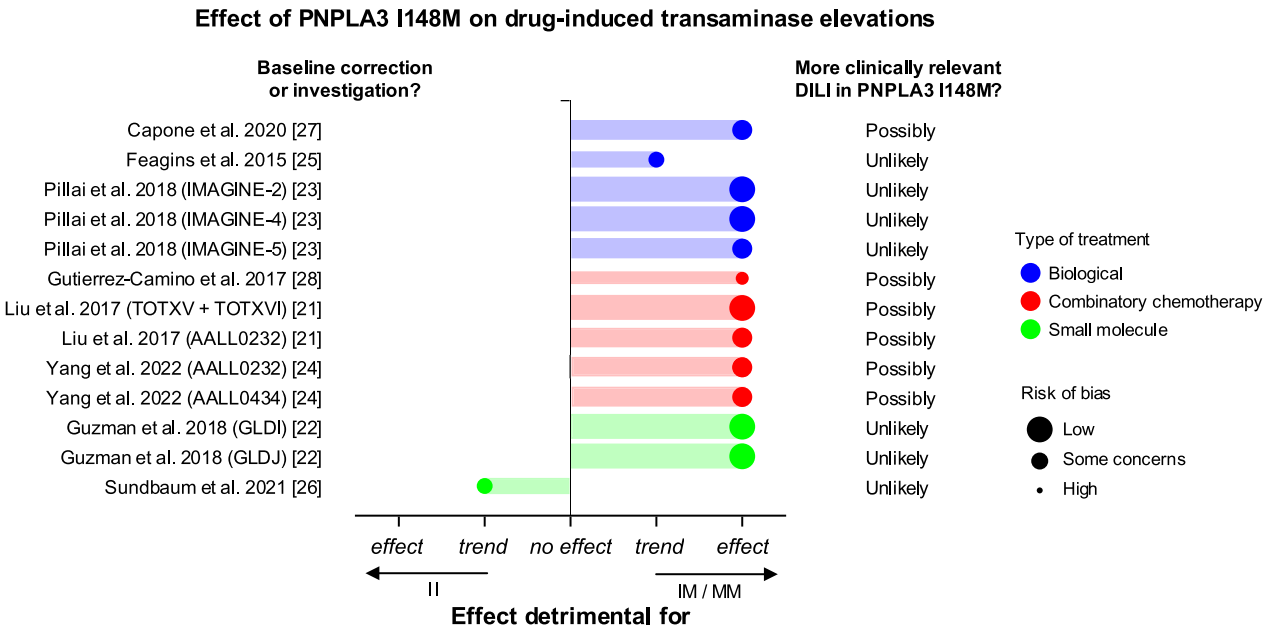

FIGURE 1 | **Narrative synthesis of results.** Some evidence suggests that carriage of a PNPLA3 I148M minor allele confers susceptibility to develop more pronounced aminotransferase elevations in response to drugs, although not all studies accounted for baseline transaminase levels. Based on the criteria of Hy's law, there is no evidence that PNPLA3 I148M increases the propensity to develop clinically relevant hepatocellular drug-induced liver injury, although it might occur more frequently in paediatric patients carrying PNPLA3 I148M following treatment with chemotherapy protocols. I and M represent a major or minor allele, respectively. PNPLA3, Patatin-like phospholipase domain-containing protein 3.

to PNPLA3 II homozygotes (OR = 1.53 [1.22–1.92], $p = 0.0002$) (Figure 2). Moderate heterogeneity was present between the studies ($\tau^2 = 0.0295$ [0.0000–0.8722]; $I^2 = 46.5\%$ [0.0–80.4]; and $Q = 7.48$, $p = 0.1127$). Asymmetry of the contour-enhanced funnel plot (Figure S2) suggested that publication bias might be present, which was supported by the addition of two studies by the trim-and-fill method to achieve symmetry. However, the impact hereof was limited as the adjusted trim-and-fill random effects estimate did not deviate from the original result (OR = 1.39 [1.12–1.72], $p = 0.0024$). Meta-regression did not reveal significant contributions from differences between PNPLA3 IM/MM and II subgroups in baseline factors such as age, sex, duration of T2DM, BMI, serum triglycerides, %Hb1Ac and transaminase levels. Moreover, in the subgroup for which LFC measurements and CFB in LFC, triglycerides and %Hb1Ac were available in both PNPLA3 genetic subgroups, inclusion of these covariates did not significantly change the outcome of the meta-analysis (Table S8).

