**Name of Journal:** *World Journal of Hepatology*

**Manuscript NO:** 57445

**Manuscript Type:** ORIGINAL ARTICLE

***Case Control Study***

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

Lee GH *et al*. Genetic variants in MAFLD

Guan Huei Lee, Wah Wah Phyo, Wai Mun Loo, Raymond Kwok, Taufique Ahmed, Asim Shabbir, Jimmy So, Calvin Jianyi Koh, Juanda Leo Hartono, Mark Muthiah, Kieron Lim, Poh Seng Tan, Yin Mei Lee, Seng Gee Lim, Yock Young Dan

**Guan Huei Lee, Wai Mun Loo, Calvin Jianyi Koh, Juanda Leo Hartono, Mark Muthiah, Kieron Lim, Poh Seng Tan, Yin Mei Lee, Seng Gee Lim, Yock Young Dan,** Division of Gastroenterology and Hepatology, Department of Medicine, National University Health System, Singapore 119228, Singapore

**Guan Huei Lee, Wah Wah Phyo, Seng Gee Lim, Yock Young Dan,** Yong Loo Lin School of Medicine, National University of Singapore, Singapore 117597, Singapore

**Raymond Kwok, Taufique Ahmed,** Department of Medicine, Khoo Teck Puat Hospital, Singapore 768828, Singapore

**Asim Shabbir, Jimmy So,** Department of Surgery, National University Health System, Singapore 119228, Singapore

**Author contributions:** Lee GH was involved in the conception and design of the study, data collection and analysis, drafting and revision of the manuscript; Phyo WW was involved in the data collection and analysis; Loo WM was involved in the drafting and revision of the manuscript; Kwok R, Ahmed T, Shabbir A, So J, Koh CJ, Hartono JL, Muthiah M, Lim K, Tan PS, Lee YM, Lim SG, and Dan YYcontributed to the data collection and revision of the manuscript.

**Supported by** National University Health System, No. NUHSRO/2013/226/CRG/08.

**Corresponding author: Guan Huei Lee, MBBS, PhD, Doctor,** Division of Gastroenterology and Hepatology, Department of Medicine, National University Health System, 1E Kent Ridge Road, NUHS Tower Block, Singapore 119228, Singapore. mdcleegh@nus.edu.sg

**Received:** July 7, 2020

**Revised:** September 28, 2020

**Accepted:** October 15, 2020

**Published online:** December 27, 2020

**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.

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

**Citation:** 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. Validation of genetic variants associated with metabolic dysfunction-associated fatty liver disease in an ethnic Chinese population. *World J Hepatol* 2020; 12(12): 1228-1238

**URL:** https://www.wjgnet.com/1948-5182/full/v12/i12/1228.htm

**DOI:** https://dx.doi.org/10.4254/wjh.v12.i12.1228

**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 independent, strong predictors of MAFLD.

**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 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 (≥ 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 (m2). 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 L 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 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 *EFXAB4B* 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 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 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.

**REFERENCES**

1 **Younossi ZM**, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology* 2016; **64**: 73-84 [PMID: 26707365 DOI: 10.1002/hep.28431]

2 **Khashab MA**, Liangpunsakul S, Chalasani N. Nonalcoholic fatty liver disease as a component of the metabolic syndrome. *Curr Gastroenterol Rep* 2008; **10**: 73-80 [PMID: 18417046 DOI: 10.1007/s11894-008-0012-0]

3 **Chalasani N**, Younossi Z, Lavine JE, Charlton M, Cusi K, Rinella M, Harrison SA, Brunt EM, Sanyal AJ. The diagnosis and management of nonalcoholic fatty liver disease: Practice guidance from the American Association for the Study of Liver Diseases. *Hepatology* 2018; **67**: 328-357 [PMID: 28714183 DOI: 10.1002/hep.29367]

4 **Farrell GC**, Wong VW, Chitturi S. NAFLD in Asia--as common and important as in the West. *Nat Rev Gastroenterol Hepatol* 2013; **10**: 307-318 [PMID: 23458891 DOI: 10.1038/nrgastro.2013.34]

