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commentary

Building bridges: PCSK7 as a NAFLD candidate gene connecting hepatic inflammation with hypertriglyceridemia[1]

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Nonalcoholic fatty liver disease (NAFLD) now ranks as the most prevalent liver disease worldwide (1), but progression from its more indolent stage of nonalcoholic fatty liver (NAFL) to advanced stages of nonalcoholic steatohepatitis (NASH) is not well understood. The accumulation of neutral lipids (principally triglycerides, TGs) within hepatocellular lipid droplets (LDs) in obese subjects with NAFL largely reflects increased de novo lipogenesis. In addition, the excessive hepatic TG burden also promotes augmented VLDL secretion and leads to systemic hypertriglyceridemia. The progressive forms of liver injury with NASH are characterized by lipotoxicity, hepatic inflammation, and fibrosis, but the mechanisms linking alterations in circulating triglycerides and LD turnover to NASH remain obscure.

In this issue of the *Journal of Lipid Research*, Dongiovanni et al. propose that upregulation of the type I membrane-bound pro-protein convertase subtilisin/kexin type 7 (PCSK7) mechanistically links hypertriglyceridemia with hepatic inflammation via hepatic iron dysmetabolism. Discovered in 1996 as one of nine secretory pro-protein convertase family members responsible for the intracellular cleavage of pro-proteins, PCSK7 is a relatively conserved protein with nearly ubiquitous tissue distribution (2). Genome-wide association study data have demonstrated an association between the PCSK7 rs236918 C risk allele, soluble transferrin receptor (3), liver cirrhosis in hemochromatosis patients (4), and hypertriglyceridemia (5, 6). Because of these prior genetic associations and evidence that excessive hepatic iron accumulation predicts hepatic inflammation and fibrosis in NASH (7, 8), Dongiovanni et al. hypothesized that PCSK7 may be "the bridge that connects lipid dysregulation with liver injury in NAFLD".

The investigators used three genetic cohorts to explore the relationship between the PCSK7 rs236918 C risk allele, iron dysmetabolism, liver injury, and hypertriglyceridemia: *1*) the Liver Biopsy Cohort (LBC), a group of European adults from Italian and Finnish referral centers who were enrolled after liver biopsy confirmed NASH diagnosis; *2*) the UK Biobank (UKBBC), a genetic dataset of UK participants; and *3*) the Dallas Heart Study (DHS), a US-based, multi-ethnic cohort of participants who reside in Dallas County, Texas. The effect of the PCSK7 rs236918 C risk variant on liver injury was estimated by measuring the association of the variant with either histologic or surrogate markers of steatosis, hepatic inflammation, and fibrosis in the three cohorts. In the LBC, there was an association between the PCSK7 rs236918 C risk allele, abnormal hepatic transaminases, and histologic lobular inflammation, although not with the sine qua non NASH histologic findings of hepatocellular ballooning or fibrosis. Perhaps counterintuitively, there was no association with steatosis in either the LBC or DHS cohorts except in LBC subjects considered high risk for progressive NAFLD or who carried the previously validated NASH genetic risk allele I148M PNPLA3 variant. Phenotypically, the PCSK7 risk variant associated with liver failure and cirrhosis in the UKBBC dataset; however, there was no stratification by PNPLA3 status in this cohort.

Dongiovanni et al. convincingly demonstrate that PCSK7 expression contributes to the TG phenotype, showing a positive association of the rs236918 C risk variant with hypertriglyceridemia in the LKB dataset. In addition, they observed an association of another low-frequency missense mutation in PCSK7 (rs142953140) with elevated circulating TGs in the DHS cohort and association of a low-frequency PCSK7 missense mutation (rs142953140) found in African Americans with lower TGs. Finally, in the UKBB, the PCSK7 risk variant was associated with cardiovascular disease and hypercholesterolemia, perhaps reflecting the previously established relationship between hypertriglyceridemia and propensity for development of atherogenic, lower density LDL particles (9).

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Using a combination of bioinformatics and gene transcript expression data, the authors propose that the PCSK7 risk variant is a gain-of-function mutation that increases hepatic PCSK7 protein expression (and secretion) by modulating expression of an 83 bp regulatory segment of long noncoding RNA (so-called "intra-PCSK7" lnc RNA). In support of this hypothesis, they observed increased hepatic expression of intra-PCSK7 in severely obese (a NAFLD-enriched population) carriers of the PCSK7 rs236918 C allele. Moreover, both hepatic and circulating PCSK7 protein expression were increased in risk allele carriers, the latter of which correlated positively with circulating TG levels in NAFLD patients.

A mechanistic examination of the authors' hypothesis in PCSK7 haploinsufficient HepG2 cells revealed impaired PCSK7 secretion, a relative reduction in the de novo lipogenic genes FAS and SREBP1, and reduced FA-induced neutral lipid accumulation compared with wild-type HepG2 cells. Haploinsufficiency also resulted in the downregulation of the inflammatory gene TNFα and of the fibrogenic gene TGFβ and its downstream effectors SMAD2/3 and SMAD4.

Although the in vitro data are bolstered by the finding in severely obese patients that hepatic PCSK7 expression correlates with FAS, TGFβ, actin, and Col1A1 gene expression, the authors' transcriptome pathway analysis adds a level of complexity to the biology of PCSK7. Specifically, they demonstrate coexpression of PCSK7 with genes involved in inflammation, epithelial mesenchymal transition, and mitosis but unlike the in vitro data, they found an inverse correlation with pathways involved in adipogenesis and FA metabolism. Those findings imply that the effects of PCSK7 on FA utilization may either be in promoting oxidation (as evidenced by the positive correlation with the β oxidative genes PPARα and CPT1 in HepG2 PCSK7 haploinsufficient cells and severely obese subjects) or in downregulating β oxidation as demonstrated by the inverse correlation of PCSK7 with oxidative phosphorylation pathways in transcriptome analysis. Understanding the relationship of PCSK7 with hepatic fat oxidation is critical as strategies that target PCSK7 could either improve or worsen the NAFLD phenotype.

Dongiovanni et al. have built a few more beams to "span the tide" (10) in NAFLD pathogenesis. What remains is to define in greater detail the link between dysregulated hepatocellular LD formation and turnover and systemic hypertriglyceridemia and the mechanisms by which PCSK7 modulates these pathways. One possibility, given the ubiquitous distribution of PCSK7, could include the role of altered intestinal chylomicron assembly and secretion. The emergence of genetically modified in vivo models would enable these physiologic questions to be answered. Nevertheless, Dongiovanni and colleagues have established an approach to move beyond genome-wide association study data to more mechanistic underpinnings of NASH development and provide strong evidence that PCSK7 should be added to the growing list of genetic modifiers at the interface of NAFL and NASH phenotypes (11).

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