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Murine Models of Alcohol Consumption: Imperfect but Still Potential Source of Novel Biomarkers and Therapeutic Drug Discovery for Alcoholic Liver Disease

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Abstract

Animal models of liver disease are fundamentally important to strengthen our knowledge and understanding of human liver diseases. Murine models of alcohol consumption are utilized to investigate alcoholic liver injury to develop new therapeutic targets. The well accepted and commonly used murine models of chronic alcohol consumption are Meadows-Cook (MC) and Lieber-DeCarli (LD). LD model is based on an isocaloric high-fat liquid diet, but mice under the MC model fed on a regular chow diet with alcohol added to the drinking water. Alcoholic liver disease in real world is frequently diagnosed in patients with obesity and high fat intake, mirroring LD diet. The overlap of the specific effect of ethanol and obesity is difficult to differentiate by clinician and pathologist. In this commentary, we will further discuss our research findings comparing MC and LD as a tool to dissect early alcohol versus increased fat intake detrimental effects on the liver. The critical analysis of these two models could provide evidence to differentiate the specific impact of alcohol on the liver from the combined influence of alcohol and diet. Ultimately, these investigations could uncover potential biomarkers and therapeutic targets for personalized type of alcoholic liver injury.

Keywords

Alcoholic liver disease (ALD); Mouse; Meadows-Cook (MC); Lieber-DeCarli (LD); Patatin-like phospholipase domain containing 3 (Pnpla3); Lipocalin-2 (Lcn2); ELOVL Fatty Acid Elongase 6 (Elov16); Sulfotransferase family 2A; member 3 (Sult2a3)

Commentary

Improving our knowledge regarding the cellular and molecular mechanisms underlying murine models of alcoholic liver injury should enhance the management and therapies of

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Authors' Contributions

KA wrote the manuscript. CA outlined and edited the manuscript and obtained the funding. The final version of the manuscript is approved by all authors.

Competing Interests

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alcoholic liver disease (ALD) seen in humans [1]. Although none of the animal models available reproduce all main aspects of human ALD, they still provide very useful tools to study and understand the molecular mechanisms of the human counterpart [2]. There are two well accepted and commonly used murine models of chronic alcohol consumption introduced by Meadows-Cook (MC) and Lieber-DeCarli (LD) [2,3]. In the MC model, alcohol is introduced in drinking water, in a final concentration increased gradually to reach 20% in most cases [2,4]. Mice kept in the MC model for up to 16 weeks; however, most studies use 12 weeks as the final readout point [3]. LD was developed to enhance the alcoholic liver injury phenotype in mice [2,5]. The LD model is based on an isocaloric liquid diet with alcohol concentration usually increased to 3.395% for the duration of 25 days to eight weeks, with four weeks as an average duration used [3]. These two murine models of chronic alcohol consumption represent a potential investigative tool to explore the specific effects of alcohol combined with the high lipid liquid diet (LD) versus solid chow (MC) or alcohol regardless of the diet type (Table 1). Our group's study [3] characterized the immune cellular, transcripts, and histological phenotypes between LD and MC models with encouraging results.

The histological phenotype in LD exposed mice with or without alcohol showed more steatosis than MC mice [2,3]. This is expected considering the liquid high fat diet in LD. This aspect of high lipid diet with alcohol (LD) which enhanced the hepatic steatosis, could represent the phenotype of alcoholic liver injury in obese people. Considering the obesity problem, at least in western societies, comparing LD and MC will help understand the molecular mechanisms of liver injury induced by alcohol alone and alcohol combined with obesity, i.e., a high fat diet.

Surprisingly, hepatic leukocytes (CD45+) are significantly higher in MC compared to LD in both alcohol-fed and control mice [3]. Further, the majority of the hepatic leukocytes were from lymphoid lineage [3], contrasting with the overall enrichment in innate immune cells of pathological features of these conditions in human. There is evidence that chronic alcohol consumption decreases T cells and B cells in both humans and animal models [8].