3.6 | Reporting Biases

Four studies did not mention registration of the study protocol. Only one registered study protocol included the investigation of pharmacogenetic predictors for treatment-related outcomes; however, it did not specify the investigation of PNPLA3 I148M (Table S9). Altogether, these factors may have contributed to selective reporting and publication bias.

4 | Discussion

DILI is a major cause of acute liver injury [3, 4], but the contributing host factors are poorly defined. In this systematic review with meta-analysis, we found no definite effect of PNPLA3 I148M on the occurrence of clinically relevant DILI, although the meta-analysis suggested that carriage of the variant could confer susceptibility to develop increased serum transaminases in response to drugs that influence lipid metabolism (i.e., adomeglivant and basal insulin peglispro in the context of T2DM). Similar results were seen in a study from the USA DILI Network, where no association between PNPLA3 I148M and the risk of development, severity, or outcome of DILI caused by 177 agents in a cohort of 832 cases and 10 397 controls was seen, although the authors noted that the variant could still be involved in DILI related to specific drugs [30]. In this context, a phenome-wide association study suggested that carriage of PNPLA3 I148M may be associated with aspirin- and ibuprofen-, but not naproxen-related DILI [31]. Because these findings were only supported by data from one out of four included cohorts, which relied on questionnaire-based self-reports as a source for the phenotypes, further investigation and independent replication are needed.

The underlying mechanisms that could explain the findings of the present study are unclear but likely lie in the context of lipid metabolism. The PNPLA3 I148M variant has been linked to elevated transaminase levels in both adults and children [10, 32], and treatments causing or contributing to hepatic lipid accumulation may hence amplify this effect. In this context, insulin is known to promote hepatic de novo lipogenesis and hyperinsulinemia, which occurs in insulin-resistant individuals, can result in hepatic steatosis [33]. In patients with insulin resistance, the PNPLA3 I148M variant has also been linked to reduced circulating very low-density lipoprotein (VLDL) particles that are smaller in size compared to those found in non-carriers of the variant [34]. A recent study suggested that

