Peer Review File

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Reviewer A

In lean individuals, nonalcoholic fatty liver disease (NAFLD) is not a benign disease, and these patients have longterm morbidity and mortality similar to those of their nonlean counterparts. Finding biomarkers for noninvasive and early detection is urgent and microRNAs (miRNAs) show potential. In the manuscript "Serum miR-4488 as a potential biomarker of lean nonalcoholic fatty liver disease", authors investigated the potential role of serum miRNAs in the detection of lean NAFLD and to explore the possible pathogenesis of lean NAFLD.

Couple questions are required to be answered before it will be accepted.

(1) What were the consequences of lean NAFLD? Please state in the introduction.

Reply: Thank you for your valuable comments. We have described the consequences of lean NAFLD in detail in the introduction. (lines 78-82)

"A systematic review published in 2020 indicated that the global prevalence of lean NAFLD was 5.1% (7) and these individuals can develop all outcomes of NAFLD (i.e., cardiovascular disease (CVD) followed by extrahepatic malignancies and liver-related complications) and have longterm morbidity and mortality similar to those of their nonlean counterparts (8)."

(2) It was advised to add related reference (DOI: 10.21037/hbsn-22-366) about intervention in nonalcoholic fatty liver disease.

Reply: Thank you for your professional comment. We have carefully reviewed the article and found that the description of this reference is not very relevant to the content of our study. Moreover, the content of our manuscript is already very complete. Therefore, we do not add this reference to the manuscript.

(3) Whether there were correlations between lipid metabolism and NAFLD? Please state in the introduction.

Reply: Once again, we sincerely thank you for your valuable comments. Because our study focused on screening potential biomarkers of lean NAFLD and predicting the possible role and molecular mechanisms of miR-4488 in lean NAFLD. In addition, we are concerned with genetic and epigenetic influences on lean NAFLD. Therefore, we briefly describe the correlation between lipid metabolism and NAFLD in the Introduction (Lines 76-78).

(4) What were the diagnostic criteria for NAFLD? Please provide supported references.

Thank you for your valuable comments. We have added the diagnostic criteria for NAFLD in the manuscript with the references cited (Lines 117-120).

" NAFLD requires: (a) evidence of hepatic steatosis either by imaging or histology, and (b) absence of other causes of hepatic fat accumulation from conditions such as significant alcohol consumption, hepatitis C, medication use, or hereditary disorders (19)."

19.Wong VW, Chan WK, Chitturi S, et al. Asia-Pacific Working Party on Non-alcoholic Fatty Liver Disease guidelines 2017-Part 1: Definition, risk factors and assessment. J Gastroenterol Hepatol 2018;33:70-85.

(5) Please list the sequences of used primers.

Thank you for your valuable comments. We supplemented the primer sequence used in the manuscript.

Table 1 Sequence of primer

Primer name	Sequence (5' to 3')
hsa- miR-4488	Forward: CGGGCAGGGGGCGGGC
	Reverse: CAGCCACAAAAGAGCACAAT
h-U6	Forward: CTCGCTTCGGCAGCACA
	Reverse:AACGCTTCACGAATTTGCGT

(6) Why to focus on miR-4488 in the study? Please state in the results.

Reply: Thank you for your valuable comments. In the results section, we explained why miR-4488 was concerned in the study. (line 214-224)

(7) What were the roles of miR-4488 in NAFLD? Please state in the discussion.

Reply: Thanks again for your valuable comments. As for the role of miR-4488 in NAFLD, our research results have been shown, and we also elaborated in the discussion section. However, since there is no research report on the role of miR-4488 in NAFLD, and this research is the first time to explore the role of miR-4488 in NAFLD based on the database, we try to quote some relevant studies in the discussion section to illustrate our results. In addition, we also explained in the discussion that the specific role of miR-4488 in NAFLD needs to be verified through more experiments.

Reviewer B

1. Please check to see if more references should be cited in the following sentences, as 'studies' are mentioned. Otherwise, 'a study' might be more appropriate.