Neutrophils and monocytes are critical components of ALD. Although their role is not yet clearly understood, neutrophils are important immune cells affected by alcohol, and their hepatic numbers are suggested to correlate with survival in alcoholic hepatitis [9, 10]. MC and LD models are murine models of early alcoholic liver injury and not models of alcoholic hepatitis; in these very early stages, we did not reveal significant changes in the numbers of hepatic neutrophils between alcohol-fed and control mice in both mouse models [3]. Surprisingly, however, in both alcohol and control, the hepatic neutrophil numbers in LD mice are decreased compared to MC [3]. Monocytes, the other counterpart innate immune cells, play an important role in ALD and potentially a cellular target for new therapeutics [11–13]. Like neutrophils, there was no difference between control and alcohol exposed mice, but mice on the MC model have higher numbers of hepatic monocytes compared to LD [3].

These data indicate that in the LC model, cellular immunological are somehow dimmed by high fat liquid diet and suggest that at least from a cellular immunological perspective, MC

model may be potentially a closer representation of human alcoholic liver injury than the LD model. Benefiting from this, our recent study using the MC model to characterize hepatic dendritic cells (DC) revealed a gender dichotomy effect of alcohol response on hepatic plasmacytoid DCs (pDC) [14]. Further, Using the MC diet for four weeks, interestingly, we recently showed an earlier increase in hepatic monocytes only in alcohol-fed female mice compared to their control counterparts [15].

Furthermore, studying transcriptomics in both models unexpectedly revealed only a limited number of genes affected specifically by alcohol, diet type, or the combination [3].

Patatin-like phospholipase domain containing 3 (Pnpla3), a gene coding for a member of lipid hydrolase enzyme [16]. Variants in *pnpla3* gene are well known as genetic risk factors for both ALD and non-alcoholic fatty liver disease (NAFLD) [17,18]. In our study by Vogle et al. [3], we found that *pnpla3* is the only gene that is upregulated in chronic alcohol exposed mice compared to their counterpart controls in both mouse models [3]. However, in the absence of alcohol exposure LD diet has a different effect: *pnpla3* was down regulated in LD control mice compared to MC controls [3]. Significant for our observation in mice, in humans counterpart, suppressive mutation of *pnpla3* tested *in vitro* and *in vivo* indicated a beneficial effect on NAFLD and therefore may represent a new therapeutic target for alcoholic induced steatosis [19–21].

Non-alcoholic steatohepatitis (NASH) overlap with alcoholic steatohepatitis (ASH) is difficult to differentiate in clinical practice due to the high prevalence of obesity in the general population and absence of specific pathological changes for these conditions [3,22]. Interestingly, studying differentially expressed genes in these two models hinted at potential biomarkers, which might help differentiate between NASH, ASH, and NASH-ASH combination [3].

Lipocalin-2 (*Lcn2*), also known as neutrophil gelatinase-associated lipocalin, is expressed by tissues and immune cells in response to inflammation [23–25]. *Lcn2* has been investigated in alcoholic liver injury models as well as human alcoholic hepatitis. In human alcoholic hepatitis with advanced fibrosis, Chen et al. investigated the intrahepatic *Lcn2* expression and serum levels of *Lcn2* [26]. Their study showed, in alcoholic hepatitis patients, a correlation between the disease severity and portal hypertension with increased hepatic *Lcn2* expression and *Lcn2* serum levels [26]. In LD mouse model, *Lcn2* exacerbated the development of ALD, and *Lcn2* knockout protected mice from ALD and liver fibrosis [26–28]. In our comparison, *Lcn2* expression was found downregulated in MC alcohol-fed mice compared to control and LD alcohol-fed mice [3]. This differential regulation of *Lcn2* pointing to a potential use to differentiate ASH, represented in the study by MC alcohol consumed, and NASH-ASH combination, represent by LD alcohol-fed. *Lcn2* was found to be a biomarker for the coexistence of liver injury not only induced by alcohol but when there is liver involvement in inflammatory arthritis [29] or in type-2 diabetes mellitus patients with hepatitis B co-infection [30].

Only a few transcripts seem to be affected by alcohol consumption in a similar way in both models and potential common target for therapies for both variants of alcoholic liver injury.