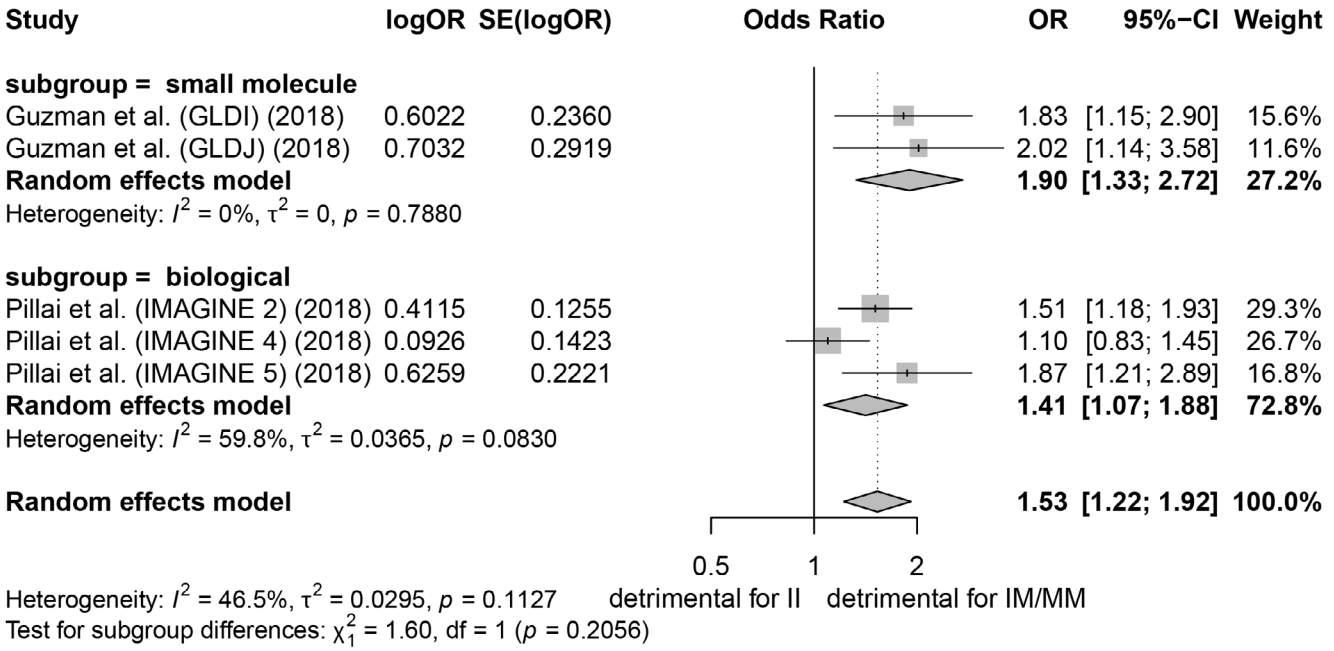

FIGURE 2 | Forest plot of the meta-analysis. The included records covered studies investigating the effect of adomeglivant (a glucagon receptor antagonist) and basal insulin peglispro in patients with type 2 diabetes mellitus. I and M represent a major or minor allele, respectively. Symbol sizes are proportional to the weight of the studies in the meta-analysis, determined by the width of their 95% confidence intervals. CI, confidence interval; OR, odds ratio; SE, standard error.

impaired lipidation of VLDL and reduced triglyceride secretion could be key contributors to PNPLA3 I148M-mediated hepatic steatosis. Under lipogenic stimulation, wild-type PNPLA3 plays a crucial role in mobilising polyunsaturated fatty acids from the triglyceride pool to synthesise phospholipids, which are in turn used for the synthesis and secretion of large, triglyceride-rich VLDLs, while this process is impaired for PNPLA3 I148M [35].

Data from multiple phase 2 and 3 trials comparing basal insulin peglispro and insulin glargine for treatment of both type 1 and type 2 diabetes suggested that basal insulin peglispro, despite being superior in lowering HbA1c, caused ALT elevations in both study populations which were more often found to be > 3-fold the ULN when compared to glargine. Moreover, in type 1 diabetes mellitus and T2DM patients previously treated with basal insulin, basal insulin peglispro caused a significant increase in LFC which was not observed in the glargine treatment group. The absence of this effect in the insulin naïve population suggests that withdrawal of basal insulin, and thus loss of suppression of peripheral lipolysis, may have contributed to this effect. A positive correlation was found between increased LFC and increases in ALT and triglycerides, irrespective of the study population [36]. Given that the prevalence of MASLD in patients with T2DM is around 65% [37], a further study investigated whether carriage of genetic polymorphisms linked to MASLD could further explain these findings. Pillai et al. [25] found that a switch from basal insulins, which suppress lipolysis, to hepatopreferential basal insulin peglispro, resulted in higher serum transaminase elevations in carriers of the PNPLA3 I148M variant. When peripheral lipolysis receives less suppression from insulin, the flux of free fatty acids to the liver increases, resulting in triglyceride re-esterification, VLDL secretion, and thus increased hepatic lipid turnover. Furthermore, systemic palmitate flux was found to be 35% higher in patients with type 1 diabetes treated with peglispro than those treated with glargine [38]. Considering that PNPLA3 I148M impairs VLDL lipidation, and that patients with MASLD who carry the PNPLA3 I148M variant benefit especially from interventions that reduce hepatic fatty acid flux such as lifestyle interventions, loss of peripheral suppression of lipolysis by conventional insulins may have an enhanced impact on hepatic steatosis in these patients [15].

