Macrophage scavenger receptor 1 mediates lipid-induced inflammation in non-alcoholic fatty liver disease

Graphical abstract

Highlights

- In human NAFLD, MSR1 is expressed in mature Kupffer cells and foamy macrophages.
- MSR1 transcript levels are associated with disease activity in patients with NAFLD.
- Mice lacking Msr1 are protected from diet-induced metabolic disorder.
- Uptake of saturated fatty acids via MSR1 results in a pro-inflammatory response.
- The SNP rs41505344 upstream of MSR1 is associated with altered serum triglycerides.

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Lay summary

Non-alcoholic fatty liver disease (NAFLD) is a chronic disease primarily caused by excessive consumption of fat and sugar combined with a lack of exercise or a sedentary lifestyle. Herein, we show that the macrophage scavenger receptor MSR1, an innate immune receptor, mediates lipid uptake and accumulation in Kupffer cells, resulting in liver inflammation and thereby promoting the progression of NAFLD in humans and mice.
Macrophage scavenger receptor 1 mediates lipid-induced inflammation in non-alcoholic fatty liver disease

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Keywords: macrophages; immunometabolism; NASH; inflammation.

Received 26 March 2021; received in revised form 5 December 2021; accepted 7 December 2021; available online 21 December 2021

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Background & Aims: Obesity-associated inflammation is a key player in the pathogenesis of non-alcoholic fatty liver disease (NAFLD). However, the role of macrophage scavenger receptor 1 (MSR1, CD204) remains incompletely understood.

Methods: A total of 170 NAFLD liver biopsies were processed for transcriptomic analysis and correlated with clinicopathological features. Msr1−/− and wild-type mice were subjected to a 16-week high-fat and high-cholesterol diet. Mice and ex vivo human liver slices were treated with a monoclonal antibody against MSR1. Genetic susceptibility was assessed using genome-wide association study data from 1,483 patients with NAFLD and 430,101 participants of the UK Biobank.

Results: MSR1 expression was associated with the occurrence of hepatic lipid-laden foamy macrophages and correlated with the...
NAFLD and Alcohol-Related Liver Diseases

degree of steatosis and steatohepatitis in patients with NAFLD. Mice lacking Msr1 were protected against diet-induced metabolic disorder, showing fewer hepatic foamy macrophages, less hepatic inflammation, improved dyslipidemia and glucose tolerance, and altered hepatic lipid metabolism. Upon induction by saturated fatty acids, MSR1 induced a pro-inflammatory response via the JNK signalling pathway. In vitro blockade of the receptor prevented the accumulation of lipids in primary macrophages which inhibited the switch towards a pro-inflammatory phenotype and the release of cytokines such as TNF-α. Targeting MSR1 using monoclonal antibody therapy in an obesity-associated NAFLD mouse model and human liver slices resulted in the prevention of foamy macrophage formation and inflammation. Moreover, we identified that rs41505344, a polymorphism in the upstream transcriptional region of Msr1, was associated with altered serum triglycerides and aspartate aminotransferase levels in a cohort of over 400,000 patients.

Conclusions: Taken together, our data suggest that MSR1 plays a critical role in lipid-induced inflammation and could thus be a potential therapeutic target for the treatment of NAFLD.

Lay summary: Non-alcoholic fatty liver disease (NAFLD) is a chronic disease primarily caused by excessive consumption of fat and sugar combined with a lack of exercise or a sedentary lifestyle. Herein, we show that the macrophage scavenger receptor MSR1, an innate immune receptor, mediates lipid uptake and accumulation in Kupffer cells, resulting in liver inflammation and thereby promoting the progression of NAFLD in humans and mice. © 2021 The Authors. Published by Elsevier B.V. on behalf of European Association for the Study of the Liver. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Introduction

With the increasing prevalence of obesity, non-alcoholic fatty liver disease (NAFLD) has become the most common chronic liver disease globally. NAFLD is characterised by excessive hepatic triglyceride accumulation and represents a series of diseased states ranging from isolated steatosis (non-alcoholic fatty liver, NAFL) to non-alcoholic steatohepatitis (NASH), identified by the presence of necro-inflammation and hepatocyte ballooning, with varying degrees of fibrosis. NAFLD is strongly linked with metabolic syndrome, i.e. dyslipidemia, hypertension, obesity and type 2 diabetes mellitus (T2DM), and currently affects 20% to 30% of the global population. Importantly, not all patients progress from NAFL to NASH and although gene signatures of more advanced fibrosing-steatohepatitis have been identified, the exact pathogenic pathways involved in the initiating phases of the disease, especially the transition from NAFL to NASH, are not fully understood.

