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THE *TM6SF2* VARIANTS, NOVEL GENETIC PREDICTORS FOR NONALCOHOLIC STEATOHEPATITIS

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Nonalcoholic fatty liver disease (NAFLD) is a spectrum of liver disease ranging from benign steatosis (hepatocellular triglyceride [TG] accumulation of >5% of liver weight) through nonalcoholic steatohepatitis (NASH, which is steatosis combined with inflammation and ballooning degeneration) to fibrosis and ultimately cirrhosis and hepatocellular carcinoma (HCC), in the absence of excessive alcohol consumption (Gastroenterology 2007;132:2191–2207; Nat Rev Gastroenterol Hepatol 2013;10:645–655). NAFLD is linked with the features of metabolic syndrome such as obesity, insulin resistance, type 2 diabetes mellitus and dyslipidemia.

Recently, 3 independent groups have reported that the single nucleotide polymorphisms (SNPs) related to transmembrane 6 superfamily member 2 (*TM6SF2*) are associated with NAFLD development. The first paper reported by Kozlitina et al. identified the association between hepatic TG content determined by proton magnetic resonance spectroscopy (^{1}H-MRS) and the *TM6SF2* variants (rs58542926) through exome-wide association study in a multiethnic, multi-ancestry, population-based cohort derived from the Dallas Heart Study (DHS; total 2,736 participants; 1,324 African Americans, 882 European Americans, 467 Hispanic, and 63 other ethnicities). This *TM6SF2* variant is an adenine-to-guanine transversion at nucleotide position 499, resulting in the replacement of the 167th glutamate by lysine (c.499A>G; p.Glu167Lys). The Glu167 of *TM6SF2* is highly conserved among mammals. This variant is more common in people of European-American (7.2%) than of Hispanic (4.7%) or African-American (3.4%) descent. The investigators determined the association of the *TM6SF2* variant with elevations in serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) as a surrogate for NASH through association studies using 3 cohorts (DHS, the Dallas Biobank and the Copenhagen study). The *TM6SF2* variant encoding p.Glu167Lys correlated with the increases in serum ALT and AST levels and the decreases in plasma levels of TG and low-density lipoprotein (LDL) cholesterols, but there was no association with the levels of plasma high-density lipoprotein (HDL) cholesterols. The elevated hepatic TG content together with the reduced plasma TG and cholesterol levels suggest TM6SF2 variant to be associated with the secretion of the very-low-density lipoprotein (VLDL). The researchers then performed functional analysis for the TM6SF2 in mouse liver by silencing *Tm6sf2* using adeno-associated viral vector that selectively infects the liver. Silencing *Tm6sf2* in the liver caused 3-fold increases in hepatic TG levels and decreases in plasma levels of TG, both LDL and HDL cholesterols and TG content of VLDL. Consistently, the VLDL secretion rates were suppressed in *Tm6sf2*-silenced mice, whereas ALT levels were unchanged. The investigators also tested the effects of a high

sucrose diet that enhances hepatic TG synthesis. TG and cholesterols were increased in the liver and decreased in plasma in *Tm6sf2*-silenced mice fed a high sucrose diet. Their results demonstrated that TM6SF2 regulates hepatic TG secretion; thus, functional impairment of TM6SF2 would increase hepatic TG content, thereby promoting NAFLD.

The second study reported by Mahdessian et al used expression quantitative trait locus analysis and showed that the rs10401969 SNP was associated with plasma lipid levels. Using 206 human liver biopsy specimens, the authors reported that the rs10401969 SNP was associated with decreased hepatic mRNA levels of *TM6SF2*. Furthermore, they found that a positive correlation between hepatic *TM6SF2* mRNA and plasma TG levels, but did not find any correlation between hepatic *TM6SF2* and plasma LDL and HDL cholesterols. The study then examined the subcellular localization and function of TM6SF2. TM6SF2 is mainly localized in the endoplasmic reticulum and endoplasmic reticulum–Golgi intermediate compartment in human hepatoma cells. The *TM6SF2* silencing in hepatoma cell lines reduced the expression of TG synthesis-related genes (*ACSS2*, *DGAT1*, *DGAT2*) and the secretion of TG-rich lipoprotein. The *TM6SF2* silencing increased the size and the number of lipid droplets whereas overexpression of full-length TM6SF2 showed a decrease in number and size of lipid droplets. Consistent with the first study (Nat Genet 2014;46:352–356), the current study also demonstrates TM6SF2 to regulate hepatic lipoprotein secretion. Moreover, TM6SF2 was found to influence hepatic TG content through gene regulation of TG synthesis.

