Serum miR-4488 as a potential biomarker of lean nonalcoholic fatty liver disease

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Background: In lean individuals, nonalcoholic fatty liver disease (NAFLD) is not a benign disease, and these patients have long-term morbidity and mortality similar to those of their nonlean counterparts. Finding biomarkers for noninvasive and early detection is urgent and microRNAs (miRNAs) show potential. The aims of this study were to investigate the potential role of serum miRNAs in the detection of lean NAFLD and to explore the possible pathogenesis of lean NAFLD.

Methods: A total of 498 patients with NAFLD and 98 healthy controls were included to compare the clinical characteristics of lean NAFLD patients [LNs: body mass index (BMI) <23 kg/m^2], nonlean NAFLD patients (NLNs: BMI ≥23 kg/m^2) and normal healthy individuals (HIs). A total of 14 serum samples were collected from 4 LNs, 6 NLNs and 4 HIs for high-throughput profiling to identify altered miRNA expression patterns in lean NAFLD. The candidate miRNA, miR-4488, was identified by filtering based on studies in a second independent cohort (31 LNs, 62 NLNs, 72 HIs) that included quantitative real-time polymerase chain reaction (qRT-PCR) analysis. Gene Ontology (GO) enrichment, Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment, and protein-protein interaction network analyses were performed to investigate the potential molecular mechanism of miR-4488 in lean NAFLD.

Results: LNs were older and had a smaller waist circumference, lower levels of alanine aminotransferase, glutamyl transpeptidase, fasting insulin, and uric acid, lower HOMA-IR score, and higher levels of total cholesterol, high-density lipoprotein cholesterol, and hemoglobin (P<0.05). The serum level of miR-4488 was increased in LNs compared with HIs (P<0.0001) and NLNs (P=0.025). miR-4488 had acceptable performance in predicting [area under the curve (AUC) =0.794, 0.698] lean NAFLD. Moreover, GO and KEGG enrichment analyses revealed that the differentially expressed target genes were mainly involved in choline metabolism in cancer, the tumor-necrosis factor (TNF) signaling pathway and the p53 signaling pathway. PPI analysis identified ARHGAP1, SLC10A1 and SIX5 as the hub genes.

Conclusions: Taken together, our findings indicate that serum miR-4488 is a potential biomarker for diagnosing and predicting the pathogenetic mechanisms of lean NAFLD.

Keywords: Biomarkers; lean nonalcoholic fatty liver disease (LN); miRNA-4488

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Introduction

Nonalcoholic fatty liver disease (NAFLD) affects one-quarter of the adult population worldwide, contributing to a very large health burden (1). Based on epidemiological statistics, a dramatic increase in the percentage of the Chinese population affected by NAFLD (from 18% to 29%) occurred within one decade (2). A substantial proportion of patients with NAFLD are lean, as defined solely by body mass index (BMI) <23 kg/m^2, epitomizing the fallout of the rapid social transformation in Asia (3). Several studies have shown that dysregulation of lipid metabolism can severely affect NAFLD (4-6). 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 long-term morbidity and mortality similar to those of their nonlean counterparts (8). However, due to the insidious onset of lean fatty liver disease, it is not easy to detect, and it is often in an advanced stage when diagnosed. Accordingly, it is urgent to explore potential biomarkers for noninvasive and early detection of lean NAFLD, preventing the misclassification of the lean subpopulation as having a benign disease.

To date, the underlying pathophysiology of lean NAFLD has not been thoroughly elucidated (9), and no approved drug therapy has been recommended (10). Genetics and epigenetics play a key role in the development of NAFLD (11). Genetic predisposition has been proposed as a pathogenic factor to better characterize this type of patient, and major candidate genes include *PNPLA3*, *TM6SF2*, *CETP* and *PEMT* (12). The most well-characterized common variant associated with lean NAFLD is the nonsynonymous variant of *PNPLA3* corresponding to p.I148M (13), although Younes *et al.* indicated that NAFLD may progress to advanced liver disease independent of *PNPLA3* genotype (14). These reports suggest that new candidate genes must be identified to more accurately recognize lean NAFLD. In addition, epigenetic changes interact with inherited risk factors to determine susceptibility to lean NAFLD (15). Gene expression regulation is provided by microRNAs (miRNAs), single-stranded noncoding RNAs composed of 20–24 nucleotides that function as regulators of gene expression and participate in the translation of proteins (16). Altered miRNA expression can thus influence the development and progression of a variety of pathophysiological processes (11). A study has 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). A study by Liu *et al.* indicated that miR-192 expression leads to progression of NAFLD (18). These miRNAs have also been proposed as diagnostic biomarkers for liver injury and potential targets for treatment (15). However, few studies have shown the role of miRNAs in detecting lean NAFLD in the absence of obesity.

In this study, we isolated miRNAs from serum and assessed their expression levels in lean patients with NAFLD (LNs), nonlean patients with NAFLD (NLNs) and normal healthy individuals (HIs). The aim of this study was to explore the potential miRNA biomarkers that could explain the pathogenetic mechanisms of lean NAFLD. We present the following article in accordance with the STARD reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-22-6620/rc).

Highlight box

Key findings

- Serum miR-4488 is a potential biomarker for diagnosing and predicting the pathogenetic mechanisms of lean NAFLD.

What is known and what is new?

- In lean individuals, NAFLD is not a benign disease, and these patients have longterm morbidity and mortality similar to those of their nonlean counterparts.
- Lean NAFLD patients were older and had a smaller waist circumference, lower levels of ALT, GGT, FINS, and UA, lower HOMA-IR score; and higher levels of TC, HDL-C, and Hb. miR-4488 was selected as the candidate biomarker for lean NAFLD.