Elevation of serum transaminases was a common side-effect observed in clinical trials investigating glucagon receptor antagonists, and liver injury was one of the key safety concerns resulting in cessation of their clinical development [39]. While the exact mechanisms behind this adverse effect remain to be elucidated, a recent study showed that a physiological increase in circulating glucagon stimulates hepatic mitochondrial oxidation in both healthy controls and individuals with liver steatosis, and several (dual) glucagon receptor agonists are currently under investigation in clinical trials for obesity, T2DM and MASLD [40–42]. In both a 6-week and 6-month trial, the glucagon receptor antagonist adomeglivant caused marked elevations of serum aminotransferases in patients with T2DM and PNPLA3 IM/MM genotypes compared to II carriers. This was not observed in the placebo or active comparator groups and thus suggests that an interaction between adomeglivant and carriage of PNPLA3 I148M may contribute to the observed transaminase elevations. Since the PNPLA3 I148M variant has been linked to hepatic mitochondrial dysfunction and increased mitochondrial stress in humans, further impairment of hepatic mitochondrial function due to blocking glucagon receptor signalling could have been one of the mechanisms that contributed to this effect [43].

In the context of cancer treatments, a recent study demonstrated that an interaction between oestrogen receptor alpha

and PNPLA3 I148M may contribute to steatotic liver disease susceptibility in women. In vitro data showed that exposure to the selective oestrogen receptor modulator tamoxifen, used for treatment of oestrogen-sensitive cancers, resulted in upregulation of PNPLA3 mRNA and protein levels, as well as accumulation of PNPLA3 I148M on intracellular lipid droplets in HepG2 cells [44]. As tamoxifen intake has been linked to the development of hepatic steatosis in patients [45], it is plausible that carriage of the PNPLA3 I148M variant may worsen the development of DILI in such cases. Unfortunately, no studies regarding tamoxifen-related DILI were found in the context of this systematic review; clinical data is needed to further investigate this possible interaction.

Some evidence was found that carriers of PNPLA3 I148M may develop excess hepatotoxicity in response to combinatory chemotherapy protocols in the paediatric population. Although these data are complex to interpret, it remains valuable to explore a possible effect of PNPLA3 I148M on increased serum transaminase levels in this context, given that individuals receiving these treatments often develop hepatotoxicity. Identifying one specific agent that was responsible for the observed transaminase elevations is unfeasible, and also other factors beyond chemotherapeutics may contribute to it, including hepatic infiltration of ALL and drugs used in supportive care, such as antibiotics [28]. The included studies by Lui et al. [27] and Gutierrez-Camino et al. [26] reported PNPLA3 I148M as a possible driver to develop elevated serum transaminases after induction therapy with the TOTXV, TOTXVI and LAL/SHOP/94/99/2005 protocols. A cross-sectional study of 300 paediatric ALL patients receiving induction therapy with UKALL 2003 protocols, including PEGylated asparaginase, vincristine and dexamethasone, confirmed these findings at the organ level by showing a significant association between PNPLA3 I148M and postinduction hepato(spleno)megaly (dominant logistic regression model, OR = 3.79 [2.29–6.28], p adjusted < 0.05) [46]. This association was independent of age, sex, ALL lineage, or the addition of daunorubicin to the protocol. The induction regimens of the aforementioned studies all included asparaginase, vincristine and a corticosteroid, with some also including methotrexate, daunorubicin, or cytarabine. Liu et al. found in their discovery cohort that postinduction ALT elevations were associated with the TOTXVI treatment protocol, which uses PEGylated asparaginase ($p = 0.025$), and additional doses of asparaginase ($p < 0.001$). Notably, grade 2–3 ALT elevations were more frequently observed in patients treated with the TOTXVI protocol than in those receiving the TOTXV protocol ($p = 5.8 \times 10^{-3}$). Moreover, the TOTXV protocol included high-dose methotrexate, while the TOTXVI protocol did not. These findings somewhat suggest that asparaginase, and particularly the more potent PEGylated form used in the TOTXVI protocol, is the predominant driver of postinduction ALT elevations in this cohort. In mice, rather than having direct hepatotoxic effects, PEGylated asparaginase was found to induce lipolysis in adipose tissue, hereby increasing fatty acid flux to the liver, resulting in steatosis. This activation of lipolysis was confirmed in an in vitro model using differentiated 3T3-L1 adipocytes, leading to increased non-esterified fatty acids in the cell culture supernatant [47]. It is plausible that the PNPLA3 I148M variant amplifies this indirect mechanism of hepatotoxicity in a similar manner as discussed for basal insulin peglispro, by increasing hepatic retention and reducing VLDL-mediated secretion of triglycerides. However, the study by Yang et al. [28] reported that the effect of PNPLA3 I148M on serum transaminase elevations was unrelated to treatment phase, suggesting that patients with this variant may have an overall increased susceptibility to hepatotoxicity, rather than an interaction with a specific treatment agent.