5 **Kim D**, Kim WR, Kim HJ, Therneau TM. Association between noninvasive fibrosis markers and mortality among adults with nonalcoholic fatty liver disease in the United States. *Hepatology* 2013; **57**: 1357-1365 [PMID: 23175136 DOI: 10.1002/hep.26156]

6 **Goh LY**, Leow AH, Goh KL. Observations on the epidemiology of gastrointestinal and liver cancers in the Asia-Pacific region. *J Dig Dis* 2014; **15**: 463-468 [PMID: 24894597 DOI: 10.1111/1751-2980.12164]

7 **Goh KL**. Changing trends in gastrointestinal disease in the Asia-Pacific region. *J Dig Dis* 2007; **8**: 179-185 [PMID: 17970873 DOI: 10.1111/j.1751-2980.2007.00304.x]

8 **Younossi ZM**, Otgonsuren M, Henry L, Venkatesan C, Mishra A, Erario M, Hunt S. Association of nonalcoholic fatty liver disease (NAFLD) with hepatocellular carcinoma (HCC) in the United States from 2004 to 2009. *Hepatology* 2015; **62**: 1723-1730 [PMID: 26274335 DOI: 10.1002/hep.28123]

9 **Wong RJ**, Cheung R, Ahmed A. Nonalcoholic steatohepatitis is the most rapidly growing indication for liver transplantation in patients with hepatocellular carcinoma in the U.S. *Hepatology* 2014; **59**: 2188-2195 [PMID: 24375711 DOI: 10.1002/hep.26986]

10 **Dongiovanni P**, Romeo S, Valenti L. Genetic Factors in the Pathogenesis of Nonalcoholic Fatty Liver and Steatohepatitis. *Biomed Res Int* 2015; **2015**: 460190 [PMID: 26273621 DOI: 10.1155/2015/460190]

11 **Bugianesi E**, Gastaldelli A, Vanni E, Gambino R, Cassader M, Baldi S, Ponti V, Pagano G, Ferrannini E, Rizzetto M. Insulin resistance in non-diabetic patients with non-alcoholic fatty liver disease: sites and mechanisms. *Diabetologia* 2005; **48**: 634-642 [PMID: 15747110 DOI: 10.1007/s00125-005-1682-x]

12 **Day CP**. From fat to inflammation. *Gastroenterology* 2006; **130**: 207-210 [PMID: 16401483 DOI: 10.1053/j.gastro.2005.11.017]

13 **Schwimmer JB**, Celedon MA, Lavine JE, Salem R, Campbell N, Schork NJ, Shiehmorteza M, Yokoo T, Chavez A, Middleton MS, Sirlin CB. Heritability of nonalcoholic fatty liver disease. *Gastroenterology* 2009; **136**: 1585-1592 [PMID: 19208353 DOI: 10.1053/j.gastro.2009.01.050]

14 **Makkonen J**, Pietiläinen KH, Rissanen A, Kaprio J, Yki-Järvinen H. Genetic factors contribute to variation in serum alanine aminotransferase activity independent of obesity and alcohol: a study in monozygotic and dizygotic twins. *J Hepatol* 2009; **50**: 1035-1042 [PMID: 19303161 DOI: 10.1016/j.jhep.2008.12.025]

15 **Romeo S**, Kozlitina J, Xing C, Pertsemlidis A, Cox D, Pennacchio LA, Boerwinkle E, Cohen JC, Hobbs HH. Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. *Nat Genet* 2008; **40**: 1461-1465 [PMID: 18820647 DOI: 10.1038/ng.257]

16 **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**: 1883-1894 [PMID: 21381068 DOI: 10.1002/hep.24283]

17 **Liu YL**, Patman GL, Leathart JB, Piguet AC, Burt AD, Dufour JF, Day CP, Daly AK, Reeves HL, Anstee QM. Carriage of the PNPLA3 rs738409 C >G polymorphism confers an increased risk of non-alcoholic fatty liver disease associated hepatocellular carcinoma. *J Hepatol* 2014; **61**: 75-81 [PMID: 24607626 DOI: 10.1016/j.jhep.2014.02.030]

