ASSOCIATION BETWEEN *PNPLA3 TM6SF2 HSD17B13* VARIANTS AND FATTY LIVER DISEASE IN PEOPLE WITH HIV AND HEPATOCELLULAR CARCINOMA

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ABSTRACT
Non-alcoholic fatty liver disease (NAFLD) is one of the main causes of chronic liver disease. The estimated prevalence of NAFLD in Thailand is around 24% to 67%. The most common causes of NAFLD are obesity, diabetes, and also viral infection. HIV infections are associated with NAFLD. Furthermore, NAFLD also showed an association with Hepatocellular carcinoma (HCC), about 4% to 27% of steatohepatitis with cirrhosis developed into HCC. Interestingly, Genetic risk factors also play a well-known role that involves in NAFLD such as *PNPLA3*, *TM6SF2*, and *HSD17B13.* The data on genetic risk factors in NAFLD with HIV and HCC is limited in Thailand. In this study, we aim to investigate the association between carriers of genetic variants of *PNPLA3*, *TM6SF2*, and *HSD17B13* and the risk of NAFLD, NAFLD with HIV and HCC. Genotyping of *PNPLA3* rs738409, *TM6SF2* rs58542926, and *HSD17B13 rs6834314* were performed in 600 blood samples including 150 samples from Healthy controls, 150 samples from NAFLD, 150 samples from NAFLD with HIV, and 150 samples from *non-B non-C* hepatocellular carcinoma (NBNC *HCC*). Patients with NAFLD, NAFLD with HIV, and NBNC HCC had an increased frequency of *PNPLA3* G allele compared to Healthy control with an odd ratio 2.43 (95% CI 1.27-4.64; P=0.01), 1.99(95% CI 1.20-3.29; P=0.007), 2.32 (95% CI 1.19-4.53; P=0.01) respectively. T allele frequencies of *TM6SF2* polymorphism were similar between healthy control and disease groups. The frequencies of *HSD17B13* GG genotypes were significantly lower in NBNC HCC group compared with the Healthy control (OR 0.41; 95%CI 0.18-0.91, P=0.03). *Conclusion* These results revealed that *PNPLA3* polymorphism was associated with fatty liver diseases in Thai population.
Keywords: SNPs, NAFLD, Polymorphism

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is one of the main causes of chronic liver disease, NAFLD is a liver dysfunction caused by the accumulation of fat in liver more than 5% of liver weight and does not relate to alcohol consumption (Eslam et al., 2018; Teng et al., 2023). The spectrum of NAFLD compasses simple hepatic steatosis to nonalcoholic steatohepatitis (Teng et al., 2023). The worldwide prevalence of NAFLD is around 6% and up to 35% (Malnick et al., 2022) and the estimated prevalence of NAFLD in Thailand is around 24% to 67% (Phisalprapa et al., 2021). The most common causes of NAFLD are obesity, insulin resistance, diabetes, and also viral infection (Akter, 2022). Various studies revealed that Chronic hepatitis B is frequently accompanied by NAFLD and could progress to more adverse liver diseases (Liu et al., 2023; Vassilopoulos et al., 2023). Other viral infections have been found to be associated with NAFLD. Interestingly, the prevalence of NAFLD in persons with HIV infection ranges from 30% to 65% (Seth & Sherman, 2019). The combination of HIV infection itself and antiretroviral therapy cloud promote the development of NAFLD (Cinque et al., 2023). NAFLD also showed an *association with* Hepatocellular carcinoma (HCC), about 4% to 27% of steatohepatitis with cirrhosis developed into HCC (Baffy et al., 2012; Dhamija et al., 2019). Multiple studies showed that genetic factors also play an important role in NAFLD. Several genetic variants such as *PNPLA3*, *TM6SF2*, and *HSD17B13* have been discovered, which mainly affect lipid metabolism leading to the development of NAFLD (Sharma & Mandal, 2022; Shi et al., 2023). *PNPLA3* acts as a lipase enzyme working to remodel phospholipids and triglycerides. The I148M variant of *PNPLA3* is strongly associated with susceptibility to NAFLD by increased lipid accumulation (Severson et al., 2016; Shi et al., 2023). Many studies reveal that the *PNPLA3* rs738409 GG genotype is associated with the progression of NAFLD in Asian cohorts. *TM6SF2* regulates the process of packing triglycerides in VLDL, The Presence of the rs58542926 variant was positively associated with the progression of NASH (Severson et al., 2016). *HSD17B13* acts as a retinoic dehydrogenase that converts retinol to retinoic acid, rs6834314 variants of *HSD17B13* were associated with reduced inflammation in patients with NAFLD (Sharma & Mandal, 2022). **As mentioned previously,** it was found that HIV-infected patients are at high risk of developing NAFLD also as HCC patients and there are a few studies on the relationship between genetic variations and the development of NAFLD in these groups. Understanding the risk factors of developing NAFLD could increase the efficiency of screening the disease and providing precision treatment to patients so this study aims to evaluate the association between genetic polymorphisms of *PNPLA3 TM6SF2* and *HSD17B13* with fatty liver disease in people with HIV and Hepatocellular Carcinoma.

