

Genomic Landscape of Murine Metabolic-dysfunction Associated Steatohepatitis (MASH):

Unveiling Key Molecular Signatures through Meta-Analysis and Fisher's Combined Probability Test of RNA-seq Data

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ABSTRACT

Introduction: Metabolic dysfunction-associated steatohepatitis (MASH) presents as a complex and multifactorial liver disorder characterized by inflammation, hepatocyte injury, and fibrosis, representing an urgent unmet medical need. Understanding the underlying genomic architecture of MASH is crucial for elucidating its pathogenesis and identifying therapeutic targets. In this study, we conducted a comprehensive meta-analysis of transcriptomics data derived from diverse murine experimental models to dissect the molecular mechanisms governing MASH progression.

Objective: This study seeks to integrate RNA-seq datasets to comprehensively characterize the molecular landscape associated with MASH. Specifically, we aim to delineate dysregulated genes and pathways associated with pathogenesis with the overarching goal of advancing the understanding of the disease and finding potential therapeutic targets.

Methods: A systematic approach was employed to curate publicly available RNA-seq datasets from GEO, ensuring stringent quality control and adherence to rigorous analytical protocols. Per-study Differential Expression Analyses using DESeq2 were conducted using an established pipeline. Subsequently, Fisher's Combined Probability Test was applied to integrate statistical evidence across datasets, facilitating the identification of molecular signatures associated with MASH.

Results: Our meta-analysis included data from 100 samples across 10 distinct murine MASH cohorts. We identified 11 genes whose expressions exhibited significant correlations with crucial pathological mechanisms including cytokine activation, lysozyme acidification, glycemic control, and insulin resistance. Importantly, these genetic signatures were intricately linked to the clinical manifestation of the disease.

Conclusion: The meta-analysis revealed a comprehensive understanding of the genetic landscape underlying MASH, shedding light on key molecular pathways driving disease progression. The identification of 11 genes associated with pivotal pathological processes underscores their potential as therapeutic targets, offering promising avenues for the development of targeted interventions.

