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Title: **Circulating MicroRNA Expression and Nonalcoholic Fatty Liver Disease in Adolescents with Severe Obesity**

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All changes in the manuscript are highlighted in yellow, both, in the Table and below and in the manuscript. All line numbers refer to the final submitted version of the manuscript.

Editor's:

I have reviewed the Peer-Review Report, full text of the manuscript, and the relevant ethics documents, all of which have met the basic publishing requirements of the World Journal of Clinical Cases, and the manuscript is conditionally accepted. I have sent the manuscript to the author(s) for its revision according to the Peer-Review Report, Editorial Office's comments and the Criteria for Manuscript Revision by Authors. Before its final acceptance, please provide and upload the following important documents: Biostatistics Review Certificate, a statement affirming that the statistical review of the study was performed by a biomedical statistician; Institutional Review Board Approval Form or Document, the primary version (PDF) of the Institutional Review Board's official approval, prepared in the official language of the authors' country; Signed Informed Consent Form(s) or Document(s), the primary version (PDF) of the Informed Consent Form that has been signed by all subjects and investigators of the study, prepared in the official language of the authors' country; STROBE Statement, an important document related to manuscript writing of observational/case control/retrospective cohort studies. Before final acceptance, uniform presentation should be used for figures showing the same or similar contents; for example, "Figure 1Pathological changes of atrophic gastritis after treatment. A: ...; B: ...; C: ...; D: ...; E: ...; F: ...; G: ...". Please provide decomposable Figures (in which all components are movable and editable), organize them into a single PowerPoint file. Please authors are required to provide standard three-line tables, that is, only the top line, bottom line, and column line are displayed, while other table lines are hidden. The contents of each cell in the table should conform to the editing specifications, and the lines of each row or column of the table should be aligned. Do not use carriage returns or spaces to replace lines or vertical lines and do not segment cell content. Please check and confirm whether the figures are original (i.e. generated de novo by the author(s) for this paper). If the picture is 'original', the author needs to add the following copyright information to the bottom right-hand side of the picture in PowerPoint (PPT): Copyright ©The Author(s) 2023. Before final acceptance, when revising the manuscript, the author must supplement and improve the highlights of the latest cutting-edge research results, thereby further improving the content of the manuscript. To this end, authors are advised to apply a new tool, the Reference Citation Analysis (RCA). RCA is an artificial intelligence technology-based open multidisciplinary citation analysis database. In it, upon obtaining search results from the keywords entered by the author, "Impact Index Per Article" under "Ranked by" should be selected to find the latest highlight articles, which can then be used to further improve an article under preparation/peer-review/revision. Please visit our RCA database for more information at: https://www.referencecitationanalysis.com/.

#	Editor's Comment	Requirement	Response
1	Biostatistics Review Certificate	A statement affirming that the statistical review of the study was performed by a biomedical statistician.	Thank you for this information. The required document is provided.
2	Institutional Review Board Approval Form or Document.	The primary version (PDF) of the Institutional Review Board's official approval, prepared in the official language of the authors' country.	Thank you for this information. Our research (manuscript ID: 88568) is secondary analysis of de-identified data from the Teen-LABS study, which means we don't have Institutional Review Board Approval Form or Signed Informed Consent Form. Instead, we can provide a letter of IRB approvement, and informed consent statement.
3	Signed Informed Consent Form(s) or Document(s), the primary version (PDF) of the Informed Consent Form	The primary version (PDF) of the Informed Consent Form that has been signed by all subjects and investigators of the study, prepared in the official language of the authors' country.	Thank you for this information. Our research (manuscript ID: 88568) is secondary analysis of de-identified data from the Teen-LABS study, which means we don't have Institutional Review Board Approval Form or Signed Informed Consent Form. Instead, we can provide a letter of IRB approvement, and informed consent statement.
4	STROBE Statement	An important document related to manuscript writing of observational/case control/retrospective cohort studies.	Thank you for this information. The required document is provided.
5	Uniform figure presentation	Uniform presentation should be used for figures showing the same or similar contents; for example, "Figure 1Pathological changes of atrophic gastritis after treatment. A: ...; B: ...; C: ...; D: ...; E: ...; F: ...; G: ...". Please provide decomposable Figures (in which all components are movable and editable), organize them into a single PowerPoint file.	Thank you for this information, we revised our figures accordingly. We additionally provided a PowerPoint file for your convenience.
6	Table format	Standard three-line tables, that is, only the top line, bottom	Thank you for this information, we

