Anuradha Supun Dassanayake[15]
Khean-Lee Goh[16]
[1]Department of Gastroenterology, S.C.B. Medical College, Cuttack 753007, Odisha, India
[2]Division of Gastroenterology and Hepatology, University of Pennsylvania, Philadelphia, Pennsylvania
[3]Division of Gastroenterology, University of Michigan Medical School, Ann Arbor, MI 48109, USA
[4]Unit of Metabolic Diseases and Clinical Dietetics, Sant'Orsola-Malpighi Hospital, "Alma Mater" University, via G. Massarenti 9, 40138 Bologna, Italy
[5]Department of Medicine, Feinberg School of Medicine, Northwestern University, Chicago, IL 60611, USA
[6]Department of Gastroenterology and Hepatology, Max Smart Super Specialty Hospital, Saket, New Delhi, 110017, India
[7]Department of Hepatology, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh
[8]Division of Gastroenterology & Hepatology, University of Virginia, Charlottesville, VA, USA
[9]National Institute for Health Research (NIHR), Nottingham Digestive Diseases Biomedical Research Centre, Nottingham University Hospital NHS Trust and University of Nottingham, Nottingham, UK
[10]Department of Medicine, Aga Khan University, Karachi, Pakistan
[11]Department of Hepatic Medicine, ANU College of Health and Medicine, Senior Staff Hepatologist, Canberra Hospital, Australian Capital Territory, Australia
[12]Sandra Atlas Bass Center for Liver Diseases & Transplantation, Department of Medicine, North Shore University Hospital/Northwell Health, 400 Community Drive, Manhasset, NY 11030, USA
[13]Department of Hepatology, Postgraduate Institute of Medical Education and Research, Chandigarh 160012, India
[14]Department of Gastroenterology and Hepatology,KIIT University, Patia, Bhubaneshwar 751024, Odisha, India
[15]Faculty of Medicine, University of Kelaniya, Kelaniya, Sri Lanka
[16]Department of Medicine, University of Malaya, Kuala Lumpur, Malaysia
*Corresponding author. Address: Department of Gastroenterology, S.C.B. Medical College, Cuttack 753007, Odisha, India. Tel.: +91 671 2505466.
E-mail address: scb_gastro_dept@hotmail.com (S.P. Singh)

Insights into contribution of genetic variants towards the susceptibility of MAFLD revealed by the NMR-based lipoprotein profiling

A new definition of metabolic dysfunction-associated fatty liver disease (MAFLD) has been proposed by a panel of international experts from 22 countries.[1] The diagnostic criteria for MAFLD are based on evidence of hepatic steatosis detected using imaging techniques, blood biomarkers/scores and/or liver histology, in addition to one of the following conditions: overweight/obesity, presence of type 2 diabetes, or evidence of metabolic dysregulation.[1] Compared with the diagnostic criteria of non-alcoholic fatty liver disease (NAFLD),[2] the definition of MAFLD excluded patients with fatty liver unrelated to metabolic dysfunction but included a large number of patients with concomitant metabolic fatty liver and other known liver diseases. Several genetic variants importantly contribute to the development of NAFLD, such as the gene variants in Patatin-like phospholipase domain-containing protein 3 (*PNPLA3*)[3] and transmembrane 6 superfamily 2 (*TM6SF2*).[4] However, their contributions to the development of MAFLD in combination with individual metabolic dysfunction status were never investigated.

