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Missense mutant PNPLA3 alters lipid droplet turnover in partnership with CGI-58

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A common missense variant rs738409 in patatin-like phospholipase domain containing 3 (*PNPLA3*^{I148M}) was identified more than a decade ago as a dominant genetic determinant of hepatic steatosis (1) and has since been replicated in multiple studies spanning nonalcoholic fatty liver disease etiology and progression (2). But how exactly this mutant gene functions to promote hepatic steatosis and progressive liver injury has been the subject of intense investigation. Earlier studies demonstrated that wild-type (I148) PNPLA3 exhibited triglyceride (TG) lipase activity in-vitro, while the mutant (I148M) variant manifested a loss of lipase activity (3). Those findings led to the suggestion that the I148M variant functioned as a loss-of-function allele and two corresponding predictions. First, that genetic deletion of *Pnpla3* would result in hepatic steatosis in knockout mice and secondly that overexpression of wild-type (WT) PNPLA3 would decrease liver fat. However, neither of these predictions turned out to be correct. Neither germline deletion of (mouse) *Pnpla3* (4) or forced overexpression of (human) PNPLA3^{WT} resulted in an obvious lipid phenotype (5). On the other hand, overexpression of the mutant human PNPLA3^{I148M} led to accumulation of lipid droplets (LDs) and an altered spectrum of TG fatty acids (5). Those findings, coupled with studies from knockin *Pnpla3*^{I148M} mice that showed increased hepatic steatosis with sucrose feeding (6), suggested an alternative explanation, namely that the mutant PNPLA3 functions as a dominant negative allele to promote hepatic steatosis. More recent findings demonstrated that catalytically defective forms of PNPLA3 (either the I148M or S47A variant) exhibit defective ubiquitylation and as a result causes PNPLA3 protein accumulation on the surface of LDs (7). These key background studies raise the question of how accumulation of the mutant PNPLA3 protein on lipid droplets exerts a dominant negative impact on lipase activity in hepatocytes?

In the current issue of Hepatology, Cohen and Hobbs (ref) begin to answer this question using a combination of cell based and mouse studies to examine the distribution and function of adipose triglyceride lipase (ATGL), the major intracellular hepatic lipase as well as its partner (and co-factor) protein comparative gene identification-58 (CGI-58). They show that forced overexpression of ATGL in hepatoma (Huh-7) cells depleted LDs (as

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expected) but then made the unexpected discovery that forced co-expression of ATGL with PNPLA3 (either WT or I148M) inhibited lipolysis. Those findings suggest that PNPLA3 overexpression (either WT or mutant) inhibits ATGL-mediated lipolysis. An important control in those studies was to demonstrate that co-expression of ATGL with another LD protein (in this case 17β-hydroxysteroid dehydrogenase 13) led to an indistinguishable pattern of LD depletion as ATGL overexpression alone, suggesting that the dominant negative phenotype with PNPLA3 overexpression is not simply a non-specific effect. So how might this be mediated?

In order to examine how both WT and mutant PNPLA3 inhibit ATGL, Cohen and Hobbs then asked if overexpression of PNPLA3 modified the availability of the ATGL cofactor CGI-58. They show that overexpression of both WT PNPLA3 and CGI-58 resulted in LD depletion (presumably as a result of activating endogenous ATGL) while overexpression of CGI-58 with catalytically 'dead' PNPLA3, either the PNPLA3 ^{I148M} or PNPLA3 ^{S47A} mutants, caused no LD depletion. They show that overexpression of PNPLA3 on LDs failed to change either the association of ATGL with CGI-58 or the distribution of ATGL across LDs. Those findings suggest that interactions between PNPLA3 and CGI-58 might in turn directly modulate ATGL function and lipase activity.

To answer this question, they turned to a line of CGI-58 liver-specific knockout mice (CGI-58^{LKO}) that were shown previously to exhibit a dramatic increase in hepatic steatosis (8). They isolated hepatic LDs from those knockout mice and found that they contain virtually no detectable PNPLA3 protein, yet exhibit unchanged levels of ATGL, implying that CGI-58 might be required for recruitment of PNPLA3 to LDs. To answer that question, they injected adenoviral vectors expressing either WT or mutant PNPLA3 into CGI-58 floxed or CGI-58^{LKO} mice and found no localization of exogenous PNPLA3 in LDs from knockout livers. In addition, they further demonstrated that adenoviral PNPLA3^{I148M} administration into CGI-58^{LKO} mice failed to increase hepatic TG content, suggesting that PNPLA3 is dependent on CGI-58 for its recruitment and/or stabilization on the surface of hepatic LDs and also that the dominant negative effects of the mutant PNPLA3 require CGI-58 expression. In a final series of experiments, the authors also show that CGI-58 and PNPLA3 can be co-immunoprecipitated in cell culture and also from mouse liver, strongly suggesting that these two lipid droplet proteins physically interact.