18 **Singal AG**, Manjunath H, Yopp AC, Beg MS, Marrero JA, Gopal P, Waljee AK. The effect of PNPLA3 on fibrosis progression and development of hepatocellular carcinoma: a meta-analysis. *Am J Gastroenterol* 2014; **109**: 325-334 [PMID: 24445574 DOI: 10.1038/ajg.2013.476]

19 **Rotman Y**, Koh C, Zmuda JM, Kleiner DE, Liang TJ; NASH CRN. The association of genetic variability in patatin-like phospholipase domain-containing protein 3 (PNPLA3) with histological severity of nonalcoholic fatty liver disease. *Hepatology* 2010; **52**: 894-903 [PMID: 20684021 DOI: 10.1002/hep.23759]

20 **Li Y**, Xing C, Cohen JC, Hobbs HH. Genetic variant in PNPLA3 is associated with nonalcoholic fatty liver disease in China. *Hepatology* 2012; **55**: 327-328 [PMID: 21898508 DOI: 10.1002/hep.24659]

21 **Shen J**, Wong GL, Chan HL, Chan HY, Yeung DK, Chan RS, Chim AM, Chan AW, Choi PC, Woo J, Chu WC, Wong VW. PNPLA3 gene polymorphism accounts for fatty liver in community subjects without metabolic syndrome. *Aliment Pharmacol Ther* 2014; **39**: 532-539 [PMID: 24417250 DOI: 10.1111/apt.12609]

22 **Speliotes EK**, Yerges-Armstrong LM, Wu J, Hernaez R, Kim LJ, Palmer CD, Gudnason V, Eiriksdottir G, Garcia ME, Launer LJ, Nalls MA, Clark JM, Mitchell BD, Shuldiner AR, Butler JL, Tomas M, Hoffmann U, Hwang SJ, Massaro JM, O'Donnell CJ, Sahani DV, Salomaa V, Schadt EE, Schwartz SM, Siscovick DS; NASH CRN; GIANT Consortium; MAGIC Investigators, Voight BF, Carr JJ, Feitosa MF, Harris TB, Fox CS, Smith AV, Kao WH, Hirschhorn JN, Borecki IB; GOLD Consortium. Genome-wide association analysis identifies variants associated with nonalcoholic fatty liver disease that have distinct effects on metabolic traits. *PLoS Genet* 2011; **7**: e1001324 [PMID: 21423719 DOI: 10.1371/journal.pgen.1001324]

23 **Hernaez R**, McLean J, Lazo M, Brancati FL, Hirschhorn JN, Borecki IB, Harris TB; Genetics of Obesity-Related Liver Disease (GOLD) Consortium, Nguyen T, Kamel IR, Bonekamp S, Eberhardt MS, Clark JM, Kao WH, Speliotes EK. Association between variants in or near PNPLA3, GCKR, and PPP1R3B with ultrasound-defined steatosis based on data from the third National Health and Nutrition Examination Survey. *Clin Gastroenterol Hepatol* 2013; **11**: 1183-1190.e2 [PMID: 23416328 DOI: 10.1016/j.cgh.2013.02.011]

24 **Sookoian S**, Castaño GO, Burgueño AL, Gianotti TF, Rosselli MS, Pirola CJ. A nonsynonymous gene variant in the adiponutrin gene is associated with nonalcoholic fatty liver disease severity. *J Lipid Res* 2009; **50**: 2111-2116 [PMID: 19738004 DOI: 10.1194/jlr.P900013-JLR200]

25 **Pirazzi C**, Adiels M, Burza MA, Mancina RM, Levin M, Ståhlman M, Taskinen MR, Orho-Melander M, Perman J, Pujia A, Andersson L, Maglio C, Montalcini T, Wiklund O, Borén J, Romeo S. Patatin-like phospholipase domain-containing 3 (PNPLA3) I148M (rs738409) affects hepatic VLDL secretion in humans and in vitro. *J Hepatol* 2012; **57**: 1276-1282 [PMID: 22878467 DOI: 10.1016/j.jhep.2012.07.030]

