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Original Research

A Cell Specific Effect of MBOAT7 MAFLD-risk Variant on Immune Cells

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Abstract

Background: Disease risk variants are likely to affect gene expression in a context- and cell-type specific manner. The membrane bound O-acyltransferase domain containing 7 (MBOAT7) rs8736 metabolic-dysfunction-associated fatty liver disease (MAFLD)-risk variant was recently reported to be a negative regulator of toll-like receptors (TLRs) signalling in macrophages. Whether this effect is generic or cell-type specific in immune cells is unknown. **Methods**: We investigated the impact of modulating TLR signaling on MBOAT7 expression in peripheral blood mononuclear cells (PBMCs). We also examined whether the rs8736 polymorphism in MBOAT7 regulates this effect. Furthermore, we measured the allele-specific expression of MBOAT7 in various immune cell populations under both unstimulated and stimulated conditions. **Results**: We show that MBOAT7 is down-regulated by TLRs in PBMCs. This effect is modulated by the *MBOAT7* rs8736 polymorphism. Additionally, we provide evidence that MBOAT7 acts primarily as a modulator of TLR signalling in mononuclear phagocytes. **Conclusion**: Our results highlight the importance of studying Genome-Wide Association Studies (GWAS) signals in the specific cell types in which alterations of gene expression are found.

Keywords: immune cells; MBOAT7; TLRs; MAFLD

1. Introduction

Metabolic dysfunction–associated fatty liver disease (MAFLD) is the leading cause of chronic liver disease and its complications. MAFLD is characterized by substantial phenotypic heterogeneity mediated by inter-individual genetic variations interacting with a variety of environmental cues. This results in the well described disparate clinical presentations and outcomes [1–4].

Although numerous genetic variants have been linked to the susceptibility to human diseases such as MAFLD, the overwhelming majority of these variants occur in non-coding portions of the genome. This suggests that they function locally to modify gene expression. Given that cis-regulatory areas often exhibit strong cell type specificity, it is probable that disease-associated mutations will impact gene expression in a manner that depends on the cellular context, specifically affecting a certain population of cell types inside the body [5,6]. The lack of association between identified risk single nucleotide polymorphisms (SNPs) in non-coding genes and the specific cell types in which they impact function has greatly impeded the advancement of functional investigations focused on determining the biological impacts of Genome-Wide Association Studies (GWAS) SNPs [7]. Hence, identifying the exact cell types in which disease risk variations alter gene expression may facilitate mechanistic and functional investigations into the genetic underpinnings of certain diseases [8,9].

A genetic variant in the membrane bound O-acyltransferase domain containing 7 (MBOAT7) gene has been identified through GWAS and candidate gene studies. This specific genetic variation has been found to have a significant impact on inflammation and fibrosis in various types of liver diseases, including alcoholic fatty liver disease, MAFLD, and viral hepatitis [10–12].

Our recent research has revealed that MBOAT7 acts as a negative regulator of Toll-Like Receptor (TLR) signaling in macrophages, and that this function is modulated by the MBOAT7 rs8736 (T) variant, which increases the risk of developing MAFLD [13].

MBOAT7 is expressed in immune cells [10], and given the involvement of various immune cell populations in MAFLD pathogenesis [14,15], it is important to determine whether the effects of the MBOAT7 rs8736 variant on gene regulation are constitutive or cell-specific. To this end, we aim to investigate the precise subtypes of immune cells that are affected by the genetic effects of MBOAT7 on gene expression regulation.

2. Methods

2.1 Immune Cell Subsets

Volunteer blood was used to isolate peripheral blood mononuclear cells (PBMCs) by employing Ficoll Paque Plus (GE Healthcare, Chicago, IL, USA). CD4, CD8, CD56, CD14, CD20 isolations were performed using spe-

cific Kits as per manufacturer procedures (STEMCELL Technologies, Vancouver, British Columbia, Canada).

2.2 Treating PBMCs with TLR Agonists

A human TLR ligand kit was obtained from Invivogen (tlrl-kit1hw, Invivogen, San Diego, CA, USA). Each individual ligand was diluted as per the manufacturer's instructions, and the cells were subsequently challenged by administering a standard concentration of 100 ng/mL of Pam3CSK4 (a TLR2 ligand), 100 ng/mL of Poly (I:C) (a TLR3 ligand), 500 ng/mL of Lipopolysaccharide (LPS) (a TLR4 ligand), and 100 ng/mL of CpG DNA (a TLR9 ligand), as previously described [13,16]. After 24 hours treatment, cells were harvested for RNA extraction and supernatants collected for further analysis.

2.3 mRNA Gene Expression

RNA Extraction and Real-time Quantitative Polymerase chain reaction (RT-qPCR)

RNA extraction and the real-time polymerase chain reaction (RT-PCR) analysis were done as described previously. RNA extraction was performed using the Qiagen miRNeasy Mini Kit (217004, QIAGEN, Hilden, Germany), following the instructions provided by the manufacturer.

