

World Journal of *Hepatology*

World J Hepatol 2020 December 27; 12(12): 1136-1371



MINIREVIEWS

- 1136 Spontaneous bacterial peritonitis due to carbapenemase-producing *Enterobacteriaceae*: Etiology and antibiotic treatment
Fiore M, Di Franco S, Alfieri A, Passavanti MB, Pace MC, Petrou S, Martora F, Leone S
- 1148 Molecular heterogeneity in intrahepatic cholangiocarcinoma
Ahn KS, Kang KJ
- 1158 Spectrum of esophageal motility disorders in patients with liver cirrhosis
Khalaf M, Castell D, Elias PS
- 1168 Metabolic associated fatty liver disease: Addressing a new era in liver transplantation
Gill MG, Majumdar A
- 1182 Liver injury in COVID-19: The hepatic aspect of the respiratory syndrome — what we know so far
Anirvan P, Bharali P, Gogoi M, Thuluvath PJ, Singh SP, Satapathy SK

ORIGINAL ARTICLE**Basic Study**

- 1198 Cyclin-dependent kinase inhibitors p21 and p27 function as critical regulators of liver regeneration following 90% hepatectomy in the rat
Moniaux N, Lacaze L, Gothland A, Deshayes A, Samuel D, Faivre J
- 1211 Pivotal role of long non-coding ribonucleic acid-X-inactive specific transcript in regulating immune checkpoint programmed death ligand 1 through a shared pathway between miR-194-5p and miR-155-5p in hepatocellular carcinoma
Atwa SM, Handoussa H, Hosny KM, Odenthal M, El Tayebi HM

Case Control Study

- 1228 Validation of genetic variants associated with metabolic dysfunction-associated fatty liver disease in an ethnic Chinese population
Lee GH, Phyo WW, Loo WM, Kwok R, Ahmed T, Shabbir A, So J, Koh CJ, Hartono JL, Muthiah M, Lim K, Tan PS, Lee YM, Lim SG, Dan YY

Retrospective Cohort Study

- 1239 Comparison between hepatocellular carcinoma prognostic scores: A 10-year single-center experience and brief review of the current literature
Campigotto M, Giuffrè M, Colombo A, Visintin A, Aversano A, Budel M, Masutti F, Abazia C, Crocè LS

Retrospective Study

- 1258 Effects of proprotein convertase subtilisin/kexin type-9 inhibitors on fatty liver
Shafiq M, Walmann T, Nutalapati V, Gibson C, Zafar Y

- 1267 Timing of paracentesis and outcomes in hospitalized patients with decompensated cirrhosis
Tocia C, Dumitru A, Alexandrescu L, Popescu R, Dumitru E

- 1276 Bioelectrical impedance vector analysis evaluates cellularity and hydration in cirrhotic patients
Fernandes SA, Leonhardt LR, da Silva DM, Alves FD, Marroni CA

- 1289 Incidental biliary dilation in the era of the opiate epidemic: High prevalence of biliary dilation in opiate users evaluated in the Emergency Department
Barakat MT, Banerjee S

Clinical Trials Study

- 1299 Effect of non-alcoholic beer, diet and exercise on endothelial function, nutrition and quality of life in patients with cirrhosis
Macías-Rodríguez RU, Ruiz-Margáin A, Román-Calleja BM, Espin-Nasser ME, Flores-García NC, Torre A, Galicia-Hernández G, Rios-Torres SL, Fernández-del-Rivero G, Orea-Tejeda A, Lozano-Cruz OA

Observational Study

- 1314 HIPPOCRATES® project: A proof of concept of a collaborative program for hepatitis C virus micro-elimination in a prison setting
Gaspar R, Liberal R, Tavares J, Morgado R, Macedo G

Prospective Study

- 1326 Subclinical proximal tubulopathy in hepatitis B: The roles of nucleot(s)ide analogue treatment and the hepatitis B virus
Brayette A, Essig M, Carrier P, Debette-Gratien M, Labrunie A, Alain S, Maynard M, Ganne-Carrié N, Nguyen-Khac E, Pinet P, De Ledinghen V, Renou C, Mathurin P, Vanlemmens C, Di Martino V, Gervais A, Foucher J, Isabelle FH, Vergniol J, Hourmand-Ollivier I, Cohen D, Duval X, Poynard T, Bardou M, Abergel A, Dao MT, Thévenot T, Hiriart JB, Canva V, Lassailly G, Aurières C, Boyer N, Thabut D, Bernard PH, Schnee M, Larrey D, Hanslik B, Hommel S, Jacques J, Loustaud-Ratti V