What is the implication, and what should change now?

- The integration of a molecular diagnosis in the clinical evaluation of patients with lean NAFLD will provide an accurate diagnosis with possible targeted therapies and may uncover novel molecular mechanisms with potential broader therapeutic implications.

Methods

Study design, patients and HIs

A total of 498 NAFLD patients and 98 HIs were enrolled between January 2020 and January 2022. The NAFLD patients consisted of two cohorts: one with BMI <23 kg/m^2 (LNs, n=98) and the other with BMI ≥23 kg/m^2 (NLNs, n=400). NAFLD requires: (I) evidence of hepatic steatosis either by imaging or histology, and (II) absence of other causes of hepatic fat accumulation from conditions such as significant alcohol consumption, hepatitis C, medication

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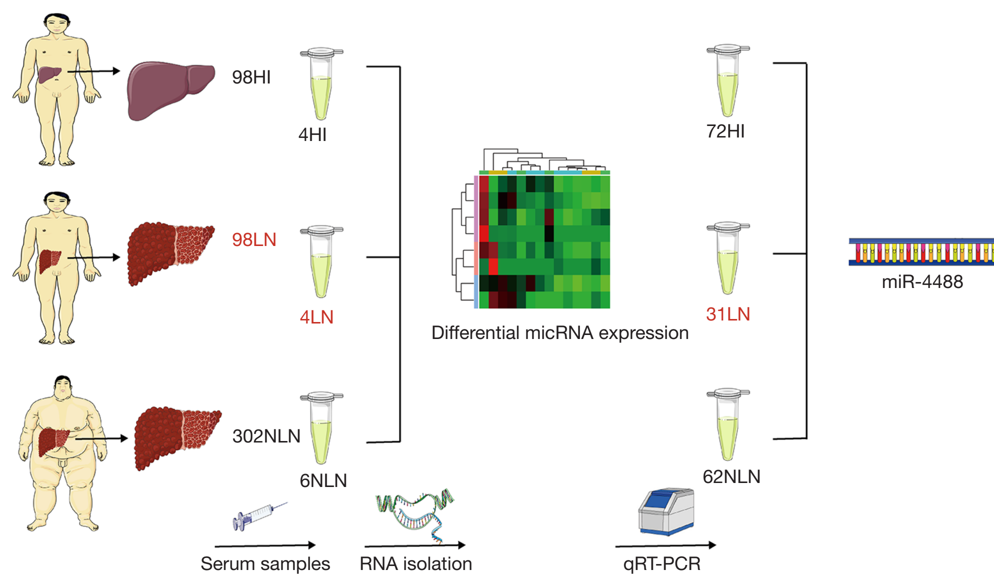

Figure 1 Flow chart of the sequencing analysis. HI, healthy individual; LN, lean nonalcoholic fatty liver disease; NLN, nonlean nonalcoholic fatty liver disease; qRT-PCR, quantitative real-time polymerase chain reaction.

use, or hereditary disorders (19). Patients with significant alcohol consumption (>20 g/day for females and >30 g/day for males) or any preexisting liver disease were excluded. Healthy control subjects were selected from individuals seeking a routine health checkup at Longhua Hospital, Shanghai University of Traditional Chinese Medicine, and who had no evidence or history of liver disease, CVD or systemic disease that would result in their exclusion as a control in this study. Documented informed consent was given by each subject, and all aspects of the study were approved by the Ethics Committee of Longhua Hospital, Shanghai University of Traditional Chinese Medicine (No. 2020LCSY080). The study was performed in accordance with the relevant guidelines and regulations and the Declaration of Helsinki (as revised in 2013).

Blood samples and RNA isolation

Peripheral blood samples were centrifuged at 1,500 ×g for 10 min at 4 ℃. Serum was separated into EP tubes and stored at −80 ℃ until further use. The serum samples were divided into three comparison groups: 4 LNs *vs.* 4 HIs, 4 LNs *vs.* 6 NLNs, and 31 LNs *vs.* 62 NLNs *vs.* 72 HIs. The first two groups were used for next-generation sequencing analysis. A total of 165 samples (31 LNs *vs.* 62 NLNs *vs.* 72 HIs) were used to verify the selected candidate biomarker (*Figure 1*). Total RNA was extracted from serum using a TRIzol Kit (Invitrogen, Life Technologies, Carlsbad, CA, USA) and an RNeasy Serum Kit (Qiagen, Hilden, Germany) following the manufacturers' instructions. The total RNA quantity and purity were confirmed with an Agilent High Sensitivity DNA Kit (Agilent Technologies, Inc., USA) using a Bioanalyzer 2100 (Agilent Technologies, Inc., USA).

Small RNA library preparation and sequence analysis

Small RNA libraries were constructed using a New England Biolabs (NEB) NEBNext Multiplex Small RNA Library Prep Kit (MA, USA) for Illumina sequencers. The qualified libraries were sequenced on the Illumina HiSeq X Ten platform (Illumina, Shanghai, China). Sequencing reads were uploaded to the Repbase database. Adapter trimming, quality control and ambiguous read sorting were performed, and the reads were then annotated using miRBase.