Growing evidence supports the view that Kupffer cells, the endogenous hepatic macrophages, are initiators of inflammation and hence contribute to NAFLD development, whilst recruited monocyte-derived macrophages are often observed in advanced stages of the disease. Hepatic macrophages are responsive to a variety of stimuli including bacterial endotoxins (such as lipopolysaccharide) but also free fatty acids (FFAs) or cholesterol. An excess of FFAs and cholesterol can cause the formation of hepatic foamy macrophages, and lead to Kupffer cell aggregates and lipogranulomas during steatohepatitis. Specifically, the intake of saturated fat has been shown to induce insulin resistance and to enhance intrahepatic triglyceride accumulation and steatohepatitis.

Palmitic acid, rather than non-saturated fatty acids (non-SFAs), has been shown to be a strong inducer of inflammation in immortalised cell lines through activation of the downstream JNK signalling pathway. Recent data show that pro-inflammatory activation of murine bone marrow-derived macrophages (BMDMs) by palmitic acid is independent of Toll-like receptor 4, yet the receptor that is responsible is still not known. Recently, we have shown that in vitro activation of the phagocytic receptor, macrophage scavenger receptor 1 (MSR1, also known as SR-A or CD204), results in pro-inflammatory macrophage polarisation through JNK activation. MSR1 is a key macrophage receptor for the clearance of circulating lipoproteins and has been implicated in atherogenesis. In irradiated low-density lipoprotein receptor-deficient mice, transplantation of Msr1+/CD36+/ monocytes reduced dietary-induced inflammation. However, the molecular mechanisms underlying hepatic macrophage activation and/or the formation of foamy macrophages in NAFLD remain poorly understood. We therefore hypothesised that MSR1 might be involved in inflammatory responses in the context of lipid overload during obesity-induced NAFLD.

Materials and methods

Patient selection

Cases were derived from the European NAFLD Registry (NCT04442334), approved by the relevant Ethical Committees in the participating centres, and all patients having provided informed consent. For the histopathological and nanoString® study, 194 formalin-fixed paraffin-embedded or frozen liver biopsies samples were obtained from patients diagnosed with histological proven NAFLD at the Freeman Hospital, Newcastle Hospitals NHS Foundation Trust, Newcastle-upon-Tyne, UK and at the Pitié-Salpêtrière Hospital, Paris, France (Table S1). For the genome-wide association study, 1,483 patients with histological proven NAFLD were included as previously described. All liver tissue samples for the histopathological and nanoString® study were centrally scored according to the semi-quantitative NASH-CRN scoring system by an expert liver pathologist (DT). Fibrosis was staged from F0 through to F4 (cirrhosis). Alternate diagnoses and aetiologies, such as excessive alcohol intake, viral hepatitis, autoimmune liver diseases and steatogenic medication use, were excluded. Viable normal human liver tissue (for the ex vivo slices) was obtained after resection from 2 adult patients treated at the University Hospitals Leuven, Leuven, Belgium. Samples were assessed by an expert liver pathologist (TR).

