JACC: BASIC TO TRANSLATIONAL SCIENCE
VOL. 1, NO. 7, 2016
ISSN 2452-302X
http://dx.doi.org/10.1016/j.jacbts.2016.09.006

JOURNAL WATCH

Genome-Wide Association Studies Candidate Gene to Dual Modifier of Nonalcoholic Steatohepatitis and Atherosclerosis

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SUMMARY

Nonalcoholic steatohepatitis is a common disease involving chronic accumulation of fat and inflammation in the liver, often leading to advanced fibrosis, cirrhosis, and cancer. It is known that nonalcoholic steatohepatitis shares many features with atherosclerosis; however, there are still no effective therapeutics. In a recent study published in *Nature*, investigators demonstrated that mice lacking a high-density lipoprotein–associated gene were surprisingly protected from both steatohepatitis and atherosclerosis through the stabilization of the liver X receptor. This work reveals a timely candidate target for 2 highly prevalent cardiovascular diseases. (J Am Coll Cardiol Basic Trans Science 2016;1:680–3)

One in 4 people in the world develop nonalcoholic fatty liver disease (NAFLD), of which about 10% progress to nonalcoholic steatohepatitis. Although obesity and insulin resistance are known pathogenic triggers, genetics and gene-environment interactions likely play critical roles in the spectrum of NAFLD and the progression to more severe sequelae. This also may partially explain why the disease prevalence varies widely among different ethnicities (e.g., Hispanics are the most susceptible and African Americans have the lowest risk).

Genome-wide association studies (GWAS) have identified both nonsynonymous and synonymous genetic variants associated with hepatic cholesterol and hepatic steatosis in or near genes, including *NCAN*, *GCKR*, *LYPLAL1*, *PNPLA3*, and *PPP1R3B* (1). Similarly, GWAS for plasma low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides have informed mechanistic links between atherosclerosis and fatty liver disease (2). These larger, multiethnic cohorts have now identified over 150 independent loci, many of which have been subjected to fine-mapping meta-analyses in nearly 200,000 individuals. The rate-limiting step remains in the functional interpretation of these loci, ultimately to identify the underlying causal variants, genes, and disease-relevant mechanisms responsible for driving the complex trait(s) (3).

For instance, a genetic variant in the intron of a gene encoding tetratricopeptide repeat domain protein 39B (TTC39B or T39) was previously associated with HDL cholesterol in 1 of these large meta-analyses; however, there were no obvious hints as to its connection to HDL regulation other than a

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Manuscript received September 28, 2016; accepted September 28, 2016.

potential scaffolding function. In a recent study published by Tall et al. in *Nature* (4), the authors employ mouse models of steatohepatitis and atherosclerosis to explore the functional roles of T39 and to pinpoint how it could represent a therapeutic target for both of these disease processes.

The authors first show that T39-deficient mice have elevated intestinal (but not liver) protein expression of liver X receptor (LXR), a known transcriptional regulator of cholesterol homeostasis. Given that LXR messenger ribonucleic acid levels were unchanged, the authors propose a post-transcriptional secondary mechanism of LXR protein. They also investigate gene expression levels of LXR targets and observe increased intestinal expression of ATP-binding cassette transporter subfamily A member 1 (*Abca1*), the major cholesterol transporter involved in HDL formation and causal gene in familial HDL deficiency Tangier disease. This intriguing observation coincided with elevated HDL cholesterol levels in T39 knockout mice that were fed a normal diet, and parallels the directionality of the human genetic variant.

Next, the authors investigated the role of T39 in steatohepatitis using mice fed a high fat/high cholesterol/high bile salt diet, and surprisingly, they found that liver-specific T39-deficient mice are protected from fatty liver, fibrosis, and mortality compared with total or intestine epithelial-specific T39 knockouts. These mice had increased hepatic HDL, liver LXR protein, and LXR-target gene expression, again due to a post-transcriptional up-regulation of LXR protein. These findings are important, as they suggest that T39 deficiency elicits protective effects in the liver specifically under inflammatory dietary conditions.