Herein, we examined the associations of several currently recognized NAFLD-related gene variants with the prevalence of NAFLD, MAFLD and non-metabolic dysfunction fatty liver disease in 4,653 participants from Shanghai Changfeng Study, which was a community-based prospective cohort study of multiple metabolic diseases in middle-aged and elderly Chinese adults.[5] Among the 6,595 participants enrolled at baseline from June 2009 to December 2012,[6] 4,653 participants with available liver ultrasonography data were genotyped using an Illumina Infinium BeadChip genotyping array (707,180 markers). The diagnosis of fatty liver disease and the values of liver fat content were determined using an ultrasound quantitative method based on the computer-aided quantification of liver ultrasound attenuation and liver-kidney contrast, which showed excellent consistency with the results of proton magnetic resonance spectroscopy (r = 0.89, $p<0.001$).[7] The prevalence of fatty liver disease in the study population was 32.6%, which consisted of 26.8% NAFLD and 5.8% alcoholic fatty liver disease (AFLD)/viral hepatitis according to the diagnostic criteria of NAFLD, or 28.0% MAFLD and 4.6% non-metabolic dysfunction fatty liver disease according to the diagnostic criteria of MAFLD.[1] We found that the risk alleles of *PNPLA3* rs738409 and *TM6SF2* rs58542926 were associated with a higher risk of NAFLD, AFLD/viral hepatitis (with liver steatosis) and MAFLD, but not non-metabolic dysfunction fatty liver, using logistic regression models assuming an additive effect of gene variants and adjusting for age, sex, BMI and the presence of type 2 diabetes (Table S1). The liver fat contents in participants with metabolic dysfunction who had at least one metabolic disorder according to the MAFLD criteria were significantly higher in the *PNPLA3* (p for trend <0.001) and *TM6SF2* (p for trend = 0.002) gene variant carriers, with age, sex, BMI and fasting blood glucose

Keywords: MAFLD; NAFLD; PNPLA3; TM6SF2; Lipoprotein profile.
Received 9 September 2020; received in revised form 15 October 2020; accepted 23 October 2020; available online 16 December 2020
https://doi.org/10.1016/j.jhep.2020.10.019

