Review Article

The Genetics of Clinical Liver Diseases: Insight into the *TM6SF2* E167K Variant

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Abstract

The transmembrane 6 superfamily member 2 (*TM6SF2*) gene E167K variant (rs58542926) was identified by exome-wide association study as a nonsynonymous single nucleotide polymorphism associated with nonalcoholic fatty liver disease. The *TM6SF2* E167K variant features a C-to-T substitution at nucleotide 499, encoding a glutamate with lysine change at codon 167 (E167K). *TM6SF2* is markedly expressed in the liver, small intestine and kidney, and has been proposed as an important risk factor for diseases associated with lipid metabolism. Subsequently, multifunctional studies of the *TM6SF2* E167K variant have been carried out in a spectrum of liver diseases, such as nonalcoholic fatty liver disease, nonalcoholic steatohepatitis, fibrosis, cirrhosis, and viral hepatitis. This review summarizes the research status of the *TM6SF2* E167K variant in different liver diseases and specific populations, and discusses the potential mechanisms of the *TM6SF2* E167K variant's role in the progression of various liver diseases.

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Introduction

In recent years, genome-wide association studies (as an accurate tool) have been used to identify the important single nucleotide polymorphisms that are related to lipid metabolism diseases.[1–6] The patatin-like phospholipase domain-containing 3 (*PNPLA3*) gene rs738409 is a well-known nonsynonymous single nucleotide polymorphism that has been identified in patients with nonalcoholic fatty liver disease (NAFLD). Many subsequent studies have demonstrated that this *PNPLA3* variant is associated with simple steatosis, severe hepatic fibrosis, cirrhosis and NAFLD-related hepatocellular carcinoma (HCC).[7–11] In 2014, Kozlitina *et al.*[12] revealed, for the first time, that the transmembrane 6 superfamily member 2 (*TM6SF2*) gene E167K variant (rs58542926) is another nonsynonymous single nucleotide polymorphism associated with NAFLD, by their conduct of a exome-wide association study. The *TM6SF2* E167K variant was also characterized in that study as the substitution of guanine by adenine at nucleotide 499, which results in the change of glutamate to lysine at codon 167 (E167K).

Human *TM6SF2* is located on chromosome 19 and encodes a protein composed of 351 amino acids.[13] Protein domain prediction has revealed that TM6SF2 contains 10 transmembrane domains.[14] Expression pattern analysis has shown that *TM6SF2* is mainly expressed in the kidney, small intestine and liver, all of which are tightly associated with lipid metabolism; the expression levels of *TM6SF2* are relative lower in most other tissues.[12] Subcellular location analysis has shown that the TM6SF2 is predominantly expressed in the intermediate compartment of the endoplasmic reticulum and endoplasmic reticulum-Golgi intermediate in HepG2 cell lines.[15] Kozlitina *et al.*[12] demonstrated that TM6SF2 is an endoplasmic reticulum membrane protein, and that the E167K variant leads to misfolding and increases the degradation of TM6SF2 in cells. The secretion of Toll-like receptors from and hepatic lipid droplet content in hepatoma HepG2 cell lines are influenced by down-regulated *TM6SF2* expression.[15] Among NAFLD patients, allele T carriers of *TM6SF2* E167K have shown a significant association with the higher hepatic triglyceride (TG) content than C allele carriers.[16]

TM6SF2 has been proposed as the important risk factor in diseases associated with lipid metabolism. Subsequently, multifunctional studies of the *TM6SF2* E167K variant have been carried out in a spectrum of liver diseases, including NAFLD, nonalcoholic steatohepatitis (NASH), fibrosis, cirrhosis, and viral hepatitis. This review summarizes the current research of the *TM6SF2* E167K variant in several clinical liver diseases and different populations (Table 1), and discusses the underlying mechanisms of the *TM6SF2* E167K variant's role in the progression of various liver diseases (Fig 1).