Quantitative real-time polymerase chain reaction (qRT-PCR) analysis for identification and validation of candidate serum miRNAs

The candidate miRNAs were further validated by qRT-PCR performed using the QuantiFast® SYBR® Green PCR Master Mix in an ABI 9700 PCR System (Applied Biosystems, Foster City, CA, USA). The relative expression

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Table 1 Sequence of primer

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

values of the target miRNAs were normalized to that of RNU6B (U6), and the differences in gene expression were analyzed using the $2^{-\Delta\Delta Ct}$ method. The primer sequence is shown in *Table 1*.

miRNA target gene prediction and pathway analysis

The TargetScan Human database (release 7.2, http://www.targetscan.org/vert_72/) and MicroRNA Target Prediction Database (miRDB; http://mirdb.org/) were used for the prediction of miRNA targets. Gene Ontology (GO; www.geneontology.org) enrichment analysis was then performed to analyze the main function of each putative target gene. Kyoto Encyclopedia Genes and Genomes (KEGG; www.genome.jp/kegg) enrichment analysis was applied to identify molecular pathways that were potentially altered.

Functional analysis of the validated or predicted targets of selected miRNAs was performed with STRING 10 (http://string-db.org/).

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics 26.0 (IBM Corp., Armonk, NY, USA), and graphs were generated with GraphPad Prism (v. 8.0.1, GraphPad Software, San Diego, CA, USA). Differential miRNA expression analysis was performed with DESeq2. Differential expression was assumed at a false detection rate <0.05. Comparisons among the three groups were performed using the nonparametric Kruskal-Wallis H test followed by the Mann-Whitney U test. Receiver operating characteristic (ROC) analysis was performed for each validated miRNA to investigate its sensitivity and specificity in distinguishing group differences. The statistical significance of the area under the curve (AUC) was assessed with the Mann-Whitney U test. A P value <0.05 was considered statistically significant. The data are presented as the mean ± standard deviation (SD) values.

Results

Clinical characteristics of the subjects

The discovery cohort consisted of 98 LNs, 302 NLNs, and 98 HIs. Their clinical characteristics are summarized in *Table 2*. The LNs were older and had a smaller waist circumference, lower levels of alanine aminotransferase, glutamyl transpeptidase, fasting insulin, and uric acid, lower HOMA-IR score, and higher levels of total cholesterol, high-density lipoprotein cholesterol, and hemoglobin (P<0.05). No statistically significant differences were observed between the LN and NLN groups with regard to systolic and diastolic blood pressures, aspartate aminotransferase, total bilirubin, triglycerides, low-density lipoprotein cholesterol, fasting plasma glucose, HbA1c glycated hemoglobin, and C-reactive protein (P>0.05).

Altered miRNA expression patterns

The heatmap of all the samples showed differentially expressed miRNAs among the three groups (*Figure 2A*). In total, 8 miRNAs showed altered expression among 4 LNs, 6 NLNs and 4 HIs. When pairwise comparisons of the overall miRNA expression profile were analyzed with a heatmap, 2 miRNAs (miR-4488 and miR-5189-5p) showed altered expression between 4 LNs and 4 HIs, and both were upregulated (*Figure 2B*). In addition, 4 miRNAs showed altered expression between 4 LNs and 6 NLNs: 2 upregulated miRNAs (miR-4488 and miR-4443) and 2 downregulated miRNAs (miR-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 2E*).

Selection and verification of a serum miRNA as a diagnostic biomarker

Subsequent analysis indicated that miR-4488 showed the same alteration patterns between LNs and HIs, and between LNs and NLNs; thus, it was selected as the candidate biomarker for lean NAFLD (*Figure 3A*). The serum level of miR-4488 (*Figure 3B*) was significantly higher in LNs than in HIs (P<0.0001). ROC curves were constructed to evaluate the diagnostic value of miR-4488 for LN, and the AUC was 0.794 [95% confidence interval (CI): 0.702–0.887] (*Figure 3C*). In addition, the serum level of miR-4488 was higher in LNs than in NLNs (P=0.025). ROC curves were constructed to evaluate the diagnostic value of miR-4488

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

Parameter	Group 1 (N=98) (LN)	Group 2 (N=302) (NLN)	Group 3 (N=98) (HI)
Sex (M/F)	46/52	208/94	46/52
Age (years)	52.34±16.79	48.45±14.50*	53.19±15.75
Waist (cm)	82.32±3.73	90.06±3.73*	79.13±4.61▲
SBP (mmHg)	123.90±8.94	125.52±8.73	124.12±8.65
DBP (mmHg)	72.97±5.24	74.11±5.48	71.97±3.24
NAFLD duration (years)	3.19±1.91	3.15±2.06	–
ALT (U/L)	29.38±23.32	36.49±27.09*	15.98±7.74▲
AST (U/L)	25.11±11.54	27.51±13.37	24.24±8.97
ALP (U/L)	70.88±23.01	71.03±16.02	69.01±15.90
GGT (U/L)	34.07±12.61	39.36±15.54*	30.11±8.21
TBIL (μmol/L)	15.60±6.77	13.97±5.19	13.43±4.87
TC (mmol/L)	5.87±1.19	5.32±1.22*	5.01±0.62▲
TG (mmol/L)	2.03±1.47	2.11±1.42	1.22±0.33▲
HDL-C (mmol/L)	1.43±0.305	1.27±0.27*	2.31±0.27▲
LDL-C (mmol/L)	3.41±1.28	3.46±0.92	2.03±0.75▲
FPG (mmol/L)	5.56±1.28	5.76±1.64	4.81±0.37▲
HbA1C (%)	5.40±0.73	5.65±0.98	5.55±0.74
FINS (mU/L)	7.69±2.52	12.54±2.45*	4.70±0.15▲
HOMA-IR	1.90±0.78	3.19±0.97*	1.00±0.07▲
UA (μmol/L)	339.74±102.50	379.97±82.14*	314.30±24.32▲
Hb (g/L)	161.94±16.23	159.43±14.56*	147.50±12.07
CRP (mg/L)	1.52±1.88	1.17±1.32	0.03±0.15▲