Taken together, the new findings from Cohen and Hobbs represent an advance in our understanding of the mechanisms underlying the dominant negative function of mutant PNPLA3. The findings suggest a model in which the increased abundance of PNPLA3^{I148M} results in sequestration of CGI-58 on LDs and as a result limits cofactor availability for activation of ATGL. The authors find no evidence for displacement of CGI-58 from LDs with PNPLA3^{I148M} overexpression, suggesting that there is a physical interaction at the LD surface that interferes with lipase activation. Those conclusions are supported by the finding that LDs from CGI-58^{LKO} mice contain no PNPLA3. That being said, the question remains how PNPLA3, CGI-58 and ATGL each find their way to hepatocyte LDs in the correct stoichiometric proportions and function to regulate LD assembly and turnover under physiological conditions of fasting or nutrient excess. It is worth noting that other studies have demonstrated that another mutant PNPLA3 (E434K) tended to decrease hepatic

steatosis in PNPLA3 I148M carriers, suggesting that yet other LD proteins may act as codominant modifiers (9).

How might these findings help shape a personalized approach to therapies targeted to mitigate progression of liver disease in patients harboring the rs738409 polymorphism? One might envision antisense compounds targeting hepatic PNPLA3 as a strategy to decrease the accumulation of mutant PNPLA3 ^{I148M} on LDs or alternatively develop strategies to augment CGI-58-ATGL interactions. The current work illuminates a path from cell biologic pathways and biochemical mechanisms to testable hypotheses that may benefit patients with liver disease.

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REFERENCES

1. 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.
2. Younossi Z, Anstee QM, Marietti M, Hardy T, Henry L, Eslam M, et al. Global burden of NAFLD and NASH: trends, predictions, risk factors and prevention. Nature reviews Gastroenterology & hepatology. 2018;15(1):11–20. [PubMed: 28930295]
3. Huang Y, Cohen JC, and Hobbs HH. Expression and characterization of a PNPLA3 protein isoform (I148M) associated with nonalcoholic fatty liver disease. The Journal of biological chemistry. 2011;286(43):37085–93. [PubMed: 21878620]
4. Basantani MK, Sitnick MT, Cai L, Brenner DS, Gardner NP, Li JZ, et al. Pnpla3/Adiponutrin deficiency in mice does not contribute to fatty liver disease or metabolic syndrome. Journal of lipid research. 2011;52(2):318–29. [PubMed: 21068004]
5. Li JZ, Huang Y, Karaman R, Ivanova PT, Brown HA, Roddy T, et al. Chronic overexpression of PNPLA3I148M in mouse liver causes hepatic steatosis. The Journal of clinical investigation. 2012;122(11):4130–44. [PubMed: 23023705]
6. Smagris E, BasuRay S, Li J, Huang Y, Lai KM, Gromada J, et al. Pnpla3I148M knockin mice accumulate PNPLA3 on lipid droplets and develop hepatic steatosis. Hepatology. 2015;61(1):108–18. [PubMed: 24917523]
7. BasuRay S, Smagris E, Cohen JC, and Hobbs HH. The PNPLA3 variant associated with fatty liver disease (I148M) accumulates on lipid droplets by evading ubiquitylation. Hepatology. 2017;66(4): 1111–24. [PubMed: 28520213]
8. Guo F, Ma Y, Kadegowda AK, Betters JL, Xie P, Liu G, et al. Deficiency of liver Comparative Gene Identification-58 causes steatohepatitis and fibrosis in mice. Journal of lipid research. 2013;54(8): 2109–20. [PubMed: 23733885]
9. Donati B, Motta BM, Pingitore P, Meroni M, Pietrelli A, Alisi A, et al. The rs2294918 E434K variant modulates patatin-like phospholipase domain-containing 3 expression and liver damage. Hepatology. 2016;63(3):787–98. [PubMed: 26605757]