

Journal Pre-proof



rs641738C>T near MBOAT7 is associated with liver fat, ALT, and fibrosis in NAFLD: a meta-analysis

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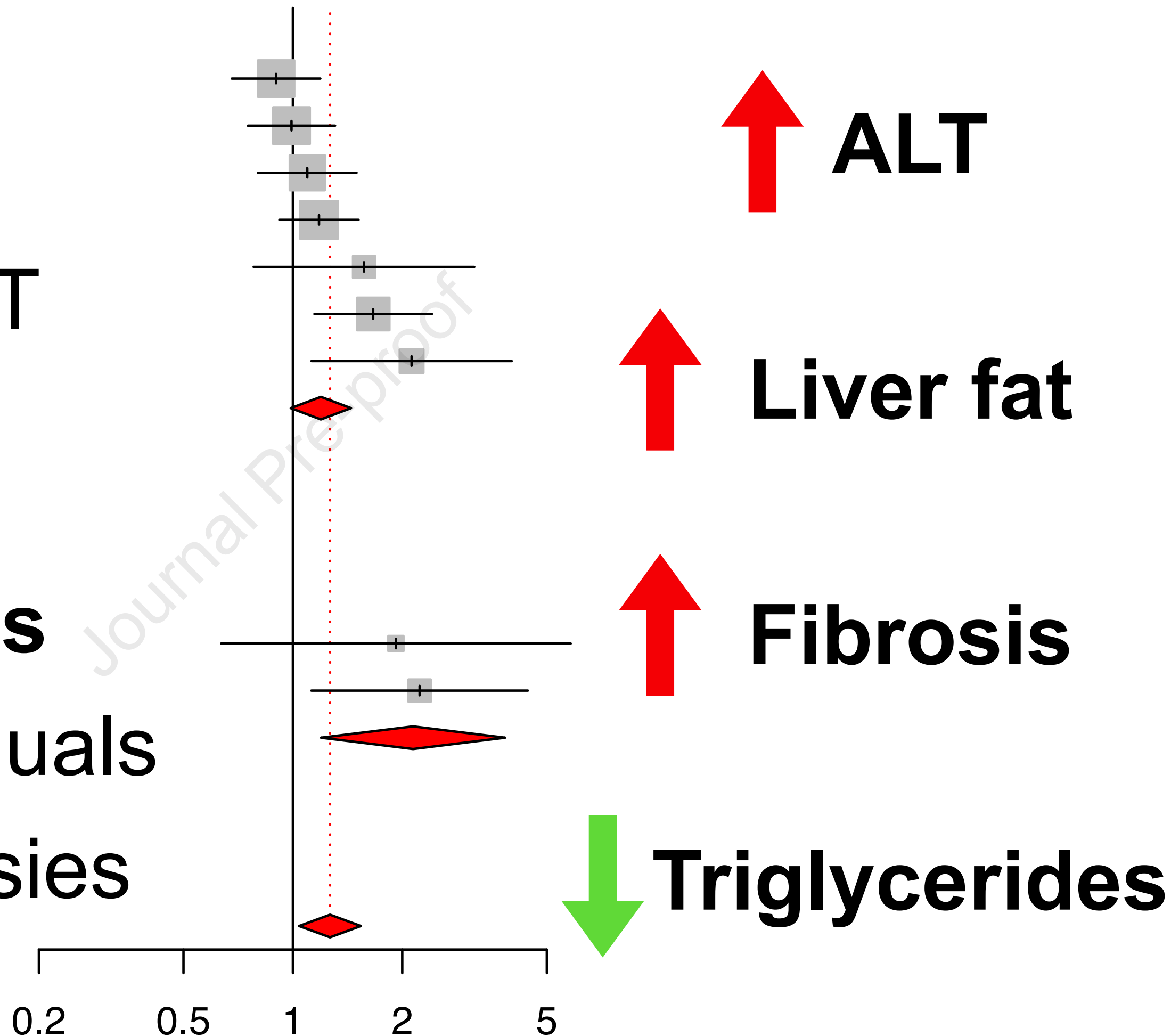
MBOAT7

rs641738 C>T
in NAFLD

Meta-analysis

>1 million individuals

9,688 liver biopsies



**rs641738C>T near MBOAT7 is associated with liver fat, ALT, and fibrosis
in NAFLD: a meta-analysis**

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Authors' contributions

Study concept and design: JPM; acquisition of data: all; analysis and interpretation of data: KT, JK, SS, LV, JPM; drafting of the manuscript: KT, JPM; critical revision of the manuscript for important intellectual content: all; statistical analysis: KT, JK, SS, LV, JPM; obtained funding: JPM, SC, NS, JKD, JK, PL, JMB, CAP, HY, KWMA, LA, QMA, ADK, TB, GSG, CH, JH, JL, PEM, TAM, NDP, SR, JIR, EKS, SS, ATH, LEW, LV, HYJ, KAY; study supervision: JPM.