Data are presented as mean ± SD of each group. *, comparison between groups 1 and 2; ▲, comparison between groups 1 and 3. LN, lean nonalcoholic fatty liver disease; NLN, nonlean nonalcoholic fatty liver disease; HI, healthy individual; SBP, systolic blood pressure; DBP, diastolic blood pressure; NAFLD, nonalcoholic fatty liver disease; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, glutamyl transpeptidase; TBIL, total bilirubin; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; FPG, fasting plasma glucose; HbA1c, glycated hemoglobin; FINS, fasting insulin; HOMA-IR, homeostatic model assessment of insulin resistance; UA, uric acid; Hb, hemoglobin; CRP, C-reactive protein; SD, standard deviation.

for LN, and the AUC was 0.698 (95% CI: 0.583–0.812) (*Figure 3D*). ROC curve analysis was performed to evaluate whether the serum level of miR-4488 could be used as a potential diagnostic biomarker for lean NAFLD.

GO and KEGG enrichment analyses and construction of the PPI network for the key target genes of miR-4488

Based on the results of RNA sequencing and subsequent validation, the TargetScan and miRDB websites were used to predict the potential target genes of miR-4488. To further understand the functions of the predicted target genes of miR-4488, the main biological processes (BPs), cellular components (CCs) and molecular functions (MFs) identified by GO enrichment analysis of the target genes of miR-4488 were summarized (*Figure 4A*). Regulation of signaling and regulation of cell communication were the two most enriched BP subcategories; intrinsic component of plasma membrane and integral component of plasma membrane were the two most enriched CC subcategories;

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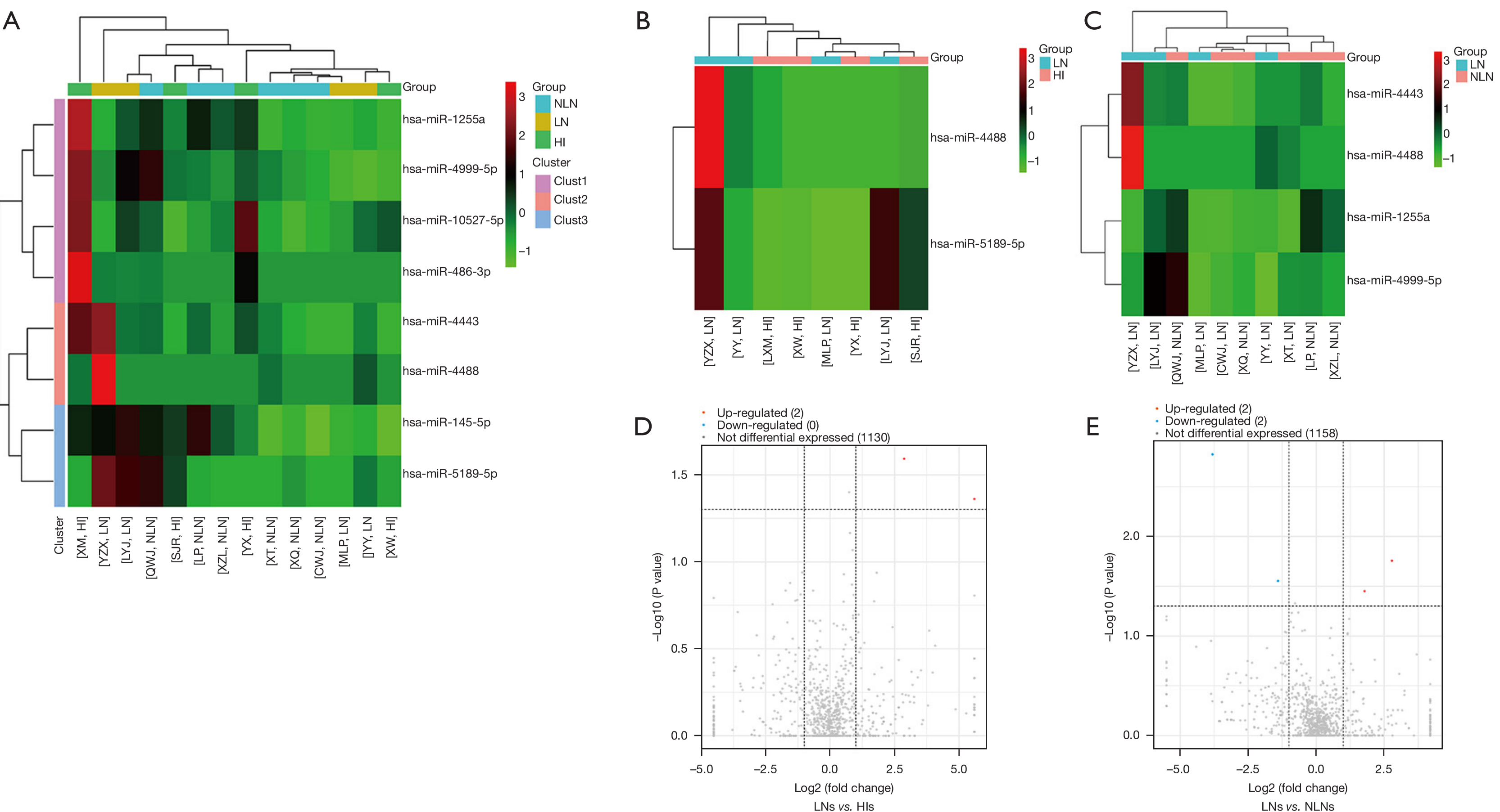

Figure 2 Results of next-generation sequencing analysis. (A) Heatmap based on Spearman rank correlation as the distance matrix indicating the microRNA (miRNA) expression profiles across the groups. When heatmaps (B,C) were plotted for pairwise group comparisons, the intensity of differentially expressed miRNAs was determined between the (B) LN and HI groups and the (C) LN and NLN groups. In each panel, the top axis shows the group of samples; the left axis shows the clustering of miRNAs; and the bottom axis shows the individuals. Volcano plot showing the differentially expressed miRNAs between 4 LNs *vs.* 4 HIs (D) and 4 LNs *vs.* 6 NLNs (E). NLN, nonlean nonalcoholic fatty liver disease; LN, lean nonalcoholic fatty liver disease; HI, healthy individual.

