Chula Med J Vol. 67 No. 4 October - December 2023;309-316 DOI : 10.14456/clmj.2023.37

Original article

Association of *PNPLA3, TM6SF2 and HSD17B13* variants with NAFLD risk in HIV and non-HIV individuals

Varis Ruamviboonsuk[a,*], Kamonchanok Moonlisarn[a], Pisit Tangkijvanich[a, b], Natthaya Chuaypen[a, b]

[a]Department of Biochemistry, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand
[b]Center of Excellence in Hepatitis and Liver Cancer, Department of Biochemistry, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand

Background: Non-alcoholic fatty liver disease (NAFLD) has multiple risk factors, including genetic risk factors. Patients with certain genetic variants are more susceptible to the disease. People living with human immunodeficiency virus (PLWH) have higher prevalence of NAFLD compared to those without human immunodeficiency virus (HIV) infection. However, the evidence on genetic factors in PLWH with NAFLD is limited.

Objectives: This study aimed to investigate whether carriers of *PNPLA3* rs738409, *TM6SF2* rs58542926, and *HSD17B13* rs6834314 had association with the risk of NAFLD, and whether PLWH with NAFLD also had similar genetic risk factors as those with NAFLD alone.

Methods: These single nucleotide polymorphisms (SNPs) were determined by using TaqMan allelic discrimination performed and analyzed by the Applied Biosystem platform in blood samples of 142 healthy individuals, 136 NAFLD patients, and 253 PLWH with NAFLD.

Results: The genotype distributions of *PNPLA3* rs738409 (CC *vs.* GG genotypes) was significantly different among the groups ($P < 0.05$), while no significant differences were found for the *TM6SF2* rs58542926 and *HSD17B13* rs6834314 genotype. The NAFLD group showed a higher frequency of *PNPLA3* GG genotype compared to healthy controls, associated with increased odds of NAFLD (OR 3.8; 95% CI 1.7 - 8.3). Comparing the NAFLD and PLWH with NAFLD groups, the NAFLD group had higher frequencies of CG and GG genotypes of *PNPLA3* and lower frequency of GG genotype of *HSD17B13*. The multivariate analysis revealed several factors that remained significantly different between the two groups, including age, controlled attenuated parameter, body mass index, alanine aminotransferase, high-density lipoprotein, gender, *PNPLA3* GG genotype, and *HSD17B13* GG genotype.

Conclusion: *PNPLA3* rs738409 was associated with NAFLD development while other SNPs were not significantly associated with NAFLD. The genotypes for PLWH with NAFLD were not significantly different from healthy controls.

Keywords: HIV, HSD17B13, non-alcoholic fatty liver disedse, PNPLA3, TM6SF2.

Non-Alcoholic Fatty Liver Disease (NAFLD) is a common chronic liver disease characterized by the accumulation of fat in the liver in the absence of excessive alcohol consumption or other chronic liver diseases. [1] It is one of the most common causes of liver disease with global prevalence of 25.2% [1, 2], and patients with NAFLD are at higher risk of non-alcoholic steatohepatitis (NASH) and, ultimately, hepatocellular carcinoma (HCC). [3]

NAFLD is associated with metabolic risk factors, such as, obesity, diabetes mellitus (DM) and dyslipidemia.[4] It is also associated with genetic risk factors, such as, single nucleotide polymorphisms (SNPs) of *patatin-like phospholipase domain-containing 3 (PNPLA3)* and *transmembrane 6 superfamily member 2 (TM6SF2)* genes. [5 - 8]

*Correspondence to: Varis Ruamviboonsuk, Department of Biochemistry, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand.
E-mail: Varis.ruam@gmail.com
Received: June 20, 2023
Revised: August 9, 2023
Accepted: September 1, 2023

The SNP of *PNPLA3* rs738409 has shown its association with increased hepatic lipid accumulation [7, 9], which is ultimately increased risk of NAFLD development. *TM6SF2* rs58542926 is also associated with increased risk of hepatic steatosis, and its effect is independent from *PNPLA3* rs738409's effect. [10, 11] Another interesting genetic variant associated with NAFLD is the variant of *hydroxysteroid 17-beta dehydrogenase 13* (*HSD17B13*) gene. This SNP had showed its potential effect as a protective factor against NAFLD development.[12]