Data availability

Raw data used in analyses is available in Supplementary Tables. Code used in analyses is available in the Supplement. Further details are available from the corresponding author upon request.

ABSTRACT

Background & Aims

A common genetic variant near *MBOAT7* (rs641738C>T) has been previously associated with hepatic fat and advanced histology in non-alcoholic fatty liver disease (NAFLD), however, these findings have not been consistently

replicated in the literature. We aimed to establish whether rs641738C>T is a risk factor across the spectrum of NAFLD and characterize its role in the regulation of related metabolic phenotypes through meta-analysis.

Methods

We performed meta-analysis of studies with data on the association between rs641738C>T genotype and: liver fat, NAFLD histology, and serum ALT, lipids, or insulin. These included directly genotyped studies and population-level data from genome-wide association studies (GWAS). We performed random effects meta-analysis using recessive, additive, and dominant genetic models.

Results

Data from 1,066,175 participants (9,688 with liver biopsies) across 42 studies were included in the meta-analysis. rs641738C>T was associated with higher liver fat on CT/MRI (+0.03 standard deviations [95% CI: 0.02 - 0.05], $p_z=4.8 \times 10^{-5}$) and diagnosis of NAFLD (OR 1.17 [95% CI 1.05 - 1.3], $p_z=0.003$) in Caucasian adults. The variant was also positively associated with presence of advanced fibrosis (OR 1.22 [95% CI: 1.03 - 1.45], $p_z=0.021$) in Caucasian adults using a recessive model of inheritance (CC+CT vs. TT). Meta-analysis of data from previous GWAS found the variant to be associated with higher ALT ($p_z=0.002$) and lower serum triglycerides ($p_z=1.5 \times 10^{-4}$). rs641738C>T was not associated with fasting insulin and no effect was observed in children with NAFLD.

Conclusion

Our study validates rs641738C>T near *MBOAT7* as a risk factor for the presence and severity of NAFLD in individuals of European descent.

LAY SUMMARY

Fatty liver disease is a common condition where fat builds up in the liver, which can cause liver inflammation and scarring (including 'cirrhosis'). It is closely linked to obesity and diabetes, but some genes are also thought to be important. We did this study to see whether one specific change ('variant') in one gene ('*MBOAT7*') was linked to fatty liver disease. We took data from over 40 published studies and found that this variant near *MBOAT7* is linked to more severe fatty liver disease. This means that drugs designed to work on *MBOAT7* may be useful for treating fatty liver disease.

Conflict of interest: Connor Emdin reports personal fees from Navitor Pharma and Novartis.

HIGHLIGHTS

- Meta-analysis of 42 studies (>1 million participants) for the role of rs641738C>T near *MBOAT7* on NAFLD
- rs641738C>T positively associated with liver fat, ALT, fibrosis, and HCC
- rs641738C>T negatively associated with serum triglycerides
- Consistent associations found in studies of Caucasian populations only

INTRODUCTION

Since the first genome-wide association study (GWAS) of liver fat[1], more than 20 genetic single nucleotide variants (SNVs) have been associated with non-alcoholic fatty liver disease (NAFLD)[2]. These studies have deepened our understanding of the condition, its heritability, and its relationship with cardio-metabolic disease.

Rs641738C>T near *MBOAT7* (membrane bound O-acyltransferase domain containing 7) was initially identified as a genome-wide significant risk variant for alcohol-related cirrhosis (odds ratio=1.35, $p=1.03 \times 10^{-9}$) [3], though not replicated in a more recent analysis[4]. It has since been implicated in the pathogenesis of NAFLD[5], hepatocellular carcinoma[6], as well as in fibrosis development in chronic hepatitis B/C[7,8], and primary sclerosing cholangitis[9]. However, unlike variants in *PNPLA3*, *TM6SF2*, and *HSD17B13*, it was not identified at genome-wide significance for liver fat or ALT[1,10,11].

Rs641738 is located a few hundred base pairs downstream of the 3' untranslated region of *MBOAT7*, which belongs to a family of genes that code for specific acyl donors and acceptors[12]. *MBOAT7* encodes lysophosphatidylinositol acyltransferase 1 (LPIAT1), which contributes to the regulation of free arachidonic acid in cells[13,14]. Rs641738C>T is associated with lower hepatic expression of *MBOAT7* at both the mRNA[15] and protein levels[5]. Given its role in inflammatory lipid pathways, most mechanistic work relating to rs641738 has focused on *MBOAT7*[16].