TM6SF2 E167K variant in NAFLD

NAFLD, as one of the most common chronic liver diseases worldwide, is characterized by liver fat deposition accompanying

Keywords: TM6SF2; SNP; NAFLD; Fibrosis; Cirrhosis; Virus hepatitis.
Abbreviations: ALD, alcoholic liver disease; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CHB, chronic hepatitis B; CHC, clinical hepatitis C; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; PNPLA3, patatin-like phospholipase domain-containing 3; TG, triglyceride; TM6SF2, transmembrane 6 superfamily member 2; VLDL, very low-density lipoprotein.
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Table 1. Summary of studies that have investigated the association of *TM6SF2* E167K with clinical liver diseases

Diseases	Study	Patients, *n*	Patient features/country	Main role of *TM6SF2* E167K in the study
NAFLD	Kozlitina, 2014[12]	4708	Subjects/America	Patients with the E167K variant possess a lower level of serum TG and low-density lipoprotein-c
	Liu, 2014[16]	1047	NAFLD patients/UK	The E167K variant was associated with advanced hepatic fibrosis or cirrhosis in NAFLD patients
	Sookoian, 2015[19]	226	NAFLD patients/ Argentina	The E167K variant was closely associated with severity of hepatic steatosis in NAFLD patients
	Zhou, 2015[20]	300	Subjects/Finland	The E167K variant could increase fat content in liver or in adipose tissue
	Holmen, 2014[21]	5643	Subjects/Norwegians	The E167K variant could decrease total cholesterol levels slightly
	Wong, 2014[23]	920	Subjects/China	E167K may not cause severe liver injury in NAFLD patients
	Wang, 2015[24]	384	NAFLD patients/China	The E167K variant was significant associated with risk of NAFLD
	Grandone, 2016[26]	1010	Obese children/Italy	The E167K variant was associated with increased liver enzymes and ultrasound-assessed hepatic steatosis in obese children
	Mancina, 2016[27]	878	Obese children/Italy	The E167K variant could enhance risk of hepatic steatosis in obese children
	Dongiovanni, 2015[28]	1201	NASH patients/Italy	The E167K variant could increase risk of progressive NASH and advanced fibrosis
	Goffredo, 2016[29]	957	Obese children and adolescents/USA	The E167K variant could increase susceptibility to hepatic steatosis and severe liver damage
	Viitasalo, 2016[30]	462	Children/Finland	Patients with the E167K variant have higher plasma ALT, and lower plasma TG, total cholesterol and low-density lipoprotein-c
	Kim, 2017[25]	2196	Subjects/Finland	E167K was associated with increased risk of type 2 diabetes, decreased liver production/secretion of VLDL, decreased cholesterol and TGs in VLDL/low-density lipoprotein-c particles in serum
ALD	Buch, 2015[38]	712	Alcohol-related cirrhosis patients/German and UK	The E167K variant was a new risk locus for alcohol-related cirrhosis
	Buch, 2016[39]	9559	Subjects/Scotland	The E167K variant could increase risk of ALD-related cirrhosis
Virus hepatitis	Coppola, 2015[42]	148	CHC patients/Italy	The E167K variant was identified as an independent risk factor of steatosis in patients with chronic hepatitis C
	Milano, 2015[43]	815	CHC patients/Italy	The E167K variant was significantly associated with cirrhosis in patients with chronic hepatitis C
	Caterina, 2016[44]	167	Human immunodeficiency virus/HCV coinfected patients/ Italy	The E167K variant was an independent indicator of severe fibrosis in human immunodeficiency virus/HCV coinfected patients

(*continued*)

Table 1. (*continued*)