People living with human immunodeficiency virus (HIV) (PLWH) exhibit a higher prevalence of NAFLD compared to those without HIV infection, with rates of 35.3% and 27.3%, respectively. [13] Furthermore, studies have demonstrated that PLWH with NAFLD are more likely to present with lower body mass index (BMI), increased hepatic steatosis, elevated serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels, and a higher risk of NASH when compared to individuals with NAFLD alone. [14, 15] The increased risk of NAFLD in PLWH might be caused by some anti-retroviral drugs (ARVs), however, nowadays, these ARVs are not used as much as in the past. Currently, there is limited research on the genetic risk factors of NAFLD in PLWH.

The aim of this study was to investigate whether carriers of *PNPLA3* rs738409, *TM6SF2* rs58542926, and *HSD17B13* rs6834314 had association with the risk of NAFLD. We also investigated the frequency of *PNPLA3, TM6SF2 and HSD17B13* variants, and their effects on NAFLD development in PLWH with NAFLD and patients with NAFLD alone.

Materials and methods

Patients and samples

Blood samples for the detection of genetic polymorphisms in this study were obtained from healthy individuals, patients with NAFLD and PLWH with NAFLD at initial presentation and were separated by centrifugation and stored at -80°C until analysis. Patients with NAFLD were randomly selected from King Chulalongkorn Memorial Hospital, Bangkok, Thailand. PLWH with NAFLD were enrolled from The HIV Netherlands Australia Thailand Research Collaboration (HIV-NAT), Bangkok, Thailand.

The inclusion criteria for patients with NAFLD alone were as follow: patients with NAFLD, age 18 years old or older, and had proton density fat fraction (PDFF) > 5.0%. The exclusion criteria for patients with NAFLD were patients with any of the following conditions: active hepatitis B virus (HBV) infection, active hepatitis C virus (HCV) infection, alcoholic liver disease, autoimmune hepatitis, cirrhosis, HCC, or with contraindication of magnetic resonance imaging (MRI).

The inclusion criteria for PLWH with NAFLD were as follow: patients living with HIV and had NAFLD, age 18 years old or older, had PDFF > 5.0%, and had viral load < 40 copies/mL. The exclusion criteria for PLWH with NAFLD were the same as those with NAFLD alone.

For the control group, we included any people with age 18 years old or older. We excluded those with any liver diseases, with HIV infection, or had contraindication of MRI.

All subjects were Asian. A total of 531 subjects were enrolled into this study: 142 healthy individuals, 136 NAFLD patients, and 253 PLWH with NAFLD.

The diagnosis of NAFLD was based on imaging studies, particularly Fibro Scan. The diagnostic criteria of NAFLD in this study was controlled attenuation parameter (CAP) of 248 dB/m or higher. Clinical and laboratory data of each participant were collected from the same date of CAP measurement. Clinical data were age, gender, BMI, CAP, and DM status. Laboratory data consisted of following biochemical blood values: serum AST, ALT, total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, and triglyceride (TG). This study have been approved by the Institute Ethics Committee (IRB no.308/66).

DNA preparation and genetic analysis

Genomic DNA was extracted from 100 µl peripheral blood mononuclear cells (PBMCs) using phenol-chloroform-isoamyl alcohol isolation method. To assess the quality of the DNA, a spectrophotometer (NanoDrop 2000c, Thermo Scientific, USA) was employed.