The real-time PCR analysis were performed on the Applied Biosystems (Waltham, MA, USA). TaqMan Fam labelled expression probes and the TaqMan™ Fast Advanced Master Mix (4444965, Thermo Fisher Scientific, Waltham, MA, USA) were used. Alternatively, forward and reverse primers specific to the *MBOAT7* gene (For: CATGCGGTACTGGAACATGA Rev: CCAGTAGGCGCTCAGCAG) were used in combination with SYBR Select Master Mix from Applied Biosystems (4312704, Applied Biosystems, Waltham, MA, USA). Glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) expression using specific primers (For: AAGGTGAAGGTCGGAGTCAAG Rev: GGGGTCATTGATGGCAACAATA) was used a control. The expression level was quantified by measuring the CT values and normalizing them to the CT value of GAPDH.

2.4 Enzyme-linked Immunosorbent Assay (ELISA)

The supernatant was analyzed for TNF-α concentration using a sandwich enzyme-linked immunosorbent assay (ELISA) following the instructions provided by the manufacturer (ab46087, Abcam, Cambridge, MA, USA).

2.5 Genotyping

Genotyping for *MBOAT7* rs8736 was performed using TaqMan genotyping probe (catalogue number 4351376), according to protocol provided by the manufacturer (Applied Biosystems®), as described previously [17].

2.6 Allele Specific Expression

Allele specific expression was measured the ratio of genomic DNA (gDNA) and complementary DNA (cDNA) from 10 individuals who were heterozygous for the variant using RT-PCR.

2.7 MBOAT7 Gene Expression in Human Tissues, Primary Hepatic Cells and PBMCs

The expression of *MBOAT7* mRNA was evaluated in 19 human tissues using a TissueScan Human Normal cDNA Array (Origene, Rockville, MD, USA), and human primary (hepatocytes, hepatic stellate cells, and hepatic sinusoidal endothelial cells) (ScienceCell) by RT-PCR. Additionally, PBMCs were also included in the analysis, following the previously reported methodology [18].

3. Results

3.1 MBOAT7 Expressed Differently in Various Human Tissues and Cells

We first assessed the expression of *MBOAT7* across panels of human tissues and cells. Although we can identify *MBOAT7* ubiquitously, although it shows different expression levels across various human tissues (Fig. 1a). *MBOAT7* is most strongly expressed in circulating PBMCs at a cellular level, with approximately 7 times higher expression compared to different subsets of liver cells namely, hepatocytes, sinusoidal endothelial cells, or hepatic stellate cells (Fig. 1b) [19].

3.2 MBOAT7 is Down-regulated by TLRs in PBMCs

Our recent study shown that MBOAT7 functions as a suppressor of TLR signaling in macrophages [13]. Herein, we investigated the impact of TLR signaling modulation on *MBOAT7* expression in PBMCs. *MBOAT7* expression was significantly down-regulated upon stimulation of TLR4 with lipopolysaccharide (Fig. 2a), an effect that was abolished by pharmacological TLR4 inhibition using TAK-242, confirming the specificity of this effect (Fig. 2b).

We next examined whether the *MBOAT7* expression differences were specific to TLR4 or spanned other toll-like receptors (TLRs) ligands. Repression of *MBOAT7* in response to stimulation of TLRs TLR1/2 and TLR9 was observed, but not in response to TLR3 (Fig. 2c).

3.3 PBMCs from rs8736 T Risk Carriers Expressed Lower Levels of MBOAT7 and Augmented TLRs-induced Secretion of Cytokines

The rs8736 polymorphism in *MBOAT7* has been linked with the entire spectrum of MAFLD [12] and we recently found it to regulate *MBOAT7* effects on TLR signalling in macrophages [13]. Therefore, we investigated whether the expression of *MBOAT7* in PBMCs is controlled in a genotype-dependent way in response to TLR signaling. We thus conducted an experiment where we stimulated PBMCs obtained from healthy individuals, then genotyped

