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Development of a Clinically Scalable Circulating microRNA Assay for Liver Fibrosis Staging in MASH

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Introduction

- Metabolic dysfunction-associated steatotic liver disease (MASLD) affects 30–35% of U.S. adults and can progress to metabolic dysfunction-associated steatohepatitis (MASH), fibrosis, cirrhosis, and liver failure.
- Liver biopsy is the gold standard for fibrosis staging but is invasive and costly. Current noninvasive methods have limitations in accuracy and accessibility.
- Circulating microRNAs (miRNAs) are promising biomarkers due to their stability and role in liver fibrogenesis.
- We evaluated a circulating miRNA assay using a one-step RT-qPCR workflow to explore its potential for noninvasive assessment of liver fibrosis in patients with MASH.

Methods

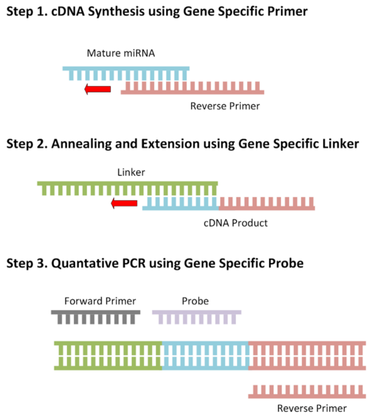
miRNA Targets

miRNA	Relevance (Biological Function / Pathway in Liver Disease)
miR-122	Injury, MASH, fibrosis, HCC → hepatocyte-specific; released during liver injury; regulates lipid metabolism
miR-34a	MASH, metabolic stress, apoptosis → p53 signaling; lipotoxicity and mitochondrial dysfunction
miR-192	MASH, fibrosis, hepatocyte function → liver-enriched; associated with metabolic dysfunction and fibrosis progression
miR-223	Inflammation, MASH activity → neutrophil/macrophage-derived; immune regulation and cytokine signaling
miR-23a	Endogenous Control / Normalizer
Cel-miR-39	Exogenous control

Patient Demographic and Clinical Characteristics

Characteristic	Obese Control (n=3)	MASLD (n=6)	MASH F0–F1 (n=8)	MASH F2+ (n=6)
Age (years), mean ± SD	35.3 ± 16.3	59.5 ± 15.7	50.9 ± 12.3	53.2 ± 7.1
Gender: Male (%) / Female (%)	0 / 100	16.7 / 83.3	62.5 / 37.5	16.7 / 83.3
Race/Ethnicity: White (%) / Hispanic (%)	66.7 / 33.3	83.3 / 16.7	87.5 / 12.5	83.3 / 16.7
CAP (dB/m), mean ± SD	NA	289.0	258.6 ± 66.0	319.5 ± 46.3
LSM (kPa), mean ± SD	NA	17.5	5.9 ± 0.9	21.7 ± 11.1

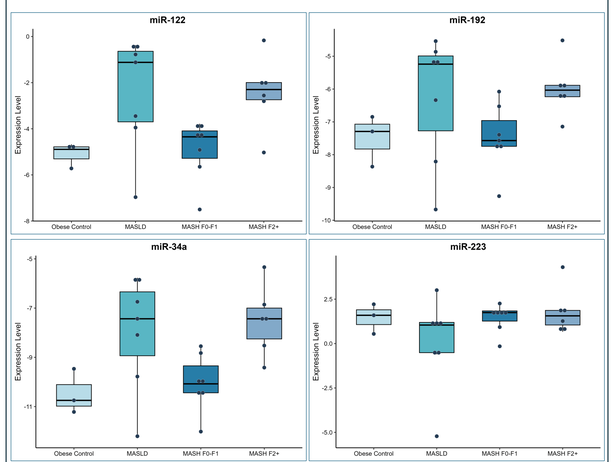
One-Step RT-PCR for miRNA Detection



The LuminoDx miRapid™ miRNA detection assay offers a single setup, single-plex or multiplex RT-qPCR solution for analyzing miRNAs. Total RNA was extracted from 100 µL of plasma per sample and analyzed using the miRapid™ RT-qPCR assay.