Animals

Male Msr1+/+ or Msr1+/− (wild-type [WT]) C57BL/6 mice were either kindly provided by Prof. Siamon Gordon, University of Oxford or obtained from Jackson Laboratories and bred in a conventional animal facility under standard conditions. Animals received human care and experimental protocols were approved by the institutional animal ethics committees at Newcastle University (PC123A338) and University of Gothenburg (294720). Mice had free access to water and were fed either standard chow (n = 10, 5 WT and 5 Msr1+/+) or 45%–high-fat and high-cholesterol diets (HFD; 820263, Special Diet Services; n = 10, 5 WT and 5 Msr1+/+) ad libitum. For the therapeutic intervention, WT mice were put on a 12-week HFD and intravenously injected with
Fig. 1. MSR1 expression in human NAFLD correlates with steatosis and steatohepatitis. (A) mRNA levels of MSR1 in a cohort of 170 histological proven NAFLD samples covering the different stages of the disease using nanoString (Mann-Whitney U test and Kruskal-Wallis with correction for multiple testing). (B) MSR1 transcript in patients stratified based on NAS ≥4 and presence of NASH (Mann-Whitney U test). (C) Receiver-operating characteristic curve showing the binary logistic model based on MSR1 transcript, MSR1 model, compared to other variables CD68 transcript, ALT and AST. (D) Immunohistochemical analysis of MSR1 in human NAFLD biopsies (n = 14), arrows indicate lipogranuloma and lipid-laden macrophages. Histopathological quantification of MSR1- and CD68-immunopositive cells in the parenchyma and portal tract (NAFL n = 4; NASH F0-2 n = 6; NASH F3-4 n = 4; one-way ANOVA or Kruskal-Wallis with correction for multiple testing). (E) Differentiation of human monocytes obtained from 5 healthy volunteers towards mature macrophages. MSR1 protein expression was assessed using FACS (n = 3, unpaired Student’s t test) and western blotting (n = 2). (F) Representative image of PLIN2+CD68+ parenchymal macrophages. Quantification was done in a cohort of 10 NAFLD samples (unpaired Student’s t test). Data are presented as mean ± SEM (*p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001, n.s., non-significant). Scale bars 100 µm. ALT, alanine aminotransferase; AST, aspartate aminotransferase; MSR1, macrophage scavenger receptor 1; NAFL, non-alcoholic fatty liver; NAFLD, non-alcoholic fatty liver; NAS, NAFLD activity score; NASH, non-alcoholic steatohepatitis.
Msr1 deficiency protects against HFD-associated metabolic dysregulation and liver damage. (A) Body weight of Msr1+/+ (WT) and Msr1−/− male aged-matched mice fed HFD for 16 weeks (n = 5 mice/experimental group). (B) eWAT and liver mass of WT and Msr1−/− male mice fed with HFD. (C) Glucose tolerance test on overnight fasted mice during the 15th week of HFD feeding. (D) Histological characterisation of livers specimens from WT and Msr1−/− mice fed a HFD for 16 weeks. (E) Representative images of morphology of the eWAT and liver from HFD-fed WT and Msr1−/− (n = 5 mice/experimental group). (G) RNA sequencing data comparing Msr1+/+ (n = 5) with baseline WT (n = 4) HFD-fed mice. Gene Ontology enrichment analysis was performed for biological processes and selected differently expressed genes were visualised with corrected p values. (H) Seahorse analysis of OCRs of liver tissue from HFD-fed WT and Msr1−/− mice (n = 4/group). Data are presented as mean ± SEM (unpaired Student’s t test or Mann-Whitney U test, or one-way ANOVA with correction for multiple testing; p values are shown for the comparisons WT and Msr1−/−; *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001, n.s., non-significant). AUC, area under the curve; eWAT, epididymal white adipose tissue; HFD, high-fat, high-cholesterol diet; Msr1, macrophage scavenger receptor 1; OCRs, oxygen consumption rates; WT, wild-type.
monoclonal rat anti-mouse Msr1 antibody (n = 8 animals, MAB1797-SP, R&D systems) or IgG control (n = 9 animals, MAB0061, R&D systems) at week 10 and 11 (0.25 mg antibody/animal).

Statistical analysis
Kolmogorov-Smirnov or the Shapiro-Wilk normality test, unpaired Student’s t test or Mann-Whitney U test, one-way ANOVA or Kruskal-Wallis test with, respectively, Tukey’s or Dunn’s post hoc multiple comparison test or Chi-Square test were performed using IBM SPSS statistics 26 or GraphPad Prism 8.4.3. A p value < 0.05 was considered significant. Binary logistic regression analysis was performed in SPSS using the backward stepwise likelihood ratio model. The model predicting high disease activity (NAFLD activity score [NAS] ≥4: NAS defined as the sum of steatosis, ballooning and lobular inflammation) was calculated as follows: MSR1_mRNA = 1.296883 + (0.003020*MSR1_mRNA).

For further details regarding the materials used, please refer to the CTAT table and supplementary information.

Results
MSR1 expression correlates with steatohepatitis activity in human NAFLD
To investigate the role of MSR1 in human NAFLD, we analysed gene expression in a cohort of 170 histologically characterised human adult liver biopsies. The cohort was stratified according to histopathological disease grade and stage, i.e. NAFL and NASH with fibrosis ranging from F0 to F4 (Table S1). Univariate analysis indicated that the MSR1 transcript was significantly associated with high steatosis, hepatocyte ballooning, presence of NASH and a NAS ≥4 (Fig. 1A,B and Table S2). Interestingly, CD68 mRNA, a marker for monocytes/macrophages, was only significantly associated with NAS ≥4 but not with any other clinicopathological features (Table S2). To further explore whether the MSR1 transcript was independently associated with high disease activity, we performed binary logistic regression analysis including the clinical variables sex, BMI, age, T2DM, alanine aminotransferase (ALT) and aspartate aminotransferase (AST), together with MSR1 and CD68 mRNA levels. Backward stepwise likelihood ratio modelling showed that MSR1 transcript levels predicted NAS ≥4 independently of CD68 mRNA or other clinical variables with an AUC of 0.735 (Fig. 1C).