In NAFLD, the rs641738C>T variant was first demonstrated to be associated with increased hepatic fat content and severity of fibrosis in individuals of European descent[5]. Proton magnetic resonance spectroscopy data from 2,736 individuals showed a modest increase in hepatic fat in those with TT-genotype (4.1%) compared to those with CT- (3.6%) or CC-genotype (3.5%, $p=0.005$). Follow-up studies of European subjects corroborated the initial findings, and suggested a role in development of hepatocellular carcinoma[17,18]. However, these results were not replicated in adults of other ancestries[5,19–21] or in children[22].

In addition, bi-allelic loss of function mutations in *MBOAT7* cause autosomal recessive mental retardation 57 (OMIM #617188) and no liver phenotype has been reported in these patients to date[14,23]. However, rare likely pathogenic (coding) variants in *MBOAT7* are associated with HCC in NAFLD[24].

In summary, the association between rs641738C>T and hepatic fat content, as well as its effects on severity of NAFLD, remain unclear. Moreover, the broader metabolic effects of this SNV, including its association with markers of insulin resistance and dyslipidaemia have not been assessed.

Understanding the broader metabolic effects of rs641738C>T is important if *MBOAT7* were to be investigated as a drug target in NAFLD.

Here, we conducted a large meta-analysis to determine if rs641738C>T influences the development or stage of NAFLD and related traits.

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METHODS

Data sources and study selection

Two data sources were included in the meta-analysis: (i) studies which looked at the effect of the variant on traits of interest by genotyping the variant; and (ii) look-up from GWAS of traits of interest.

Studies were sourced through: Medline, Embase, HuGe Navigator, Web of Science, bioRxiv, and medRxiv. The search terms used were: “(*MBOAT7* or membrane-bound-o-acyltransferase) or (rs641738 or rs626283) or (*TMC4*)”. In addition, HuGe Navigator Phenopedia was searched using terms related to liver disease (Supplementary Methods). There were no restrictions on date or language. The search was completed on 28th July 2020. Reference lists of publications were also reviewed.

A separate search was conducted for all potentially relevant GWAS through: GWAS Catalogue[25], Phenoscanner[26], Type 2 diabetes knowledge portal[27], and Cardiovascular disease knowledge portal[28] (Supplementary Methods).

After removal of duplicates, titles and abstracts were screened for eligibility independently by two authors (investigators), with inclusion/exclusion criteria applied to potentially eligible full texts.

HuGENet guidelines[29] were followed throughout and MOOSE reporting guidelines[30] were used. This study was prospectively registered on PROSPERO Database of Systematic Reviews (CRD42018105507) available from:

http://www.crd.york.ac.uk/PROSPERO/display_record.php?ID=CRD42018105

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Inclusion and exclusion criteria

Studies were included if genotyping of rs641738C>T (or rs626283G>C [$R^2 > 0.98$ in European and American populations[31]] / rs2576452C>T [$R^2 = 0.92$ in Guzman *et al.* [32]], which are in strong linkage disequilibrium with rs641738C>T) was conducted and data on one of the outcomes of interest were reported. Narrative review articles, *in vitro* studies, and investigations involving animals, fish, and invertebrates were excluded. Studies which investigated liver disease of other aetiologies were also excluded. There was no restriction on ethnicity or ancestry. Types of studies eligible for inclusion were: case-control, cohort, genome-wide association studies, systematic reviews, and meta-analyses. Pre-print and abstract publications were not eligible for inclusion. Several studies reported on the same cohort (or patient sample) in more than one article. In these instances, data only from the larger of the overlapping cohorts were included in analyses. A full list of overlapping cohorts and articles is in Supplementary Table 1.

Data collection

Details on the recruitment of controls and cases were obtained from each study and, where necessary, clarified by discussion with the study's authors. In particular, it was noted when cases and controls were not recruited from the same population or clinics.

Hepatic steatosis or NAFLD (as diagnosis) was evaluated as a dichotomous variable where radiological (liver ultrasound, controlled attenuation parameter [CAP, with cut-off >248dB/m], CT, MRI) or histological assessment were used. Hepatic fat content was collected as a continuous variable from CT, MRS, MRI, PDFF. Non-invasive assessment of hepatic fat content was also assessed using semi-quantitative scoring in the Fenland cohort, as previously described[33], and using CAP.

Individual participant-level histology data were extracted according to the NASH Clinical Research Network scoring system[34] and, where not otherwise diagnosed by a pathologist's assessment, NASH was defined using the Fatty Liver Inhibition of Progression (FLIP) algorithm[35]. The above data were collected for each genotype separately (CC, CT, and TT).