ELOVL Fatty Acid Elongase 6 (Elov16) gene codes an enzyme involved in elongating 16 carbon saturated and unsaturated fatty acids yielding 18 carbon fatty acids [31,32]. Sulfotransferase family 2A, member 3 (Sult2a3) gene express the enzymes involved in catalyzing the sulfate conjugation for many hormones and neurotransmitters [33]. Elov6 and Sult2a3 were upregulated by alcohol consumption in MC and LD compared to controls [3]. This upregulation, regardless of the different diets between the two mouse models, suggests the potential of using them as alcohol-specific therapeutic targets. There are no studies published so far regarding their involvement in alcoholic liver disease pathogenesis. The four genes and their potential biomarker/therapeutic utilization is described in Table 2.

Another layer of complexity is brought by the sexbased differential gene expression on somatic tissues, liver specifically [34]. Pnpla3 variant was found to be a risk factor for reduced survival of males with primary sclerosing cholangitis [35]. Lcn2 has shown a sex specific difference in hepatic steatosis in a mice study [36]. In a diversity outbred mice study, Sult2a3 was assigned to female liver co-regulated cluster [34]. We do not know at present if Elov16 is the subject of similar modulation.

In summary, our study strongly suggests that at least in the early stages, alcohol effect on the murine liver are mechanistically quite different and model specific. Whether with increasing time of alcohol exposure, this mechanistic difference converges or remains quite distinct, highlights the importance of continuing these studies. These studies suggesting diversity of ALD needs be validated in human as well. The results of such studies might surprise us in a way that alcoholic hepatitis in a slim person may not be the same disease from a mechanistic perspective as in an obese person, and therefore, they should not be treated the same, in spite of their similar histological characteristics.

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References

1. Ohashi K, Pimienta M, Seki E. Alcoholic liver disease: A current molecular and clinical perspective. *Liver Research*. 2018;2(4):161–72. [PubMed: 31214376]
2. Nevzorova YA, Boyer-Diaz Z, Cubero FJ, Gracia-Sancho J. Animal models for liver disease - A practical approach for translational research. *Journal of Hepatology*. 2020;73(2):423–40. [PubMed: 32330604]
3. Vogle A, Qian T, Zhu S, Burnett E, Fey H, Zhu Z, et al. Restricted immunological and cellular pathways are shared by murine models of chronic alcohol consumption. *Scientific Reports*. 2020;10(1):2451. [PubMed: 32051453]
4. Meyerholz DK, Edsen-Moore M, McGill J, Coleman RA, Cook RT, Legge KL. Chronic alcohol consumption increases the severity of murine influenza virus infections. *Journal of Immunology*. 2008;181(1):641–8.
5. Lieber CS, DeCarli LM, Sorrell MF. Experimental methods of ethanol administration. *Hepatology*. 1989;10(4):501–10. [PubMed: 2673971]