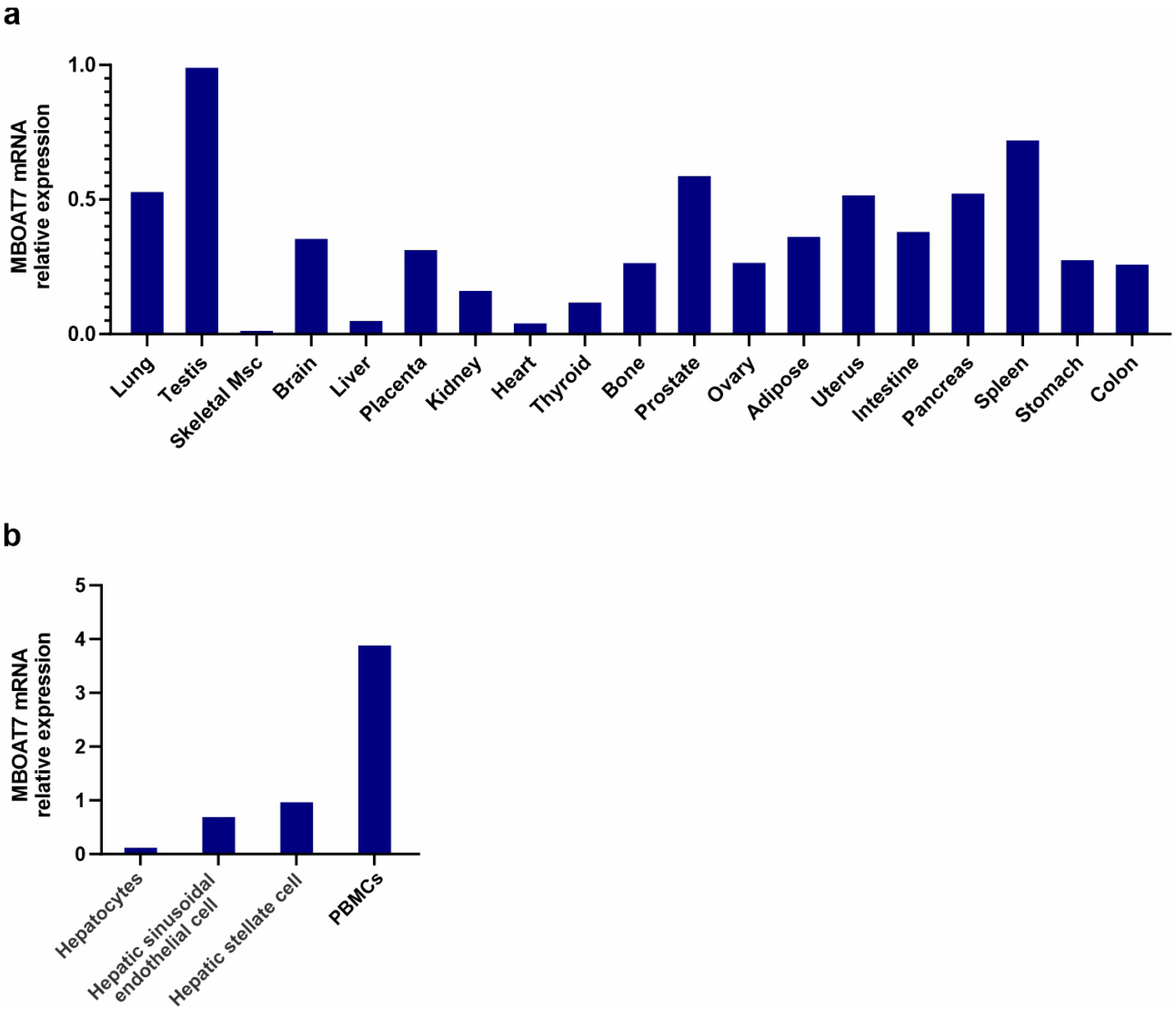

Fig. 1. *MBOAT7* expression in Various Human Tissues and Cells. (a) Expression of *MBOAT7* mRNA in 19 human tissues. (b) *MBOAT7* mRNA expression in human primary hepatic cell types and PBMCs. Gene expression was assessed by RT-PCR and normalized to *GAPDH*. *MBOAT7*, membrane bound O-acyltransferase domain containing 7; PBMCs, peripheral blood mononuclear cells; *GAPDH*, glyceraldehyde 3-phosphate dehydrogenase.

these cells for a specific genetic variant called rs8736. Next, we used an allele-specific test to analyze the expression levels in individuals who were heterozygous for the rs8736 variant, which is associated with MAFLD. This experiment was conducted under basal conditions and following stimulation of TLRs. At baseline, individuals with the rs8736 TT genotype exhibited lower levels of *MBOAT7* mRNA expression in their PBMCs compared to individuals with the CC genotype (Fig. 3a); this differential effect was further enhanced on TLRs stimulation (Fig. 3b). Thus, *MBOAT7* expression attenuates upon TLRs challenge, while PBMCs from rs8736 T risk carriers express less *MBOAT7* of the levels observed in CC carriers.

We investigated if the rs8736 polymorphism in *MBOAT7*, which is linked to MAFLD, affects the down-regulation of *MBOAT7* in PBMCs by TLRs in a genotype-dependent manner. Specifically, we examined whether the cytokine secretion triggered by TLRs in primary human PBMCs is influenced by the *MBOAT7* genotype. In line with the reduced *MBOAT7* expression in PBMCs of individuals with the rs8736 TT risk genotype, those with the rs8736 T risk genotype produced a higher amount of TNF-α when their TLRs were stimulated, compared to individuals with the CC genotype (Fig. 3c). These findings suggest that individuals with the MBOAT7 rs8736 minor allele homozygotes produce more pronounced inflammatory responses in these specific circumstances.