Participant demographics and characteristics meta-data were collected from each study, including: sex, age, ethnicity, presence of type 2 diabetes, body mass index (BMI). Where possible, individual patient-level data was obtained. The authors of 59 studies were contacted for additional data or clarification, of whom 49 replied. Data from 11 potentially relevant studies could not be included, which are listed in the Supplementary Methods.

Additional details regarding cohorts with genome-wide data, the Avon Longitudinal Study of Parents and Children (ALSPAC) [36–38] data extracted from the UK BioBank, quality assessment, and statistical analysis is found in the Supplementary Methods.

RESULTS

Database search identified 1167 articles (Supplementary Fig. 1), of which 44 articles were included: 42 primary studies (Supplementary Tables 2-4), one systematic review and one meta-analysis (Supplementary Table 5).

In total, 1,066,175 individuals (5,711 children) were included in the meta-analysis. Most studies were in adults (32/42, 76%) and in individuals from predominantly Caucasian populations (26/42, 62%). Of the 42 included studies, 14 studies (9,688 participants, hereof 584 children) reported data on liver histology.

Studies were generally of high quality, though in five studies[11,22,39–41] (four in adults and one in children) the control group was recruited from a different population or sample to the cases (Supplementary Table 3).

One previous meta-analysis was included[42], which used data from 5 case-control studies to assess the effect of rs641738C>T on diagnosis of NAFLD. The meta-analysis included 2,560 cases and 8,738 controls and found no evidence of an association between this variant and diagnosis of NAFLD (Supplementary Table 5). One previous systematic review[43] found positive associations between rs641738C>T in adults of Caucasian, Hispanic, and African American descent with limited data in children (Supplementary Table 5).

Liver fat, NAFLD, and severe steatosis in adults

Seven studies (29,679 participants) reported data on hepatic fat as a continuous variable assayed by CT or MRI. On meta-analysis, rs641738C>T was associated with higher liver fat in studies in Caucasian populations using an additive model of inheritance, with a per T-allele change of β 0.034 (95% CI 0.018, 0.051), $p_z=4.8 \times 10^{-5}$) standard deviations in inverse-normalized liver fat (Figure 1), whilst no consistent effect was observed in non-Caucasian populations. A similar trend was observed using a dominant model of inheritance in studies of Caucasian populations: mean difference in hepatic fat +0.18% ((95% CI 0.2, 0.34), $p_z=0.04$, Supplementary Table 6).

Given the difference in sensitivity and specificity of modalities used to assess liver fat, a sub-analysis by modality of imaging was performed. No significant differences were observed between studies using CT, MRI, or MRS for quantification of liver fat (Supplementary Figure 2).

A similar trend was observed using CAP and semi-quantitative ultrasound to assess steatosis severity in 12,224 adults (β 0.02 (95% CI -0.002, 0.04), , $p_z=0.08$, Supplementary Figure 3).

Data from a range of diverse modalities was used to assess the effect of this variant on diagnosis of NAFLD, to reflect real-world diagnostic practice. rs641738C>T was associated with NAFLD as a trait (OR 1.15 (95% CI 1.05, 1.26), $p_z=0.002$) using a recessive model of inheritance (Figure 2) but not using additive or dominant models (Supplementary Table 7). The effect was only observed in studies of Caucasian populations (OR 1.17 (95% CI 1.05,

1.3), $p_z=0.003$). Sub-group analysis by modality of diagnosis found the 95% confidence intervals for all modalities overlapped, except for MRI-PDFF, which had only one study (Supplementary Figure 4). The association remained after excluding four studies where there was a lack of similarity between cases and controls (OR 1.19 (95% CI 1.07, 1.33), $p_z=0.0017$) using a recessive model of inheritance.

However, Egger's test suggested evidence of study distribution (publication) bias ($p=0.013$) and when using the Trim and Fill method to account for this bias, the positive association remained but was attenuated (OR 1.11 (95% CI 1.01, 1.23), $p_z=0.037$, Supplementary Figure 5).

In patients with NAFLD, data from eight studies (6,206 participants) rs641738C>T was not significantly associated with the presence of severe steatosis (S1-2 vs. S3) on liver biopsy (OR 1.08 (95% CI 0.78, 1.5), $P_z=0.64$, Table 1 & Supplementary Figure 6).

Histological NASH in adults

Data from nine studies (7,719 participants) found that rs641738C>T was not associated with the presence of NASH on biopsy in adults (OR 1.24 (95% CI 0.96, 1.36), $p_z=0.128$ Supplementary Figure 7).