It has been known that synthetic LXR agonists induce fatty liver and excess triglycerides through up-regulation of the lipogenic transcription factor, sterol regulatory element-binding protein (SREBP)-1c. However, here the authors show that T39-deficient mice have reduced fatty liver, triglycerides, LDL cholesterol, and atherosclerotic lesions in the *Ldl* receptor knockout/high-fat diet mouse model. They identify a mechanism for this through a dramatic reduction of SREBP-1 processing and LXR-alpha-mediated increase in enzymes controlling phosphatidylcholine synthesis and incorporation of polyunsaturated fatty acids into different phospholipid species. They further demonstrate that T39 deficiency stabilizes LXR protein in hepatocytes by inhibiting ubiquitin-mediated proteasomal degradation. Interestingly, these convincing data may explain why mice lacking T39 are protected from fatty liver disease (**Central Illustration**).

TRANSLATIONAL RELEVANCE

Although tremendous progress has been made in understanding the environmental risk factors for fatty liver disease, the causes of the nonalcoholic forms (NAFLD and nonalcoholic steatohepatitis) are still unknown, and there are still no approved treatments available. This study exploits natural human variation from GWAS and disease-relevant mouse models to directly test the causality of a previously unsuspected target, TTC39B (T39). As a modifier of the master regulator of cholesterol, LXR, targeted inhibition of T39 may represent a unique strategy to simultaneously reduce both fatty liver disease and atherosclerosis.

Selective-LXR modifiers have been elusive since the discovery of potent synthetic LXR agonists in the early 2000s. These full agonists target both LXR-alpha and -beta and dramatically inhibit atherosclerosis, but resulted in unfavorable elevation of serum and hepatic triglycerides, due to SREBP-1c–mediated lipogenesis (5). Clinical trials of other compounds (e.g., LXR-623) showed beneficial effects on reverse cholesterol pathway genes ABCA1 and ATP-binding cassette transporter subfamily G member 1 (ABCG1); however, adverse neurological side effects limited their utility. By targeting a protein that directly complexes with and regulates the stability of LXR-alpha while inhibiting SREBP-1c processing, T39 inhibition is an attractive therapeutic approach to promote a favorable hepatic lipid profile and block atherosclerosis.