Histopathological analysis showed that MSR1 was predominantly expressed in resident liver macrophages, i.e. Kupffer cells, rather than infiltrating monocyte-derived macrophages located in the portal tract, as visualised by the MSR1 and CD68 immunostaining (Fig. 1D and Fig. S1A,B). This was confirmed by immunofluorescent double staining (Fig. S1C). While the number of infiltrating portal CD68-immunopositive cells increased with disease progression, no significant differences were found for infiltrating MSR1-positive cells (Fig. 1D). These results were supported by publicly available single-cell RNA sequencing data indicating that MSR1 expression was mainly restricted to the Kupffer cell population whereas CD68 was also expressed in monocyte populations (Fig. S2A,B).15 Moreover, when differentiating monocytes from healthy individuals towards mature macrophages, we observed an increase in MSR1 protein expression (Fig. 1E). Notably, MSR1 immunopositivity was also seen in lipogranulomas and lipid-laden macrophages throughout the spectrum of NAFLD (Fig. 1D and Fig. S1A). Using the marker perlipin 2 (PLIN2) to visualise intracellular lipid droplets, immunofluorescence analysis showed that lipid droplets accumulate in Kupffer cells (Fig. 1F). Furthermore, a significant increase in parenchymal CD68+ PLIN2+ cells was observed in patients with NAFLD stratified based on NAS ≥4 or steatosis grade ≥2 (Fig. 1F).

Taken together, these human data demonstrate a positive correlation of MSR1 transcript and protein levels with NAFLD disease activity and the occurrence of hepatic-resident lipid-laden macrophages in the presence of excessive lipids.

Msr1 deficiency protects against diet-induced metabolic dysregulation and liver damage in mice
To further investigate how Msr1 functionally contributes to the development of obesity-related NAFLD, we subjected Msr1−/− mice (n = 5) and their corresponding Msr1+/+ (n = 5 WT) age-matched male counterparts to a HFD for 16 weeks. Upon HFD feeding, Msr1-deficient mice displayed increased total body weight, an increase in liver and epididymal white adipose tissue weight and increased food intake compared to WT (Fig. 2A,B, Fig S3A,B). Furthermore, HFD-fed Msr1−/− mice exhibited improved glucose uptake from blood, higher serum leptin, lower concentrations of circulating FFAs and enhanced fatty acid accumulation in adipocytes (Fig. 2C, Fig. S3C,D). Consistently, the adipocytes in HFD-fed Msr1−/− mice were larger than in WT mice, suggesting an increased adiposity and fat storage in the absence of Msr1 (Fig. 2D–F). Although no murine models accurately recapitulate all histological features of human steatohepatitis, histological and transcriptomic features of liver fibrosis were clearly attenuated by Msr1 deficiency upon HFD feeding (Fig. 2D–F). Sixteen weeks of regular diet did not result in any histological differences between the livers of WT and Msr1−/− mice (Fig. S3E), while WT mice on HFD displayed a significant higher hepatic fibrosis stage, sinusoidal fibrosis and increased collagen deposition (Fig. 2D–F, Fig. S3F) compared to the Msr1+/+ mice. Next, we characterised the livers of HFD-fed WT and HFD-fed Msr1−/− mice by high-throughput RNA sequencing analysis. The analysis revealed 728 differentially expressed genes (Table S3). Gene Ontology analysis of differentially expressed genes highlighted an enrichment for genes correlating to biological processes including “innate immune response”, “phagocytosis” and “lipid metabolic process” (Fig. 2G, Fig. S3G,H). HFD-fed Msr1−/− mice displayed reduced hepatic transcript expression of inflammatory cytokines (including Axl, Ccl5, Il1b, Spp1), pro-inflammatory immune cell markers (Ccr5, Cd14, Cd44, S100a8, S100a9), markers for hepatic stellate cell activation (Sos9, Pdgfb) and members of the Tnfα signalling pathway (Ripk3, Tnfαp2, Tnfαp82) when compared with WT mice (Fig. 2G). Furthermore, Msr1−/− mice on HFD showed a shift in gene expression associated with lipid metabolism, with genes including Acox1, Acox2, Apoe, Ces1d, Hsd17b11, Pla2g6 and Ppara increasing, and genes such as Fabp5, Lpcat2, Lpl, Pla2g7 and Pnpla3 decreasing (Fig. 2G). Functionally, the measured mitochondrial oxygen consumption rate in viable liver samples of HFD-fed Msr1−/− mice was approximately 50% higher compared to that in WT mice, indicating enhanced metabolic function (Fig. 2H). Taken together, these results demonstrate that Msr1 deficiency increases body weight but protects against features of the metabolic syndrome, including liver inflammation and fibrosis, while modulating hepatic lipid metabolism.
Mr1 deficiency prevents formation of pro-inflammatory foamy macrophages in vivo