Fibrosis in adults

Liver biopsy data on presence of advanced fibrosis was available from eight studies (7,692 adults). Our primary outcome, presence of advanced fibrosis in

adults (stage F0-2 versus stage F3-4), showed a borderline positive association with rs641738C>T in Caucasian populations (OR 1.22 (95% 1.03, 1.45), $p_z=0.021$)(Figure 3). In addition, two studies used ICD-codes (International Statistical Classification of Diseases and Related Health Problems) in the UKBB cohort to identify individuals with NAFLD and advanced fibrosis or cirrhosis[44,45]. Both found positive associations below genome-wide significance: for example, using an additive model of inheritance Emdin *et al.* found the association between rs641738C>T and cirrhosis as β 1.22 (SE 0.06, $P=0.03$), using an additive genetic model.

Data from nine studies (8,389 participants), found that presence of any fibrosis (F0 versus F1-4) was also borderline positively associated with rs641738C>T overall (OR 1.27 (95% 1.04, 1.54), $p_z=0.018$) as well as in non-Caucasian populations as a sub-group (Supplementary Figure 8).

Development of hepatocellular carcinoma

Four cohorts (2,328 participants, 228 cases of NAFLD-HCC) reported on development of HCC in patients with NAFLD. rs641738C>T was associated with increased odds of HCC in NAFLD only when using a dominant model (CC vs. CT+TT) of inheritance (OR 1.64 (95% CI 1.18, 2.27), $p_z=0.003$, Figure 4).

Effect on alanine aminotransferase (ALT)

Data from GWAS using log-transformed ALT (609,794 participants) were available for meta-analysis to investigate the role of rs641738C>T on ALT.

The variant showed a positive association with ALT (β 0.004 (95% CI 0.002, 0.007), $p_z=0.002$), which on sub-analysis was observed in Caucasian populations but not in non-Caucasian populations (Figure 5 & Supplementary Table 7).

Additionally, in the UKBB cohort, rs641738C>T was associated with a small, but statistically significant ($P=2.0 \times 10^{-8}$) increase in un-transformed ALT: 0.18 IU/L higher ALT per T-allele in this variant (Supplementary Table 8).

In the remaining cohort and case-control studies included in the meta-analysis (15,208 adults), rs641738C>T was not found to be significantly associated with a change in ALT, for example mean difference using a recessive model (CC+CT vs. TT) +0.32 IU/L (95% CI -0.06, 0.7), $p_z=0.08$, Supplementary Table 9) in Caucasian populations.

Effect on serum lipids and insulin

Data from GWAS using log-transformed serum triglycerides (850,241 participants) found that rs641738C>T was associated with lower triglycerides (β -0.01 (95% CI -0.018, -0.006), $p_z=1.5 \times 10^{-4}$), which on sub-analysis was observed in Caucasian populations but not in non-Caucasian populations (Supplementary Fig. 9). Similar findings were obtained from meta-analysis of cohort and case control studies, particularly using an additive model (β -0.03 (95% CI -0.05, -0.01), $p_z=0.00091$.), Supplementary Table 9).

Data from GWAS (852,409 participants) found rs641738C>T to be positively associated with total cholesterol, in Caucasian populations (β 0.007 (95% CI 0.003, 0.01), $p_z=2.1 \times 10^{-4}$), which was not observed in non-Caucasian populations (Supplementary Fig. 10). A borderline positive association was also observed between rs641738C>T and high-density lipoprotein (HDL) cholesterol (β 0.009 (95% CI 0.001, 0.02), $p_z=0.02$), Supplementary Table 7). There was no effect on fasting insulin levels found in population-level GWAS (β 0.009 (95% CI -0.03, 0.04), $p_z=0.64$), Supplementary Table 7). However, a negative association was observed using data from cohort and case-control studies with a dominant genetic model (mean difference -1.4 pmol/L (95% CI -2.1, -0.65), $p_z=0.004$), Supplementary Table 9).

Effect of rs641738C>T on paediatric NAFLD

Data from ten studies (5,711 children) was used in the meta-analysis. rs641738C>T was not significantly associated with the diagnosis of NAFLD, liver fat content, stage of liver histology, or serum biochemistry (Supplementary table 10).

Meta-regression shows interaction between rs641738C>T and type 2 diabetes

Finally, we aimed to determine whether baseline participant characteristics influenced the association of rs641738C>T on histological outcomes using meta-regression. There was a negative association with presence of type 2 diabetes and effect size for NASH vs. NAFL (β -1.8 (standard error 0.65), $p=0.006$, Supplementary Figure 11A). A similar negative trend with type 2

diabetes was observed for severe steatosis (S1-2 vs. S3, β -2.6 (standard error 1.5), $p=0.08$) and presence of fibrosis (F0 vs. F1-4, β -1.5 (standard error 0.8), $p=0.06$, Supplementary Table 11). In addition, effect size for any fibrosis was greater in cohorts with an older mean age (β 0.05 (standard error 0.02), $p=0.014$, Supplementary Figure 11D).