Diseases	Study	Patients, *n*	Patient features/country	Main role of *TM6SF2* E167K in the study
	Petta, 2016[45]	694	CHC patients/Italy	E167K was not associated with severity of liver damage in terms of steatosis or fibrosis in CHC patients
	Eslam, 2016[46]	3260	CHC patients and health control/Europe	The E167K variant could promote steatosis and abnormal lipid metabolism, in part, in patients, impacting CHC and CHB viral load to different degrees in hepatitis B patients
HCC	Liu, 2014[16]	99	HCC patients/Northern Europe	The E167K variant was significantly associated with the development of HCC by univariate analysis
	Falleti, 2016[52]	511	Cirrhotic patients/Italy	The *TM6SF2* E167K and *PNPLA3* I148M variants were more likely be found in the patients with HCC

a systemic insulin resistance. Patients with NAFLD present oxidative hepatocellular damage and a varying degree of inflammation (i.e. NASH), which could progress to fibrosis and cirrhosis, or even to HCC.[17]

Abundant research on the *TM6SF2* E167K variant in NAFLD patients has been reported since the variant was found. Anstee *et al.*[18] summarized some of the previous research findings and concluded that TM6SF2 could regulate hepatic lipid efflux, with deletion or mutation of *TM6SF2* resulting in a reduced secretion of hepatic lipoprotein (very low density lipoproteins (VLDL), TG, and APOB), an increased accumulation of hepatocellular lipid droplets, and a higher TG level. Sookoian and colleagues[19] conducted a study in 226 Argentinean NAFLD patients (diagnosed by histopathological evidence), and the results showed a close association between the *TM6SF2* E167K variant and the severity of hepatic steatosis (diagnosed by liver biopsy). The influence of *TM6SF2* E167K variant has been found to be independent of sex, body mass index (BMI) and age, as well as the effect of the *PNPLA3* I148M variant. Another study of a Finnish population found that the *TM6SF2* E167K variant could increase fat content in the liver or in adipose tissue, but that the insulin sensitivity in these tissues was not decreased.[20] A study of Norwegians showed that the *TM6SF2* E167K variant is associated with a slight decrease in total cholesterol levels, but has no effect on the levels of high-density lipoprotein-c and total TG.[21] Finally, Kozlitina *et al.*[12] showed that patients with the *TM6SF2* E167K variant possess a lower level of serum TG and low-density lipoprotein-c, as compared to health controls in a large cohort study.

Many early studies of non-Asian populations observed a significant effect of the *TM6SF2* E167K variant on NAFLD, in both adults and children.[22] To confirm whether this variant also increases the risk of NAFLD in Asians (particularly in East Asians), Wong *et al.*[23] investigated the effect of the E167K variant on a Chinese cohort of NAFLD patients; the results showed that the variant ratio of *TM6SF2* was low in the Chinese population and that E167K may not cause severe liver injury in this population. Due to the lower number of subjects included in that study, the conclusion needs further investigation to be confirmed. Later, Wang *et al.*[24] performed a case-control study of a community-based Han Chinese population and found a significant relationship ($P < 0.001$) between the *TM6SF2* E167K variant and the risk of NAFLD, despite there being a low variant ratio of *TM6SF2*. Kim *et al.*[25] investigated the genotype of *TM6SF2* E167K and serum tyrosine levels in nondiabetic statin-naïve participants. The authors found that *TM6SF2* E167K was associated with increased risk of type II diabetes, decreased liver production/secretion