CASE REPORT

- 1341 Safety and efficacy of sofosbuvir/velpatasvir/voxilaprevir in post-liver transplant patients with previous direct-acting antiviral failure: Six case reports
Higley C, Hsu CC, Smith C, Nadella S, Lalos AT
- 1349 Successful hepatic resection for recurrent hepatocellular carcinoma after lenvatinib treatment: A case report
Yokoo H, Takahashi H, Hagiwara M, Iwata H, Imai K, Saito Y, Matsuno N, Furukawa H
- 1358 Hepatitis E virus re-infection accelerates hepatocellular carcinoma development and relapse in a patient with liver cirrhosis: A case report and review of literature
Lin XN, Lin QX, Li SM, Xie KP, Hou J, Chen R

LETTER TO THE EDITOR

- 1367 Autophagy related protein 9A increase in hepatitis B virus-associated hepatocellular carcinoma and the role in apoptosis
Kimkong I, Kunanopparat A

ABOUT COVER

Editor-in-Chief of *World Journal of Hepatology*, Dr. Nikolaos Pyrsopoulos, MD, PhD, MBA, FACP, AGAF, FAASLD, FRCP, FACC, currently serves Professor of Medicine, Professor of Physiology, Pharmacology and Neuroscience, and Chief of Gastroenterology & Hepatology at New Jersey Medical School and the Medical Director of Liver Transplantation for University Hospital (United States). Dr. Pyrsopoulos is board certified in the areas of Internal Medicine, Gastroenterology and Transplant Hepatology. Dr. Pyrsopoulos is a Fellow of the American College of Physicians, American Gastroenterological Association, Royal College of Physicians at Edinburgh, and American Association of the Study of Liver Diseases. He is also a member of various medical associations, such as the European Association of the Study of the Liver, American Society of Gastrointestinal Endoscopy, and American Society of Transplantation, among others. (L-Editor: Filipodia)

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WJH mainly publishes articles reporting research results and findings obtained in the field of hepatology and covering a wide range of topics including chronic cholestatic liver diseases, cirrhosis and its complications, clinical alcoholic liver disease, drug induced liver disease autoimmune, fatty liver disease, genetic and pediatric liver diseases, hepatocellular carcinoma, hepatic stellate cells and fibrosis, liver immunology, liver regeneration, hepatic surgery, liver transplantation, biliary tract pathophysiology, non-invasive markers of liver fibrosis, viral hepatitis.

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Case Control Study

Validation of genetic variants associated with metabolic dysfunction-associated fatty liver disease in an ethnic Chinese population

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Abstract

BACKGROUND

Genetic factors play an important role in the pathogenesis and development of metabolic dysfunction-associated fatty liver disease (MAFLD).

AIM

To study the association of single nucleotide polymorphisms (SNPs), previously identified in Western populations, with the risk of MAFLD in a Singapore Chinese population and their interactions with environmental and medical risk factors.

METHODS

A retrospective case-control study was conducted with 72 MAFLD cases and 72 controls with no hepatic steatosis on computed tomography, magnetic resonance imaging, or controlled attenuation parameter score. Subjects were recruited from two tertiary hospitals. Genetic alleles such as *NCAN*, *GCKR*, *LYPLAL1*, *PNPLA3*, *PPP1R3B*, *FDFT1*, *COL13A1*, *EFCAB4B*, *PZP*, and *TM6SF2* were genotyped using the TaqMan® Predesigned SNP Genotyping Assay.

Institutional review board

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RESULTS

Weight and body mass index (BMI) were 1.2-times higher in patients (70.6 kg, 95% confidence interval [CI]: 57.1-84.1 *vs* 60.8 kg, 95%CI: 48.5-73.1, $P < 0.001$ and 26.9 kg, 95%CI: 23-40.8 *vs* 23.3 kg 95%CI: 19-27.6, $P < 0.001$ respectively). The prevalence of diabetes mellitus in patients was 40.3% and 20.8% in controls ($P = 0.011$). Patients had higher mean triglycerides than controls ($P < 0.001$). *PNPLA3* GG was more likely to be associated with MAFLD (43.4% CC *vs* 69.7% GG, $P = 0.017$, and 44.8% CG *vs* 69.7% GG, $P = 0.022$). In multivariable analysis, hypertriglyceridemia (odds ratio [OR]: 2.04 95%CI: 1.3-3.1, $P = 0.001$), BMI (OR: 1.2 95%CI: 1.1-1.4, $P < 0.001$) and *PNPLA3* GG (OR: 3.4 95%CI: 1.3-9.2, $P = 0.014$) were associated with MAFLD (area under the receiver operating characteristic curve of 0.823).

CONCLUSION

Among the Chinese population of Singapore, *PNPLA3* homozygous GG allele is a strong predictor of MAFLD, whereas *LYPLAL1*, *GCKR*, *FDFT1*, *COL13A1*, *PZP*, and *TM6SF2* are not significantly associated. Hypertriglyceridemia, high BMI, and *PNPLA3* GG are independent predictors of MAFLD.