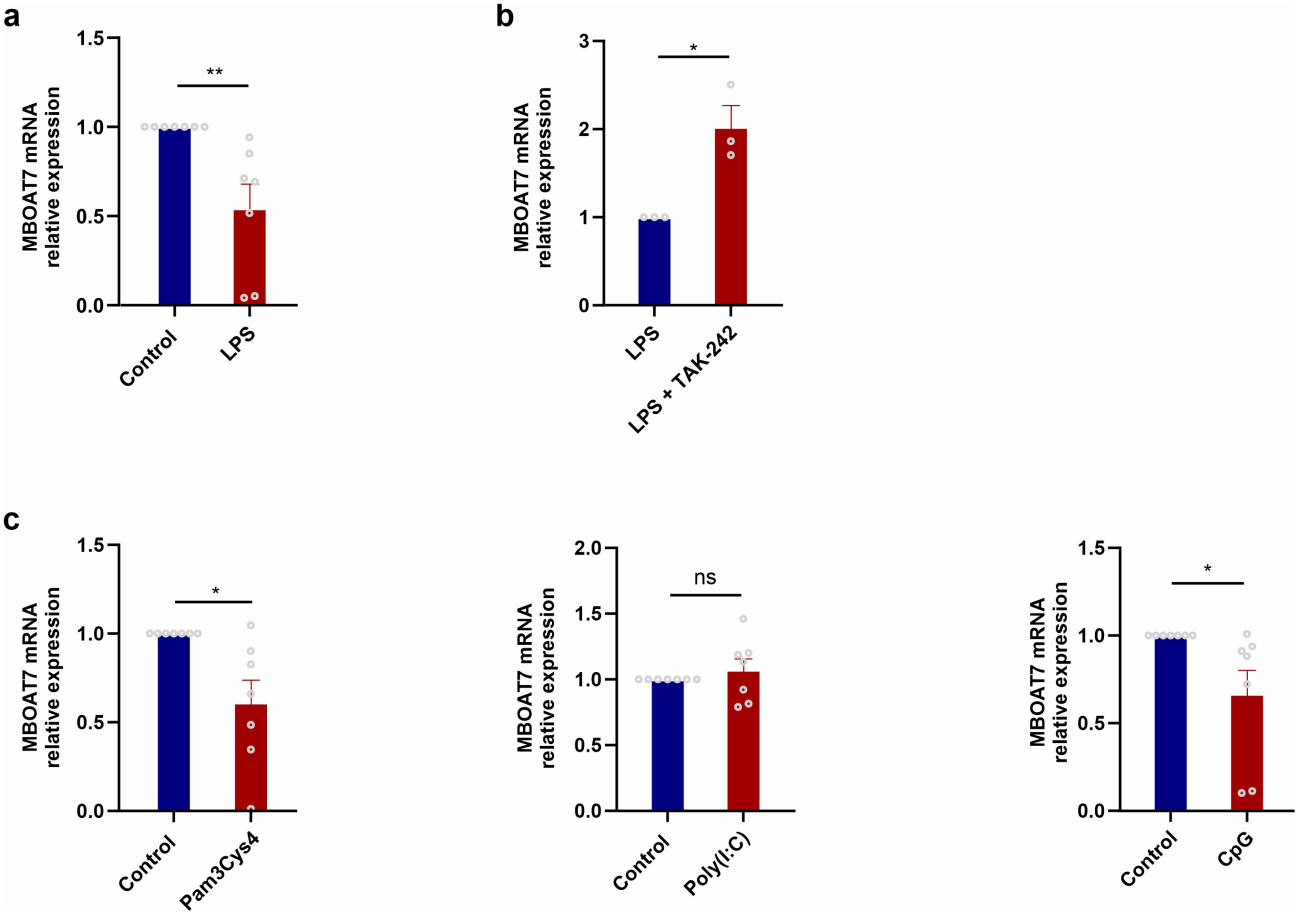

Fig. 2. PBMCs from rs8736 heterozygous carriers (n = 7). (a) were left untreated or challenged with LPS and/or (b) a specific TLR4 inhibitor (TAK-242), (c) challenged with (Pam3Cys4 [TLR1/2], Poly (I:C) [TLR3], and CpG [TLR9]) for 24 hours. *MBOAT7* mRNA expression analyzed by PCR and normalized to *GAPDH*. Values are means with Standard Error of the Mean (SEM) depicted as vertical bars. *ns*, not significant, $*p < 0.05$, $**p < 0.01$; determined by 2-tailed Student's *t* test. LPS, Lipopolysaccharide; TLR, toll-like receptors.