Next, we asked whether the lipid-laden environment is a proximal stimulus leading to Mr1-mediated inflammation in the liver and adipose tissue, which may explain the observed metabolic dysfunction. In agreement with our human data, histopathological analysis of the liver and adipose tissue from HFD-fed Mr1⁻/⁻ mice showed no hepatic lipogranuloma and very few foamy macrophages compared to their WT counterparts, demonstrated by F4/80 immunostaining (Fig. 3A). Moreover, Mr1⁻/⁻ mice displayed lower Il6 and Tnfa gene expression in the liver and epididymal white adipose tissue (Fig. 3B-D). Furthermore, Mr1 deficiency impaired pro-inflammatory activation of isolated adipose tissue macrophages and hepatic-associated macrophages as shown by lower gene transcripts of Tnfa and Il6 (Fig. 3E-G). Altogether, these results show that Mr1 mediates HFD-induced hepatic and adipose tissue inflammation and facilitates macrophage activation towards a pro-inflammatory phenotype.

Triggering of Mr1 by lipids induces JNK-mediated pro-inflammatory activation of macrophages

We next investigated the underlying mechanism of Mr1-mediated lipid-induced inflammation. We reasoned that Mr1 is directly responsible for lipid uptake in macrophages, leading to an inflammatory response independent from other cell types. In this regard, we measured the uptake of SFA (palmitic acid) and non-SFA (oleic acid) in Mr1⁻/⁻ and WT BMDMs by quantifying Oil-red-O staining using confocal microscopy (Fig. 4A-C, Fig. S4A). The analysis revealed that Mr1 facilitates the uptake of both SFA and non-SFA but only SFA induced enhanced levels of Tnfa and Il6 transcripts in BMDMs (Fig. 4D). Furthermore, blocking the Mr1 receptor with a monoclonal antibody reduced the expression of Tnfa and Il6, and reduced the phosphorylation of JNK in response to SFA treatment (Fig. 4E,F). In line with these data, pharmacological inhibition of JNK phosphorylation abrogated the induction of Tnfa and Il6 pro-inflammatory gene expression upon SFA treatment (Fig. 5B). Similarly, using primary Mr1⁻/⁻ hepatic macrophages or WT ones treated with...
monoclonal antibody resulted in reduced lipid uptake, reduced expression of Tnfa and reduced JNK phosphorylation (Fig. 4G-J).

To extend these findings, we co-cultured Hepa1-6 cells with BMDMs or primary hepatocytes with hepatic macrophages, which resulted in a comparable response (Fig. S5A-E). The data indicate that SFA-induced triggering of Msr1 regulates JNK-mediated pro-inflammatory activation of macrophages in the absence of lipopolysaccharide.

Therapeutic inhibition of MSR1 reduces the release of TNFA

To investigate the therapeutic potential of targeting MSR1 in the treatment of NAFLD, we applied an antibody-based intervention using NAFLD mouse models and ex vivo human liver slices. WT mice were fed a HFD for 12 weeks and were administered 2 doses of monoclonal rat anti-mouse Msr1 antibody (n = 8 animals) or isotype-matched IgG control (n = 9 animals) at week 10 and 11 by intravenous injection. Antibody treatment did not result in any weight difference or changes in glucose or insulin levels at week 12 (Fig. S6). Notably, histological assessment did show reduced hepatic fibrosis and sinusoidal/peri-cellular fibrosis in anti-Msr1-treated mice compared to the IgG control mice, while steatosis grade, hepatocyte ballooning and lobular inflammation remained unchanged (Fig. 5A,B). In addition, F4/80 immunostaining showed a reduction in occurrence of hepatic foamy macrophages and lipogranulomas upon treatment, which translated into reduced surface area positivity of F4/80-positive cells (Fig. 5B,C). Furthermore, treated animals showed reduced expression of Tnfa transcript in liver samples and isolated hepatic macrophages (Fig. 5D-E).