The targeted single nucleotide polymorphisms (SNPs), namely *PNPLA3* rs738409, *TM6SF2* rs58542926, and *HSD17B13* rs6834314, were genotyped using a real-time polymerase chain reaction (PCR) protocol based on TaqMan assays. The reaction mixture included 4 µL of 2.5 x master mix (5 PRIME, Germany), 0.25 µL of 40X primers and probes mixture TaqMan SNP Genotyping Assay (assay ID:C_7241_10, Applied Biosystems, USA), 50-100

ng of genomic DNA, and nuclease-free water, bringing the final volume to 10 µL. Real-time PCR was performed on a QuantStudio3 Real-time PCR system (Applied Biosystems, USA) following the manufacturer's instructions. The procedure involved an initial denaturation at 95 °C for 10 min, followed by 50 amplification cycles consisting of denaturation at 92 °C for 10 s and annealing/extension at 60 °C for 1 min. Fluorescent signals (FAM and VIC) were acquired at the end of each cycle. To ensure accurate data interpretation, positive and negative controls were included in every experiment. The allelic discrimination plot was analyzed using Quant Studio TM software (version 2.2, Applied Biosystems, USA).

Statistical analysis

Statistical analysis was performed by the Statistical Package for The Social Sciences (SPSS) software for MacOS 29.0 (SPSS Inc., Chicago, IL, USA). Demographic data were expressed as median and interquartile range. Group comparisons were assessed using the Chi-square test or Fisher's exact test for categorical variables, while the Mann-Whitney U test or Student's *t* - test was employed for quantitative variables. Hardy-Weinberg equilibrium (HWE) was determined using Pearson's Chi-square. Associations of different genetic polymorphisms with NAFLD risk were assessed under genotype and allele frequency models. Odd ratio (OR) with 95% confidence interval (CI) between each group were determined. The logistic regression analysis was performed to assess the factors associated with NAFLD. Clinical and laboratory parameters were dichotomized according to their median for univariate and multivariate analysis.[16] Statistical significance was set at a threshold of $P < 0.05$, indicating results with a significant impact.

Results

From a total of 531 subjects, we divided into three distinct groups: healthy controls, NAFLD, and PLWH with NAFLD. Demographic data were then compared by using median and interquartile range of each parameter, which were summarized in Table 1. All parameters (age, BMI, AST, ALT, total cholesterol, HDL cholesterol, LDL cholesterol, triglyceride, CAP, gender, DM status) were significantly different among three groups ($P < 0.05$). The NAFLD group possessed the highest age, BMI and CAP among all groups, while PLWH with NAFLD group had the highest AST, ALT and TG than other groups.

Table 1. Demographic data.

	Control (142)	NAFLD (136)	PLWH with NAFLD (253)	*P* - value
Age, years	50.0 (44.0 - 56.0)	58.0 (47.0 - 65.0)	48.0 (37.5 - 55.0)	<0.001
BMI, kg/m^2	22.2 (20.2 - 24.3)	26.9 (24.9 - 30.0)	25.0 (23.2 - 28.0)	<0.001
AST, IU/L	19.0 (15.0 - 23.0)	23.0 (20.0 - 31.0)	26.0 (20.5 - 33.5)	<0.001
ALT, IU/L	16.0 (13.0 - 22.0)	27.0 (21.0 - 51.0)	36.0 (26.0 - 53.0)	<0.001
TC, mg/dL	217.5 (200.0 - 249.0)	184.0 (162.0 - 210.0)	190.0 (167.0 - 223.0)	<0.001
HDL, mg/dL	64.0 (54.0 - 75.3)	49.0 (42.0 - 55.0)	42.0 (34.0 - 51.0)	<0.001
LDL, mg/dL	136.0 (118.8 - 162.3)	115.50 (94.8 - 147.3)	115 (92.0 - 137.0)	<0.001
TG, mg/dL	82.0 (65.0 - 107.3)	118.0 (96.0 - 140.0)	144.5 (96.0 - 212.3)	<0.001
CAP, dB/m	206.0 (192.8 - 217.0)	309.5 (286.5 - 340.0)	285.0 (260.5 - 308.5)	<0.001
Male	17.0	72.0	179.0	
Female	125.0	64.0	74.0	<0.001
No DM	132.0	99.0	187.0	
DM	10.0	37.0	66.0	<0.001

Results for age, BMI, AST, ALT, TC, HDL, LEL, TG and CAP are expressed as median (Q1 - Q3).
ALT, alanine aminotransferase; AST, aspartate aminotransferase;
BMI, body mass index; CAP, controlled attenuation parameter; DM, diabetes mellitus; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; TC, total cholesterol; TG, triglyceride.