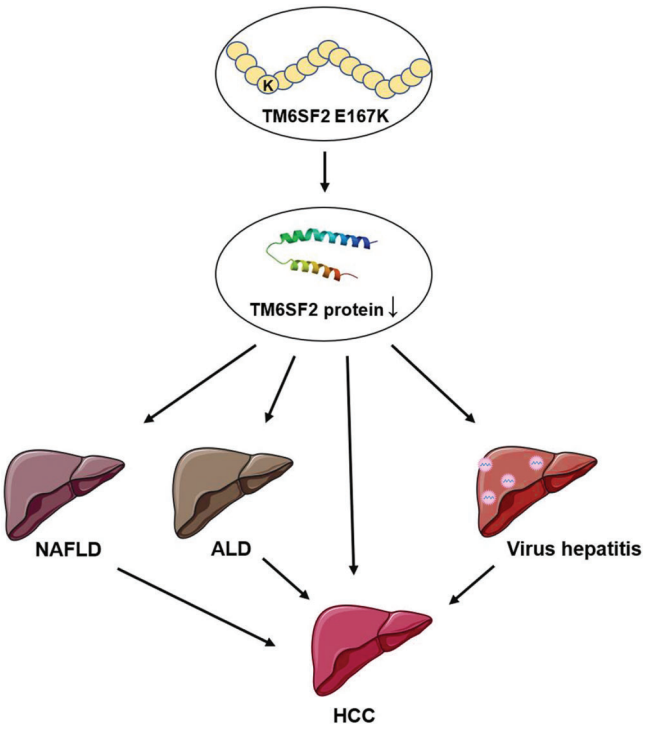

Fig. 1. The potential mechanism of *TM6SF2* E167K in clinical liver diseases. The E167K variant accelerates protein degradation. Reduced TM6SF2 protein levels could lead to the development of NAFLD, ALD, viral hepatitis, and HCC.

of VLDL, and decreased cholesterol and TGs in VLDL/low-density lipoprotein particles in serum; moreover, increased tyrosine levels were regarded as the potential mechanisms of *TM6SF2* E167K in the risk of NAFLD.

The collective results presented above suggest that frequency of the *TM6SF2* E167K variant and effects of the E167K variant on the risk of NAFLD may be inconsistent among different nations and ethnicities. More case-control studies of Asian populations need to be conducted to confirm the effects of the *TM6SF2* E167K variant on the development of NAFLD.

TM6SF2 E167K variant in youths with NAFLD

As in adults, is the E167K variant related to risk of NAFLD in youths? To clarify this question, Grandone *et al.*[26] evaluated the potential effects of the E167K variant on liver enzyme levels in obese children, for the first time; the results showed a close correlation of the E167K variant with increased liver enzymes and ultrasound-assessed hepatic steatosis in obese children. That same year, Mancina *et al.*[27,28] reported the observation of the E167K variant increasing the risk of hepatic steatosis in obese children, and that patients with the E167K variant show an increased susceptibility to hepatic steatosis, independent of other risk factors, including existence of the *PNPLA3* I148M variant. In addition, Goffredo *et al.*[29] observed that the minor allele of the E167K variant increased susceptibility to hepatic steatosis and severe liver damage in obese children and adolescents. Finally, Viitasalo *et al.*[30] performed a cross-sectional study of 462 Caucasian children whose ages ranged from 6 to 9 years, with the aim of testifying to the relationship of the E167K variant with levels of liver enzymes and lipid profiles. The results indicated that patients with the E167K variant have a higher plasma alanine aminotransferase (ALT) level and lower plasma TG, total cholesterol and low-density lipoprotein-c level in childhood. These findings agree with the results of the previous studies in adults.

TM6SF2 E167K variant and progression of NAFLD

Accumulated studies suggest that the E167K variant is tightly related to the progression of NAFLD. In two histologically characterized cohorts encompassing steatosis, steatohepatitis, fibrosis and cirrhosis, Liu *et al.*[14,16] demonstrated that the E167K variant was associated with advanced hepatic fibrosis or cirrhosis, and that the effects of E167K were independent of BMI, age, type 2 diabetes mellitus and *PNPLA3* I148M presence. Simple steatosis is considered a nonprogressive liver disease, with only 10–20% of NAFLD patients progressing to NASH.[31] While NAFLD is generally regarded as a benign disease, NASH possesses the possibility to deteriorate to cirrhosis and HCC.[32]

Yoon *et al.*[33] summarized the relationship of the E167K variant to the risk of NAFLD, concluding that the E167K variant may result in the development of NASH through regulation of the metabolism of cholesterol in hepatocytes. In 2014, Kozlitina and colleagues[12] ascertained the correlation between the *TM6SF2* E167K variant and the increased serum ALT and AST levels through relevant studies in three cohorts (The Dallas Biobank and the Copenhagen study). The serum ALT and AST levels could be regarded as a potential marker of liver injury and NASH. In addition, Liu *et al.*[16] investigated the correlation between the E167K variant and the severity of histological NASH and NAFLD-related liver fibrosis; the results showed an independent effect of the variant and NASH and fibrosis. In 2015, Dongiovanni *et al.*[28] investigated 1201 patients with suspected NASH who were diagnosed by liver biopsy, and reported that the E167K variant could increase the risk of NASH and advanced fibrosis.