In line with this, apart from some specific lipid metabolism-related effects mediated by PNPLA3 I148M, a higher burden of pre-existing MASLD in the PNPLA3 I148M groups could have made these individuals more prone to develop hepatotoxicity [48, 49]. A large study including 4837 individuals with suspected MASLD and controls investigated the risk of suspected DILI caused by the top 10 medications that most frequently cause DILI. Suspected DILI was defined as ALT > 200 IU/L, alkaline phosphatase > 250 IU/L and/or total bilirubin > 2.5 mg/dL within 3 months of drug prescription at two or more consecutive laboratory tests. Suspected DILI occurred in 0.8% of the group with suspected MASLD, which was significantly higher compared to the controls without MASLD (0.2%), suggesting that MASLD can be a relevant condition predisposing to hepatotoxicity [50]. The relation between DILI and PNPLA3 I148M could therefore be bidirectional, with some drugs enhancing liver steatosis and pre-existing liver disease predisposing to develop DILI [51]. Indeed, pre-existing liver disease can also alter drug pharmacokinetics and MASLD has been specifically linked to clinically important reductions in expression of CYP2C19, which is responsible for the metabolism of many drugs [52, 53]. Thus, although PNPLA3 is not a pharmacogene, it is plausible that the resulting liver-related effects caused by the I148M variant can indirectly influence pharmacokinetics and DILI potential of drugs [53, 54].

Moreover, several studies have reported a synergistic interaction between PNPLA3 I148M and BMI when it comes to elevated serum ALT and hepatic steatosis [55, 56]. Notably, in both children and adults with steatotic liver disease, BMI above 30 kg/m^2 was found to confer an exponential increase in the relationship between PNPLA3 I148M and risk for advanced fibrosis [57]. Whether this interaction also affects the risk for hepatocellular DILI remains unclear. In the meta-regression, the mean difference between PNPLA3 IM/MM and II in baseline BMI did not significantly affect the odds of enlarged serum aminotransferase elevations (estimate = −0.4026, adjusted $p = 0.196$), nor did baseline BMI in the PNPLA3 IM/MM group (estimate = −0.0979, adjusted $p = 0.211$). In a study regarding treatment with basal insulin peglispro in insulin-naïve patients with T2DM, CFB in LFC was found to correlate negatively with baseline BMI. The same study did report a positive correlation between CFB in LFC and CFB in BMI, irrespective of previous insulin treatment, suggesting that it is not the patients with a high baseline BMI that are at risk but rather the patients who exhibit an increase in this parameter during treatment [36]. Unfortunately, CFB BMI data per PNPLA3 I148M genetic subgroup were not available for any of the studies in the meta-regression. Interestingly, in the GLDJ study, patients with PNPLA3 MM genotype had a lower baseline BMI (II/IM/MM, mean BMI: 32.6, 31.4, 26.9 kg/m, p-value not declared), yet they exhibited larger elevations in ALT compared to patients with PNPLA3 II and IM genotypes after treatment with adomeglivant (Δ mean CFB MM vs. II ≈28 IU/L, vs. IM ≈20 IU/L) [22]. Similarly, carriers of the PNPLA3 I148M variant