6. Brandon-Warner E, Schrum LW, Schmidt CM, McKillop IH. Rodent models of alcoholic liver disease: of mice and men. *Alcohol*. 2012;46(8):715–25. [PubMed: 22960051]
7. Lamas-Paz A, Hao F, Nelson LJ, Vazquez MT, Canals S, Gomez Del Moral M, et al. Alcoholic liver disease: Utility of animal models. *World Journal of Gastroenterology*. 2018;24(45):5063–75. [PubMed: 30568384]
8. Pasala S, Barr T, Messaoudi I. Impact of Alcohol Abuse on the Adaptive Immune System. *Alcohol Research : Current Reviews*. 2015;37(2):185–97. [PubMed: 26695744]
9. Szabo G, Saha B. Alcohol's Effect on Host Defense. *Alcohol research: Current Reviews*. 2015;37(2):159–70.
10. Altamirano J, Miquel R, Katoonizadeh A, Abraldes JG, Duarte-Rojo A, Louvet A, et al. A histologic scoring system for prognosis of patients with alcoholic hepatitis. *Gastroenterology*. 2014;146(5):1231–9 e1–6. [PubMed: 24440674]
11. Ju C, Mandrekar P. Macrophages and Alcohol-Related Liver Inflammation. *Alcohol Research : Current Reviews*. 2015;37(2):251–62. [PubMed: 26717583]
12. Tacke F. Targeting hepatic macrophages to treat liver diseases. *Journal of Hepatology*. 2017;66(6):1300–12. [PubMed: 28267621]
13. Guillot A, Tacke F. Liver Macrophages: Old Dogmas and New Insights. *Hepatology communications*. 2019;3(6):730–43. [PubMed: 31168508]
14. Alharshawi K, Fey H, Vogle A, Klenk T, Kim M, Aloman C. Sex specific effect of alcohol on hepatic plasmacytoid dendritic cells. *International Immunopharmacology*. 2021;90:107166. [PubMed: 33199233]
15. Alharshawi K, Fey H, Vogle A, Klenk T, Kim M, Aloman C. Alcohol Consumption Accumulation of Monocyte Derived Macrophages in Female Mice Liver Is Interferon Alpha Receptor Dependent. *Frontiers in Immunology*. 2021;12(1609).
16. Kienesberger PC, Oberer M, Lass A, Zechner R. Mammalian patatin domain containing proteins: a family with diverse lipolytic activities involved in multiple biological functions. *Journal of Lipid Research*. 2009;50 Suppl:S63–8. [PubMed: 19029121]
17. Stickel F, Hampe J. Genetic determinants of alcoholic liver disease. *Gut*. 2012;61(1):150–9. [PubMed: 22110053]
18. Grimaudo S, Pipitone RM, Pennisi G, Celsa C, Camma C, Di Marco V, et al. Association Between PNPLA3 rs738409 C>G Variant and Liver-Related Outcomes in Patients With Nonalcoholic Fatty Liver Disease. *Clinical Gastroenterology and Hepatology : The Official Clinical Practice Journal of the American Gastroenterological Association*. 2020;18(4):935–44 e3. [PubMed: 31419571]
19. Krawczyk M, Liebe R, Lammert F. Toward Genetic Prediction of Nonalcoholic Fatty Liver Disease Trajectories: PNPLA3 and Beyond. *Gastroenterology*. 2020;158(7):1865–80 e1. [PubMed: 32068025]
20. Schwartz BE, Rajagopal V, Smith C, Cohick E, Whissell G, Gamboa M, et al. Discovery and Targeting of the Signaling Controls of PNPLA3 to Effectively Reduce Transcription, Expression, and Function in Pre-Clinical NAFLD/NASH Settings. *Cells*. 2020;9(10).
21. Dong XC. PNPLA3-A Potential Therapeutic Target for Personalized Treatment of Chronic Liver Disease. *Frontiers in Medicine*. 2019;6:304. [PubMed: 31921875]
22. Horvath B, Allende D, Xie H, Guirguis J, Jeung J, Lapinski J, et al. Interobserver Variability in Scoring Liver Biopsies with a Diagnosis of Alcoholic Hepatitis. *Alcoholism, Clinical and Experimental Research*. 2017;41(9):1568–73.
23. Kjeldsen L, Johnsen AH, Sengelov H, Borregaard N. Isolation and primary structure of NGAL, a novel protein associated with human neutrophil gelatinase. *The Journal of Biological Chemistry*. 1993;268(14):10425–32. [PubMed: 7683678]
24. Chan P, Simon-Chazottes D, Mattei MG, Guenet JL, Salier JP. Comparative mapping of lipocalin genes in human and mouse: the four genes for complement C8 gamma chain, prostaglandin-D-synthase, oncogene-24p3, and progesterone-associated endometrial protein map to HSA9 and MMU2. *Genomics*. 1994;23(1):145–50. [PubMed: 7829063]
25. Cowland JB, Borregaard N. Molecular characterization and pattern of tissue expression of the gene for neutrophil gelatinase-associated lipocalin from humans. *Genomics*. 1997;45(1):17–23. [PubMed: 9339356]