3.4 MBOAT7 rs8736 Variant Regulates the Gene Expression Level on Cell-specific Manner

In order to identify the specific cell type accountable for the variations in cytokine production, we undertook the isolation of monocytes, T-cells (CD4 and CD8), B-cells (CD20), and NK-cells (CD56), which were subsequently exposed to LPS. We quantified the allele-specific expression of *MBOAT7* under both unstimulated and stimulated conditions. Monocytes derived from individuals with the rs8736 TT genotype exhibited lower levels of *MBOAT7* mRNA compared to individuals with the CC genotype, both under normal conditions (Fig. 4a) and when stimulated with TLR4 (Fig. 4b). NK cells exhibited a contrasting trend; cells derived from individuals with the rs8736 TT genotype displayed elevated levels of *MBOAT7* mRNA compared to individuals with the CC genotype, both at baseline and upon TLR4 stimulation. There was no noticeable variation in allele-specific expression between T- and B cells before and after TLR4 stimulation, as shown in Fig. 4a,b.

4. Discussion

MAFLD has a substantial heritable component. Nevertheless, most known risk variants explain only a small fraction of heritability and SNPs linked to MAFLD are mainly located in noncoding regions of the genome that include a high concentration of enhancer elements. These enhancer elements are frequently unique to distinct cell types [20]. The current challenge lies in establishing the connection between disease-associated regions and the genes they impact, as well as determining the precise cell types where there is variation in gene expression and the direction of these effects. This then will allow us to identify the specific mechanisms and biological pathways involved in patients who are genetically vulnerable. SNPs in the *MBOAT7* gene have been associated with an increased risk of inflammation and fibrosis in viral and non-viral liver diseases [10–12]. *MBOAT7* is highly expressed in various types of immune cells and was recently found to regulate the signalling of TLR-induced pro-inflammatory genes in macrophages [13]. Nevertheless, the precise immune cell subtype that

