Reviewer #1:

1. A new nomenclature has been established for what was known to be Non-alcoholic fatty liver disease (NAFLD) - metabolic (dysfunction) associated fatty liver disease (MAFLD). Please replace the term with the new one in the manuscript.
Thank you for pointing out the change in nomenclature. The term has been replaced in this revision.

2. Can the conclusion that more progressive forms of NAFLD can be predicted by PNPLA3 genotyping in Singapore Chinese population be drawn from this study?
This sample size and patient selection criteria of this study was not designed/powered to differentiate between simple hepatic steatosis and more progressive forms of MAFLD.

3. It is mentioned that the effect size for SNPs other than PNPLA3 is likely to be too small to be clinically significant. What is the effect size of PNPLA3 gene polymorphism on MAFLD?
PNPLA3 G allele is 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)].

4. How was the sample size calculated?
We estimated that a sample size of 72 cases and 72 controls would provide 90% power at an alpha of 0.05 to detect an odds ratio of 3.0, assuming that the effect allele frequency is 0.34 among controls. (Ref: 1. Speliotes EK, Yerges-Armstrong LM, Wu J, Hernaez R, Kim LJ, et al. (2011) Genome-wide association analysis identifies variants associated with nonalcoholic fatty liver disease that have distinct effects on metabolic traits. PLoS Genet 7: e1001324. 2. Fleiss, Statistical Methods for Rates and Proportions)

Reviewer #2:

The aim considers causality: `to evaluate the impact of single nucleotide polymorphisms (SNPs), previously identified in Western populations, and environmental and medical risk factors on the risk for NAFLD in a Singapore Chinese population.` I would suggest that associations would be more acceptable…
Thank you for pointing out the implication in the choice of wording. We have revised the aim to "study the associations of…" as suggested.

Methods. Why did Authors focused on ethnic-Chinese population? Is any evidence on specific development of NAFLD in this ethic group? Group in heterogeneous according to the treatment options. Confounding factors although considered, like comorbidities, smoking, alcohol, etc. largely affect the records. Number of diseases can affect liver function. Comparison to overall population?
It is known that the frequency of single nucleotide polymorphisms can differ significantly between different ethnic groups, and the SNPs for MAFLD has not been well-studied in different Asian populations. In Singapore, we have a multiracial society, with >75% who are of Han Chinese descent. With limited funding available, we had decided to focus on the Chinese subjects first, to minimise the potential confounding effects of SNP variations as a result of heterogeneity introduced by ethnic diversity. Other known confounding factors listed were taken into account in the multivariate analysis.

Why did Authors consider only imaging data (CT, MRI) - `no hepatic steatosis on CT, MRI or CAP score`… Did authors use ultrasound visualization / sonoelastography (other than fibroscan)? Liver biopsy. Adhere point to the existing evidence. If Authors used CT, US, visceral fat measurement could be added? As well as other organs evaluation (relevant and minimally needed), e.g., kidney, parameters of metabolic syndrome.
We accepted both imaging and liver biopsy reports for the diagnosis of hepatic steatosis (or no steatosis). We accepted CAP score (measured by fibroscan machine) but not the more subjective diagnosis of fatty liver by standard ultrasound scan of the liver, which has a higher inter-operator variability. Visceral fat measurement is not regularly performed in our centre. Parameters of metabolic syndrome were included in our analysis (Table 1).

Cohort is too small to make initial conclusions in the matter, in particular for specific pattern in Chinese population.
We acknowledged that the sample size is a limitation (page 12, last paragraph) of our study. The study does indicate that some of the alleles identified in the Western population, are rare in the Han Chinese population, and that other SNP markers may not be associated with MAFLD in our population. It points to the need for larger, population-specific, validation studies if such SNPs are to be applied.

The following issues should be (more) carefully considered and discussed as follows: Authors found that `The subjects who had NAFLD were more likely to have body mass index higher than 24.9 kg/m2.` Why BMI was not inclusion criteria? Stratification on body weight, any relevant findings…
It is known that in the Asian population, including Singapore, non-obese patient with MAFLD is fairly common. Thus we did not include BMI levels in the inclusion criteria. The sample size does not permit meaningful stratification by body weight.

The issues need to be explained. Patients stratification: • Stratification of metabolic syndrome / obesity/ liver diseases. • Obese vs non-obese vs low BMI cohorts; • Gender, age; • History of receiving drugs (min/max doses), drugs overuse; • Diet; • Next point is the phenotype of individuals, e.g., microbiota ?
These are indeed very important factors to evaluate. Many of these are analysed in our study (Table 1). Stratification into so many different variables are not reliable statistically due to the sample size limitation. Other factors, such as diet and microbiota, are subjects of interest in our group, and will be included in future studies.