To further investigate whether inhibition of MSR1 prevents the formation of foamy macrophages and release of TNFA in humans, we collected human liver slices with normal morphology from 2 different patients (2 biological replicates per condition for each patient sample). The samples were incubated with a polyclonal anti-human MSR1 antibody prior to culturing them with a mixture of oleic acid (2 mM) and palmitic acid (1 mM) combined with anti-MSR1 antibody for 16 h (Fig. 5F). Treatment with the antibody reduced the surface area positivity.
of Kupffer cells as shown by CD68 immunostaining (Fig. 5G,H). Moreover, lipid-induced release of TNF-α into the culture medium was reduced upon anti-MSR1 antibody treatment (Fig. 5I). Overall, our in vivo and ex vivo results show that therapeutic inhibition of MSR1 prevents the formation of foamy macrophages and the release of TNF-α.

Relevance of polymorphisms in MSR1 region to NAFLD and metabolic traits

Next, we asked whether genetic variants in MSR1 are associated with susceptibility to NAFLD and if there is an association with transcriptional regulatory mechanisms controlling MSR1 expression. Using previously published genomics data encompassing a cohort of 1,483 European Caucasian patients with histologically proven NAFLD and 17,781 European general-population controls, we identified 4 single nucleotide polymorphisms (SNPs) in or around the MSR1 locus with p values <5*10^{-6}, with rs41505344 as the most significant (p = 1.64*10^{-4}) (Fig. 6A and Table S4). Quantitative trait analysis for rs41505344 in 430,101 patients enrolled in the UK Biobank showed a significant correlation with serum triglycerides and AST levels, even after adjustment for age, sex, BMI, centre, batch and the first 10 principal components (Table 1).

Our human data indicated that MSR1 is expressed in the liver on mature endogenous macrophages rather than on infiltrating monocyte-derived macrophages. To unravel transcriptional regulatory mechanisms of MSR1, we used publicly available RNA sequencing data comparing human monocytes with differentiated macrophages, which identified 1,208 differentially expressed genes, with MSR1 mRNA expression increased in the recovered monocytes, that are predicted to regulate the expression of MSR1. By motif enrichment analysis using iRegulon, we identified 8 differentially expressed transcription factors, upregulated in human macrophages compared to monocytes, that are predicted to regulate the expression of MSR1: BHDE4I, ETV5, HMGN3, MAF, MITF, NR1H3, THRA and ZNF562 (Fig. 6B, Table S5). To verify whether these transcription factors bind any regulatory regions near the MSR1 gene, and in particular the rs41505344 SNP locus, we investigated chromatin-immunoprecipitation sequencing data for these proteins. MITF, MAF, THRA and NR1H3 proved to bind in the vicinity of the rs41505344 locus, suggesting an indirect role for the SNP in the transcriptional regulation of MSR1 (Fig. 6C). When assessing the rs41505344 genotype in our nanoString cohort, a significant correlation with serum triglycerides and AST levels, even after adjustment for age, sex, BMI, centre, batch and the first 10 principal components (Table 1).
increase in MSR1 transcript levels was observed in patients carrying the SNP (Fig. S7).

Taken together, these results suggest that the frequency of variants potentially affecting MSR1 expression during monocyte-macrophage differentiation, which could influence features of obesity-related diseases, is increased in patients with NAFLD.

**Discussion**

In this study, we provide evidence that MSR1 is important for the uptake of lipids in macrophages, leading to an inflammatory response and metabolic changes throughout the body. In a setting of lipid overload, MSR1 deficiency not only led to reduced hepatic inflammation and changes in hepatic lipid metabolism but it also reduced circulating fatty acids, increased lipid storage in the adipose tissue and improved glucose tolerance, highlighting the importance of the liver-adipose tissue axis in NAFLD and the metabolic syndrome.17 Our data demonstrated that MSR1 was expressed in tissue-resident macrophages, i.e. Kupffer cells, rather than in infiltrating monocytes, and that its expression increases as human monocytes differentiate towards mature macrophages.16,18 The association between MSR1 mRNA and disease activity in our study would suggest that there is an ongoing differentiation from infiltrating monocytes towards macrophages during NASH. Although portal inflammation is associated with advanced NAFLD, lobular inflammation has been reported to predict fibrosis progression in human NAFLD, suggesting that disease progression is driven by tissue-resident macrophages rather than infiltrating monocytes.19 Our results support this as Msr1 deficiency in HFD-fed mice tempered the lipid-induced inflammatory response in the liver, by reducing the expression of Axl, Il1b, S100a8/a9 and Spp1 but also Cd44. Cd44 expression has been associated with NASH in human and mouse, and is crucial for homing of monocytes into the damaged liver, suggesting that lipid accumulation in tissue-resident macrophages via MSR1 is a trigger to recruit immune cells.20 This is in line with a previous study reporting that Kupffer cell depletion by clodronate liposomes reduces infiltration of inflammatory cells, mainly monocytes, into the livers of mice on a 22-week
Table 1. Correlation of rs41505344 SNP with clinical features in participants from the UK Biobank (N = 430,101).