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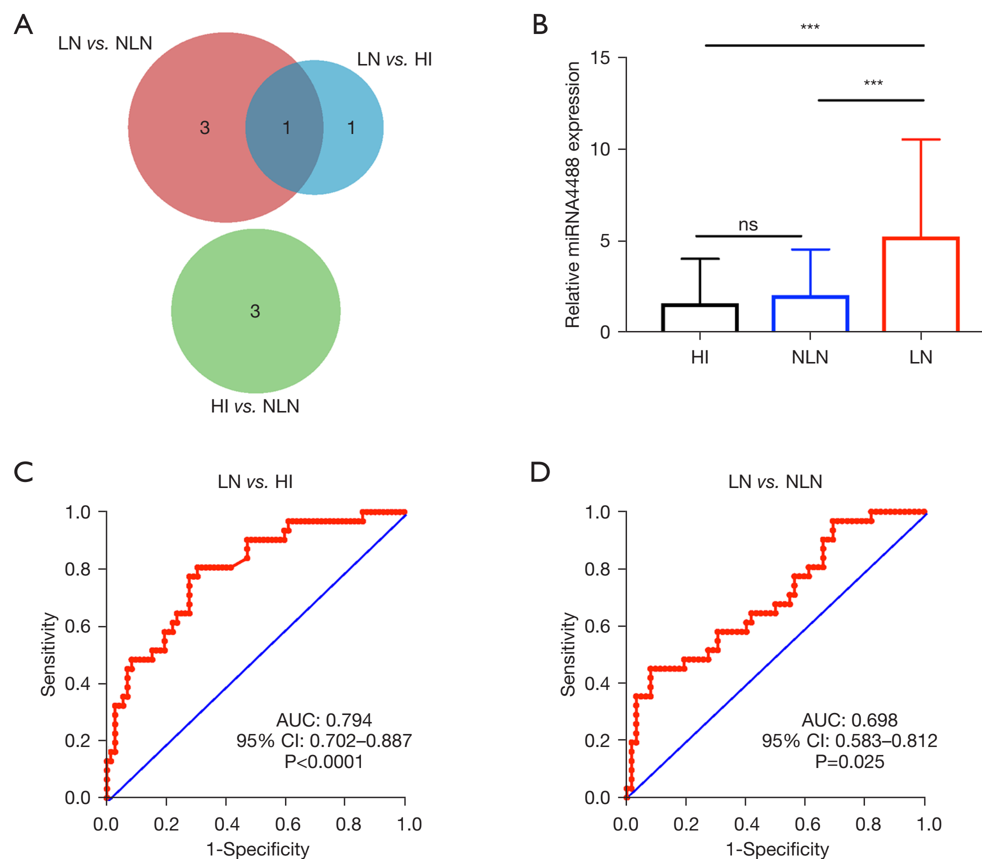

Figure 3 Serum level of microRNA, miR-4488, in the validation cohort (31 LNs *vs.* 62 NLNs *vs.* 72 HIs) by qRT-PCR. Venn diagram showing that miR-4488 had the same alteration patterns between LNs and HIs and between LNs and NLNs (A). The expression of miR-4488 was significantly higher in LNs than in HIs (P<0.0001) and NLNs (P=0.025) (B). ROC curve analysis showed that miR-4488 had diagnostic value in the LN *vs.* HI comparison; the AUC was 0.794 (95% CI: 0.702–0.887) (C). ROC curve analysis showed miR-4488 had diagnostic value in the LN *vs.* NLN comparison; the AUC was 0.698 (95% CI: 0.583–0.812) (D). ***, P<0.001. ns, not significant; HI, healthy individual; LN, lean nonalcoholic fatty liver disease; NLN, nonlean nonalcoholic fatty liver disease; qRT-PCR, quantitative real-time polymerase chain reaction; ROC, receiver operating characteristic; AUC, area under the curve; CI, confidence interval.

and GTPase binding was the most enriched MF subcategory.

To better understand the biological functions of the predicted target genes, KEGG enrichment analysis was performed to reveal the main pathways in which the candidate target genes may be involved. The top 20 statistically significantly enriched pathways were identified and are shown in *Figure 4B*. Choline metabolism in cancer (hsa05231), the tumor-necrosis factor (TNF) signaling pathway (hsa04668), p53 signaling pathway (hsa04115), circadian entrainment (hsa04713) and basal cell carcinoma (hsa05217) were the top five enriched pathways (*Figure 4B*).

Protein-protein interaction (PPI) network analysis is a viable tool to understand MFs and disease mechanisms. A PPI network with 30 nodes was constructed (*Figure 4C*), and the most important modules were then screened. Among the genes in these modules, *ARHGAP1*, *SLC10A1*, *SIX5*, *WTIP*, *CTNNA1*, *BCL7A*, *MYOD1*, *GLB1L2*, *ASPA*, and *SLC9A3R2* were the 10 genes with the closest connections to other nodes.