Distribution of SNPs

The genotype frequencies of each SNP in this study were not deviated from HWE ($P > 0.05$), as shown in Table 2. The genotype distributions of *PNPLA3* rs738409 was significantly different comparing among three groups ($P = 0.001$). However, for each genotype of *TM6SF2* rs58542926 and *HSD17B13* rs6834314, there were no significant difference among groups ($P = 0.508$ and 0.279, respectively), as summarized in Table 3.

Association of SNPs in NAFLD patients and healthy controls

Our findings indicated a significantly higher frequency of the *PNPLA3* GG genotype in NAFLD patients compared with healthy controls ($P = 0.001$). Furthermore, the G allele frequency was significantly higher in the NAFLD group than the control group ($P = 0.002$). Individuals with the GG genotype exhibited increased odds of developing NAFLD compared to those with the CC genotype (OR 3.8; 95% CI 1.7 - 8.3, $P = 0.001$). Additionally, those carrying G allele had higher odds of NAFLD development than those with C allele (OR 3.1; 95% CI 1.2 - 2.4, $P = 0.003$). However, no significant differences were observed between genotypes of the *TM6SF2* and *HSD17B13* genetic variants.

Association of SNPs in PLWH with NAFLD and healthy controls

The genotyping results showed that the frequencies of *PNPLA3* rs738409, *TM6SF2* rs5854292 and *HSD17B13* rs6834314 were not significantly different between PLWH with NAFLD and healthy controls ($P = 0.185$, 0.118 and 0.667, respectively).

Association of SNPs in NAFLD patients and PLWH with NAFLD

The frequencies of CG and GG genotypes of *PNPLA3* in the NAFLD group were significantly higher than PLWH with NAFLD group ($P = 0.007$), whereas the frequency of GG genotype of *HSD17B13* was significantly lower than the other group ($P = 0.031$). The analysis revealed that subjects in the PLWH with NAFLD group with CG and GG genotypes of *PNPLA3* exhibited lower odds of NAFLD compared to those in the NAFLD group (OR 0.6; 95% CI 0.4 - 1.0, and OR 0.4; 95% CI 0.3 - 0.8, respectively). Additionally, individuals in PLWH with NAFLD group with GG genotype of *HSD17B13* had higher odds of NAFLD than those in the NAFLD group (OR 2.3, 95% CI 1.1 - 4.8).

Independent and additive effects of SNPs associated with NAFLD and HIV

Each factor with significant difference between each group were then categorized into binary variables based on their respective median. Comparisons between NAFLD group and PLWH with NAFLD group revealed significant differences in age, CAP, BMI, ALT, HDL, gender, *PNPLA3*, and *HSD17B13* genotypes ($P < 0.05$). Multivariate analysis further demonstrated that the following factors maintained their significant differences between NAFLD and PLWH with NAFLD groups: age (OR 0.3; 95% CI 0.2 - 0.5), CAP (OR 0.2; 95% CI 0.1 - 0.4), BMI (OR 0.2; 95% CI 0.1 - 0.4), ALT (OR 2.5; 95% CI 1.3 - 4.8), HDL (OR 0.3; 95% CI 0.2 - 0.5), *PNPLA3* GG genotype (OR 0.4; 95% CI 0.2 - 0.8) and *HSD17B13* GG genotype (OR 2.7; 95% CI 1.1 - 6.5). Detailed results from both univariate and multivariate analysis were summarized in Table 4.

Table 2. Hardy-Weinberg equilibrium (HWE) testing.

Single nucleotide polymorphisms	Genotype	Observed N	Expected N	*P* - value
PNPLA3	CC	235.0	222.9	
rs738409	CG	218.0	242.3	0.074
	GG	78.0	65.9	
TM6SF2	CC	411.0	406.3	
rs58542926	CT	107.0	116.3	0.217
	TT	13.0	8.3	
HSD17B13	AA	207.0	208.8	
rs6834314	AG	252.0	248.3	0.944
	GG	72.0	73.8	

Table 3. Genotype and allele frequencies of the SNPs in the healthy controls, NAFLD and NAFLD+HIV groups.