<table>
<thead>
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<th>Characteristic</th>
<th>Total</th>
<th>Control</th>
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<th>p value Beta CI</th>
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<td>Age, years, mean ± SD</td>
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<td>23.5±14.1 (3.04–495)</td>
<td>23.6±14.1 (3.1–495)</td>
<td>23.5±14.3 (3.04–472)</td>
<td>23.3±13.1 (3.82–286)</td>
</tr>
<tr>
<td>AST, U/L</td>
<td>26.2±10.6 (3.3–947)</td>
<td>26.2±10.5 (3.3–947)</td>
<td>26.2±11 (3.3–227)</td>
<td>8.29E−10</td>
</tr>
<tr>
<td>Glucose, mM</td>
<td>5.12±1.21 (1–36.8)</td>
<td>5.12±1.2 (1.1–36.8)</td>
<td>5.12±1.23 (1–32.7)</td>
<td>5.12±1.18 (1.8–22.3)</td>
</tr>
<tr>
<td>Cholesterol, mM</td>
<td>5.71±1.14 (0.601–15.5)</td>
<td>5.71±1.14 (1.71–13.3)</td>
<td>5.68±1.13 (2.39–12.3)</td>
<td>3.55±1.58 (0.628–11)</td>
</tr>
<tr>
<td>LDL, mM</td>
<td>3.57±0.87 (0.266–9.8)</td>
<td>3.57±0.87 (0.266–9.8)</td>
<td>3.57±0.868 (0.751–9.61)</td>
<td>3.55±0.862 (1.22–7.64)</td>
</tr>
<tr>
<td>HDL, mM</td>
<td>1.45±0.382 (0.219–4.4)</td>
<td>1.45±0.382 (0.226–4.4)</td>
<td>1.46±0.382 (0.219–4.13)</td>
<td>1.45±0.38 (0.628–11)</td>
</tr>
</tbody>
</table>

Moreover, we found some evidence that the genetic variant promotes adipose tissue therapy to treat NASH by reducing hepatic inflammation. We demonstrated the feasibility of using targeted monoclonal antibodies to treat NASH by reducing hepatic inflammation. Furthermore, our results showed that the absence of Msr1 induced a change in hepatic expression of genes associated with lipid metabolism, including an increase in Ppara, with concordantly increased mitochondrial oxygen consumption and ameliorated glucose tolerance in HFD-fed mice. Peroxisome proliferator-activated receptors (PPAR) are nuclear receptors that play key roles in metabolic homeostasis and inflammation. Selective Kupffer cell depletion has been reported to activate Ppara signalling in hepatocytes while resulting in overall reduced levels of hepatic triglycerides in mice fed a 45% HFD. Furthermore, hepatocyte-restricted Ppara deletion in mice impaired liver lipid metabolism, leading to increased plasma FFAs. In human adult patients with non-cirrhotic NASH, the pan-PPAR agonist lanifibranor induced NASH resolution after 24 weeks of treatment in a phase IIb randomised, placebo-controlled, double-blind study. Taken together, the effects of Msr1 deficiency on liver metabolism, triglycerides and circulating FFAs observed in this study could in part be explained by altered Ppara signalling in the liver.

This study showed that Msr1 can facilitate the uptake of SFAs, such as palmitic acid, as well as non-SFAs, such as oleic acid, independently of other receptors. Yet, only SFAs could induce the release of TNFs through phosphorylation of JNK in macrophages, which is in line with previous reports. In our Msr1−/− HFD-fed mice, we observed lower hepatic Tnfα expression as well as lower serum Tnfα. Furthermore, therapeutic blocking of Msr1 in vivo or ex vivo reduced foamy macrophage formation and the release of TNFs. Tnfα has a pleiotropic effect as it can sensitize hepatocytes to apoptosis and it can stimulate hepatic lipid synthesis while reducing Ppara expression. Furthermore, Tnfα affects glucose homeostasis in adipocytes and promotes lipolysis in cultured adipocytes, which could explain the obese phenotype in our Msr1−/− HFD-fed mice.