Key Words: Single nucleotide polymorphism; *PNPLA3*; Genotyping; Metabolic dysfunction-associated fatty liver disease; Non-alcoholic steatohepatitis; Hypertriglyceridemia; Body mass index; Waist-hip ratio; Screening; Hepatic steatosis

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Core Tip: A number of genetic variations (known as single nucleotide polymorphism, SNPs) are reportedly associated with metabolic dysfunction-associated fatty liver disease (MAFLD), mostly by studies from Europe and America. This study examined 10 of the most important SNPs in a Chinese population in Singapore, and found that 1 such variation, the *PNPLA3* GG variation, is strongly linked to MAFLD, whereas the rest are not significantly associated. *PNPLA3*, together with high triglyceride and elevated body mass index, are found to be independent, strong predictors of MAFLD.

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INTRODUCTION

Metabolic dysfunction-associated fatty liver disease (MAFLD) is increasingly recognized as a leading cause of liver morbidity and mortality, and has emerged as the most common chronic liver disease^[1]. The increasing prevalence of MAFLD is associated with the epidemic surge in obesity and metabolic syndrome^[2]. The estimated prevalence of MAFLD in Asia is about 27.4%^[1]. MAFLD is a clinicopathological spectrum that consists of hepatic steatosis and non-alcoholic steatohepatitis (NASH), with up to 20% of NASH patients progressing to cirrhosis and end-stage liver complications^[3-5]. This heralds an expectant future epidemic of hepatocellular carcinoma (HCC) caused by NASH, potentially overshadowing the role of viral hepatitis in the development of HCC^[6-8]. Reflecting this trend, NASH is the most rapidly growing indication for liver transplantation in the United States, increasing 4-fold from 2002 to 2012, and is poised to become the leading indication^[9].

The pathogenesis of MAFLD is multifactorial and complex, and both genetic and epigenetic factors appear to play vital roles in its development. These factors interact with environmental, dietary, and metabolic risk factors, which all contribute to the development of MAFLD and the risk of disease progression. Different genes encode proteins involved in the regulation of lipid metabolism in the liver^[10]. Excess of hepatic

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triglycerides (TGs) associated with insulin resistance is a key mechanism in MAFLD pathophysiology. Adipose tissue insulin resistance is linked with lower circulating adipokines and this leads to increased lipolysis with resultant oxidative stress, lipotoxicity and apoptosis, thus inducing NASH^[11,12].

A multitude of studies have demonstrated a heritable component and familial clustering as important factors in MAFLD. The majority of these studies have been published in the western population. First-degree relatives of individuals with MAFLD have conferred a higher risk of the disease compared to the general population^[13]. Twin studies have shown that genetic factors contribute up to 60% of variability in alanine aminotransferase (ALT) levels and hepatic fat content in subjects^[14]. Over the years, multiple genetic single nucleotide polymorphisms (SNPs) associated with MAFLD have been studied. *PNPLA3* is the major gene associated with hepatic TG content, increasing susceptibility to more aggressive forms of MAFLD and HCC^[15-18]. Specifically, the variant allele rs738409 C>G or I148M of *PNPLA3* is deemed an important genetic factor for a predisposition towards progressive MAFLD and increases hepatic TG accumulation^[15]. The genetic association with *PNPLA3* is also evident in several Asian studies and is estimated to be present in 13%-19% of the general population^[19-21]. In a Chinese study, *PNPLA3* had a stronger correlation with hepatic steatosis in non-obese individuals without metabolic syndrome^[20]. Other SNPs associated with progressive MAFLD include *TM6SF2*, *LYPLAL1*, *NCAN*, *APOB*, *MBOAT7*, *LPIN1*, *GCKR*, *ENPP1*^[22]. The effect size and allele frequency of these genetic variants have shown much diversity across different ethnicities leading to racial and ethnic differences in MAFLD prevalence^[23]. To date, these genetic variants have not been evaluated in our local population.

In this study, we evaluated the association of SNPs, previously identified in Western populations, and environmental and medical risk factors with the risk of MAFLD in a Singapore Chinese population.

MATERIALS AND METHODS

The study was conducted using a retrospective case-control design recruiting subjects from the National University Hospital (NUH) and Khoo Teck Puat Hospital in Singapore. All participants gave informed consent and the study was approved by the Institutional Review Board.

Inclusion criteria

Fatty liver was identified based on computed tomography (CT) or magnetic resonance imaging (MRI) or histopathology of liver (> 20% steatosis) or controlled attenuation parameter (CAP) score. CAP is a non-invasive tool for the detection of hepatic steatosis but is limited by body mass index (BMI). CAP value of S2-3 ($\geq 34\%$ steatosis) is considered as positive hepatic steatosis and S0 (< 5% steatosis) as no steatosis. The enrolled subjects in this study were Singapore residents of Chinese ancestry. These subjects also do not have other chronic liver etiology. The control group will either have a CT or MRI scan or CAP score showing the absence of hepatic steatosis (performed for non-liver disease indications) within 1 year of enrolment into the study.