Results

Circulating miRNA Expression Across Liver Disease Groups

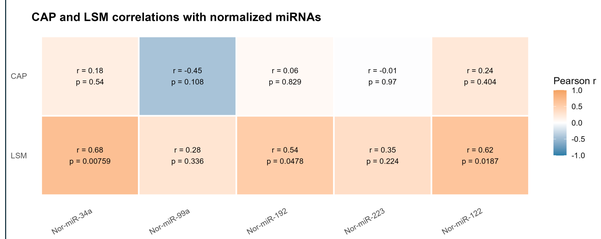


Differential miRNA Expression Between Disease Groups

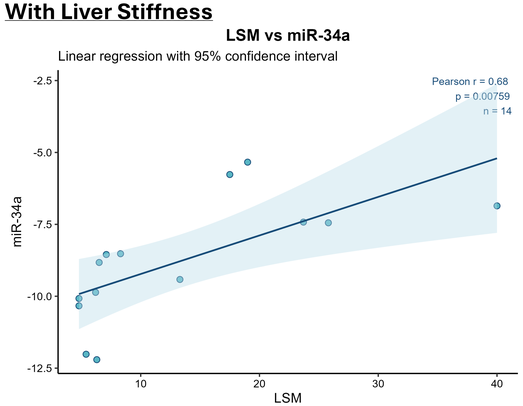
Marker	*p* Value (MASLD vs. MASH)	*p* Value (MASH F0-F1 Vs. F2+)
miR-34a	0.267	0.008
miR-192	0.428	0.018
miR-223	0.096	0.830
miR-122	0.154	0.027

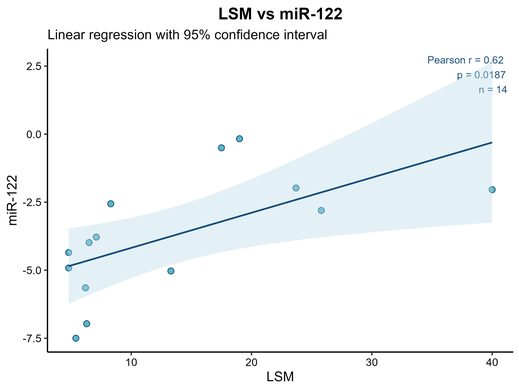
Between-group comparisons were performed using the Wilcoxon rank-sum test. Reported p-values are unadjusted.

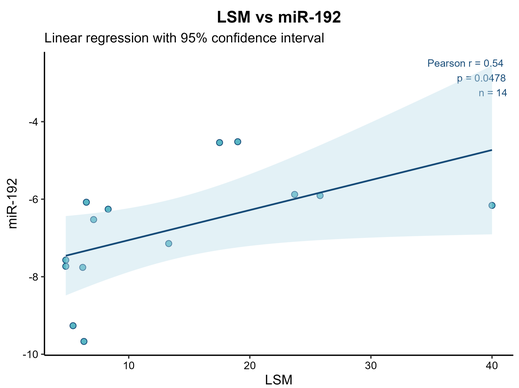
Associations Between Circulating miRNAs and Liver Stiffness



Correlations of miR-34a, miR-192, and miR-122 With Liver Stiffness







Conclusion

- This pilot study supports the feasibility of circulating miRNA analysis using a one-step RT-qPCR workflow for noninvasive assessment of fibrosis in MASH.
- Three circulating miRNAs, miR-34a, miR-192, and miR-122, showed exploratory differences between MASH F0–F1 and F2+ groups.
- Positive correlations between these miRNAs and liver stiffness measurements provide additional preliminary evidence of their association with fibrosis severity.
- Larger, independent studies are warranted to validate these findings and assess the potential clinical utility of this scalable miRNA-based assay for fibrosis risk stratification in MASLD/MASH.