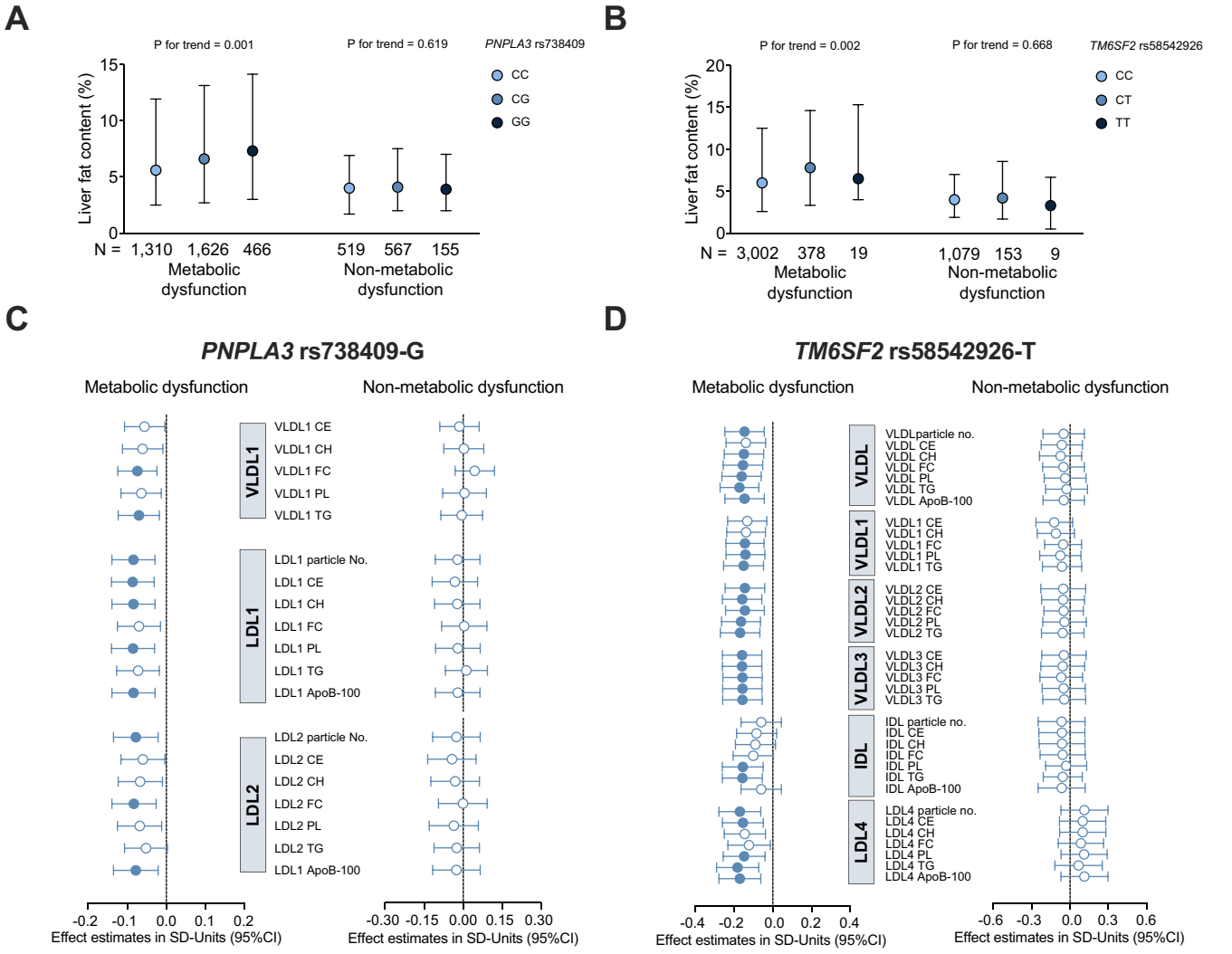

Fig. 1. Effect of *PNPLA3/TM6SF2* variants on LFC and lipoprotein profile in participants divided by metabolic status. LFC in participants carrying (A) *PNPLA3* rs738409 C>G and (B) *TM6SF2* rs58542926 C>T alleles, with/without metabolic dysfunction. Data are presented as median ± IQR and transformed to normality before analysis. Levels of significance: $p<0.05$ (generalized linear model adjusting for age, sex, BMI and FBG). Effect estimates of (C) *PNPLA3* and (D) *TM6SF2* variant on representative differential lipoproteins. The effect estimates with 95% CI were standardized in SD-units. Level of significance: solid dots $p<0.0071$ (generalized linear model adjusting for age, sex, BMI and FBG). FBG, fasting blood glucose; LFC, liver fat content.

adjusted (Fig. 1A,B). The liver fat contents in participants without metabolic dysfunction showed no significant difference between *PNPLA3/TM6SF2* gene variant carriers and non-carriers (Fig. 1A,B). A recent study reported that adiposity augments the genetic risk of NAFLD conferred by multiple loci.[8] Our current study further indicated that the presence of any metabolic dysfunction, including but not limited to adiposity, may be a prerequisite for the deleterious effect of multiple genetic variants on the development of liver steatosis, and *PNPLA3* rs738409 and *TM6SF2* rs58542926 gene variants were associated with the development of MAFLD in Chinese adults.

To further examine the metabolic status-dependent association between the gene variants and fatty liver disease, we assessed the associations of *PNPLA3/TM6SF2* variants with serum lipoprotein profiles using nuclear magnetic resonance (NMR) in participants with or without metabolic dysfunction. Each lipoprotein component was transformed to normality via rank-based inverse normal transformation before analysis, and the effect of each risk allele on lipoprotein profiling was evaluated using a generalized linear model (GLM). Model 1 was adjusted for age, sex and BMI, and Model 2 was adjusted for model 1 variables plus fasting blood glucose to further exclude the effect of metabolic confounders. We considered statistical significance at $p<0.0071$ (0.05/7), where 7 is the number of principal components explaining 95% of the variation in the NMR lipoprotein profile data. All the comparison results are listed in Tables S2-5. Notably, in participants with metabolic dysfunction, the *PNPLA3* variant was associated with lower free cholesterol and triglycerides concentrations in very low-density lipoprotein 1 (VLDL1) and the *TM6SF2* variant was associated with a reduction in most VLDL, VLDL1, VLDL2 and VLDL3 components (Fig. 1C,D), which indicated reduced hepatic triglyceride export from the liver, consistent with the function of PNPLA3 and TM6SF2 explored in animal models.[9,10] Several recent studies on the impact of genetic variants on the lipoprotein profile showed that polyunsaturated fatty acids (PUFAs) were depleted in the VLDL triglycerides in *PNPLA3* variant carriers,[11] and the