Exclusion criteria

Patients with secondary causes of steatosis including alcohol abuse, total parenteral nutrition, hepatitis B and hepatitis C virus infection, and the use of drugs known to precipitate hepatic steatosis were excluded. In addition, patients with any of the following diseases such as autoimmune hepatitis, drug-induced liver disease, primary biliary cirrhosis, and primary sclerosing cholangitis were excluded from participation in this study.

Clinical data collection

Subjects underwent an assessment comprising anthropometric measurements and a questionnaire on health-related behaviors such as smoking and alcohol drinking habits. Smokers are defined as subjects who had smoked at least 100 cigarettes during their lifetime and are henceforth classified as smokers *vs* non-smokers. With regards to drinking history, the study was confined to men and women who drank less than 140 g or 70 g of alcohol per week respectively. Body measurements including weight and height were measured in a standardized fashion by a trained examiner. BMI was calculated as follows: Weight (kg) divided by height squared (m^2). Overweight and/or

obesity were defined as having a BMI > 23 kg/m² and > 28 kg/m² respectively. Venous blood samples were drawn for biochemical and genotyping analyses. Total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), TG, fasting plasma glucose (FPG), and liver function tests were measured by standard clinical laboratory techniques in NUH Biochemistry Laboratory. Reduced HDL-C, hypertriglyceridemia, and raised FPG were diagnosed according to the International Diabetes Federation consensus worldwide definition of the metabolic syndrome.

SNP genotyping

Blood samples obtained from the subjects were centrifuged at 1500 rpm for 10 min. The buffy coat layer was separated and transferred into a 1.5-mL centrifuge tube. Genomic DNA was extracted from the concentrated lymphocytes of the buffy coat using the QIAamp DNA Mini Kit (Qiagen, Hilden, German). The related genetic alleles such as *NCAN*, *GCKR*, *LYPLAL1*, *PNPLA3*, *PPP1R3B*, *FDFT1*, *COL13A1*, *EFCAB4B*, *PZP*, and *TM6SF2* were genotyped using the TaqMan® Predesigned SNP Genotyping Assay (Applied Biosystems, Foster City, CA, United States) on a step one real-time PCR instrument (Applied Biosystems). The total reaction volume for each well was 10 µL containing 5 µL universal mastermix (Applied Biosystems), 0.5 µL assay mix, 3.5 µL distilled water, and 1 mL genomic DNA. The plate was set up at 95 °C holding stage for 20 s, 45 cycles of 95 °C denaturation for 3 s and 60 °C annealing for 20 s and run on a fast reaction (40 min for each run). Negative controls were introduced for every run to ensure genotyping quality.

The pathologist, radiologist, and laboratory technologist, who performed the tests for hepatic steatosis and SNPs, were blinded to the patients' participation in this study. The laboratory team performing and interpreting the SNP assay was blinded to the identity and grouping of the patients and samples.

Statistical analysis

The data were analyzed using SPSS (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY, United States) and $P < 0.05$ was considered statistically significant. All values are presented as the mean ± standard deviation (SD) for continuous data and as frequency and percent for categorical data. For normally distributed variables (age, BMI, waist circumference, ALT, HDL cholesterol, LDL cholesterol, TC, TG, and systolic blood pressure), two independent sample *t*-test was performed to compare between NASH and simple steatosis. Categorical variables were compared between cases and controls using Chi-square or Fisher's exact test, where appropriate. Multivariable logistic regression analysis was performed to investigate the association of risk factors and SNPs with MAFLD outcome. Area under the curve, positive predictive value and negative predictive value were assessed using receiver operating characteristic analysis.

RESULTS

In total, 150 patients were screened initially; however, 6 were lost to follow-up after recruitment and were removed from the analysis; 72 MAFLD subjects and 72 control subjects completed the study. Among the cases, the methods used to diagnose fatty liver disease were CT (54%); biopsy (24%); combination of ultrasound, laboratory results and clinical features (11%); MRI (10%); and CAP score (1%). Majority (99%) of controls were identified based on CT and MRI results. The mean age was 57 years and 61 years for the NAFLD and control group respectively. 59.7% of the patients in each group were male. 11.8% were smokers. None of the patients in both groups drank more than the 140 g (male) or 70 g (female) of alcohol per week.

The subjects who had MAFLD were more likely to have BMI higher than 24.9 kg/m² (27, 95% confidence interval [CI]: 23-31 *vs* 23, 95% CI: 19-28, $P < 0.001$). While waist-hip ratio was similar between the two groups (0.94, 95% CI: 0.9-1.0 *vs* 0.95, 95% CI: 0.8-1.1), lipid profiles were significantly higher in the MAFLD group than in the control group. Mean TC in MAFLD group was 4.4 (95% CI: 2.9-5.8) while in control was 3.4 (95% CI: 1.2-5.7), $P = 0.0032$, and mean TG in MAFLD group was 1.7 (95% CI: 0.8-2.7) while in control was 1.0 (95% CI: 0.1-1.98), $P < 0.0012$, (Table 1). 36% of MAFLD patients were taking lipid-lowering agents while only 24% in the control group were. Similar proportion of each group had hypertension (35% in MAFLD group *vs* 31% in control group, $P = 0.594$) and were on anti-hypertensive medication. There were 40% of patients who had diabetes mellitus in MAFLD group compared to 21% in the