LITERATURE REVIEWS

Patatin-like phospholipase domain-containing proteins 3; PNPLA3

The PNPLA3 gene is significantly related to fatty liver disease. The protein from the PNPLA3 gene acts as a lipase enzyme, converting triglycerides into fatty acids in hepatocytes. The PNPLA3 gene variation associated with NAFLD is at the rs738409 position, which is a substitution change from base C to G, causing an amino acid change from Isoleucine to Methionine at position 148, causing the protein to lose its function (Basu Ray, 2019). From the previous research that studied the relationship of genetic variation or SNPs (Single nucleotide polymorphisms) between the PNPLA3 gene at position rs738409, it was found that people with the GG genotype had a risk of developing NAFLD more than people who carry the CC genotype. In a study by Busca et al. of HIV-infected patients with NAFLD in Spain, it was found that people with the G allele were more likely to develop NAFLD than those with the CC genotype (Busca et al., 2022; Mazo et al., 2019).

TM6SF2 transmembrane 6 superfamily member 2; TM6SF2

TM6SF2 protein is expressed in the ER-Golgi intermediate compartment in hepatocytes. The protein plays a role in fat metabolism by promoting the delivery of triglyceride-rich lipoproteins outside the liver cells. Genetic variation of TM6SF2 is associated with the development of NAFLD. From many studies, it has been found that people with genetic variation of TM6SF2 at position rs58542926 cause the lost function of a protein, making it unable to transport fatty acid and reducing the amount of VLDL secretion from liver cells. A fatty acid that cannot be exported outside the cells will accumulate in hepatocytes, leading to NAFLD (Chen et al., 2015; Luo et al., 2022; Mahdessian et al., 2014). A study by Li et al. in a Chinese population focusing on the association of genetic variation in the TM6SF2 gene at the rs58542926 position found that people with the CT and TT genotypes had a 1.368% higher risk of developing NAFLD than healthy controls (Li et al., 2019).

17-Beta Hydroxysteroid Dehydrogenase 13; HSD17B13

HSD17B13 belongs to the group of 17β-hydroxysteroid dehydrogenase, It acts as an enzyme involved in the metabolism of fat and cholesterol. The HSD17B13 gene is located on chromosome 4 and is highly expressed in the liver. The protein from the HSD17B13 gene is expressed on the surface of lipid droplets. Genetic variation of the HSD17B13 gene at position rs6834314 results in the protein losing its function (Amangurbanova et al., 2023; Mashek, 2021; Ting et al., 2021). In a study by Ting El on the relationship between the rs6834314 variation and the occurrence of NAFLD in an Asian population consisting of Malaysians, Chinese, and Indians, it was found that people with the G allele had a lower risk for developing NAFLD than those carry with the A allele. The analysis suggests that the G allele appears to be a protective factor in NAFLD (Ting et al., 2021).