secretion of lipoprotein was significantly reduced in *TM6SF2* variant carriers.[12] Our current study further indicated that the *PNPLA3* gene variant tended to reduce the triglycerides in VLDL1, and the *TM6SF2* variant widely reduced the secretion of VLDL1, VLDL2 and VLDL3. In addition, LDL1 and LDL2 particle numbers and the cholesterol components and ApoB-100 concentrations in LDL1 and LDL2 were reduced in *PNPLA3* variant carriers. LDL4 particle number and its cholesterol ester, phospholipid, triglyceride and ApoB-100 concentrations, as well as the triglycerides and phospholipids in IDL, were significantly reduced in *TM6SF2* variant carriers. These alterations may contribute to the reduced cardiovascular mortality reported in *PNPLA3* or *TM6SF2* gene variant carriers.[13] However, the whole serum lipoprotein profile showed no significant alterations in *PNPLA3* or *TM6SF2* variant carriers in the absence of metabolic dysfunction, which may partially explain the disconnect between *PNPLA3/TM6SF2* variants and fatty liver disease in participants without metabolic dysfunction.

These findings, together with previous mechanistic studies in animals,[9,10] suggest that metabolic dysfunction status may be a prerequisite for the contribution of *PNPLA3/TM6SF2* variants to the development of liver steatosis. The genetic risk of fatty liver disease in patients with AFLD/viral hepatitis was easy to neglect under the diagnostic criteria of NAFLD, and the diagnostic criteria of MAFLD would better represent the population who needed an evaluation of the genetic risk for fatty liver disease.

Financial support
This work was supported by the Shanghai Municipal Science and Technology Major Project (2017SHZDZX01 to X. Gao), the key basic research grants from Science and Technology Commission of Shanghai Municipality (grant number 16JC1400500 to X. Gao), the Shanghai Municipal Science and Technology Commission Foundation (grant number 16411954800 to X. Gao).

Conflict of interest
The authors declare no conflicts of interest that pertain to this work.

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

Authors' contribution
The study was designed by Xin Gao, Huiru Tang and Mingfeng Xia; the data were acquired by Mingfeng Xia and Huailuan Zeng; Statistical analysis was performed by Mingfeng Xia and Hailuan Zeng and guided by Sijia Wang and Huiru Tang; Xin Gao provided funding for the project; Huiru Tang and Sijia Wang provided the techinical support; Drafting of the manuscript were done by Mingfeng Xia and Hailuan Zeng; Critical revision of the manuscript was performed by Xin Gao, Huiru Tang and Sijia Wang.

Data availability statement
Data are available from the corresponding authors upon reasonable request.

Ethics approval and consent to participate
This study was approved by the Research Ethics Committee of Zhongshan Hospital, Fudan University (No. 2008-119). Each participant provided written informed consent.

Acknowledgement
We would like to thank Dr. Huandong Lin (Zhongshan Hospital, Fudan University, China) for his contribution in data collection and management, Dr Qingxia Huang (Fudan University, China) for her contribution in lipoprotein profile test, Drs. Jieyu Ge and Wenjie Xu (Chinese Academy of Sciences) for their contribution in the genotyping of the study participants and Dr. You Zhou (School of Medicine, Cardiff university, Cardiff, United Kingdom) for critical reading of the manuscript.

Supplementary data
Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhep.2020.10.019.