26 **Valenti L**, Rametta R, Ruscica M, Dongiovanni P, Steffani L, Motta BM, Canavesi E, Fracanzani AL, Mozzi E, Roviaro G, Magni P, Fargion S. The I148M PNPLA3 polymorphism influences serum adiponectin in patients with fatty liver and healthy controls. *BMC Gastroenterol* 2012; **12**: 111 [PMID: 22898488 DOI: 10.1186/1471-230X-12-111]

27 **Polyzos SA**, Toulis KA, Goulis DG, Zavos C, Kountouras J. Serum total adiponectin in nonalcoholic fatty liver disease: a systematic review and meta-analysis. *Metabolism* 2011; **60**: 313-326 [PMID: 21040935 DOI: 10.1016/j.metabol.2010.09.003]

28 **Valenti L**, Al-Serri A, Daly AK, Galmozzi E, Rametta R, Dongiovanni P, Nobili V, Mozzi E, Roviaro G, Vanni E, Bugianesi E, Maggioni M, Fracanzani AL, Fargion S, Day CP. Homozygosity for the patatin-like phospholipase-3/adiponutrin I148M polymorphism influences liver fibrosis in patients with nonalcoholic fatty liver disease. *Hepatology* 2010; **51**: 1209-1217 [PMID: 20373368 DOI: 10.1002/hep.23622]

29 **Wei JL**, Leung JC, Loong TC, Wong GL, Yeung DK, Chan RS, Chan HL, Chim AM, Woo J, Chu WC, Wong VW. Prevalence and Severity of Nonalcoholic Fatty Liver Disease in Non-Obese Patients: A Population Study Using Proton-Magnetic Resonance Spectroscopy. *Am J Gastroenterol* 2015; **110**: 1306-14; quiz 1315 [PMID: 26215532 DOI: 10.1038/ajg.2015.235]

30 **Shen J**, Wong GL, Chan HL, Chan RS, Chan HY, Chu WC, Cheung BH, Yeung DK, Li LS, Sea MM, Woo J, Wong VW. PNPLA3 gene polymorphism and response to lifestyle modification in patients with nonalcoholic fatty liver disease. *J Gastroenterol Hepatol* 2015; **30**: 139-146 [PMID: 25040896 DOI: 10.1111/jgh.12656]

31 **Sevastianova K**, Kotronen A, Gastaldelli A, Perttilä J, Hakkarainen A, Lundbom J, Suojanen L, Orho-Melander M, Lundbom N, Ferrannini E, Rissanen A, Olkkonen VM, Yki-Järvinen H. Genetic variation in PNPLA3 (adiponutrin) confers sensitivity to weight loss-induced decrease in liver fat in humans. *Am J Clin Nutr* 2011; **94**: 104-111 [PMID: 21525193 DOI: 10.3945/ajcn.111.012369]

32 **Beer NL**, Tribble ND, McCulloch LJ, Roos C, Johnson PR, Orho-Melander M, Gloyn AL. The P446L variant in GCKR associated with fasting plasma glucose and triglyceride levels exerts its effect through increased glucokinase activity in liver. *Hum Mol Genet* 2009; **18**: 4081-4088 [PMID: 19643913 DOI: 10.1093/hmg/ddp357]

33 **Petta S**, Miele L, Bugianesi E, Cammà C, Rosso C, Boccia S, Cabibi D, Di Marco V, Grimaudo S, Grieco A, Pipitone RM, Marchesini G, Craxì A. Glucokinase regulatory protein gene polymorphism affects liver fibrosis in non-alcoholic fatty liver disease. *PLoS One* 2014; **9**: e87523 [PMID: 24498332 DOI: 10.1371/journal.pone.0087523]

34 **Chalasani N**, Guo X, Loomba R, Goodarzi MO, Haritunians T, Kwon S, Cui J, Taylor KD, Wilson L, Cummings OW, Chen YD, Rotter JI; Nonalcoholic Steatohepatitis Clinical Research Network. Genome-wide association study identifies variants associated with histologic features of nonalcoholic Fatty liver disease. *Gastroenterology* 2010; **139**: 1567-1576, 1576.e1-1576.e6 [PMID: 20708005 DOI: 10.1053/j.gastro.2010.07.057]

35 **Wong VW**, Wong GL, Tse CH, Chan HL. Prevalence of the TM6SF2 variant and non-alcoholic fatty liver disease in Chinese. *J Hepatol* 2014; **61**: 708-709 [PMID: 24824280 DOI: 10.1016/j.jhep.2014.04.047]

**Footnotes**

**Institutional review board statement:** The study was approved by the Institutional Review Board.

**Informed consent statement:** All patients gave informed consent.

**Conflict-of-interest statement:** The authors declare that there is no conflict of interest.

**Data sharing statement:** No additional data available.

**STROBE statement:** The authors have read the STROBE Statement—checklist of items, and the manuscript was prepared and revised according to the STROBE Statement—checklist of items.