From the literature review, the conceptual framework can be drawn as shown in Figure 1.

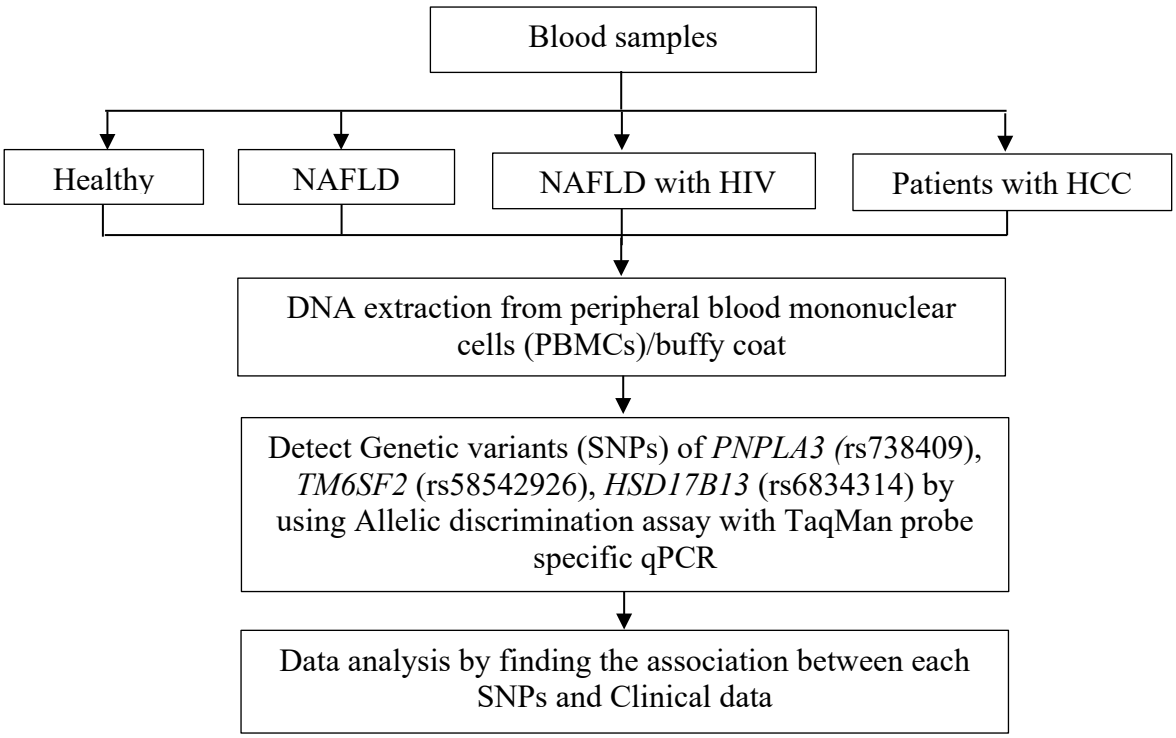

Figure 1 Conceptual Framework

RESEARCH METHODOLOGY

A total of 600 blood samples that enrolled in this study were divided into four groups including 150 samples from Healthy controls, 150 samples from NAFLD, 150 samples from NAFLD with HIV, and 150 samples from *non-B non-C* hepatocellular carcinoma (NBNC *HCC*). Blood samples of Healthy controls, NAFLD and NBNC *HCC* were randomly collected from King Chulalongkorn Memorial Hospital and Blood samples of NAFLD with HIV were collected from **The HIV Netherlands Australia Thailand Research Collaboration (HIV-NAT), Bangkok Thailand.**

DNA samples were extracted from PBMCs using phenol-chloroform-isoamyl alcohol isolation method. Genotyping of *PNPLA3* rs738409, *TM6SF2* rs58542926, and *HSD17B13 rs6834314* were performed by TaqMan® SNP Genotyping Assays using QuantStudio™3 Real-Time PCR System (ThermoFisher Scientific, US). Real-time PCR results were plotted into Allelic discrimination plot for interpretation of DNA genotyping.