References
Author names in bold designate shared co-first authorship

[1] **Eslam M**, **Newsome PN**, Sarin SK, Anstee QM, Targher G, Romero-Gomez M, et al. A new definition for metabolic dysfunction-associated fatty liver disease: an international expert consensus statement. J Hepatol 2020;73(1):202–209.
[2] Chalasani N, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K, et al. The diagnosis and management of non-alcoholic fatty liver disease: practice guideline by the American Gastroenterological Association, American Association for the Study of Liver Diseases, and American College of Gastroenterology. Gastroenterology 2012;142(7):1592–1609.
[3] Romeo S, Kozlitina J, Xing C, Pertsemlidis A, Cox D, Pennacchio LA, et al. Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. Nat Genet 2008;40(12):1461–1465.
[4] Kozlitina J, Smagris E, Stender S, Nordestgaard BG, Zhou HH, Tybjærg-Hansen A, et al. Exome-wide association study identifies a TM6SF2 variant that confers susceptibility to nonalcoholic fatty liver disease. Nat Genet 2014;46(4):352–356.
[5] Gao X, Hofman A, Hu Y, Lin H, Zhu C, Jeekel J, et al. The Shanghai Changfeng Study: a community-based prospective cohort study of chronic diseases among middle-aged and elderly: objectives and design. Eur J Epidemiol 2010;25(12):885–893.
[6] Lin HD, Li Q, Hu Y, Zhu CW, Ma H, Gao J, et al. The prevalence of multiple non-communicable diseases among middle-aged and elderly people: the Shanghai Changfeng Study. Eur J Epidemiol 2017;32(2):159–163.
[7] Xia MF, Yan HM, He WY, Li XM, Li CL, Yao YZ, et al. Standardized ultrasound hepatic/renal ratio and hepatic attenuation rate to quantify liver fat content: an improvement method. Obesity (Silver Spring) 2012;20(2):444–452.
[8] Stender S, Kozlitina J, Nordestgaard BG, Tybjærg-Hansen A, Hobbs HH, Cohen JC. Adiposity amplifies the genetic risk of fatty liver disease conferred by multiple loci. Nat Genet 2017;49(6):842–847.
[9] Wang Y, Kory N, BasuRay S, Cohen JC, Hobbs HH. PNPLA3, CGI-58, and inhibition of hepatic triglyceride hydrolysis in mice. Hepatology 2019;69(6):2427–2441.
[10] Smagris E, Gilyard S, BasuRay S, Cohen JC, Hobbs HH. Inactivation of Tm6sf2, a gene defective in fatty liver disease, impairs lipidation but not secretion of very low density lipoproteins. J Biol Chem 2016;291(20):10659–10676.
[11] **Luukkonen P**, **Nick A**, Hölttä-Vuori M, Thiele C, Isokuortti E, Lallukka-Brück S, et al. Human PNPLA3-I148M variant increases hepatic retention of polyunsaturated fatty acids. JCI Insight 2019;4(16):e127902.
[12] Trépo E, Valenti L. Update on NAFLD genetics: from new variants to the clinic. J Hepatol 2020;72(6):1196–1209.
[13] Meffert PJ, Repp KD, Völzke H, Weiss FU, Homuth G, Kühn JP, et al. The PNPLA3 SNP rs738409:G allele is associated with increased liver disease-associated mortality but reduced overall mortality in a population-based cohort. J Hepatol 2018;68(4):858–860.

Mingfeng Xia[1,†]
Hailuan Zeng[1,†]
Sijia Wang[3,4]
Huiru Tang[2,**]
Xin Gao[1,*]

JOURNAL
OF HEPATOLOGY

[1]Department of Endocrinology and Metabolism, Zhongshan Hospital and Fudan Institute for Metabolic Diseases, Fudan University, Shanghai, China
[2]State Key Laboratory of Genetic Engineering, Zhongshan Hospital and School of Life Sciences, Human Phenome Institute, Metabonomics and Systems Biology Laboratory at Shanghai International Centre for Molecular Phenomics, Fudan University, Shanghai 200438, China
[3]CAS Key Laboratory of Computational Biology, CAS-MPG Partner Institute for Computational Biology, Shanghai Institute of Nutrition and Health, University of Chinese Academy of Sciences, Chinese Academy of Sciences, China
[4]Center for Excellence in Animal Evolution and Genetics, Chinese Academy of Sciences, Kunming 650223, China
*Corresponding author. Address: Department of Endocrinology and Metabolism, Zhongshan Hospital and Fudan Institute for Metabolic Diseases, 180 Fenglin Road, Shanghai, 200032, China. Tel.: +86 21 64041990-8021, Fax: +86 21 64037269.
**Corresponding author. Address: 825 Zhangheng Rd, 200438, China. Tel:86-21-31247625.
E-mail addresses: Huiru_tang@fudan.edu.cn (H. Tang), happy20061208@126.com (X. Gao)

† These authors contribute equally to the work.