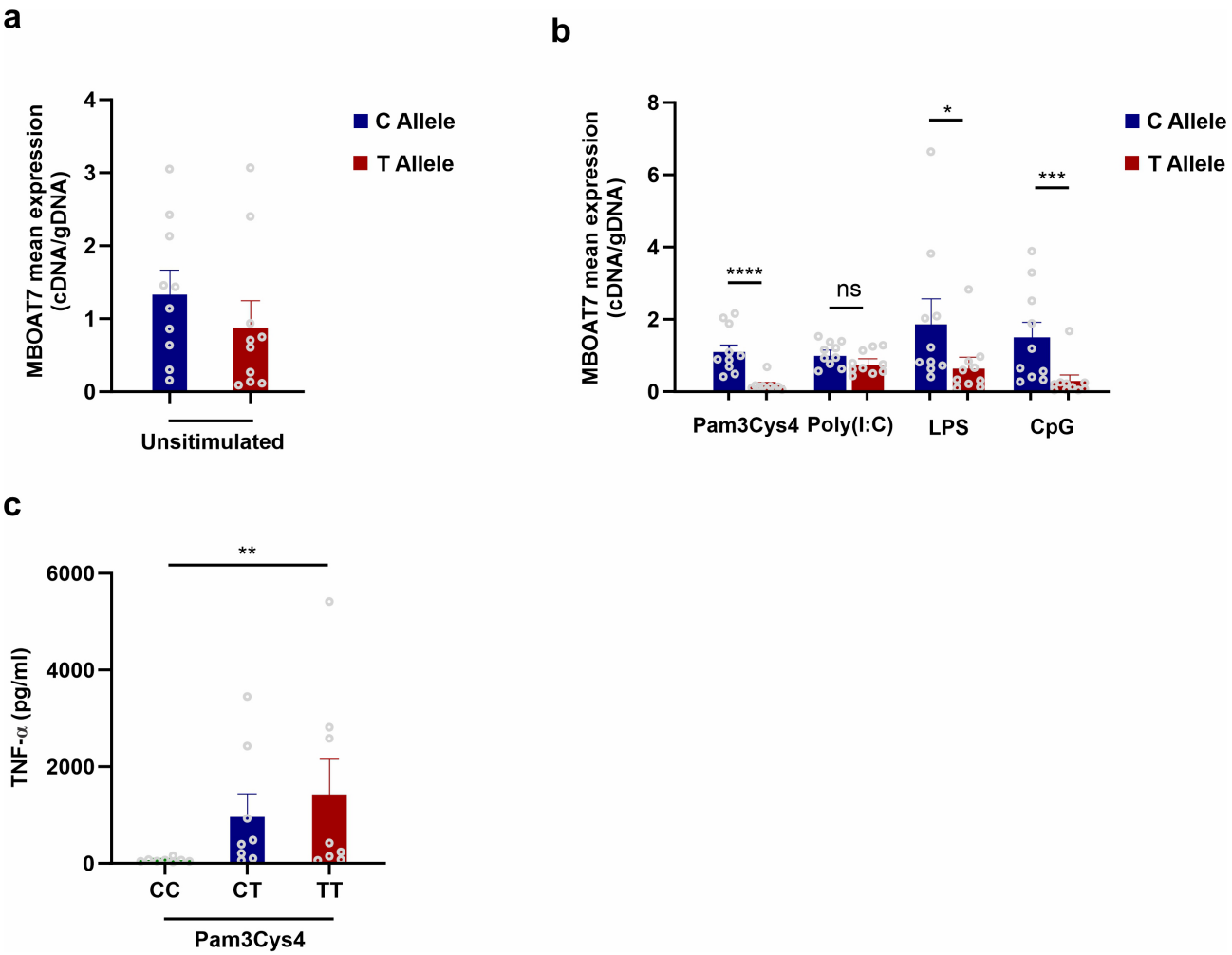

Fig. 3. Allele-specific expression was evaluated in PBMCs obtained from subjects who were heterozygous carriers of rs8736. The PBMCs were either (a) unstimulated or (b) stimulated for 24 hours with various doses of Pam3Cys4 (recognized by TLR1/2), Poly (I:C) (recognized by TLR3), LPS (recognized by TLR4), and CpG (recognized by TLR9). (c) The secretion of TNF-α was measured and compared based on the rs8736 genotype following stimulation with Pam3Cys4 [TLR1/2]. The sample size for each group ranged from 8 to 10 individuals. Statistical significance was determined using a 2-tailed Student's *t*-test or one-way ANOVA, and multiple comparisons were corrected using Bonferroni correction. Significance was denoted as *ns*, not significant, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.

is predominantly influenced by the MBOAT7 MAFLD-risk mutations remains undefined. Accurate data is crucial for studying the genetic origins of diseases like MAFLD, where many immunological and structural cells found in the liver are believed to play a role in its development. *MBOAT7* is expressed at higher levels in PBMCs and primary immune cell types compared to non-immune cells such hepatocytes, sinusoidal endothelial cells, or hepatic stellate cells, as previously documented [19].

Based on the data we've gathered, it appears that in healthy individuals with a disease-associated SNP, PBMCs stimulated by TLRs exhibit decreased expression of *MBOAT7* mRNA and an increased release of proinflammatory cytokines compared to individuals in lower-risk groups. Interestingly, the overproduction of these cytokines seems to be triggered by various TLRs, except for TLR3, which specifically signals through the TIR-domain-containing adaptor-inducing interferon-β (TRIF).

The rs8736 variant of *MBOAT7* has been shown to regulate gene expression in a cell-specific manner. In monocytes from healthy individuals with a disease-associated SNP, *MBOAT7* mRNA levels were lower compared to both baseline and TLR-stimulated monocytes. Conversely, no genotype-dependent effect on gene expression was observed in T and B cells. Interestingly, NK cells from the same individuals exhibited higher *MBOAT7* expression. Notably, individuals with the disease-associated SNP demonstrated an increased production of cytokines in PBMCs. NK cells play a bifunctional role in the development and progression of liver disease, which includes both