RESEARCH RESULTS

Demographic data of Healthy controls, NAFLD, NAFLD with HIV, and patients with NBNC-HCC are presented in Table 1. Patients in NAFLD and NBNC-HCC were significantly older than Healthy controls. There were significant differences between male and female distribution in this study. There were more females than males in Healthy controls but fewer females in NAFLD, NAFLD with HIV, and NBNC-HCC. There is no significant difference in BMI between HIV group and NBNC-HCC. There was no significant difference in AST serum level between NAFLD group and NAFLD with HIV but both had lower serum AST compared with NBNC-HCC However, there was no significant difference observed in mean serum ALT among disease groups. HIV-group had significantly lower CAP compared with NAFLD. The distribution of genotypes in each gene did not deviate from the Hardy-Weinberg equilibrium ($P > 0.05$). Minor allele frequency (MAF) of each variant were presented in Table 1.

Distribution of the SNPs in Healthy Control and Patients related to NAFLD are shown in Table 2. Patients with NAFLD, NAFLD with HIV, and NBNC HCC had an increased frequency of *PNPLA3* G allele compared to Healthy control with an odd ratio 2.43 (95% CI 1.27-4.64; $P = 0.01$), 1.99 (95% CI 1.20-3.29; $P = 0.007$), 2.32 (95% CI 1.19-4.53; $P = 0.01$) respectively. In addition, NAFLD with HIV group had a higher frequency of CG+GG genotype compared with NAFLD group. For *TM6SF2* polymorphism, the genotype distribution and T allele frequencies were similar between groups. The data revealed that the frequencies of *HSD17B13* GG genotypes were significantly lower in NBNC HCC group compared with the Healthy control (OR 0.41; 95% CI 0.18-0.91, $P = 0.03$).

Table 1 Baseline characteristics of Healthy controls NAFLD, NAFLD with HIV, and NBNC-HCC

Characteristics	**Healthy controls (n=150)**	**NAFLD (n=150)**	**NAFLD with HIV (n=150)**	**NBNC-HCC (n=150)**	***P***
Age(years)	50 ± 8	57 ± 13	49±10	68±12	<0.001*
Sex (%)					
Male	18 (12.0)	76 (50.7)	102 (68.0)	110 (73.3)	<0.001*
Female	132 (88.0)	73 (49.3)	48(32.0)	40(26.7)	
Body mass index(BMI)	22.6±3.2	27.6 ± 4.1	24.9±3.5	24.6±4.0	<0.001*
AST (IU/L)	20±7	27 ±13	32±35	53±43	<0.001*
ALT (IU/L)	19±11	37 ±24	45±32	43±49	<0.001*
CAP (dB/m)		306±30	271±17		<0.001*
PNPLA3 rs738409					
CC/CG/GG, n	66/64//20	53/58/39	44/85/21	47/70/33	<0.001*
MAF (G allele)	0.35	0.45	0.42	0.45	

Characteristics	Healthy controls (n=150)	NAFLD (n=150)	NAFLD with HIV (n=150)	NBNC-HCC (n=150)	*P*
TM6SF2 rs58542926					
CC/CT/TT, n	119/30/1	119/28/3	111/34/5	113/31/6	0.55
MAF (T allele)	0.11	0.11	0.15	0.14	
HSD17B13 rs6834314					
AA/AG/GG, n	56/73/21	62/68/20	51/78/21	72/67/11	0.25
MAF (G allele)	0.38	0.36	0.40	0.30	

Data express as mean ± standard deviation or n (%), differences between groups were tested by one-way ANOVA as appropriate, * P < 0.05