Table 1 Patients' demographics and clinical characteristics

	NAFLD patient, <i>n</i> = 72	Control, <i>n</i> = 72	Total, <i>n</i> = 144	<i>P</i> value
Clinical characteristics				
Age	57 (46-68)	61 (48-74)	59 (46-71)	0.037 ²
Male, %	43 (59.7%)	43 (59.7%)	86 (59.7%)	1.0 ¹
BMI, kg/m ²	27 (23-31)	23 (19-28)	25 (21-30)	< 0.001 ²
Waist-hip ratio	0.94 (0.9-1.0)	0.95 (0.8-1.1)	0.9 (0.8-1.0)	0.587 ²
Smoker, %	5 (6.9%)	12 (16.7%)	17 (11.8%)	0.071 ¹
DM, %	29 (40.3%)	15 (20.8%)	44 (30.6%)	0.011 ¹
Hypertension, %	25 (34.7)	22 (30.6%)	47 (32.6%)	0.594 ¹
On anti-diabetic treatment	26 (36.1)	14 (19.4)	40 (27.8)	0.026 ¹
On lipid-lowering treatment	26 (36.1)	17 (23.6)	43 (29.9)	0.101 ¹
Laboratory measures				
Serum lipid levels and fasting glucose				
Total cholesterol, mmol/L	4.4 (2.9-5.8)	3.4 (1.2-5.7)	3.9 (1.9-5.8)	0.0032 ²
LDL-C, mmol/L	2.5 (1.5-3.5)	2.1 (0.7-3.5)	2.3 (1.1-3.6)	0.0492 ²
HDL-C, mmol/L	1.3 (0.6-1.9)	1.0 (0.3-1.7)	1.6 (0.5-1.8)	0.0292 ²
TG, mmol/L	1.7 (0.8-2.7)	1.0 (0.1-1.98)	1.4 (0.4-2.4)	< 0.0012 ²
FPG, mmol/L	6.1 (5.6-6.6)	5.9 (5.5-6.3)	6.0 (5.7-6.3)	0.4592 ²
Liver function test				
Albumin, g/L	40.9 (32.1-49.7)	41.0 (34.6-47.3)	40.9 (33.3-48.6)	0.97422
Bilirubin, mmol/L, Median	13.1 (4.5-21.7), Median 11.0	14.7 (-15.6-44.97), Median 9.0	13.9 (-8.3-36.1), Median 10.0	0.6672 ²
AST, U/L	37.1 (16.7-57.5)	26.2 (7.4-44.9)	31.7 (11.4-51.98)	0.0012 ²
ALT, U/L	46.2 (15.5-76.8)	22.2 (8.2-36.1)	34.3 (7.6-60.9)	< 0.0012 ²
ALP, U/L	87.6 (36.2-138.9)	77.4 (39.95-114.8)	82.5 (37.4-127.6)	0.1792 ²
INR	0.9 (0.5-1.2)	0.8 (0.2-1.3)	0.8 (0.3-1.3)	0.3732 ²
Platelet as 10 ⁹ /L	223 (130-315)	256 (162-350)	240 (146-334)	0.0402 ²

Categorical variables were in number (%) and continuous variables in range (95% confidence interval).

¹Chi-squared test.

²Student's *t*-test. ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; BMI: Body mass index; DM: Diabetes mellitus; HDL-C: High-density lipoprotein cholesterol; FPG: Fasting plasma glucose; INR: International normalized ratio; LDL-C: Low-density lipoprotein cholesterol; NAFLD: Nonalcoholic fatty liver disease; TGs: Triglycerides.

control group ($P = 0.011$). However, there was no difference in fasting glucose between the two groups (6.1, 95%CI: 5.6-6.6 *vs* 5.9, 95%CI: 5.5-6.3) since most patients were treated with anti-diabetic medication. Both aspartate aminotransferase (AST) and ALT levels were observed to be higher in MAFLD patients than in the controls (Table 1).

A total of 9 SNPs were tested and *PNPLA3* G allele is more likely to be associated with MAFLD compared to C allele (GG *vs* CC OR: 3.0 [95%CI: 1.2-7.5] and GG *vs* CG OR: 2.83 [95%CI: 1.1-6.9]). Some homozygous alleles of *NCAN*, *PPP1R3B*, and *EFCAB4B* that were proven significantly associated with MAFLD in the west are found to be absent among the study population. For instance, *NCAN* homozygous TT allele, *PPP1R3B* homozygous GG allele and *EFCAB4B* homozygous TT allele were not found in any of the recruited MAFLD and non-MAFLD participants. Furthermore, there were some alleles in which each was present only among the minority (< 3%) of the study population, particularly for allele *PPP1R3B* AG, *EFCAB4B* CT, *PZP* CT, *LYPLAL1* TT and *TM6SF2* TT. Despite the high presentation among the MAFLD patient group, four SNPs were not significantly associated with the presence of MAFLD. Two of the insignificant SNPs could be due to small sample sizes as mentioned above in *EFCAB4B* CT *vs* CC (odds ratio [OR]: 3.09, 95%CI: 0.31-30.40) and

PPP1R3B AA *vs* AG (OR: 3.09, 95%CI: 0.31-30.4). Another two insignificant alleles were *COL13A1* AA *vs* GG (OR: 2.3, 95%CI: 0.4-13.1) and *PZP* CC *vs* TT (OR: 2.0, 95%CI: 0.9-4.4) (Figure 1).