Yet more evidence that MAFLD is more than a name change

To the Editor:

In their elegant study in 4,653 Chinese patients, Xia *et al.*[1] provide robust evidence that the presence of metabolic dysfunction, including but not limited to adiposity, is a prerequisite for the deleterious impacts of the *PNPLA3* rs738409 and *TM6SF2* rs58542926 risk alleles on hepatic steatosis and lipoprotein profiles. They conclude that the MAFLD definition has dual advantages compared to the old NAFLD definition: i) it better captures the population who would benefit from an evaluation of genetic risks for fatty liver and ii) it overcomes the issue that the role of the variants was easy to neglect in those with alcoholic fatty liver disease/viral hepatitis *etc.*, under the NAFLD definition. This and other reports add to mounting evidence demonstrating the superiority of the MAFLD criteria for identifying patients at high-risk of hepatic and extrahepatic complications, emphasising that the re-definition extends far beyond a mere name change.[2–8]

Singh *et al.*[9] have some comments on our work[10,11] to which we would like to respond.

The authors argue in favour of a name and a diagnosis of exclusion (NAFLD), stating that in medicine, this has been practiced since time immemorial. This is right in many instances when the pathophysiological basis of a disease is unknown when first described, but once clarified, a change is needed. The change from "non-A, non-B" to hepatitis C is an exemplar.[12] Interestingly, the article by Dr. Reuben from 2002 that they refer too[13] stated that while "nonalcoholic fatty liver disease" – and its acronyms NAFL and NAFLD – "encompasses all possible histologic forms of the syndrome" it does not "articulate well… and sounds more like military terms for a blunder than a liver disease"! Yet, despite these very early caveats and repeated acknowledgement of the same, clinical inertia has impeded and stymied the correction process. After 4 decades, hepatology would be ill served by further delays, particularly as MAFLD more accurately reflects current understanding of pathophysiology, and cold hard scientific evidence (rather than opinion) can be brought to bear on the debate.[7]

Simply put, a diagnosis by exclusion is a diagnosis with "no means of objective proof".[14] This lack of "objective proof" implicitly brings heterogeneity, which consequently impedes the development of rational, evidence-based therapies. The approach brings confusion, resulting in increased healthcare costs, wastage of time and acts as a barrier to effective care.[15] This is what we see in "NAFLD" with clinical trials increasingly attempting to correct for tremendous variability in disease progression by only including individuals with advanced histological forms of the disease. In contrast, MAFLD identifies patients with advanced fibrosis and metabolic risk.[2–7] Similarly, irritable bowel syndrome (IBS) considered a diagnosis of exclusion has now moved to a "positive diagnosis".[16] Notably, studies have shown that providers who still believe IBS is a diagnosis of exclusion ordered 1.6x more tests and consumed $364 more per patient ($p<0.0001$), while experts were less likely than nonexperts to endorse IBS as a diagnosis of exclusion (8% *vs.* 72%; $p<0.0001$)[15]. The authors' particular example of Non-"Hodgkin Lymphoma" is not appropriate. The atypical B-cell blasts in Non-Hodgkin Lymphoma (NHL) simulate the Hodgkin Reed-Sternberg cells, leading to a mistaken diagnosis of classical Hodgkin lymphoma.[17] Thus, NHL is an entirely appropriate definition with a definite set of positive clinical, morphological, immunophenotypic, genetic and molecular diagnostic criteria.[17] It is not surprising that real-world data reveals a diagnostic gap in NAFLD[18] with a recent study that included mainly academic hepatologists suggesting that clinical practice patterns for the management of steatohepatitis frequently deviate considerably from practice guidelines.[19] A path to precision medicine in fatty liver would not be possible under the guise of NAFLD. The shift to MAFLD is likely the first pivotal step towards it.

Singh *et al.* mention that 'NAFLD' is diagnosed based on the presence of fatty liver, without significant amounts of alcohol consumption and not having any other causes of liver disease or competing causes of steatosis, as per AASLD guidelines.[20] Then, is it logical to use the term NAFLD despite having other liver diseases or the continuous use of alcohol as is happening currently and is the basis of most population-based studies which only exclude an arbitrary amount of alcohol?[21–23] Is it not time to correct this ambiguity?

The authors question the degree of assertion and the rationale for why the European Liver Patients Association (ELPA)

Received 22 December 2020; accepted 23 December 2020; available online 13 December 2020
https://doi.org/10.1016/j.jhep.2020.12.025