Discussion

Emerging data suggest that NAFLD is present in a considerable proportion of lean individuals, as described by the previous study (20). Lean NAFLD used to be considered a benign disease, and clinicians always ignored its therapy and management (21). Considering that lean NAFLD

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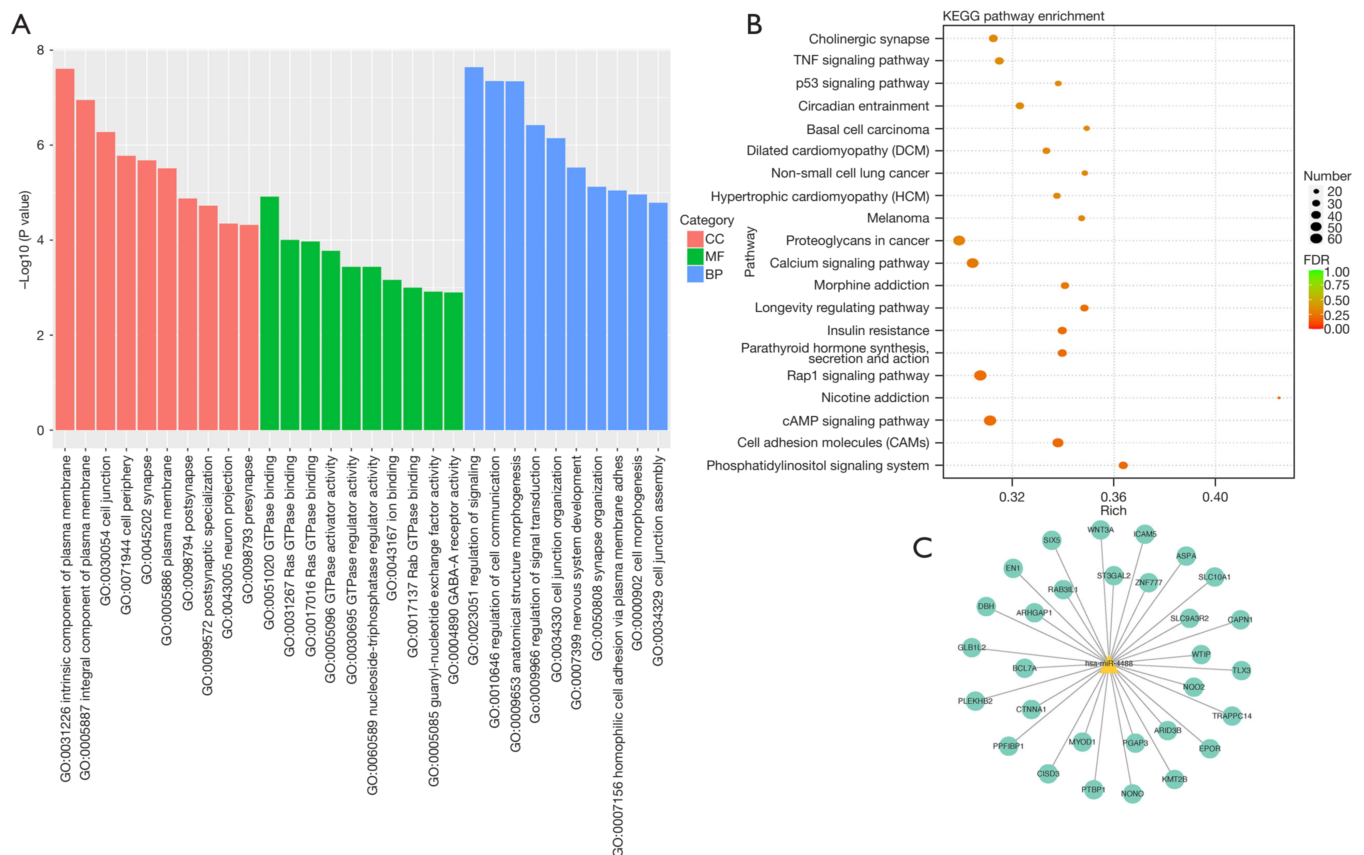

Figure 4 Bioinformatic analysis of the predicted target genes of microRNA, miR-4488. GO enrichment analysis of the predicted target genes of miR-4488 (A). KEGG enrichment analysis of the predicted target genes of miR-4488 revealed the top 20 most enriched KEGG pathways (B). Protein-protein interaction network of the predicted target genes of miR-4488. The nodes are proteins that were predicted as the targets of miR-4488, and the edges represent the functional associations (C). CC, cellular component; MF, molecular function; BP, biological process; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.

patients can develop the full spectrum of liver damage that characterizes nonlean NAFLD (22), finding more accurate biomarkers for noninvasive and early detection of lean NAFLD is essential. Many efforts have been made to explain the progression of NAFLD according to genes and miRNAs (23). However, the pathophysiological mechanisms underlying NAFLD development in lean subjects are not entirely understood (24). Our data showed that serum miR-4488 may have potential for noninvasive and early detection of lean NAFLD. The serum miR-4488 level was significantly higher in LNs than in NLNs and HIs.

Very recently, the range of miRNA applications has broadened as they are increasingly used in different clinical settings for early disease detection and monitoring of disease progression. A study by Fang *et al.* (25) indicated that a reduction in miR-4488 expression induces venous endothelia cell inflammation via the COX-2/NFκB pathway, showing high potential for preventing venous graft disease. miR-4488 participates in autophagy by targeting the N-acetyl transferase 8-like (NAT8L) protein and affects mitochondrial function (26). miR-4488 is highly downregulated in adeno-associated virus transduction associated with hepatocellular carcinoma (27). Huang *et al.* (28) found a new miRNA signaling pathway in vascular endothelial cell autophagy and inflammation, showing that NAT8L was downregulated as an important target of miR-4488. However, the function of miR-4488 in lean NAFLD is unclear.