We performed multivariable analysis of risk factors and SNPs, independent predictors of MAFLD were hypertriglyceridemia (OR: 2.3 95%CI: 1.3-4.1), BMI (OR: 1.2 95%CI: 1.1-1.4) and *PNPLA3* GG (OR: 4.1 95%CI: 1.3-12.9) (Table 2). This model has a cut-off value of 0.47 (based on Youden index) that can predict 73.8% of positive cases with an area under the receiver operating characteristic curve (AUROC) of 0.823 (95%CI: 0.73-0.91, $P < 0.001$) (Figure 2). Without the *PNPLA3*, the model performance in the prediction of MAFLD was lower with the AUROC of 0.789 (95%CI: 0.71-0.86, $P < 0.001$).

DISCUSSION

In this study, *PNPLA3* GG allele was the only SNP found to be associated with progressive MAFLD in the Singaporean Chinese population. The surprising finding is none of the other SNP variants, including *LYPLAL1*, *GCKR*, *FDFT1*, *COL13A1*, *PZP*, and *TM6SF2*, which were identified in the Western population, demonstrate a similar association. On further analysis, the *PNPLA3* GG variant, elevated BMI, and hypertriglyceridemia were independent predictors of MAFLD.

The *PNPLA3* I148M variant is associated with increased severity of MAFLD, with a higher susceptibility to NASH, fibrosis, and HCC^[24]. The I148M mutation confers double the risk for HCC for each variant allele^[17]. *PNPLA3* I148M predisposes to lower hepatic very-low-density lipoprotein (VLDL)^[25] and also lower levels of adiponectin^[26] which result in inflammation leading to the development of NASH^[27]. Furthermore, this association is independent of predisposition to hepatic steatosis, insulin resistance, dyslipidemia or obesity^[15]. A study of Italian and United Kingdom patients who carried the *PNPLA3* GG genotype had a 3.3-fold risk of MAFLD compared to those with the CC genotype. This data is in line with our results of the predisposition of homozygous GG allele to MAFLD. The GG phenotype was linked with higher LDL levels, fasting insulin levels, homeostasis model assessment of insulin resistance (HOMA-IR) score. However, there was no association with type 2 diabetes. These subjects also had significantly higher ALT levels in comparison with those who possessed the CC or CG genotypes^[28]. In Asians, there is a higher prevalence of the entity that is non-obese MAFLD defined as those with a BMI of less than 25 kg/m². There is some evidence indicating a greater influence of the *PNPLA3* variant in the non-obese MAFLD subjects. Wei *et al*^[29] reported a higher proportion of non-obese MAFLD patients with the *PNPLA3* variant (78.4%) compared with the obese MAFLD group (59.8%) in a population-based study from Hong Kong. But the mechanism linking the two is incompletely understood. On the contrary, these results were not replicated in our study.

Polymorphism in the *PNPLA3* gene may also play an important role in the management of MAFLD as there is data suggesting that lifestyle modification may be more beneficial in this group compared to other genotypes. The effect of degree of weight loss on intrahepatic TG content and liver enzyme levels was more pronounced in MAFLD patients with the homozygous GG allele than in subjects with the homozygous CC allele^[30,31]. Hence, lifestyle modification and weight loss can be strongly advocated in our patient cohort with a predominance of the GG genotype which may result in more optimal outcomes.

Variants in the *GCKR* gene (rs1260326 and rs780094) increase MAFLD susceptibility by inducing lipogenesis *via* activation of hepatic glucose uptake^[22,32]. In a study on Italian MAFLD patients, *GCKR* rs780094 C>T was associated with higher serum TG levels and severity of liver fibrosis^[33]. However, there was no observed association between the *GCKR* SNP and our study cohort. The *FDFT1* gene is involved in the regulation of cholesterol biosynthesis and the rs2645424 SNP has been shown to demonstrate a positive correlation with the MAFLD activity score (NAS)^[34]. Several genes with different SNPs including *COL13A1* and *EFCAB4B* were associated with lobular inflammation in NASH whereas *PZP* SNPs had an association with serum AST levels^[34]. A large GWAS study detected variants in *NCAN* (rs2228603) and *LYPLAL1* correlating with histologic lobular inflammation and fibrosis, but not in *PPP1R3B* which was associated with liver steatosis only^[22]. Another major genetic determinant of MAFLD is *TM6SF2* which is associated with reduced hepatic secretion of VLDL and increased risk of myocardial infarction. However, a study from Hong Kong showed a prevalence of only 0.4% of Chinese who had the variant which conferred a higher risk