in the pooled IMAGINE trials exhibited a slightly lower BMI at baseline (II/IM/MM, mean BMI: 32.92, 32.34, 32.38, $p = 0.03$) yet exhibited a significantly higher CFB in ALT, AST and LFC after treatment with basal insulin peglispro [25]. It should be noted that for all studies in the meta-analysis, the mean BMI was above 30 kg/m^2 in both the PNPLA3 II and PNPLA3 IM/MM groups. Further, Liu et al. reported a positive correlation between postinduction ALT elevations and BMI percentile, which remained significant after adjustment for PNPLA3 I148M carriage ($p = 0.0027$). Nevertheless, data regarding the interaction between PNPLA3 I148M and either baseline or CFB BMI on drug-induced serum aminotransferase elevations are scarce, and further investigation is needed.

Our study should be interpreted in the context of its limitations. While elevations in ALT and AST are sensitive signals of hepatocellular injury, especially AST is an unspecific parameter and can be increased because of, for example, cardiac toxicity and muscle damage. In line with this, there was insufficient data to cover all parameters of Hy's law as a more specific measure of potential severe hepatocellular DILI [7, 8], although most studies did not report on severe DILI cases. Second, the included studies were not primarily powered to detect differences between PNPLA3 I148M subgroups and were retrospective in nature, which are sources of bias that could not be controlled for. Third, the relatively small number of studies, along with the heterogeneity of their summary measures, are additional possibly biasing factors, while severe DILI is rare and most compounds had a relatively safe toxicity profile [1, 2]. Last, not all studies ascertained the presence of MASLD or MASH that could have been more prevalent in the people carrying PNPLA3 I148M, which could have conferred an increased risk to develop hepatotoxicity [53, 54].

5 | Conclusion

There is low to no evidence that carriage of PNPLA3 contributes to clinically relevant hepatocellular DILI; although it may amplify drug-induced transaminase elevations attributed to adomeglivant and basal insulin peglispro, which are both known to influence liver lipid metabolism in individuals with metabolic impairment. A higher prevalence or severity of MASLD may have mediated the observed effect of PNPLA3 I148M on the more pronounced increase of transaminases in response to these drugs.

Author Contributions

A.G.: conceptualization, formal analysis (lead), investigation, methodology, visualisation, writing – original draft preparation. L.M.: formal analysis (supporting), investigation, methodology, writing – review and editing. H.H.: methodology, writing – review and editing. J.M.S.: methodology, writing – review and editing. R.M.R.: methodology, writing – review and editing. T.V.: methodology, supervision, writing – review and editing. J.B.: conceptualization, formal analysis (supporting), methodology, supervision, writing – review and editing.

Ethics Statement

The systematic review is registered in the PROSPERO register (CRD42024557488).

Conflicts of Interest

J.B. reports research funding from Colgate-Palmolive outside the submitted work. H.H. reports research funding from AstraZeneca, EchoSens, Gilead, Intercept, MSD, Novo Nordisk and Pfizer. He has served as a consultant for AstraZeneca and Novo Nordisk and has been or is part of hepatic events adjudication committees for Arrowhead, Boehringer Ingelheim, KOWA and GW Pharma. J.M.S. reports consulting for Alentis, Alexion, Altimmune, Astra Zeneca, 89Bio, Bionorica, Boehringer Ingelheim, Gilead Sciences, GSK, Ipsen, Inventiva Pharma, Madrigal Pharmaceuticals, Lilly, MSD, Northsea Therapeutics, Novartis, Novo Nordisk, Pfizer, Roche, Sanofi and Siemens Healthineers. speaker honorarium from AbbVie, Academic Medical Education (AME), Boehringer Ingelheim, Echosens, Forum für Medizinische Fortbildung (FOMF), Gilead Sciences, MedicalTribune, MedPublico GmbH, MedScape, Novo Nordisk, Madrigal Pharmaceuticals, Stockholder options: AGED diagnostics and Hepta Bio. The other authors declare no potential conflicts of interest.

Data Availability Statement

Data sharing not applicable to this article as no new data were generated for this study. All data used to perform the meta-analysis are available within the paper and Appendix S1.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Appendix S1:** liv70287-sup-0001-AppendixS1.zip.