To predict the function of miR-4488 in lean NAFLD, we performed GO and KEGG enrichment analyses. Several enriched pathways were identified, among which choline metabolism in cancer, the TNF signaling pathway, p53

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signaling pathway, circadian entrainment, and basal cell carcinoma were the top five. Activated choline metabolism, the TNF signaling pathway, and basal cell carcinoma are associated with hallmarks of carcinogenesis and tumor progression (29). Another pathway related to hepatocyte apoptosis identified in our study was the p53 signaling pathway (30). A previous study demonstrated that p53/DRAM-mediated mitophagy is a primary inducer of apoptosis in mild hepatosteatosis, whereas p53-induced BAX expression induces apoptosis mainly in severe hepatosteatosis (31). Circadian entrainment was another related pathway identified by the present study. A recent study revealed that morning circadian misalignment was associated with metabolic dysregulation in girls who were obese (32). Schwerbel *et al.* revealed that members of the immunity-related GTPase family act as regulators of hepatic fat accumulation, with links to autophagy (33). Overexpression of the *Ifgga2* gene was shown to reduce hepatic lipid storage and could be a therapeutic target for fatty liver disease (33). Thus, miR-4488 may influence the progression of lean NAFLD by participating in these signaling pathways.

Moreover, our PPI network analysis revealed that miR-4488 regulatory targets relevant to lean NAFLD included *ARHGAP1*, *SLC10A1*, *SIX5*, *CTNNA1*, and *WTIP*. There are limited reports on these genes: studies on *ARHGAP1* and the PI3K/AKT pathway in breast cancer (34), *SLC10A1* and metabolic changes in hepatoblastoma cells (35), *DMAHP/SIX5* in myotonic dystrophy (36), *CTNNA1* in gastric and breast cancer (37), and *WTIP* and the Hippo pathway in hepatocellular carcinoma (38). The precise mechanisms still need to be fully described. However, the precise mechanism by which miR-4488 acts by regulating these targets in NAFLD still needs to be validated by more experiments.

To the best of our knowledge, ours is the first study to examine the correlation between the serum levels of miRNAs and lean NAFLD. However, significant correlations remain to be found between miR-4488 and its target genes. Further studies are needed in the future to clearly explain the pathophysiology and provide novel options for therapy.

Future translational research in this field may provide new diagnostic and therapeutic approaches to treat lean NAFLD, considering the miRNA-driven regulation of this disease. However, several limitations still exist. First, the diagnosis of sarcopenic obesity based solely on BMI is controversial. Waist circumference or body composition analysis should be used in future studies to better define lean NAFLD. Second, the cohort in the present study was relatively small, so the results still need to be verified in a larger population with other specific phenotypes.

Collectively, the integration of a molecular diagnosis in the clinical evaluation of patients with lean NAFLD will provide an accurate diagnosis with possible targeted therapies and may uncover novel molecular mechanisms with potential broader therapeutic implications.

Conclusions

In summary, this study showed that the serum level of miR-4488 was increased in LNs compared with HIs and NLNs. miR-4488 may be a potential biomarker for diagnosing and predicting the pathogenetic mechanisms of lean NAFLD.

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Footnote

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://atm.amegroups.com/article/view/10.21037/atm-22-6620/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Documented informed consent was given by each subject, and all aspects of the study were approved by the Ethics Committee of Longhua Hospital, Shanghai University of Traditional Chinese Medicine (No. 2020LCSY080). The study was performed in accordance with the relevant guidelines and regulations and the Declaration of Helsinki (as revised in 2013).

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References

1. Eslam M, Newsome PN, Sarin SK, et al. A new definition for metabolic dysfunction-associated fatty liver disease: An international expert consensus statement. J Hepatol 2020;73:202-9.
2. Zhou F, Zhou J, Wang W, et al. Unexpected Rapid Increase in the Burden of NAFLD in China From 2008 to 2018: A Systematic Review and Meta-Analysis. Hepatology 2019;70:1119-33.
3. Eslam M, Sanyal AJ, George J, et al. MAFLD: A Consensus-Driven Proposed Nomenclature for Metabolic Associated Fatty Liver Disease. Gastroenterology 2020;158:1999-2014.e1.
4. Tian S, Zhao H, Song H. Shared signaling pathways and targeted therapy by natural bioactive compounds for obesity and type 2 diabetes. Crit Rev Food Sci Nutr 2022. [Epub ahead of print]. doi: 10.1080/10408398.2022.2148090.
5. Guetterman TC, Fetters MD, Creswell JW. Integrating Quantitative and Qualitative Results in Health Science Mixed Methods Research Through Joint Displays. Ann Fam Med 2015;13:554-61.
6. Zhou S, You H, Qiu S, et al. A new perspective on NAFLD: Focusing on the crosstalk between peroxisome proliferator-activated receptor alpha (PPARα) and farnesoid X receptor (FXR). Biomed Pharmacother. 2022;154:113577.
7. Ye Q, Zou B, Yeo YH, et al. Global prevalence, incidence, and outcomes of non-obese or lean non-alcoholic fatty liver disease: a systematic review and meta-analysis. Lancet Gastroenterol Hepatol 2020;5:739-52.
8. Targher G, Byrne CD, Tilg H. NAFLD and increased risk of cardiovascular disease: clinical associations, pathophysiological mechanisms and pharmacological implications. Gut 2020;69:1691-705.
9. Younes R, Bugianesi E. NASH in Lean Individuals. Semin Liver Dis 2019;39:86-95.
10. Skubic C, Drakulić Ž, Rozman D. Personalized therapy when tackling nonalcoholic fatty liver disease: a focus on sex, genes, and drugs. Expert Opin Drug Metab Toxicol 2018;14:831-41.
11. Del Campo JA, Gallego-Durán R, Gallego P, et al. Genetic and Epigenetic Regulation in Nonalcoholic Fatty Liver Disease (NAFLD). Int J Mol Sci 2018.
12. Ren TY, Fan JG. What are the clinical settings and outcomes of lean NAFLD? Nat Rev Gastroenterol Hepatol 2021;18:289-90.
13. Vilarinho S, Ajmera V, Zheng M, et al. Emerging Role of Genomic Analysis in Clinical Evaluation of Lean Individuals With NAFLD. Hepatology 2021;74:2241-50.
14. Younes R, Govaere O, Petta S, et al. Caucasian lean subjects with non-alcoholic fatty liver disease share long-term prognosis of non-lean: time for reappraisal of BMI-driven approach? Gut 2022;71:382-90.
15. Gjorgjieva M, Sobolewski C, Dolicka D, et al. miRNAs and NAFLD: from pathophysiology to therapy. Gut 2019;68:2065-79.
16. Boon RA, Vickers KC. Intercellular transport of microRNAs. Arterioscler Thromb Vasc Biol 2013;33:186-92.
17. Wang X, He Y, Mackowiak B, et al. MicroRNAs as regulators, biomarkers and therapeutic targets in liver diseases. Gut 2021;70:784-95.
18. Liu XL, Pan Q, Cao HX, et al. Lipotoxic Hepatocyte-Derived Exosomal MicroRNA 192-5p Activates Macrophages Through Rictor/Akt/Forkhead Box