Table 2 Multivariable analysis of the environmental factors and single nucleotide polymorphisms

	Odds ratio	95%CI		P value
		Lower	Upper	
TG, mmol/L	2.338	1.3	4.1	0.003
BMI, kg/m ²	1.243	1.1	1.4	0.003
<i>PNPLA3</i> GG vs CC	4.146	1.3	12.9	0.014

BMI: Body mass index; CI: Confidence interval; TGs: Triglycerides.

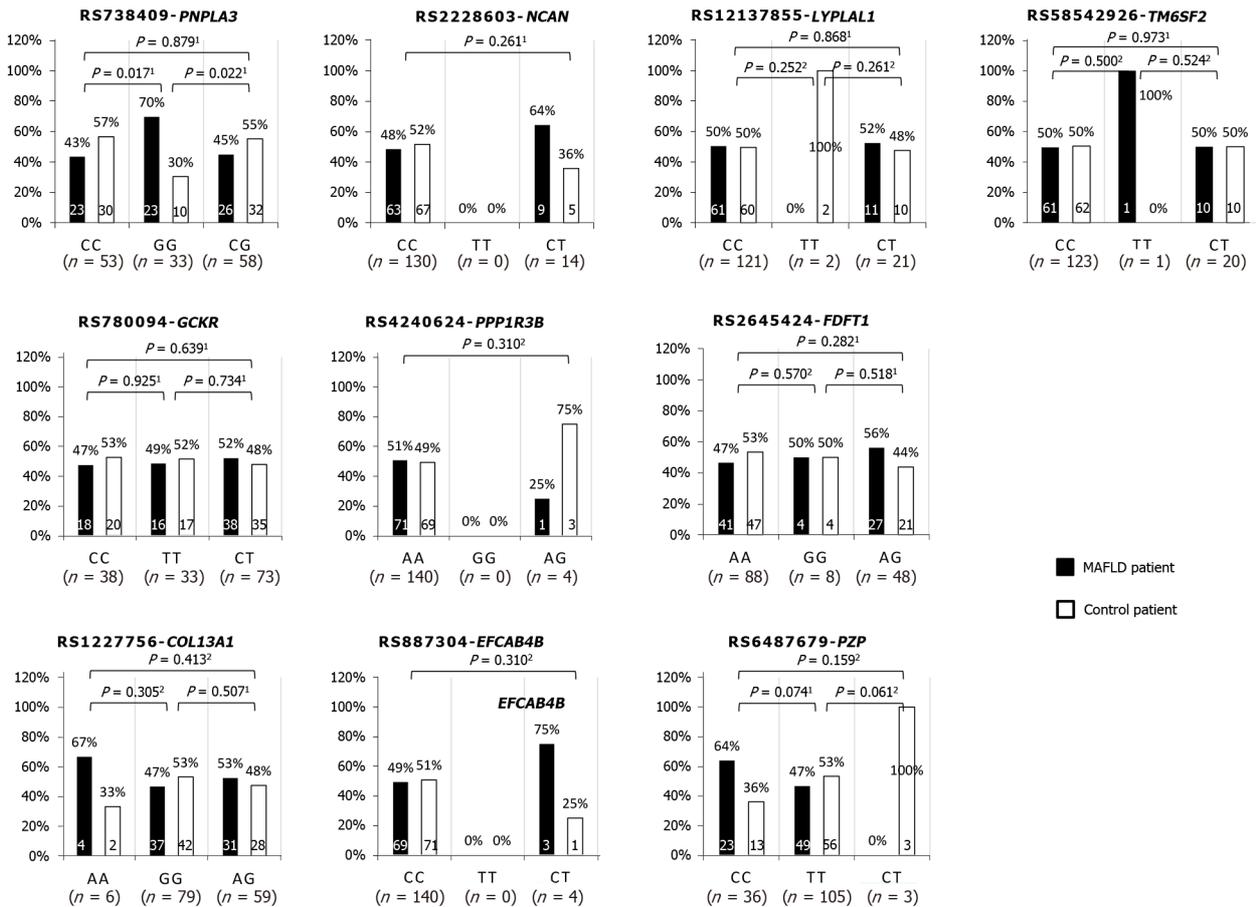


Figure 1 Single nucleotide polymorphisms in patients with or without metabolic dysfunction-associated fatty liver disease. The bar charts represent the percent of each allele comparing metabolic dysfunction-associated fatty liver disease (MAFLD) patients (blue) and controls (green). ¹Chi-squared test; ²Fisher's exact test.

of MAFLD, indicating a potentially limited role of this gene in Asian MAFLD^[35]. Overall, none of these genetic SNPs except for *PNPLA3* were related to MAFLD development in our study population.