	Healthy Control n (%)	NAFLD n (%)	PLWH with NAFLD n (%)	*P*-value	HC vs NAFLD OR (95% CI)	*P*-value	HC vs PLWH with NAFLDOR (95% CI)	*P*-value	NAFLD vs PLWH with NAFLD OR (95% CI)	*P*-value
PNPLA3	rs738409									
CC	63 (44.4)	47 (34.6)	125 (49.4)		1	-	1	-	1	-
CG	68 (47.9)	58 (42.6)	92 (36.4)	0.001	1.1 (0.7 - 1.9)	0.509	0.7 (0.4 - 1.1)	0.085	0.6 (0.4 - 1.0)	0.031
GG	11 (7.7)	31 (22.8)	36 (14.2)		3.8 (1.7 - 8.3)	0.001	1.7 (0.8 - 3.5)	0.185	0.4 (0.2 - 0.8)	0.007
MAF	0.16	0.22	0.16							
Major C	194	152	342	0.002	1	-	1	-	1	-
Minor G	90	120	164		3.1 (1.2 - 2.4)	0.003	1.0 (0.8 - 1.4)	0.208	0.6 (0.5 - 0.8)	0.001
TM6SF2	rs58542926									
CC	112 (78.9)	107 (78.7)	192 (75.9)		1	-	1	-	1	-
CT	29 (20.4)	26 (19.1)	52 (20.6)	0.508	1.0 (0.5 - 1.7)	0.833	1.1 (0.7 - 1.7)	0.863	1.1 (0.7 - 1.9)	0.687
TT	1 (0.7)	3 (2.2)	9 (3.6)		3.1 (0.3 - 30.7)	0.325	5.3 (0.7 - 42.0)	0.118	1.7 (0.4 - 6.3)	0.448
MAF	0.05	0.06	0.07							
Major C	253	240	436	0.449	1	-	1	-	1	-
Minor T	31	32	70		0.7 (0.6 - 1.8)	0.752	1.3 (0.8 - 2.1)	0.24	1.2 (0.8 - 1.9)	0.415
HSD17B13	rs6834314									
AA	56 (39.4)	58 (42.6)	93 (36.8)		1	-	1	-	1	-
AG	65 (45.8)	67 (49.3)	120 (47.4)	0.279	1.0 (0.6 - 1.6)	0.985	1.1 (0.7 - 1.7)	0.644	1.1 (0.7-1.7)	0.625
GG	21 (14.8)	11 (8.1)	40 (15.8)		0.5 (0.2 - 1.1)	0.102	1.2 (0.6 - 2.1)	0.667	2.3 (1.1 - 4.8)	0.031
MAF	0.19	0.16	0.20							
Major A	177	183	306	0.171	1	-	1	-	1	-
Minor G	107	89	200		0.5 (0.6 - 1.1)	0.222	1.1 (0.8 - 1.5)	0.609	1.3 (1.0 - 1.8)	0.061

HC, healthy control; MAF, minor allele frequency; NAFLD, non-alcoholic fatty liver disease; OR, odds ratio; 95% CI = 95% confidence intervene

Table 4. Logistic Regression for NAFLD *vs.* NAFLD+HIV.