**Open-Access:** This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: http://creativecommons.org/Licenses/by-nc/4.0/

**Manuscript source:** Unsolicited manuscript

**Peer-review started:** July 9, 2020

**First decision:** September 11, 2020

**Article in press:** October 15, 2020

**Specialty type:** Gastroenterology and hepatology

**Country/Territory of origin:** Singapore

**Peer-review report’s scientific quality classification**

Grade A (Excellent): 0

Grade B (Very good): 0

Grade C (Good): C, C, C

Grade D (Fair): D

Grade E (Poor): 0

**P-Reviewer:** Bubnov R, Ferrarese A, Liao R, Smolić M **S-Editor:** Huang P **L-Editor:** Filipodia **P-Editor:** Wu YXJ

**Figure Legends**



**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.



**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%.

**Table 1 Patients’ demographics and clinical characteristics**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **NAFLD patient, *n* = 72** | **Control, *n* = 72** | **Total, *n* = 144** | ***P* 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%) | 1.01 |
| BMI, kg/m2 | 27 (23-31) | 23 (19-28) | 25 (21-30) | < 0.0012 |
| Waist-hip ratio | 0.94 (0.9-1.0) | 0.95 (0.8-1.1) | 0.9 (0.8-1.0) | 0.5872 |
| Smoker, % | 5 (6.9%) | 12 (16.7%) | 17 (11.8%) | 0.0711 |
| DM, % | 29 (40.3%) | 15 (20.8%) | 44 (30.6%) | 0.0111 |
| Hypertension, % | 25 (34.7) | 22 (30.6%) | 47 (32.6%) | 0.5941 |
| On anti-diabetic treatment | 26 (36.1) | 14 (19.4) | 40 (27.8) | 0.0261 |
| On lipid-lowering treatment | 26 (36.1) | 17 (23.6) | 43 (29.9) | 0.1011 |
| 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) | 0.00322 |
| LDL-C, mmol/L | 2.5 (1.5-3.5) | 2.1 (0.7-3.5) | 2.3 (1.1-3.6) | 0.04922 |
| HDL-C, mmol/L | 1.3 (0.6-1.9) | 1.0 (0.3-1.7) | 1.6 (0.5-1.8) | 0.02922 |
| TG, mmol/L | 1.7 (0.8-2.7) | 1.0 (0.1-1.98) | 1.4 (0.4-2.4) | < 0.00122 |
| FPG, mmol/L | 6.1 (5.6-6.6) | 5.9 (5.5-6.3) | 6.0 (5.7-6.3) | 0.45922 |
| 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, mol/LMedian | 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 | 0.66722 |
| AST, U/L | 37.1 (16.7-57.5) | 26.2 (7.4-44.9) | 31.7 (11.4-51.98) | 0.00122 |
| ALT, U/L | 46.2 (15.5-76.8) | 22.2 (8.2-36.1) | 34.3 (7.6-60.9) | < 0.00122 |
| ALP, U/L | 87.6 (36.2-138.9) | 77.4 (39.95-114.8) | 82.5 (37.4-127.6) | 0.17922 |
| INR | 0.9 (0.5-1.2) | 0.8 (0.2-1.3) | 0.8 (0.3-1.3) | 0.37322 |
| Platelet as 109/L | 223 (130-315) | 256 (162-350) | 240 (146-334) | 0.04022 |

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.

**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/m2 | 1.243 | 1.1 | 1.4 | 0.003 |
| *PNPLA3* GG *vs* CC | 4.146 | 1.3 | 12.9 | 0.014 |

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