		line, and column line are displayed, while other table lines are hidden; The contents of each cell in the table should conform to the editing specifications, and the lines of each row or column of the table should be aligned; Do not use carriage returns or spaces to replace lines or vertical lines and do not segment cell content.	revised our tables accordingly.
7	Copyright of figures	Please check and confirm whether the figures are original (i.e. generated de novo by the author(s) for this paper). If the picture is 'original', the author needs to add the following copyright information to the bottom right-hand side of the picture in PowerPoint (PPT): Copyright ©The Author(s) 2023	Thank you for acknowledging this information. These figures are original and copyright information are added.

Reviewer 1:

So far, many studies have reported the relationship between microRNA and NAFLD, but there is poor consistency in the global published research and evaluation of human liver miRNA expression. There is limited research on the human liver. This study identified new circulating miRNAs and analyzed their expression in different pathological features of NAFLD, which have mechanisms to promote or alleviate the progression of NAFLD. This is a new exploration and has good innovation. This study is of great significance for the diagnosis and treatment of NAFLD. As a contributor to the pathogenesis of human NAFLD, novel miRNAs are expected to serve as biomarkers for the noninvasive diagnosis and staging of NAFLD or hepatocellular carcinoma, or as targets for drug therapy, thereby preventing or reversing disease progression. The novel miRNA discovered in this study provides a new direction for targeted therapy of NAFLD. Due to the different types and quantities of miRNA expression at different stages of NAFLD, as well as differences in gender and whether obesity is present (such as obesity with NAFLD or lean individuals with NAFLD), further research is needed. Due to the relatively small sample size of this study, gender stratification studies were not conducted. Additionally, it would be better if specific miRNAs that reflect disease progression or deterioration could be identified.

#	Reviewers Comment	Response	Changes in manuscript
1	Due to the relatively small sample size of this study, gender stratification studies were not conducted.	Thank you for this comment. We acknowledge that the limited sample size in our study, with a notable disproportion in gender distribution. Specifically, we only had 36 male participants. As a result, we did not conduct stratification analysis by sex. We expanded our discussion of sample size issue in manuscript.	Discussion: Line: 464-467 Given our specific focus on adolescents with obesity, who are at high risk of developing NAFLD(Loomis et al., 2016; Marchesini et al., 2003; Xanthakos et al., 2015), and the limitations arising from our small sample size, a validation for generalizability is imperative. The limitation in sample size is an inherent consequence of our methodological choice to employ liver biopsy for NAFLD diagnosis. While liver biopsy ensures accurate and definitive diagnosis, its invasiveness and cost present challenges in expanding the participant pool(Spengler & Loomba, 2015). Studies with larger and more diverse populations would facilitate more robust and conclusive findings regarding NAFLD–miRNA associations.
2	Additionally, it would be better if specific miRNAs that reflect disease progression or deterioration could be identified.	Thank you for your valuable feedback and suggestions. We added a discussion of miRNA that may reflect NAFLD progressions.	Discussion: Line: 386-394: Additionally, our analysis revealed decreased levels of miR-1296-5p, miR-1301-5p, miR-199b-5p, miR-411-5p, and miR-6885-3p in NASH compared

			to NAFL, while these levels were elevated in NAFLD compared to individuals without NAFLD. These miRNAs also demonstrated predominantly negative associations with fibrosis, lobular inflammation and ballooning (Figure 2). Notably, the downregulation of miR-411-5p aligned with a recent study by Wan et al.(Wan et al., 2022), which reported decreased expression of serum miR-411-5p in persons with NASH. Collectively, the distinct expression patterns observed across various NAFLD features suggest that these miRNAs may serve as potential biomarkers for NAFLD progression.