Factors	Category	Univariateanalysis	*P*-value	Multivariateanalysis	*P*-value
Age, years	≥ 50 *vs.* < 50	0.343 (0.2 - 0.5)	< 0.001	0.308 (0.2 - 0.5)	< 0.001
BMI, kg/m^2	≥ 24.75 *vs.* < 24.75	0.309 (0.2 - 0.5)	< 0.001	0.193 (0.1 - 0.4)	< 0.001
AST, IU/L	≥ 23 *vs.* < 23	1.759 (1.2 - 2.7)	0.009	1.134 (0.6 - 2.0)	0.675
ALT, IU/L	≥ 27 *vs.* < 27	2.640 (1.7 - 4.1)	< 0.001	2.504 (1.3 - 4.8)	0.005
TC, mg/dL	≥ 198 *vs.* < 198	1.241 (0.8 - 1.9)	0.321		
HDL, mg/dL	≥ 49 *vs.* < 49	0.316 (0.2 - 0.5)	< 0.001	0.284 (0.2 - 0.5)	< 0.001
LDL, mg/dL	≥ 121 *vs.* < 121	0.684 (0.5 - 1.1)	0.077		
TG, mg/dL	≥ 114 *vs.* < 114	1.401 (0.9 - 2.2)	0.123		
Gender	Male *vs.* Female	2.150 (1.4 - 3.3)	< 0.001	1.162 (0.7 - 2.1)	0.607
DM	No *vs.* DM	0.944 (0.6 - 1.5)	0.811		
CAP, dB/m	≥ 275 *vs.* < 275	0.259 (0.2 - 0.4)	< 0.001	0.226 (0.1 - 0.4)	< 0.001
PNPLA3	CC *vs.* CG	0.596 (0.4 - 1.0)	0.031	0.598 (0.3 - 1.1)	0.077
	CC *vs.* GG	0.437 (0.2 - 0.8)	0.006	0.403 (0.2 - 0.8)	0.014
HSD17B13	AA *vs.* AG	1.117 (0.7 - 1.7)	0.625	1.203 (0.7 - 2.1)	0.501
	AA *vs.* GG	2.269 (1.1 - 4.8)	0.031	2.69 (1.1 - 6.5)	0.028

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CAP, controlled attenuation parameter; DM, diabetes mellitus; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; TC, total cholesterol; TG, triglyceride.

Discussion

This study aimed to compare three groups with different NAFLD status: healthy controls, patients with NAFLD alone, and PLWH with NAFLD. The findings provided an overview of the demographic data and genetic associations among these groups. The observed variations in age, BMI, AST, ALT, total cholesterol, HDL cholesterol, LDL cholesterol, TG levels, CAP values, gender distribution and presence of DM between groups were likely attributable to their association with NAFLD risk factors. It should be noted that CAP was utilized as a diagnostic criterion in this study, thus resulting in expected differences between individuals with NAFLD and healthy controls.

Our study revealed interesting results comparing healthy controls with subjects with NAFLD alone. Previously, *PNPLA3* rs738409 has been extensively studied, and is known to play an important role in lipid metabolism [17] and hepatic steatosis. [5] The association between *PNPLA3* rs738409 and an increased risk of NAFLD observed in our study aligned with previous research studies in European and Asian study cohorts. [7, 18, 19] This might hint that the frequency of *PNPLA3* rs7389409 was similar across Asian and European population.

Additionally, the G minor allele of *PNPLA3* rs738409 was associated with an increased risk of NAFLD, which also aligned with a previous study by Paternostro R, *et al.* [19] The G allele of *PNPLA3* rs738409 could alter the normal function of PNPLA3 protein in hepatocytes which exhibits triacylglycerol hydrolase activity. Therefore, this G allele could promote increased triglyceride concentration in hepatocytes by lowering the function of this protein. This supports the role of *PNPLA3* genetic variations as a genetic risk factor of NAFLD.

On the other hand, comparing between healthy controls and those with NAFLD alone, our study did not find significant associations between NAFLD and the SNPs of *TM6SF2* and *HSD17B13* genes, while these genes have also been implicated in NAFLD development in previous studies. [19-22] Although there was not statistically significant, there was a slight trend indicating an increasing risk of NAFLD development as the heterozygotes of *TM6SF2* had higher odds compared to major allele homozygotes, and the minor allele homozygotes had even higher odds. A similar trend on decreasing risk of NAFLD can also be found with *HSD17B13* rs6834314.[12] This trend aligns with the results from a previous study in Asian ethnic group by Sookoian S, *et al*. on *TM6SF2,*[11] in European ethnic group by Paternostro R, *et al.*, [19] and in Chinese ethnic group by Ting YW, *et al.* on *HSD17B13.*[23] This trend suggests a potential genetic predisposition that might contribute to the risk of NAFLD development, and might suggest that the frequency of these variants might be similar among

the mentioned ethnic groups. The lack of association observed in our study may be attributed to the smaller sample size compared to other studies with more subjects.[12] Future studies with larger sample sizes should be necessary to validate the significance of this trend.