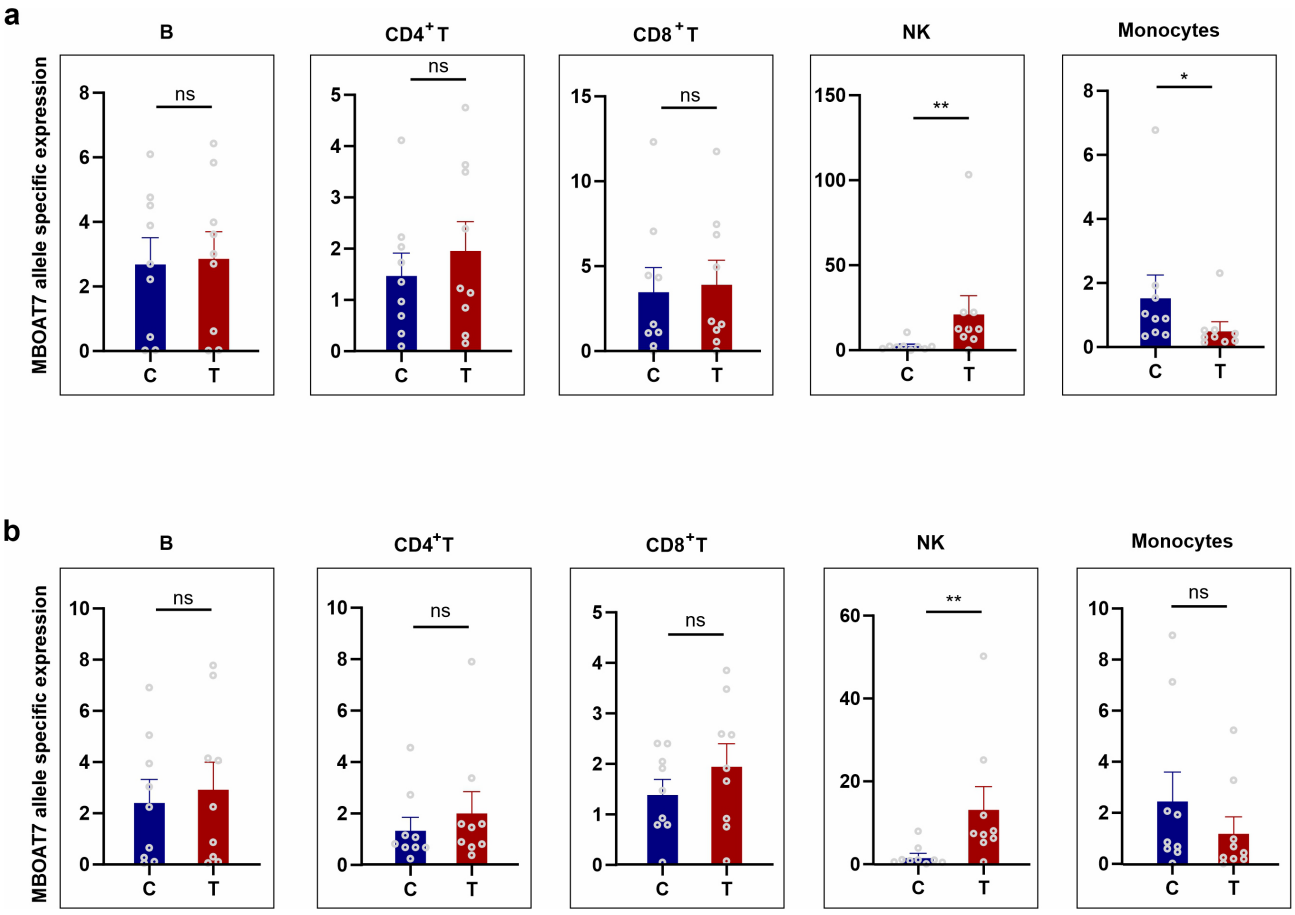

Fig. 4. Allele-specific expression was analyzed in B cells, CD4$^+$ cells, CD8$^+$ cells, NK cells, and monocytes isolated from individuals who were heterozygous carriers of rs8736 (n = 9). The analysis was conducted in two conditions: (a) unstimulated cells, and (b) cells challenged with LPS for 24 hours. Values are means with SEM depicted as vertical bars. Statistical significance was determined using a 2-tailed Student's *t*-test, with *ns*, not significant, *$p < 0.05$ and **$p < 0.01$ indicating significance levels.

immunostimulatory and immunosuppressive effects leading to profibrotic and anti-fibrotic effects. A regulatory network and crosstalk between macrophages and NK cells have also been described [21]. Further studies are required to clarify the role of MBOAT7 in NK cells.

The present study reveals that the impact of the MBOAT7 MAFLD-risk variants is confined to specific primary immune cell types, particularly mononuclear phagocytes, in a cell-specific manner. These observations are in line with our prior data on macrophages as well as our predictions based on cell-specific methylation QTLs (mQTL) of *MBOAT7* risk variants in blood and monocytes that plays a pivotal role in regulating the effect of rs8736 on mRNA stability and, consequently, the expression of *MBOAT7* [13]. These results may have important implications for the development of targeted therapies for metabolic disorders, particularly in populations with high prevalence of *MBOAT7* risk variants. It is anticipated that further investigations into the underlying mechanisms of this cell-specific effect will shed light on the role of MBOAT7 in metabolic diseases.

5. Conclusion

In conclusion, our findings demonstrate that the expression of *MBOAT7* is significantly affected by the *MBOAT7* MAFLD-risk SNPs in primary mononuclear phagocytes. Our findings emphasize the significance of examining GWAS signals in the particular cell types where changes in gene expression occur.

Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author Contributions

Formal analysis was performed by ZP, JA, and AB; Methodology was designed by JG, ME and ZP; Resources were handled by ME and JG; Study concept and design was provided by ME; Writing—original draft was done by ZP and ME; Writing—review and editing was done by ZP, JG and ME. All authors contributed to editorial changes in the manuscript. All authors read and approved the fi-

nal manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The patient samples were obtained for research purposes from January 2019 to January 2020. The study received ethical approval from the Sydney West Area Health Service and the Human Research Ethics Committee (HREC/17/WMEAD/433) at the University of Sydney. Written informed consent was obtained from all patients, including for genetic testing.

Acknowledgment

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Conflict of Interest

The authors declare no conflict of interest.

References

[1] Chan KE, Koh TJL, Tang ASP, Quek J, Yong JN, Tay P, *et al*. Global Prevalence and Clinical Characteristics of Metabolic-associated Fatty Liver Disease: A Meta-Analysis and Systematic Review of 10 739 607 Individuals. The Journal of Clinical Endocrinology and Metabolism. 2022; 107: 2691–2700.