The third study reported by Liu et al analyzed the relationship between the *TM6SF2* rs58542926 SNP and NASH-associated fibrosis/cirrhosis and HCC. Utilizing a (n = 349) “discovery” and (n = 725) “validation” cohort of patients with biopsy-proven NAFLD, the authors found that the *TM6SF2* rs58542926 SNP was associated with both the severity of NASH (necroinflammation and ballooning hepatocytes) and advanced liver fibrosis (advanced fibrosis [F2–F4] versus mild fibrosis [F0–F1]) in patients with NAFLD. Although combining the 2 cohorts showed that homozygous individuals have an increased incidence of NAFLD-related HCC, these findings were no longer significant after the adjustment of age, gender, BMI, and type 2 diabetes mellitus to the regression models. Taken together, these 3 studies provide evidence that the TM6SF2 variant is associated with the development of NAFLD/NASH through regulation of lipid metabolism.

Comment

The prevalence of NAFLD is increasing worldwide. Only 10%–20% of NAFLD patients progress to NASH (Clin Liver Dis 2004;8:521–533; Nat Rev Gastroenterol Hepatol 2013;10:686–690). Simple steatosis is considered a nonprogressive (or at minimal risk of progression) entity. In contrast, NASH has the potential to progress to liver fibrosis and cirrhosis. Moreover, NASH in the presence of cirrhosis significantly increases the risk of development of HCC. Currently, there is no effective, US Food and Drug Administration–approved treatment for NASH. The hallmark of the treatment of NASH is lifestyle interventions, such as weight loss, dietary modification, and exercise; however, lifestyle interventions are not effective in the long term and sustained benefits are not seen. Vitamin E has been shown to be effective in the treatment of NASH, but is limited owing to long-

term safety concerns related to the potential for increasing cardiovascular risks and the risk of prostate cancer in men (Curr Opin Gastroenterol 2014; 30:223–237). Pioglitazone is also effective in the treatment of NASH, but is limited owing to weight gain seen with continued therapy (Aliment Pharmacol Ther 2012; 35:66–75). Therefore, better therapies are needed for the treatment of NASH. In addition, we must establish useful biomarkers to diagnose an early stage of NASH and discover genetic predictors for individuals who would progress to cirrhosis and HCC.

Romeo et al previously identified a single, highly significant association of increased hepatic TG levels with the variant of the patatin-like phospholipase domain-containing 3 (*PNPLA3*) gene (Nat Genet 2008;40:1461–1465). The *PNPLA3* variant (rs738409 encoding Ile148Met) is a non-synonymous cytosine-to-guanine nucleotide substitution mutation that results in an isoleucine to methionine amino acid change at residue 148. Their later study tested a candidate-gene approach in a separate cohort of 592 patients with histologically characterized NAFLD (PLOS Genet 2011;7:e1001324). The study identified the variants in or near *PNPLA3* (rs738409). The genes with the variants encode neurocan (*NCAN*; rs2228603), protein phosphatase 1 regulatory subunit 3B (*PPP1R3B*; rs4240624), glucokinase regulatory protein (*GCKR*; rs780094), and lysophospholipase-like protein 1 (*LYPLAL1*; rs12137855). To elucidate the functional variants at these loci, Kozlitina et al recently performed an exome-wide association study of liver TG content in a DHS cohort (Nat Genet 2014; 46:352–356). The study found 3 variants that are associated with higher hepatic TG levels; 2 variants were located within *PNPLA3* (rs738409 and rs2281135), an established locus for NAFLD, and 1 was in *TM6SF2* (rs58542926), a gene with hitherto unknown function. The correlation of the *TM6SF2* variant encoding p.Glu167Lys with the increase in serum ALT and AST levels suggests the *TM6SF2* variant to be involved in liver injury, which are the similar findings seen in individuals with the *PNPLA3* variant encoding p. Ile148Met (Nat Genet 2008;40:1461–1465; Am J Hum Genet 2008;83:520–528). Kozlitina et al also found that the *TM6SF2* variant encoding p.Glu167Lys causes protein mis-folding and degradation, resulting in the reduction of the levels of TM6SF2 protein (Nat Genet 2014;46:352–356).