This study had limitations. The general limitations of case-control design are well-documented. The sample size may not be large enough to detect the effects of some of the genetic polymorphisms tested, but the effect sizes will likely be too small for clinical application. Despite our best effort to match the control group with the MAFLD subjects, we were not able to completely balance all known risk factors and associated co-morbidities linked to MAFLD. Very few patients with high BMI in our population have completely normal findings for liver steatosis measurement, and also free from other features of metabolic syndrome. As a result, BMI and corresponding risk factors, such as diabetes mellitus and hyperlipidemia are more prevalent in the MAFLD group. The other major limitation is the lack of liver histology in the study population to have the gold standard diagnosis of hepatic steatosis and steatohepatitis. Surrogate markers, including CAP score (on FibroScan), CT, and MRI, are accepted. To

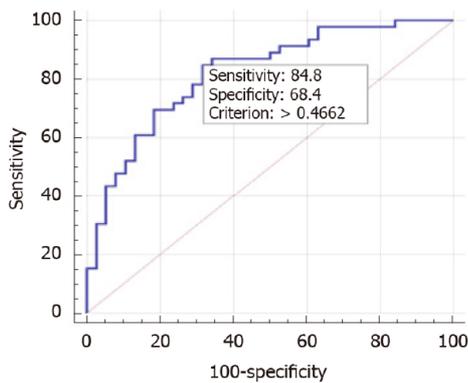


Figure 2 Area under curve of the regression model including *PNPLA3* GG. Area under curve by receiver operating characteristic is 0.823 (95% confidence interval: 0.73-0.91, $P < 0.001$). Regression equation $-7.01 + 0.849$ (triglycerides) $+ 0.218$ (body mass index) $+ 1.422$ (*PNPLA3* GG). Positive predictive value = 72.1%. Negative predictive value = 65.4%.

avoid diagnostic uncertainties, we used more stringent criteria for defining hepatic steatosis. For CAP score, we accepted only subjects with S2 and above into the MAFLD group and only S0 into the control group. For histopathology, we included only those patients with higher liver steatosis of 20% or more into the MAFLD group and < 5% into the control group.

CONCLUSION

In conclusion, we demonstrated that the genetic variant in *PNPLA3* is associated with an increased risk of MAFLD in the Singaporean Chinese population, but not with the other studied SNPs. While it is possible that with a much larger study population, some of these MAFLD-related SNPs may reach statistical significance, their effect size is likely to be much smaller than *PNPLA3*, and are unlikely to inspire lifestyle changes in affected individuals. Together with the other factors of TG level and BMI, *PNPLA3* can potentially be used as a predictive tool to identify individuals in the community, who are at risk of more progressive forms of MAFLD for targeted close surveillance and early weight loss interventions. The influence of genetic variation can be translated into more precise clinical management, which should be tailored to each individual population in the country.

ARTICLE HIGHLIGHTS

Research background

Metabolic dysfunction-associated fatty liver disease (MAFLD) is increasingly recognized as a leading cause of liver morbidity and mortality and has emerged as the most common chronic liver disease globally. The estimated prevalence of MAFLD in Asia is about 27.4%. Genetic factors play an important role in the pathogenesis and development of MAFLD.

Research motivation

A number of genetic variations (known as single nucleotide polymorphism, SNP) have been reported to be associated with MAFLD, mostly by studies from Europe and America. This study examines 10 of the most important SNPs in the Chinese population in Singapore.

Research objectives

To study the association of SNPs, previously identified in Western populations, with the risk for MAFLD in the Singapore Chinese population and their interactions with environmental and medical risk factors.

Research methods

This is a retrospective case-control study of 72 MAFLD cases and 72 controls with no

hepatic steatosis on imaging or controlled attenuation parameter score. Subjects were recruited from two tertiary hospitals in Singapore.

Research results

PNPLA3 GG was more likely to be associated with MAFLD (43.4% CC vs 69.7% GG, $P = 0.017$, and 44.8% CG vs 69.7% GG, $P = 0.022$). In multivariable analysis, hypertriglyceridemia (OR: 2.04 95%CI: 1.3-3.1, $P = 0.001$), body mass index (BMI, OR: 1.2 95% confidence interval [CI]: 1.1-1.4, $P < 0.001$) and *PNPLA3* GG (odds ratio [OR]: 3.4 95%CI: 1.3-9.2, $P = 0.014$) were associated with MAFLD (area under the receiver operating characteristic curve of 0.823).

Research conclusions

This study showed that *PNPLA3* GG allele was the only SNP associated with progressive MAFLD in the Singaporean Chinese population. The *PNPLA3* GG variant, elevated BMI, and hypertriglyceridemia were independent predictors of MAFLD.

Research perspectives

PNPLA3, along with triglyceride level and BMI, can potentially be used as a predictive tool to identify and risk-stratify affected individuals in the community for early intervention and targeted surveillance.

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