The accumulated studies have shown a significant relationship between the E167K variant and the progression of NAFLD, but the underlying mechanism of the *TM6SF2* E167K variant in the progression of NAFLD remains unclear.[34]

TM6SF2 E167K variant in alcoholic liver disease (ALD)

ALD is a common cause of liver-related mortality in the world, as excessive alcohol consumption is sustained and growing; ALD cases account for about 75% of alcohol-related mortality.[35,36] ALD composes a spectrum of liver disease, ranging from steatosis, steatohepatitis and fibrosis to cirrhosis, that is similar to the progression of NAFLD.[37] Unlike NAFLD, only few studies have referred to the correlation of the E167K variant and the risk of ALD. A genome-wide association study in alcohol-related cirrhotic European patients and subsequent studies in two independent European cohorts have shown the E167K variant to be a new risk locus for alcohol-related cirrhosis ($P = 7.98\times10^{-10}$).[38] Buch *et al.*[39] conducted a genome-wide association study to screen the candidate genes associated with the risk of ALD; the results first showed that the E167K variant increased the risk of ALD-related cirrhosis by 1.4-fold ($P = 0.022$). When the influence of the E167K variant was analyzed by a multivariate analysis considering the potential confounding factors (sex, age, BMI, and type 2 diabetes mellitus), a modest role was observed in the patients with ALD-related cirrhosis.

The above-cited results have suggested that the E167K variant is a new additional risk locus for ALD-related cirrhosis, but still lack large enough numbers of studied populations to confirm the influence of the E167K variant in ALD patients. More subjects of different races should be included to validate the relationship of the E167K variant and the risk of ALD. The detailed mechanism of the E167K variant in the progressive of ALD also needs to be illuminated in the future.

TM6SF2 E167K variant in viral hepatitis

Hepatitis C is a major health problem around the world, and is the main cause of liver disease that could progress to cirrhosis and HCC.[40] Most hepatitis C virus (HCV) infections occur by blood transfusions and drug injection.[41] In 2015, Coppola *et al.*[42] investigated the relationship of the E167K variant and liver steatosis in patients with chronic hepatitis C, for the first time. The results showed that the liver steatosis scores in 18 E167K variant carriers were higher than in 130 patients with the *TM6SF2* wild-type. A general linear model analysis was also performed in that study and the E167K variant was found to be an independent risk factor of steatosis ($P = 0.0376$).

Milano *et al.*[43] conducted a cross-sectional cohort study in 815 Italian clinical hepatitis C (CHC) patients to investigate the effect of the E167K variant on the score of steatosis, necroinflammation and fibrosis. The results showed that the *TM6SF2* E167K variant was an independent risk factor of cirrhosis. After further controlling for steatosis and necroinflammation in the analysis, the *TM6SF2* E167K variant was significantly associated with cirrhosis. These results suggested that the E167K variant is tightly associated with steatosis and fibrosis, as an independent risk factor impacts the liver damage in patients with CHC.

For human immunodeficiency virus/HCV coinfected patients, Caterina *et al.*[44] reported that the E167K variant was an independent indicator of severe fibrosis, but the variant appeared to be independently associated with severe steatosis only for those patients without HCV-genotype 3. Furthermore, Petta *et al.*[45] found no obvious effect of E167K on the severity of liver damage in terms of steatosis or fibrosis, in their study of 694 Caucasian genotype 1 CHC patients. The results also showed a low prevalence of the E167K variant in the population with genotype 1 CHC.