Comparing between subjects with NAFLD alone and PLWH with NAFLD, our study showed interesting results. The frequencies of *PNPLA3* rs738409 and *HSD17B13* rs6834314 homozygous minor allele genotypes were significantly lower in PLWH with NAFLD than patients with NAFLD alone. The multivariate analysis showed that age, BMI, serum ALT and HDL levels, *PNPLA3* GG genotype and *HSD17B13* GG genotype remained associated with risk of NAFLD. Age, BMI, serum HDL level and *PNPLA3* GG genotype were lower among PLWH with NAFLD, which were consistent with previous studies by Vodkin I, *et al*. [14] Additionally, the genetic frequencies of these genes were also not significantly different between PLWH with NAFLD and healthy controls. These findings suggested that factors other than these specific genetic variants might contribute to the development of NAFLD among PLWH. However, with the limited number of research studies in PLWH with NAFLD and these genetic variants, it is rather challenging to bring in the conclusion, especially in other ethnic populations. Further research focusing on the genetic factors associated with NAFLD in PLWH is necessary, and the inclusion of a cohort comprising PLWH with and without NAFLD would be beneficial for future studies in this area.

This study evaluated subjects with NAFLD alone, PLWH with NAFLD, and healthy controls altogether. Cases and controls were identified from the same standard. However, there were some limitations in this study. The main weaknesses of this study include its' retrospective design and cross-sectional nature. The relatively small sample size affected our analysis. *TM6SF2* rs58542926 had a very small minor allele frequency, and with our small sample size, we found very few homozygous minor allele genotypes, thus we could see only some trend but not statistical significance.

Conclusion

PNPLA3 GG genotype was associated with higher NAFLD risk. However, in individuals living with HIV (PLWH), additional factors related to the disease and external influences may contribute to the development of NAFLD. To obtain more conclusive results, future studies should include larger sample sizes and conduct more comprehensive analyses to determine the relative importance of various factors in NAFLD development among PLWH.

Acknowledgements

We thank all members of the Center of Excellence in Hepatitis and Liver Cancer, Department of Biochemistry, Faculty of Medicine, Chulalongkorn University for their collaborative effort during data collection and participation in the study.

Conflict of interest statement

Each of the authors has completed an ICMJE disclosure form. None of the authors declare any potential or actual relationship, activity, or interest related to the content of this article.

Data sharing statement

The present review is based on the reference cited. Further details, opinions, and interpretation are available from the corresponding authors on reasonable request.

References

1. Powell EE, Wong VW, Rinella M. Non-alcoholic fatty liver disease. Lancet. 2021;397(10290):2212-24.
2. Anstee QM, McPherson S, Day CP. How big a problem is non-alcoholic fatty liver disease? BMJ 2011;343: d3897.
3. Shah PA, Patil R, Harrison SA. NAFLD-related hepatocellular carcinoma: The growing challenge. Hepatology 2023;77:323-38.
4. Eslam M, Sanyal AJ, George J. MAFLD: A consensus-driven proposed nomenclature for metabolic associated fatty liver disease. Gastroenterology 2020;158:1999-2014.e1.
5. Sookoian S, Pirola CJ. Meta-analysis of the influence of I148M variant of patatin-like phospholipase domain containing 3 gene (PNPLA3) on the susceptibility and histological severity of nonalcoholic fatty liver disease. Hepatology 2011;53:1883-94.
6. Smagris E, BasuRay S, Li J, Huang Y, Lai KM, Gromada J, et al. Pnpla3I148M knockin mice accumulate PNPLA3 on lipid droplets and develop hepatic steatosis. Hepatology 2015;61:108-18.
7. Arslanow A, Stokes CS, Weber SN, Grünhage F, Lammert F, Krawczyk M. The common PNPLA3 variant p.I148M is associated with liver fat contents as quantified by controlled attenuation parameter (CAP). Liver Int 2016;36:418-26.