Although current efforts to develop drug therapies for NAFLD primarily focus on ameliorating the specific histological features of the disease (i.e. steatohepatitis or fibrosis), it is important to remember that NAFLD is part of a multi-system metabolic disease state and so agents that offer more broad metabolic or cardiovascular benefits would be highly attractive. Our data indicate that by targeting Msr1, one would not only reduce lipid-induced inflammation in the liver but also improve dyslipidaemia and increase lipid storage in adipocytes. In addition, we demonstrated the feasibility of using targeted monoclonal antibody therapy to treat NASH by reducing hepatic inflammation. Moreover, we found some evidence that the genetic variant rs41505344 in Msr1 was associated with serum triglycerides and ALT in a large cohort of over 400,000 patients. Though the SNP in Msr1 was not strongly associated with susceptibility to NAFLD, we found that several transcription factors regulating the expression of Msr1 bound in the locus and that the SNP was associated with changes in Msr1 transcript levels, indicating a role for rs41505344 during macrophage differentiation.

This study has several limitations. We used a global knock-out mouse model and focused on the early phases of NAFLD by using a relatively short-term diet of 16 weeks. To further investigate the liver-adipose tissue axis, a Kupffer cell-specific Msr1 knock-out or a conditional Msr1 knock-out mouse model challenged with a long-term diet would provide more information on advanced NAFLD. Furthermore, we mainly explored the role of SFAs in macrophages, but this does not exclude that exosomes or oxidised LDL can have an additive effect on the inflammatory response.
response, nor have we explored the synergetic function of other scavenger receptors such as CD36 or TREM2.

This study showed that the scavenger receptor MSR1, as part of the innate immune system, is a critical sensor for lipid homeostasis, highlighting the importance of the liver-adipose tissue axis. With the prevalence of obesity increasing globally, it is crucial that we understand how our immune system reacts when challenged with over-nutrition. Understanding and therapeutically influencing macrophage immunometabolism could help us treat features of the metabolic syndrome, such as dyslipidaemia, NAFLD and T2DM.

Abbreviations
ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMDMs, bone marrow-derived macrophages; FFAs, free fatty acids; HFD, high-fat, high-cholesterol diet; MSR1, macrophage scavenger receptor 1; NAFL, non-alcoholic fatty liver; NAFLD, non-alcoholic fatty liver; NAS, NAFLD activity score; NASH, non-alcoholic steatohepatitis; PPAR, peroxisome proliferator-activated receptors; SFA, saturated fatty acid; SNPs, single nucleotide polymorphisms; T2DM, type 2 diabetes mellitus; WT, wild-type.

Financial support
This study has been supported by the EPos (Elucidating Pathways of Steatohepatitis) consortium funded by the Horizon 2020 Framework Program of the European Union under Grant Agreement 634413, the LITMUS (Liver Investigation: Testing Marker Utility in Steatohepatitis) consortium funded by the Innovative Medicines Initiative (IM2) Program of the European Union under Grant Agreement 777377, which receives funding from the EU Horizon 2020 programme and EFPIA, and the Newcastle NIHR Biomedical Research Centre (to QMA), the Newcastle University start-up funding and the Wellcome Trust Investigator Award (215542/Z/19/Z) (to MT), Knut och Alice Wallenberg Foundation Wallenberg Centre for molecular and translational medicine, University of Gothenburg, Sweden and Åke Wirbergs Research funding #M18-0121 (to AH), Cancer-Society Stichting tegen Kanker (to JW), European NAFLD Registry: a real-world longitudinal cohort study of nonalcoholic fatty liver disease. Nat Rev Gastroenterol Hepatol 2019;16:145–159. The authors would like to thank the Newcastle Bioimaging Unit, the Newcastle Molecular Pathology Core Facility and the Newcastle Molecular Pathology Node Proximity Laboratory for their technical support.

Conflict of interest
The authors have no potential conflicts (financial, professional or personal) directly relevant to the manuscript.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions
OG and AH conceived the study. Study design, manuscript drafting and funding: AH, OG, MT and QMA. Manuscript preparation: AH, OG, SKP, MT, QMA. In vivo experiments: AH, SKP, OBG and NML. In vitro experiments: AH, OG and SKP. Human ex vivo experiments: OG, MVH, TR. Histopathology: OG, MVH, TR and DT. Nanostring analysis: OG. Bioinformatics: OG and JW. GWAS analysis: RD, HJC, AKD. eQTL UKBiobank data: RMM, OJ, SR. All authors contributed to data collection and interpretation, and critically revised the manuscript for intellectual content.

Data availability statement
To review GEO accession GSE163471:

Acknowledgments
The authors would like to thank the Newcastle Bioimaging Unit, the Newcastle University Genomics Core Facility, the Newcastle NanoString Core Facility and the Newcastle Molecular Pathology Node Proximity Laboratory for their technical support.

Supplementary data
Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhep.2021.12.012.

References
Author names in bold designate shared co-first authorship


Journal of Hepatology 2022 vol. 76 1001–1012