Table 2 Genotype distribution and minor allele frequencies (MAF) of the SNPs

Polymorphisms	Healthy control (n=150)	NAFLD (n=150)	NAFLD with HIV (n=150)	NBNC HCC (n=150)	Healthy Control vs NAFLD		Healthy control vs NAFLD with HIV		Healthy Control vs NBNC HCC		NAFLD vs NAFLD with HIV		NAFLD vs NBNC HCC	
					OR (95%CI)	P	OR (95%CI)	P	OR (95%CI)	P	OR (95%CI)	P	OR (95%CI)	P
PNPLA3 rs738409														
CC	66	53	44	47	1	-	1	-	1	-	1	-	1	-
CG	64	58	85	70	1.13	0.64	1.99	0.007*	1.54	0.10	1.76	0.03*	1.36	0.25
GG	20	39	21	33	2.43	0.01*	1.58	0.22	2.32	0.01*	0.65	0.20	0.95	0.88
CG + GG	84	97	106	103	1.44	0.13	1.58	0.23	1.72	0.02*	1.31	0.27	1.20	0.46
TM6SF2 rs58542926														
CC	119	119	111	113	1	-	1	-	1	-	1	-	1	-
CT	30	28	34	31	0.93	0.81	1.21	0.49	1.09	0.77	1.30	0.36	1.17	0.60
TT	1	3	5	6	3.00	0.344	5.36	0.13	6.32	0.09	1.78	0.43	2.11	0.30
CT + TT	31	31	39	37	1.00	1.00	1.35	0.28	1.23	0.41	1.35	0.28		
HSD17B13 rs6834314														
AA	56	62	51	72	1	-	1	-	1	-	1	-	1	-
AG	73	68	78	67	0.84	0.49	1.17	0.53	0.71	0.17	1.39	0.18	0.85	0.50
GG	21	20	21	11	0.86	0.69	1.10	0.80	0.41	0.03*	1.27	0.50	0.47	0.07
AG + GG	94	88	99	78	0.85	0.47	1.16	0.55	0.65	0.06	1.37	0.19	0.76	0.25

Data expressed as n (%), OR odds ratio, CI confidence interval, *P<0.05

DISCUSSION & CONCLUSION

Genetic factors have shown a concrete association with NAFLD. Many studies showed that carrying genetic polymorphism of *PNPLA3* and *TM6SF2* leads to an increase in hepatic fat accumulation (Trepo & Valenti, 2020). On the other hand variations of *HSD17B13* have found to be related with reduced risk of liver-related complications but in some studies did not show the protective effect of *HSD17B13* variants in NAFLD. The finding in this study showed the association of genetic variants including *PNPLA3* rs738409, *TM6SF2* rs58542926, and *HSD17B13* rs6834314 in a cohort of patients related to NAFLD and patients with HCC. The variant of PNPLA3 especially rs738409 effect the lipolytic enzyme and lipid metabolism. In addition, Carrying GG genotype could be associated with NAFLD (Tai et al., 2024). The result from this study could be concluded that a genetic variant of *PNPLA3* rs738409 might be an increased risk of progression in NAFLD and several studies have proven this association (Rosso et al., 2023; Tai et al., 2024). TM6SF2 is involved in the triglyceride-VLDL secretion pathway. The rs58542926 T allele in Genetic variant of *TM6SF2* showed a positive correlation with liver steatosis. In this study did not find any significant association in *TM6SF2* rs58542926 between each group. Ting et al revealed that loss of function of *HSD17B13* rs6834314 variants was inversely associated with NAFLD in Asian patients Cohorts. *HSD17B13* function is not completely clear but it is probably associated with lipid droplets and may be involved in the lipid metabolism (Ting et al., 2021). In this study, Focusing on a variation of *HSD17B13* rs6834314 revealed that there was no significant difference between other groups except NBNC HCC and the healthy control group. In summary, G allele in the genetic variant of *PNPLA3* was associated with a higher risk of being NAFLD and NBNC HCC. For Recommendations Large sample sizes should be considered for future studies to evaluate the precise effects of *PNPLA3 TM6SF2* and *HSD17B13* genotypes on hepatic steatosis in Thai population.

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