8. Arab JP, Arrese M, Trauner M. Recent insights into the pathogenesis of nonalcoholic fatty liver disease. Annu Rev Pathol 2018;13:321-50.
9. Bruschi FV, Claudel T, Tardelli M, Caligiuri A, Stulnig TM, Marra F, et al. The PNPLA3 I148M variant modulates the fibrogenic phenotype of human hepatic stellate cells. Hepatology 2017;65:1875-90.
10. Liu YL, Reeves HL, Burt AD, Tiniakos D, McPherson S, Leathart JB, et al. TM6SF2 rs58542926 influences hepatic fibrosis progression in patients with non-alcoholic fatty liver disease. Nat Commun 2014;5:4309.
11. Sookoian S, Castaño GO, Scian R, Mallardi P, Fernández Gianotti T, Burgueño AL, et al. Genetic variation in transmembrane 6 superfamily member 2 and the risk of nonalcoholic fatty liver disease and histological disease severity. Hepatology 2015;61:515-25.
12. Abul-Husn NS, Cheng X, Li AH, Xin Y, Schurmann C, Stevis P, et al. A Protein- Truncating HSD17B13 Variant and Protection from chronic liver disease. N Engl J Med 2018;378:1096-106.
13. Maurice JB, Patel A, Scott AJ, Patel K, Thursz M, Lemoine M. Prevalence and risk factors of nonalcoholic fatty liver disease in HIV-monoinfection. AIDS 2017; 31:1621-32.
14. Vodkin I, Valasek MA, Bettencourt R, Cachay E, Loomba R. Clinical, biochemical and histological differences between HIV-associated NAFLD and primary NAFLD: a case-control study. Aliment Pharmacol Ther 2015;41:368-78.
15. Papagianni M, Tziomalos K. Non-Alcoholic Fatty Liver Disease in Patients with HIV Infection. AIDS Rev. 2018;20:171-3.
16. Pourhoseingholi MA, Baghestani AR, Vahedi M. How to control confounding effects by statistical analysis. Gastroenterol Hepatol Bed Bench 2012;5:79-83.
17. Park J, Zhao Y, Zhang F, Zhang S, Kwong AC, Zhang Y, et al. IL-6/STAT3 axis dictates the PNPLA3-mediated susceptibility to non-alcoholic fatty liver disease. J Hepatol 2023;78:45-56.
18. Dai G, Liu P, Li X, Zhou X, He S. Association between PNPLA3 rs738409 polymorphism and nonalcoholic fatty liver disease (NAFLD) susceptibility and severity: A meta-analysis. Medicine (Baltimore) 2019;98: e14324.
19. Paternostro R, Staufer K, Traussnigg S, Stättermayer AF, Halilbasic E, Keritam O, et al. Combined effects of PNPLA3, TM6SF2 and HSD17B13 variants on severity of biopsy-proven non-alcoholic fatty liver disease. Hepatol Int. 2021;15:922-33.
20. Wang X, Liu Z, Peng Z, Liu W. The TM6SF2 rs58542926 T allele is significantly associated with non-alcoholic fatty liver disease in Chinese. J Hepatol 2015;62: 1438-9.
21. Kozlitina J, Smagris E, Stender S, Nordestgaard BG, Zhou HH, Tybjærg-Hansen A, et al. Exome-wide association study identifies a TM6SF2 variant that confers susceptibility to nonalcoholic fatty liver disease. Nat Genet 2014;46:352-6.
22. Seko Y, Yamaguchi K, Tochiki N, Yano K, Takahashi A, Okishio S, et al. Attenuated effect of PNPLA3 on hepatic fibrosis by HSD17B13 in Japanese patients with non-alcoholic fatty liver disease. Liver Int 2020;40:1686-92.
23. Ting YW, Kong AS, Zain SM, Chan WK, Tan HL, Mohamed Z, et al. Loss-of-function HSD17B13 variants, non-alcoholic steatohepatitis and adverse liver outcomes: Results from a multi-ethnic Asian cohort. Clin Mol Hepatol 2021;27:486-98.