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Transcription Factor O1 Signaling in Nonalcoholic Fatty Liver Disease. Hepatology 2020;72:454-69.
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.
20. Ito T, Ishigami M, Zou B, et al. The epidemiology of NAFLD and lean NAFLD in Japan: a meta-analysis with individual and forecasting analysis, 1995-2040. Hepatol Int 2021;15:366-79.
21. Francque S, Wong VW. NAFLD in lean individuals: not a benign disease. Gut 2022;71:234-6.
22. Chen F, Esmaili S, Rogers GB, et al. Lean NAFLD: A Distinct Entity Shaped by Differential Metabolic Adaptation. Hepatology 2020;71:1213-27.
23. Eslam M, Valenti L, Romeo S. Genetics and epigenetics of NAFLD and NASH: Clinical impact. J Hepatol 2018;68:268-79.
24. Maier S, Wieland A, Cree-Green M, et al. Lean NAFLD: an underrecognized and challenging disorder in medicine. Rev Endocr Metab Disord 2021;22:351-66.
25. Fang SY, Huang CW, Huang TC, et al. Reduction in MicroRNA-4488 Expression Induces NFκB Translocation in Venous Endothelial Cells Under Arterial Flow. Cardiovasc Drugs Ther 2021;35:61-71.
26. Pessentheiner AR, Pelzmann HJ, Walenta E, et al. NAT8L (N-acetyltransferase 8-like) accelerates lipid turnover and increases energy expenditure in brown adipocytes. J Biol Chem 2013;288:36040-51.
27. Arumugam S, Mary B, Kumar M, et al. Analysis of hepatic and retinal cell microRNAome during AAV infection reveals their diverse impact on viral transduction and cellular physiology. Gene 2020;724:144157.
28. Huang S, Lu W, Ge D, et al. A new microRNA signal pathway regulated by long noncoding RNA TGFB2-OT1 in autophagy and inflammation of vascular endothelial cells. Autophagy 2015;11:2172-83.
29. Sonkar K, Ayyappan V, Tressler CM, et al. Focus on the glycerophosphocholine pathway in choline phospholipid metabolism of cancer. NMR Biomed 2019;32:e4112.
30. Tomita K, Teratani T, Suzuki T, et al. p53/p66Shc-mediated signaling contributes to the progression of non-alcoholic steatohepatitis in humans and mice. J Hepatol 2012;57:837-43.
31. Liu K, Lou J, Wen T, et al. Depending on the stage of hepatosteatosis, p53 causes apoptosis primarily through either DRAM-induced autophagy or BAX. Liver Int 2013;33:1566-74.
32. Simon SL, McWhirter L, Diniz Behn C, et al. Morning Circadian Misalignment Is Associated With Insulin Resistance in Girls With Obesity and Polycystic Ovarian Syndrome. J Clin Endocrinol Metab 2019;104:3525-34.
33. Schwerbel K, Kamitz A, Krahmer N, et al. Immunity-related GTPase induces lipophagy to prevent excess hepatic lipid accumulation. J Hepatol 2020;73:771-82.
34. Yang C, Zhang G, Zhang Y, et al. Exosome miR-134-5p restrains breast cancer progression via regulating PI3K/AKT pathway by targeting ARHGAP1. J Obstet Gynaecol Res 2021;47:4037-48.
35. Wang J, Tian R, Shan Y, et al. Metabolomics study of the metabolic changes in hepatoblastoma cells in response to NTCP/SLC10A1 overexpression. Int J Biochem Cell Biol 2020;125:105773.
36. Korade-Mirnics Z, Tarleton J, Servidei S, et al. Myotonic dystrophy: tissue-specific effect of somatic CTG expansions on allele-specific DMAHP/SIX5 expression. Hum Mol Genet 1999;8:1017-23.
37. Clark DF, Michalski ST, Tondon R, et al. Loss-of-function variants in CTNNA1 detected on multigene panel testing in individuals with gastric or breast cancer. Genet Med 2020;22:840-6.
38. Mao J, Tian Y, Wang C, et al. CBX2 Regulates Proliferation and Apoptosis via the Phosphorylation of YAP in Hepatocellular Carcinoma. J Cancer 2019;10:2706-19.

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