development of steatosis, the key early feature of NAFLD. The lack of association of *ATG7* V471A with steatosis found in the human NAFLD cohort by Baselli *et al.* further supports the notion that impaired hepatic autophagy is not critical for steatosis.

Defective hepatic autophagy impairs the removal of damaged mitochondria and protein aggregates that can lead to increased oxidative stress, hepatocyte death and genome instability resulting in liver fibrosis and tumorigenesis.[5,6] Patients that have chronic liver diseases including NAFLD and HCC may have combined multiple gene mutations such as *PNPLA3* and *ATG7* that contribute to distinctive stages of liver pathogenesis. Therefore, the findings from Baselli *et al.*'s study seem to support the notion that loss-of-function *ATG7* variants may not affect hepatic lipid accumulation, but rather promote the severity of NAFLD, cirrhosis and HCC in humans who may also have *PNPLA3* or other gene variants that affect lipid metabolism. However, it is also likely that the ATG7 V471A mutant protein may have unknown scaffold functions that could regulate lipid metabolism independently of autophagy. Future work using *ATG7* V471A knockin mice may help to further clarify its function in the pathogenesis of NAFLD.

Financial support

Conflict of interest

The authors disclose no conflicts.

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

Authors' contributions

HMN, SW and MK performed experiments. WXD conceived and drafted the manuscript and all authors read the manuscript. We thank Dr. Yssa Rodriguez 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.2022.05.015.

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Reply to: "Lack of hepatic autophagy promotes severity of liver injury but not steatosis"

ATG7 genetic variants behave as fatty liver disease progression modifiers

To the Editor:
We read with great pleasure the comment by Ding *et al.* on our recent study reporting that rare genetic variants impairing the function of autophagy related-7 (*ATG7*) predispose individuals at risk of fatty liver disease (FLD) associated with metabolic dysfunction (MAFLD) to the development of severe fibrosis and hepatocellular carcinoma.[1,2] In our study, we firstly highlighted an enrichment in rare mutations in *ATG7* in patients with severe MAFLD compared to healthy individuals. We then validated the impact of rare *ATG7* variants on liver disease in the population-based UK Biobank cohort, in a cohort of individuals with metabolic dysfunction, and in a large liver biopsy

Table 1. Associations between *ATG7* p.V471A genotype and liver steatosis grade in subgroup at risk for FLD from the liver biopsy cohort.

Group	Sample size	*ATG7* genotype	
		OR (95% CI)	adj *p* value
T2DM, yes	506	0.89 (0.49–1.59)	0.70
BMI > 30	1,219	1.09 (0.72–1.65)	0.68
PRS-HFC, High	1,071	1.18 (0.79–1.75)	0.42

Statistical analysis was performed by ordinal logistic regression using the R software v4.0.3. Models were adjusted by sex and age. When appropriate models were also adjusted by BMI, T2DM, and the *PNPLA3* p.I148M, *TM6SF2* p.E167K, *MBOAT7* rs641738, and *GCKR*, p.P446L genotypes. All genotypes were investigated under an additive model, *p* <0.05 were considered statistically significant. *ATG7*, autophagy-related 7; FLD, fatty liver disease; OR, odds ratio; PRS-HFC, polygenic risk score hepatic fat content; T2M, type 2 diabetes mellitus.

cohort (LBC). Overall, the low-frequency p.V471A *ATG7* and *ATG7* mutational burden were associated with hepatocellular ballooning, severe FLD, and hepatocellular carcinoma at a population level.[1]

We previously showed that hepatic fat accumulation has a causal role in the progression of liver damage,[3] and autophagy is involved in hepatic lipid catabolism in hepatocytes.[4] Therefore, an important question was whether the impact of *ATG7* variants was mediated by hepatocellular fat accumulation.

In response to our study, Ding *et al.* reported that liver-specific *Atg5* or *Atg7* knockout models were more prone to liver inflammation and fibrosis but were protected from fasting- or partial hepatectomy-induced steatosis,[2,5,6] suggesting the predisposition to liver damage in carriers of ATG7 mutations was not mediated by intracellular fat accumulation. Consistently, we did not observe any association between p.V471A and other *ATG7* variants with steatosis grade in the LBC.[1] Furthermore, the p.V471A variant was not associated with steatosis grade in patients at higher risk for FLD progression (Table 1) due to either type 2 diabetes (T2M) or obesity (BMI >30), or otherwise genetically predisposed to FLD (high polygenic risk score for hepatic fat content[3] [PRS-HFC]). Conversely, the p.V471A variant was associated with an increased risk of ballooning independently of the steatosis grade.[1] These associations were largely confirmed in the UK Biobank, where the p.V471A variant was not associated with steatosis but rather with increased aspartate aminotransferase levels and the loss-of-function variant predisposed severely obese individuals to liver cancer.[2]