In contrast to hepatitis C, there was few data on the role of the *TM6SF2* E167K variant in chronic hepatitis B (CHB). Recently, Eslam *et al.*[46] carried out a detailed analysis in 3260 CHB patients, nonviral liver patients, and healthy controls. The results showed that the E167K variant promotes steatosis and abnormal lipid metabolism, in part by altering the expression of *TM6SF2* and *MTTP*, and impacted CHC and CHB viral load to different degrees; the association of the *TM6SF2* E167K variant with fibrosis in hepatitis B patients was unclear.

Undoubtedly, the E167K variant is significantly associated with viral hepatitis, influencing the severity of liver damage in patients with hepatitis B virus or HCV. The detailed mechanism of the E167K variant in the progression of viral hepatitis and the spread of viruses needs further study.

TM6SF2 E167K variant in HCC

HCC is the sixth most common cancer and the third cause of cancer-related morbidity and mortality worldwide.[47] The main risk factors of HCC include NASH, alcohol intake, ingestion of the fungal metabolite aflatoxin B1, and hepatitis B virus and HCV infections.[48–50] Increasing evidence has shown that patients with NAFLD are prone to progression to HCC, which could be affected by the *PNPLA3* I148M variant without the presence of cirrhosis.[11,51]

In 2014, Liu *et al.*[16] were the first to report a significant association of the E167K variant with development of HCC, by their univariate analysis of a cohort of 99 consecutive Northern European Caucasian NAFLD patients with or without HCC. But, the significance disappeared when the relationship was investigated by multivariate analysis incorporated with the known risk factors, including BMI, sex, age, type 2 diabetes mellitus and presence of cirrhosis (P = 0.42). Subsequently, Falleti *et al.*[52] conducted a study to assess the interaction between *TM6SF2* E167K and *PNPLA3* I148M in the development of HCC. The results showed that the *TM6SF2* E167K and *PNPLA3* I148M variants were more likely to be found in patients with HCC, and the same for alcohol-related cirrhosis (P = 0.0007), but not in those with viral cirrhosis. This indicated that *TM6SF2* E167K in connection with *PNPLA3* I148M could be regarded as potential genetic risk factors for the progression of HCC in alcohol-related cirrhosis.

A recent research showed that the *TM6SF2* E167K variant affected the cell cycle of the HCC cell line HEPA 1–6, possibly through up-regulated expression of cyclinD1, P53 and Rb, and down-regulated expression of P27. The abnormal cell cycle disordered energy metabolism, thereby promoting the progression of HCC.[53]

It's certain that the E167K variant affects the development of HCC, but the detailed mechanism remains unclear. Further studies should be conducted to reveal the effects of the *TM6SF2* E167K variant on the development of HCC that has progressed from alcohol-related and nonalcohol-related liver disease.

Conclusions

In summary, despite a low frequency of the minor allele being observed, the *TM6SF2* E167K variant shows a significant association with steatosis, fibrosis, cirrhosis and HCC in various liver diseases of different etiologies (i.e. abnormal lipid metabolism, alcohol intake, hepatitis B virus and HCV infection). Identification of the *TM6SF2* E167K variant in more liver diseases will contribute to establishment of new genetic predictors for the accurate and noninvasive diagnosis of NAFLD, ALD, viral hepatitis and HCC, and will help develop convenient methods of initiating early preventive and therapeutic strategies for liver disease in the seeming healthy population. However, there have been rare data on the role of *TM6SF2* E167K in chronic hepatitis B and HCC. In addition, compared with considering *TM6SF2* alone as a predictor for the clinical liver diseases, advocation for the establishment of a large well-phenotyped cohort with prospective follow-up is more significant. In the future, more liver disease-related polymorphisms should be identified to create a joint prediction system for the early diagnosis and treatment of clinical liver diseases.

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Conflict of interest

The authors have no conflict of interests related to this publication.

Author contributions

Drafted the initial and final manuscript (XZ, SL), supervised the project (QD, YX), contributed to study concept and design, acquisition of data, analysis and interpretation of data, and critical revision of the manuscript (XZ, SL, QD, YX, SX).

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