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DISCUSSION

Identification of genetic variants associated with NAFLD has the potential to inform pre-clinical research and our understanding of hepatic metabolism. In this meta-analysis we have validated rs641738C>T near *MBOAT7* as a risk factor for the full spectrum of NAFLD in Caucasian adults.

A two-stage GWAS initially identified rs641738C>T as a genome-wide significant locus for alcohol-related cirrhosis[3]. *MBOAT7* was a potentially interesting target as an enzyme involved in (phospho)lipid metabolism, conceptually similar to other SNVs at GWAS-significance in alcoholic and non-alcoholic liver disease, namely *TM6SF2* and *PNPLA3*. Later studies found the variant to influence the full spectrum of fatty liver disease, from steatosis to NASH, to fibrosis, cirrhosis and HCC[5,17]. However, these associations have not been consistently replicated in the literature[19]. We conducted a meta-analysis to firmly establish the association of rs641738C>T with the presence and severity of NAFLD, and associated metabolic traits.

Main findings

We found that rs641738C>T was associated with higher liver fat content, higher ALT, and with higher odds of NAFLD diagnosis, fibrosis, and HCC, particularly in Caucasian adults and in the homozygous 'TT' genotype. The effects sizes of rs641738C>T reported here are small compared to those of *PNPLA3* p.I148M and *TM6SF2* p.E167K, the two strongest steatogenic variants[46]. Also, the magnitude of change in alanine aminotransferase is small relative to that associated with variants in *PNPLA3*, *HSD17B13*,

MTARC1, and *TM6SF2*. This may account for the absence of this variant (or others near *MBOAT7*) from GWAS for NAFLD in the general population[1,10,11,45,47] the effect size (and associated p-value) was too small to be identified as significant genome-wide. The marginal positive effect on hepatic triglyceride content may suggest this variant acts through alterations in the composition of hepatic lipid, as well as quantity[17]. This is consistent with pre-clinical data on lipotoxicity, where the composition of hepatic fats influence development of NASH. On the other hand, a recent Mendelian randomization study using these variables as instruments to assess causality of fatty liver in determining fibrosis has shown the effect of steatosis highly correlates with fibrosis in all the genetic variables indicating that quantity of lipid rather than quality may be more important[48]. Functional studies are needed to understand the relationship between quality/quantity of fat and hepato-toxic/-protective mechanism in causing progression of disease.

The function of this variant is still relatively poorly understood and there is conflicting evidence as to whether rs641738C>T is associated with changes in hepatic expression of *MBOAT7*. Results from the GTEx Consortium show a strong negative association with T-allele[15], which is supported by data from Schadt *et al.*[49]. *MBOAT7* protein expression correlated with mRNA in liver biopsies from Mancina *et al.*[5] but this finding was not replicated by Sookoian *et al.*[19]. *MBOAT7* encodes LPIAT1, a 6 transmembrane domain protein involved in acyl-chain remodelling of membranes that influence intracellular membrane composition and circulating phosphatidylinositols[50]. Further, recent metabolite profiling data implicates *MBOAT7* as the causal gene for

this SNV[51]. Moreover, *TMC4* was found with a low expression in the liver[5] that is consistent with no mechanistic data supporting its role in NAFLD.

The hypothesis that *MBOAT7* is the causal gene underlying the association with liver disease at the locus is supported by the observation that mice deficient for *MBOAT7* have altered hepatic concentrations of polyunsaturated phosphatidylinositol[50]. Similarly, metabolite data from humans is strongly suggestive that rs641738C>T reduces *MBOAT7* function[52]. In addition, two independent groups have found that loss of *MBOAT7* (but not *TMC4*) increases the severity of NAFLD in mice fed a high-fat diet[53,54].

These analyses suggest that rs641738C>T impacts the severity of NAFLD through a recessive model of inheritance, though some analyses (e.g. liver fat and ALT) were suggestive of a role using an additive genetic model. Other genetic variants are known to impact on all-cause mortality in a recessive manner, notably variants that perturb *HFE*[44]. Further mechanistic work is required to understand the extent to which the haplo-insufficient state affects hepatocyte function.

We found no evidence of an effect of rs641738C>T on insulin resistance: the key driver of hepatic steatosis, as determined by unaltered fasting insulin concentrations. GWAS meta-analyses of type 2 diabetes have implicated p.I148M in *PNPLA3* and p.E167K in *TM6SF2* as significant risk loci (albeit with very modest effect size as compared to their effects on liver disease)[55] and Mendelian randomization studies indicate a causal role in determining

insulin resistance mediated by the degree of liver damage[48,56]. Similarly, these two variants are associated with reduced risk of coronary artery disease; whilst our analysis did find lower serum triglycerides to be associated with this variant it has not been associated with lower rates of cardiovascular disease[57]. However, we did observe a negative association between effect size and prevalence of diabetes on meta-regression, potentially suggesting that this variant has the greatest effect in less insulin-resistant individuals.