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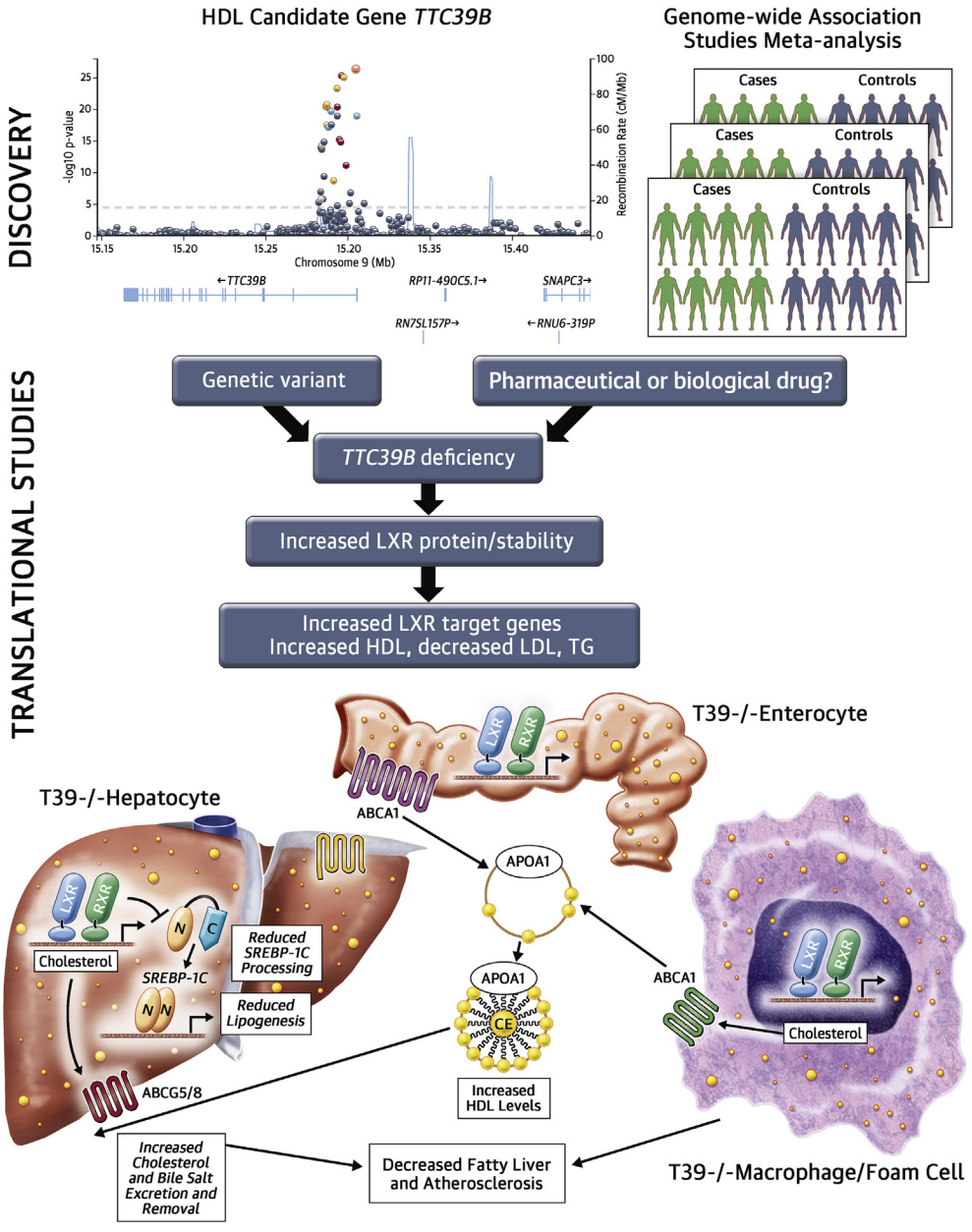
CENTRAL ILLUSTRATION **Schematic of GWAS to Functional Validation of TTC39B HDL Locus Using Relevant Mouse Models**



Miller, C.L. et al. J Am Coll Cardiol Basic Trans Science. 2016;1(7):680–3.

(Top) Regional association plot for lead single nucleotide polymorphism **(orange circle)** and variants in linkage disequilibrium associated with high-density lipoprotein (HDL) levels at *TTC39B* locus on chromosome 9. Negative log p values are shown on the left axis, and recombination rate **(blue line)** is shown on the right axis. **(Middle)** Genetic variation at *TTC39B* or potential drug targeting TTC39B leads to TTC39B (T39) deficiency and post-transcriptional increase/stabilization of liver X receptor (LXR) protein levels. This results in increased LXR target gene expression and increased HDL cholesterol, but reduced low-density lipoprotein (LDL) cholesterol and triglyceride (TG) levels. **(Bottom)** Pathways targeted as a result of T39 deficiency in mouse liver hepatocytes, intestine enterocytes, and atherosclerotic artery macrophage/foam cells. LXR protein binds deoxyribonucleic acid along with its obligate partner, retinoid X receptor (RXR) activating transcription of target genes, such as ABCA1 (major cholesterol efflux transporter) involved in formation of HDL, or ABCG5/8 (major biliary cholesterol transporters). Sterol regulatory element-binding protein (SREBP-1c) processing to the active deoxyribonucleic acid binding complex is reduced by altered phosphatidylcholine metabolism, thus preventing activation of lipogenic gene expression. Ultimately, both hepatosteatosis and atherosclerosis are reduced by T39 deficiency.

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KEY WORDS **atherosclerosis, fatty liver, genome-wide association studies, LXR, steatohepatitis**