Reviewer 2:

Specific Comments to Authors: This study indicates the differential expression of circulating miRNAs in adolescent NAFLD, suggesting that they may become diagnostic and prognostic biomarkers for NAFLD. However, there are two shortcomings. Firstly, the sample size is small and research needs to be conducted in a larger and more diverse populations. The second issue is that there has been no molecular mechanism validation of differentially expressed circulating miRNAs through cytology or animal experiments.

#	Reviewers Comment	Response	Changes in manuscript
1	Firstly, the sample size is small and research needs to be conducted in a larger and more diverse populations.	Thank you for your comment. We expanded our discussion of sample size limitations in this study.	Discussion: Line: 464-467 Given our specific focus on adolescents with obesity, who are at high risk of developing NAFLD(Loomis et al., 2016; Marchesini et al., 2003; Xanthakos et al., 2015), and the limitations arising from our small sample size, a validation for generalizability is imperative. The limitation in sample size is an inherent consequence of our methodological choice to employ liver biopsy for NAFLD diagnosis. While liver biopsy ensures accurate and definitive diagnosis, its invasiveness and cost present challenges in expanding the participant pool(Spengler & Loomba, 2015). Studies with larger and more diverse populations would facilitate more robust and conclusive findings regarding NAFLD–miRNA associations.

2	The second issue is that there has been no molecular mechanism validation of differentially expressed circulating miRNAs through cytology or animal experiments.	Thank you for your insightful comment and suggestion. While we acknowledge this limitation, we reviewed experimental studies and expanded our discussion on potential mechanisms associated with the identified miRNAs in NAFLD. Additionally, we have provided a summary of current findings on miRNA pathways in NAFLD based on experimental research, presented in Table 3.	Result: Table 3. Discussion: Line: 397-399 An increasing body of research have investigated the associations between NAFLD and miRNA expression, yet little is known about mechanisms underlying the dysregulation of circulating miRNA in NAFLD patients. Line: 409-434 Experimental studies provide valuable insights into the molecular mechanisms underlying associations between NAFLD and miRNA expression while minimizing confounding variables intrinsic to human observational studies. Particularly, miR-122, a highly expressed hepatic miRNA in hepatocytes, is associated with NAFLD progression by regulating lipid metabolism[23]. For example, Long et al.[74] revealed that miR-122 inhibited liver kinase B1/AMP-activated protein kinase signaling pathway, which further induced hepatic lipogenesis and steatosis in NAFLD. Additionally, the inhibition of miR-122-5p may suppress the inflammation and oxidative stress damage in NAFLD[75]. Given the observed upregulation of circulating miR-122 and downregulation of hepatic miR-122 in NASH patients[30, 36], the elevated circulating miR-122-5p across NAFLD features in our study might be released by hepatocytes, as a protective mechanism against NAFLD progression[23]. Furthermore, we identified downregulation of plasma miR-146a-5p,

			miR-181a-5p, and miR-22-3p in individuals with NAFLD, which is supported by experimental studies of miRNA. For example, miR-146a targeted complex subunit 1 (MED1) to attenuate lipid accumulation and alleviate NAFLD progression in mice[76]. Additionally, miR-181a was found to downregulate peroxisome proliferator-activated receptor-α (PPARα) and mediate lipid metabolisms in NAFLD in human liver cells[77]. Moreover, miR-22 is a pivotal regulator of lipid and glucose metabolism, playing a crucial role in mitigating NAFLD progression in mice[78]. For example, miR-22 inhibited sirtuin-1 and regulated gluconeogenesis in NAFLD[79]. We also observed increased expression of miR-125b-2-3p in both NAFLD and lobular inflammation, while studies indicated that miR-125b promoted the NF-κB-mediated inflammatory response in NAFLD[80]. Furthermore, we observed positive associations between liver fibrosis and expression of miR-34a-5p and miR-375, while experimental research suggested that both miR-34a-5p and miR-375 could alleviate liver fibrosis[81, 82]. Together these experimental data support a plausible biological mechanism of NAFLD-miRNA association (Table 3).

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