Based on these 3 papers, it can be proposed that the function of TM6SF2 is to promote VLDL secretion, which is associated with a decrease in hepatic TG content. Although the mouse study with the silencing of hepatic *Tm6sf2* done by Kozlitina et al showed an increase in hepatic TG levels and a decrease in plasma TG levels and VLDL secretion rates, the elevation of serum ALT was not observed (Nat Genet 2014;46:352–356). In addition, Mahdessian et al also showed that the *TM6SF2*-silenced hepatoma cells exhibited decreased TG secretion and increased lipid accumulation, whereas *TM6SF2* silencing had no effect on cell damage and proliferation (Proc Natl Acad Sci U S A 2014;111: 8913–8918). The inconsistent findings between human study and mouse experiments may be owing to either the different time period of NASH development in humans versus mice (years vs weeks or months) or could be owing to the inability of the mouse model to completely mimic human NASH. Disease progression in NAFLD causes excessive lipid accumulation in hepatocytes, which is known to increase the susceptibility to both cytokines and stress-induced hepatocyte death (Gastroenterology 2010;139:323–334; Hepatology 2014;59:483–495).

Because *Tm6sf2*-silenced mice used in the Kozlitina et al study were fed a normal chow for 2 week or a high sucrose diet for 4 weeks, it did not provide sufficient duration of exposure to develop “full-blown” histologic features of human NASH, including ballooning degeneration, Mallory–Denk body formation, and perisinusoidal fibrosis. Further mouse studies with much longer observation and/or feeding with a high-fat diet may be required for the determination of the detrimental effect of *TM6SF2* silencing in hepatocytes.

In contrast with the study from Kozlitina et al, Liu et al found no correlation between the *TM6SF2* rs58542926 SNP and hepatic steatosis (Nat Commun 2014;30;5:4309). Several possibilities are considered. First, the study of Kozlitina et al used 3 cohorts with 3 different ethnicities, whereas the study of Liu et al used 2 cohorts mainly of European descent. Differences in the ethnicity between the study populations could influence the discrepancy in study findings. Also the dietary and environmental factors could affect. Second, Kozlitina et al used MRS-based hepatic fat quantification, whereas Liu et al used histologically assessed hepatic steatosis. MRS is better in quantifying liver fat; however, liver histology is less sensitive owing to the subjective nature of steatosis grade on liver biopsy examination (Hepatology 2013;58:1930–1940; Hepatology 2012;56: 922–932). Third, because the study of Liu et al focused on liver fibrosis/cirrhosis, the cohorts might include advanced NASH patients with “burnt-out” NASH in which hepatic fat content is often reduced (Aliment Pharmacol Ther 2012;36:22–29).

In addition to the regulation of the VLDL secretion, another function of TM6SF2 is also suggested. Kozlitina et al showed that the *Tm6sf2*-silenced mice had dramatically decreased plasma cholesterol (TC) levels. In another study, TM6SF2 was also identified to regulate plasma TC levels (Nat Genet 2014;46:345–351). Mice overexpressing human TM6SF2 had the increased levels TC and LDL cholesterol on fasting and mice with knockdown of *Tm6sf2* showed a significant reduction of plasma TC levels after fasting (Nat Genet 2014;46:345–351). Several reports have pointed to the key role of cholesterol in liver inflammation and NASH development. Dietary cholesterol and increased cholesterol metabolism are associated with the development and the severity of NASH (Hepatology 2008;48:474–486; Cell Metab 2012;15:665–674). Mechanistically, free cholesterol-accumulated hepatocytes increase the susceptibility to Fasand tumor necrosis factor-α–mediated death (Cell Metab 2006;4:185–198) and free cholesterol-treated hepatic stellate cells increases the sensitivity to transforming growth factor-β–mediated hepatic stellate cell activation through Toll-like receptor-4 and Bambi-dependent manner (Gastroenterology 2012;142:152–164). Collectively, *TM6SF2* may contribute to NASH progression through regulation of cholesterol metabolism in hepatocytes.

These studies reported the relationship between the *TM6SF2* variant and NAFLD. However, the mechanistic link between the *TM6SF2* variant and its function in reducing lipoprotein secretion still remain obscure. Further investigations on the functions of *TM6SF2* through molecular approach and genetic animal model are critical for providing insight into *TM6SF2*, not only as a new genetic predictor and biomarker, but also as a new potential target for the treatment of NAFLD/NASH.

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