[2] Eslam M, Sanyal AJ, George J, International Consensus Panel. MAFLD: A Consensus-Driven Proposed Nomenclature for Metabolic Associated Fatty Liver Disease. Gastroenterology. 2020; 158: 1999–2014.e1.

[3] Eslam M, El-Serag HB, Francque S, Sarin SK, Wei L, Bugianesi E, *et al*. Metabolic (dysfunction)-associated fatty liver disease in individuals of normal weight. Nature Reviews. Gastroenterology & Hepatology. 2022; 19: 638–651.

[4] Alharthi J, Gastaldelli A, Cua IH, Ghazinian H, Eslam M. Metabolic dysfunction-associated fatty liver disease: a year in review. Current Opinion in Gastroenterology. 2022; 38: 251–260.

[5] Roadmap Epigenomics Consortium, Kundaje A, Meuleman W, Ernst J, Bilenky M, Yen A, *et al*. Integrative analysis of 111 reference human epigenomes. Nature. 2015; 518: 317–330.

[6] Bayoumi A, Grønbæk H, George J, Eslam M. The Epigenetic Drug Discovery Landscape for Metabolic-associated Fatty Liver Disease. Trends in Genetics: TIG. 2020; 36: 429–441.

[7] Eslam M, George J. Genetic and epigenetic mechanisms of NASH. Hepatology International. 2016; 10: 394–406.

[8] Schmiedel BJ, Singh D, Madrigal A, Valdovino-Gonzalez AG, White BM, Zapardiel-Gonzalo J, *et al*. Impact of Genetic Polymorphisms on Human Immune Cell Gene Expression. Cell. 2018; 175: 1701–1715.e16.

[9] Eslam M, George J. Genetic Insights for Drug Development in NAFLD. Trends in Pharmacological Sciences. 2019; 40: 506–516.

[10] Thabet K, Asimakopoulos A, Shojaei M, Romero-Gomez M, Mangia A, Irving WL, *et al*. MBOAT7 rs641738 increases risk of liver inflammation and transition to fibrosis in chronic hepatitis C. Nature Communications. 2016; 7: 12757.

[11] Thabet K, Chan HLY, Petta S, Mangia A, Berg T, Boonstra A, *et al*. The membrane-bound O-acyltransferase domain-containing 7 variant rs641738 increases inflammation and fibrosis in chronic hepatitis B. Hepatology (Baltimore, Md.). 2017; 65: 1840–1850.

[12] Mancina RM, Dongiovanni P, Petta S, Pingitore P, Meroni M, Rametta R, *et al*. The MBOAT7-TMC4 Variant rs641738 Increases Risk of Nonalcoholic Fatty Liver Disease in Individuals of European Descent. Gastroenterology. 2016; 150: 1219–1230.e6.

[13] Alharthi J, Bayoumi A, Thabet K, Pan Z, Gloss BS, Latchoumanin O, *et al*. A metabolic associated fatty liver disease risk variant in MBOAT7 regulates toll like receptor induced outcomes. Nature Communications. 2022; 13: 7430.

[14] Alharthi J, Latchoumanin O, George J, Eslam M. Macrophages in metabolic associated fatty liver disease. World Journal of Gastroenterology. 2020; 26: 1861–1878.

[15] Huby T, Gautier EL. Immune cell-mediated features of non-alcoholic steatohepatitis. Nature Reviews. Immunology. 2022; 22: 429–443.

[16] Alharthi J, Pan Z, Gloss BS, McLeod D, Weltman M, George J, *et al*. Loss of metabolic adaptation in lean MAFLD is driven by endotoxemia leading to epigenetic reprogramming. Metabolism: Clinical and Experimental. 2023; 144: 155583.

[17] Eslam M, Leung R, Romero-Gomez M, Mangia A, Irving WL, Sheridan D, *et al*. IFNL3 polymorphisms predict response to therapy in chronic hepatitis C genotype 2/3 infection. Journal of Hepatology. 2014; 61: 235–241.

[18] Bayoumi A, Jalil I, Metwally M, Adams LA, Aller R, García-Monzón C, *et al*. Genetic variation in the TLL1 gene is not associated with fibrosis in patients with metabolic associated fatty liver disease. PLoS ONE. 2020; 15: e0243590.

[19] Meroni M, Longo M, Fracanzani AL, Dongiovanni P. MBOAT7 down-regulation by genetic and environmental factors predisposes to MAFLD. EBioMedicine. 2020; 57: 102866.

[20] Eslam M, Valenti L, Romeo S. Genetics and epigenetics of NAFLD and NASH: Clinical impact. Journal of Hepatology. 2018; 68: 268–279.

[21] Yang M, Vanderwert E, Kimchi ET, Staveley-O'Carroll KF, Li G. The Important Roles of Natural Killer Cells in Liver Fibrosis. Biomedicines. 2023; 11: 1391.