A strength of this meta-analysis is the large number of individuals with liver biopsy-derived phenotypic data as well as use of population-based GWAS data. The larger number of included studies and participants is likely to account for the different conclusions reached in this study compared to the previous meta-analysis by Xia *et al.*[42].

Limitations and quality of evidence

An important practical consideration is the population frequency of this variant in different ethnicities. The mean allelic frequency of the effect (T) allele is highly variable: from 0.24 in East Asians compared to 0.53 in those of South Asian ancestry[58]. Moreover, the majority of studies included in this meta-analysis used self-reported ethnicity, rather than genetic ancestry.

Though this analysis did include data from individuals of multiple ethnicities (and genetic ancestries) we only found evidence of an effect of this variant in Caucasian individuals. This is consistent with the initial discovery and it is likely that rs641738C>T is a proxy for the true causal variant. However, due to

differences in patterns of linkage disequilibrium, we cannot exclude the possibility that a different nearby locus is associated with liver-related phenotypes in individuals of other genetic ancestries.

A limitation of using meta-analysis for a single variant is the lack of adjustment for population stratification. When further genome-wide data are available, a formal GWAS meta-analysis may be able to address this.

We found significant differences between adult and paediatric histological analyses. Whilst there were fewer clinical events (e.g. with advanced fibrosis) in children, the analyses did not show a trend congruous with those in adults. Paediatric NAFLD has a different histological phenotype to that of adults (with prominent periportal inflammation) and it is therefore plausible that this is a true lack of association in children with NAFLD.

Data from multiple diagnostic or imaging modalities were combined in several analyses. Though we observed minimal heterogeneity between modalities, these techniques have differing accuracy for diagnosis of steatosis, which has the potential to affect results. The sub-group analysis of hepatic fat by modality suggested a marginally greater effect size in studies using MRS, which is regarded as a highly sensitive technique. There is potential that through inclusion of other modalities (e.g. CT) we have underestimated the effect size associated with this variant.

The magnitude of effect observed across all associations is small in comparison to other well-established variants. The clinical relevance of rs738409C>G in *PNPLA3* has been validated with hard end-points[59] but large cohorts will be required to prospectively demonstrate the clinical risk associated with this variant near *MBOAT7*.

Though there was minimal heterogeneity across included studies, there was evidence of publication bias but the effect on diagnosis of NAFLD appeared to persist after attempting to account for this. Also of note, the numbers of individuals with NAFLD and HCC were comparatively low, also limiting the power to assess for an association of this variant with non-cirrhotic HCC, as has been previously reported[6]. The HCC analysis was also unique in only demonstrating an effect in the dominant, rather than recessive, model of inheritance. Further work in this area may improve the accuracy of effect estimates.

Conclusions

rs641738C>T near *MBOAT7* is positively associated with liver fat, ALT, and histological severity in Caucasian adults with NAFLD, but negatively associated with serum triglycerides and with relatively small effect sizes throughout. These data validate this locus as significant in the pathogenesis of NAFLD.

ABBREVIATIONS

ALSPAC, Avon Longitudinal Study of Parents and Children; ALT, alanine aminotransferase; BMI, body mass index; CAP, controlled attenuation parameter; CT, computed tomography; GWAS, genome-wide association study; *HFE*, homeostatic iron regulator protein; HOMA-IR, homeostatic model assessment of insulin resistance; HSD17B13, 17 β -Hydroxysteroid dehydrogenase type 13; *MBOAT7*, membrane bound O-acyltransferase domain containing 7; *MTARC1*, Mitochondrial amidoxime reducing component 1; OR, odds ratio; MRI-PDFF, magnetic resonance imaging-proton density fat fraction; MRS, magnetic resonance spectroscopy; *PNPLA3*, patatin-like phospholipase domain containing protein 3; SNV, single nucleotide variant; *TM6SF2*, transmembrane 6 superfamily member 2; *TMC4*, transmembrane channel-like 4; UKBB, UK BioBank.

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Author names in bold designate shared co-first authorship.