26. Chen J, Argemi J, Odena G, Xu MJ, Cai Y, Massey V, et al. Hepatic lipocalin 2 promotes liver fibrosis and portal hypertension. *Scientific Reports*. 2020;10(1):15558. [PubMed: 32968110]
27. Wieser V, Tymoszyk P, Adolph TE, Grander C, Grabherr F, Enrich B, et al. Lipocalin 2 drives neutrophilic inflammation in alcoholic liver disease. *Journal of Hepatology*. 2016;64(4):872–80. [PubMed: 26682726]
28. Cai Y, Jogasuria A, Yin H, Xu MJ, Hu X, Wang J, et al. The Detrimental Role Played by Lipocalin-2 in Alcoholic Fatty Liver in Mice. *The American Journal of Pathology*. 2016;186(9):2417–28. [PubMed: 27427417]
29. Jia J, Yang L, Cao Z, Wang M, Ma Y, Ma X, et al. Neutrophil-derived lipocalin-2 in adult-onset Still's disease: a novel biomarker of disease activity and liver damage. *Rheumatology*. 2021;60(1):304–15. [PubMed: 32766690]
30. Shah Nawaz W, Suhail N, Siddiqui MAI, Yasmeen S, Fatima SS. Does Lipocalin-2 Affect Metabolic Syndrome in Hepatic Infections? *Cureus*. 2020;12(8):e10040. [PubMed: 32983730]
31. Moon YA, Shah NA, Mohapatra S, Warrington JA, Horton JD. Identification of a mammalian long chain fatty acyl elongase regulated by sterol regulatory element-binding proteins. *The Journal of biological chemistry*. 2001;276(48):45358–66. [PubMed: 11567032]
32. Matsuzaka T, Shimano H, Yahagi N, Yoshikawa T, Amemiya-Kudo M, Hasty AH, et al. Cloning and characterization of a mammalian fatty acyl-CoA elongase as a lipogenic enzyme regulated by SREBPs. *Journal of Lipid Research*. 2002;43(6):911–20. [PubMed: 12032166]
33. Weinshilboum RM, Otterness DM, Aksoy IA, Wood TC, Her C, Raftogianis RB. Sulfation and sulfotransferases 1: Sulfotransferase molecular biology: cDNAs and genes. *FASEB journal: official publication of the Federation of American Societies for Experimental Biology*. 1997;11(1):3–14. [PubMed: 9034160]
34. Melia T, Waxman DJ. Genetic factors contributing to extensive variability of sex-specific hepatic gene expression in Diversity Outbred mice. *PLoS One* 2020;15(12):e0242665. [PubMed: 33264334]
35. Friedrich K, Rupp C, Hov JR, Steinebrunner N, Weiss KH, Stiehl A, et al. A frequent PNPLA3 variant is a sex specific disease modifier in PSC patients with bile duct stenosis. *PLoS One*. 2013;8(3):e58734. [PubMed: 23505555]
36. Chella Krishnan K, Sabir S, Shum M, Meng Y, Acin-Perez R, Lang JM, et al. Sex-specific metabolic functions of adipose Lipocalin-2. *Molecular Metabolism*. 2019;30:30–47. [PubMed: 31767179]

Table 1:

Summary description of Meadows-Cook (MC) and Lieber-DeCarli (LD).

Feature	MC	LD	References
Goal of the model	The simplest model developed to investigate the effect of chronic alcohol exposure on the immune system	Developed to enhance the liver injury phenotype to study the initial stages of ALD	[2,3,5-7]
Model concept	Regular chow diet with alcohol added to drinking water	High fat liquid diet with added alcohol	
Duration average	12 weeks	4 weeks	

Table 2:

Brief description of potential biomarkers genes.

Gene	Literature	Our Finding	Biomarker/ Therapeutic target
Pnpla3	Variants of it are well known genetic risk factors for both ALD and NAFLD [17, 18]	The only gene that is upregulated after chronic alcohol exposure in both mouse models and downregulated in LD controls compared to MC controls [3]	Potential common therapeutic target for alcoholic induced steatosis [19–21]
Lcn2	It is associated with increase in liver injury severity in mouse and human [26–28]	Downregulated in MC alcohol-fed mice compared to MC control and LD alcohol-fed mice [3]	Potential use to differentiate ASH from NASH-ASH combination [3,29,30]
Elov/6 & Sult2a3	No studies regarding their involvement in ALD pathogenesis [3]	Upregulated by alcohol consumption in both MC and LD compared to controls [3]	Potential use as target and markers of alcohol-specific immunopathology [3]