I recommend to reconsider structure for named avoiding bias, speculative hypotheses and conclusions in the matter; and rather focus on clear associations between particular genetic and phenotype markers.
Thank you for highlighting, and we acknowledge that this is study of association between genetic (specific SNPs) and phenotype (hepatic steatosis).

Recommendations for prevention and management future research plan including stratification patients to groups under risk is needed. Extensive limitation paragraphs should be added.
As mentioned, future studies are planned to include stratification of patients to groups under risk. We have added to the limitation paragraph as suggested.

Reviewer #3:

1. Hypertriglyceridemia, high BMI and PNPLA3 GG are independent predictors of NAFLD. Whether do the combination of the three indicators have more powerful predictive ability in NAFLD.
Yes. Based on the regression equation, having a combination of the 3 indicators would be associated with a higher likelihood of MAFLD.

2. For 72 NAFLD cases, what kinds of therapies were performed?
We did not collect the data on their subsequent treatment for their fatty liver disease.

3. How long is the follow-up? During the follow-up, how did the authors monitor these patients?
This is a case-control study with no patient follow-up. The patients are managed by their respective physicians.

4. AUROC were 0.823 and 0.789 with and without the PNPLA3, respectively. What are the sensitivity and specificity?
GG vs CC: Sensitivity 0.70, Specificity 0.57
GG vs CG: Sensitivity 0.70, Specificity 0.55

Reviewer #4:
What criteria were adopted for diagnosis of NAFLD? Moreover, diagnosis of steatosis has been heterogeneously made – with histology, MRI, CT scan, combination of both…Moreover, only 1% used

CAP for diagnosis. This heterogeneity could be an important bias. - 99% controls were identified as having no steatosis using only radiological features. How many were identified as having no steatosis using liver biopsy? - How many patients fulfil criteria for NASH? How many for metabolic syndrome? –
In this study, we identified fatty liver based on CT or MRI or CAP score or histopathology of liver (>20% steatosis). Liver biopsy, combined with a history of no or low alcohol use, have been the gold standard for diagnosing NAFLD. However, in real life, it is seldom performed due to the risk of the procedure. CT and MRI liver, as surrogate imaging modalities for hepatic steatosis, were specific[1] enough that they had been accepted in many previous large scale studies on genetic polymorphism of NAFLD. CAP scores, using the cut-offs chosen, also has high specificity when validated against liver histopathology for fatty liver. We acknowledged that this approach is less ideal than having a single gold standard test for subject selection, and cautioned the readers as one of the major limitations of the study. The control patients did not undergo liver biopsy. 47.2% of the MAFLD patients fulfil criteria for NASH. Overall, 43.8% of our study population had metabolic syndrome.

[1]MRI and CT offer the ability to provide semi-quantitative estimation of degree of steatosis, with sensitivities and specificities of up 98%. (Torres DM, Harrison SA (2008) Diagnosis and therapy of nonalcoholic steatohepatitis. Gastroenterology 134:1682-1698).

This was a retrospective, case-control study. Therefore, terms as "enrolled" should be avoided.
We agree. The term was deleted.

The Authors said that the BMI cut-offs were 23 and 28 for overweight and obesity, respectively. However, in the result section they used different cut-offs. –
There were not "different cut-offs" in the results section of this study. The BMI was analysed as a continuous variable with no categorisation into "overweight" or "obesity" categories. The only other time a BMI cut-off was mentioned was in Discussion (page 11, paragraph 2), where we cited another reference "In Asians, there is a higher prevalence of the entity that is non-obese NAFLD defined as those with a BMI of less than 25 kg/m^{2}". This should not be confused to mean that the study used different cut-offs to define obesity.

Result section: SD for age is missing; total cholesterol, triglycerides…units are missing; PNPLA3: p value is missing
The p value of PNPLA3 is 0.014 (Table 2).

Considering multivariate analysis, the Authors demonstrated that serum TG was an independent predictor of NAFLD. However, as they acknowledged, some patients (36% and 23%, respectively) have been treated with lipid lowering agents. Therefore, it is difficult to provide a clinical value to this result at multivariate analysis.
We agree this could be a limitation of this study. However, the effect of some subjects being on lipid-lowering agents is that the impact of untreated hypertriglyceridemia is likely be greater than the calculated value based on the multivariate analysis. Clearly, larger studies where patients with high TG, and can be stratified into treatment and non-treatment group, will be useful to determine the real impact. But there may be ethical issue to such design as well.

Introduction: Patatin instead of palatin
Thank you for pointing out the spelling error. It is corrected in the revised version.