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Tables

Outcome	Genetic model	Sub-analysis	No. of studies	Heterogeneity		Effect summary	
				I ²	p _Q	OR [95% CI]	p _Z
NAFLD diagnosis (control vs NAFLD)	Recessive	Overall	17	0.25	0.17	1.15 (1.05, 1.26)	0.0018
NAFLD diagnosis (control vs NAFLD)	Recessive	Non-Caucasian	5	0	0.46	1.1 (.9, 1.34)	0.343
NAFLD diagnosis (control vs NAFLD)	Recessive	Caucasian	12	0.38	0.09	1.17 (1.05, 1.3)	0.0033
Severe steatosis (S1-2 vs S3)	Recessive	Overall	8	0.67	0	1.08 (.78, 1.5)	0.642
Severe steatosis (S1-2 vs S3)	Recessive	Non-Caucasian	1	NA	NA	1.11 (.39, 3.16)	0.852
Severe	Recessive	Caucasian	7	0.72	0	1.08	0.676

steatosis (S1-2 vs S3)						(.76, 1.54)	
NASH (NAFL vs NASH)	Recessive	Overall	9	0.33	0.15	1.14 (.96, 1.36)	0.128
NASH (NAFL vs NASH)	Recessive	Non-Caucasian	3	0	0.58	1.24 (.81, 1.9)	0.324
NASH (NAFL vs NASH)	Recessive	Caucasian	6	0.53	0.06	1.14 (.93, 1.41)	0.213
Any fibrosis (F0 vs F1-4)	Recessive	Overall	9	0.52	0.03	1.27 (1.04, 1.54)	0.0183
Any fibrosis (F0 vs F1-4)	Recessive	Non-Caucasian	2	0	0.82	2.14 (1.2, 3.84)	0.0105
Any fibrosis (F0 vs F1-4)	Recessive	Caucasian	7	0.51	0.06	1.19 (.99, 1.45)	0.068
Advanced fibrosis (F0-2 vs F3-4)	Recessive	Overall	8	0	0.65	1.2 (1.02, 1.42)	0.027
Advanced fibrosis (F0-2 vs F3-4)	Recessive	Non-Caucasian	2	0	0.64	.96 (.5, 1.85)	0.911

F3-4)							
Advanced fibrosis (F0-2 vs F3-4)	Recessive	Caucasian	6	0	0.5	1.22 (1.03, 1.45)	0.0206
HCC (NAFLD-HCC vs NAFLD no-HCC)	Recessive	Overall	4	0	0.95	1.4 (.99, 1.98)	0.056

Table 1. Summary of results in adults from meta-analyses for dichotomous outcomes. Meta-analyses were performed using random effects with subgroup analysis for Caucasian and Non-Caucasian populations. Additive, recessive, and dominant genetic models were tested for all outcomes. Results using a recessive model of inheritance (CC+CT vs. TT) are shown for all outcomes, except for HCC, where a dominant model (CC vs. CT+TT) is shown. Due to use of three genetic models, critical p-value for effect summary is $p_z < 0.017$. Full results (with all genetic models) are in Supplementary Table 5. CI, confidence interval; HCC, hepatocellular carcinoma; NAFL, non-alcoholic fatty liver; NASH, non-alcoholic steatohepatitis; OR, odds ratio.

FIGURE LEGENDS

Fig. 1. The effect of rs641738C>T on liver fat. Data from 29,679,916 individuals with CT, or MRI, or MRS liver fat. rs641738C>T positively associated with liver fat in Caucasian populations, where data represents standard deviation change in normalized liver fat per T-allele. CI, confidence interval; UKBB, UK BioBank.

Fig. 2. rs641738C>T is associated with higher odds of diagnosis of NAFLD. Data from 52,173,333,263 adults (11,301,971 cases and 40,872,233,550 controls) with radiologically or histologically defined steatosis for presence versus absence of NAFLD. CI, confidence interval; LBC, Liver Biopsy Cohort; OR, odds ratio.

Fig. 3. The effect of rs641738C>T on presence of advanced fibrosis in adult patients with NAFLD. Data from 7,692,621 adults (1,214,828 cases and 6,478,538 controls) with biopsy-proven NAFLD comparing advanced fibrosis (F3-4) versus F0-2, using a recessive model of inheritance (CC+CT vs. TT). CI, confidence interval; LBC, Liver Biopsy Cohort; OR, odds ratio.

Fig. 4. rs641738C>T is associated with higher odds of NAFLD-HCC. Data from 2,328 adults with NAFLD assessing for the presence versus absence of HCC, using a dominant model of inheritance (CC vs. CT+TT).

Fig. 5. rs641738C>T is positively associated with alanine aminotransferase (ALT) in Caucasian populations in genome-wide association studies (GWAS). Meta-analysis of GWAS summary statistics from 609,794 participants for the association between rs641738C>T on logarithmically-transformed ALT using linear regression. CI, confidence interval; UKBB, UK BioBank.

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