Several studies have shown that miR-122, the most highly expressed miRNA in the human liver, accelerates NAFLD progression, whereas miR-122 and miR-223 ameliorate it (17).

Emerging data suggest that NAFLD is present in a considerable proportion of lean individuals, as described by numerous studies worldwide (20).

We have modified it.

2. There is no 'LNL' or 'NAFLS' in Figure 1.

Figure 1 Flow chart of the sequencing analysis. HI, healthy individual; LN, lean nonalcoholic fatty liver disease; LNL, nonlean nonalcoholic fatty liver disease; NAFLS, nonalcoholic fatty liver disease; qRT-PCR, quantitative real-time polymerase chain reaction.

We have modified it.

3. There is no Figure 1E, and Figure 2E should be cited in the main text.

1255a and miR-4999-5p) (Figure 2C). A volcano plot revealed the differentially expressed miRNAs between 4 LNs *vs.* 4 HIs (Figure 2D) and 4 LNs *vs.* 6 NLNs (Figure 1E).

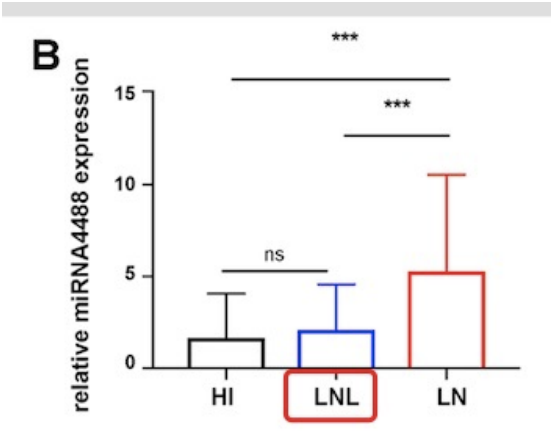
We have modified it.

4. There is no *** in Figure 2.

differentially expressed miRNAs between 4 LNs *vs.* 4 HIs **(D)** and 4 LNs *vs.* 6 NLNs **(E)**. ***, $P <$ 0.001. HI, healthy individual; LN, lean nonalcoholic fatty liver disease; NLN, nonlean nonalcoholic fatty liver disease.

We have modified it.

5. Please check to see if 'LNL' should be 'NLN' in Figure 3. And please define '***'.



LN, lean nonalcoholic fatty liver disease; LNL, nonlean nonalcoholic fatty liver disease; qRT-PCR, quantitative real-time polymerase chain reaction; ROC, receiver operating characteristic; AUC, area under the curve.

We have modified it.

6. There are 302 NLNs in Table 2. Please check.

#Results

##Clinical characteristics of the subjects

The discovery cohort consisted of 98 LNs, 400 NLNs, and 98 HIs. Their clinical characteristics are summarized in Table 2. The LNs were older and had a smaller

Table 2 Clinical characteristics of the lean (LN) and nonlean (NLN) cohorts as well as controls (HI)

Parameter	Group 1 (LN)	Group 2 (NLN)	Group 3 (HI)
Participants (n)	98	302	98
Sex (M/F)	46/52	208/94	46/52

We have modified it.

7. There is no LNL or BMI in Table 2. Please check and revise.

Data are presented as mean ± standard deviation (SD) of each group. *, comparison between groups 1 and 2. ▲, comparison between groups 1 and 3. LN, lean nonalcoholic fatty liver disease; LNL, nonlean nonalcoholic fatty liver disease; HI, healthy individual; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CRP, C-reactive protein;

DBP, diastolic blood pressure; FINS, fasting insulin; GGT, glutamyl transpeptidase; Hb, hemoglobin; HbA1c glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment of insulin resistance; LDL-C, low-density lipoprotein cholesterol; NAFLD, nonalcoholic fatty liver disease; SBP, systolic blood pressure; UA, uric acid.

We have modified it.