On the other hand, we confirmed that *ATG7* regulates intracellular lipid content in several *in vitro* models. Indeed, *ATG7* KO by RNAi in human hepatoma cell lines and primary hepatocytes facilitated intracellular fat accumulation. *Vice versa*, overexpression of wild-type *ATG7* but not the p.V471A mutant protein protected against lipid accumulation. Similarly, human HepG2 hepatoma cells with stable *ATG7* KO or carrying the p.V471A variant in homozygosis showed defective autophagy and were more prone to developing steatosis.[2] Taken together, our data suggest that autophagy protects against steatosis *in vitro* and are in line with previously published evidence describing lipo-autophagy in hepatocytes.[4]

The underlying explanation for the discrepancy between the epidemiological and *in vitro* findings remains unclear. Of note, *ATG7* is strongly conserved in humans and the p.V471A represents the only coding variant with an allelic frequency >0.01 in population studies.[7] In our models, the p.V471A variant resulted in lower ATG7 expression and activity.[1] In the LBC, only a handful of patients carried the variant in homozygosis, all of whom displayed liver steatosis. Furthermore, in the Liver-Bible-2021 cohort of individuals with metabolic dysfunction, *ATG7* genetic variability was associated with steatosis (controlled attenuation parameter >275db/m, odds ratio 1.90, 95% CI 1.06-3.42, adjusted *p* = 0.029).[1]

Taken together, rare *ATG7* variants and the low-frequency p.V471A variant appear to act mostly as modifiers of FLD progression, whereas their impact on hepatic fat accumulation remains elusive. We cannot rule out that *ATG7* variants exert a mild predisposition towards steatosis development, as observed in patients with metabolic dysfunction. However, available data suggest that their impact on liver disease progression is not fully accounted for by defective lipo-autophagy leading to the accumulation of intracellular fat. Instead, we observed that defective autophagy leads to the accumulation of p62 and ballooning degeneration.[1]

Indeed, autophagy plays a multifaceted role in hepatocytes, being implicated in lipid droplet turnover but also in the clearance of protein aggregates and damaged mitochondria.[2] Concerning non-parenchymal cells, *Atg7* deficiency in hepatic stellate cells protected mice from liver fibrosis,[8] while *Atg7* KO was associated with more severe disease in liver endothelial cells and Kupffer cells.[9] Additional studies are required to clarify: a) the extent to which the impact of the p.V471A variant on liver disease is the result of functional impairment of *ATG7* in hepatocytes *vs.* other cell types; b) to pinpoint the exact mechanism, possibly involving defective clearance of damaged mitochondria and protein aggregates[5,6]; c) to clarify the common pathogenic mechanism and differences leading to hepatic and neurological diseases in carriers of *ATG7* mutations.

Financial support

Conflict of interest

The authors declare that they have no conflict of interest relevant to the present study. LV has received speaking fees from MSD, Gilead, AlfaSigma and AbbVie, served as a consultant for Gilead, Pfizer, AstraZeneca, Novo Nordisk, Intercept, Diatech Pharmacogenetics and Ionis Pharmaceuticals, and received research grants from Gilead. SR has served as a consultant for AstraZeneca, Celgene, Sanofi, Amgen, Akcea Therapeutics, Camp4, AMbys, Medacorp and Pfizer in the past 5 years, and received research grants from AstraZeneca, Sanofi and Amgen.

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

Authors' contributions

LV, SR and GB conceptualized the study. GB performed the analyses. GB and LV drafted the manuscript. LV and SR reviewed the manuscript and supervised the study.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhep.2022.07.026.

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2022 International Autoimmune Hepatitis Group non-response criteria in autoimmune hepatitis: A too early endpoint?

To the Editor:

We have read with great interest the manuscript by Pape *et al.*[1] regarding an international consensus on response criteria and treatment endpoints in autoimmune hepatitis (AIH). The main goal was to standardize the criteria used among studies, enabling comparisons and the generation of more robust evidence. In this paper, the International Autoimmune Hepatitis Group (IAIHG) defined complete biochemical response (CBR) as normalisation of aminotransferases and IgG

after no more than 6 months. The inability to achieve this endpoint was defined as insufficient response. Response was defined as ≥50% decrease of serum aminotransferases within 4 weeks after initiation of treatment.

Another recent paper from Pape *et al.*[2] had previously reported that patients with a significant decrease of aminotransferases after 8 weeks of treatment (rapid responders: defined as a decrease of ≥80% in level of aspartate aminotransferase [AST]) were more likely to achieve normalization of aminotransferases at week 26 and 52 ($p < 0.001$).

Herein, we aim to validate the new IAIHG criteria and the 8-week response criteria in our cohort. We performed a