RESEARCH FINDINGS

Data Curation from Public Repositories

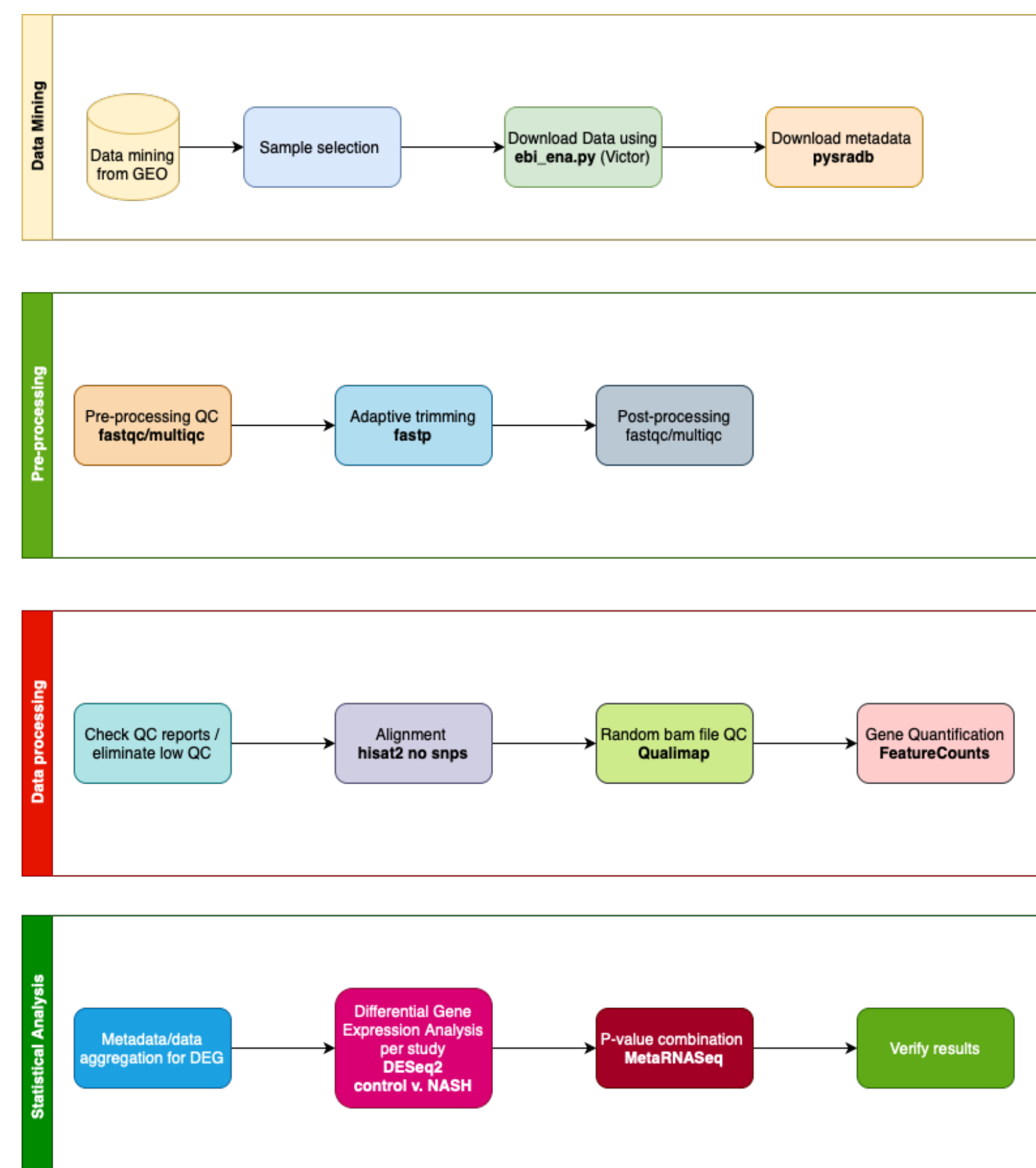


Figure 1. Application of Data Analysis Pipeline for Publicly Available Data

The methodological framework presented encompasses pre-processing, processing, and subsequent statistical analysis, with a strong emphasis on data quality control. By adhering to stringent standards, the study ensures the reliability and validity of the results obtained from the analysis of publicly available data.

Murine MASH Models

STUDY	MASH STRATEGY	TREATMENT TIME (WEEKS)
SRP271805	HFHC diet (Research Diet #D12331) starting at week 8 - euthanasia: week 16	8
SRP294364	HFCD-HF/G (D12492: 60% Kcal fat, with drinking water containing 23.1 g/L fructose and 18.9 g/L glucose. Start: week 9 - euthanasia: week 33	24
SRP297093	L-amino-defined high (60 kcal %) fat diet with 0.1 % methionine and no added choline (CDAHFD, Research Diet A06071302). Start: week 7 - euthanasia: 12	5
SRP297093	fructose-palmitate-cholesterol (FPC) diet (Research Diet, D17020104) with drinking water containing 42 g/L glucose and fructose (55 % / 45 %, w/w)	12
SRP297106	fructose-palmitate-cholesterol (FPC) diet (Research Diet, D17020104) with drinking water containing 42 g/L glucose and fructose (55 % / 45 %, w/w)	12
SRP348024	modified ALIOS diet (Envigo, TD.170428)	36 (9 months)
SRP348369	NASH model (in revision)	N/A
SRP367936	high fat diet (HFD) for 16 weeks to induce obesity	16
SRP427948	DEN-ALIOS diet	10
SRP107196	Western Diet (WD) containing 21.1% fat, 41% Sucrose, and 1.25% Cholesterol by weight (Teklad diets, TD. 120528) and a high sugar solution (23.1g/L d-fructose (Sigma-Aldrich, G8270) and 18.9 g/L d-glucose (Sigma-Aldrich, F0127)). CCl4 (Sigma-Aldrich, 289116-100ML) of 0.2 µl (0.32 µg)/g or corn oil injected intraperitoneally once/week	12 or 24

Table 1. Murine MASH models in each study analyzed

For each study to be considered in the meta-analysis, liver tissue samples required to present steatosis, ballooning and fibrosis, with histologically confirmed samples in the original study. In total, MASH N=100, Control N=80

Fisher's Combined Probability Test (Meta-Analysis) Method

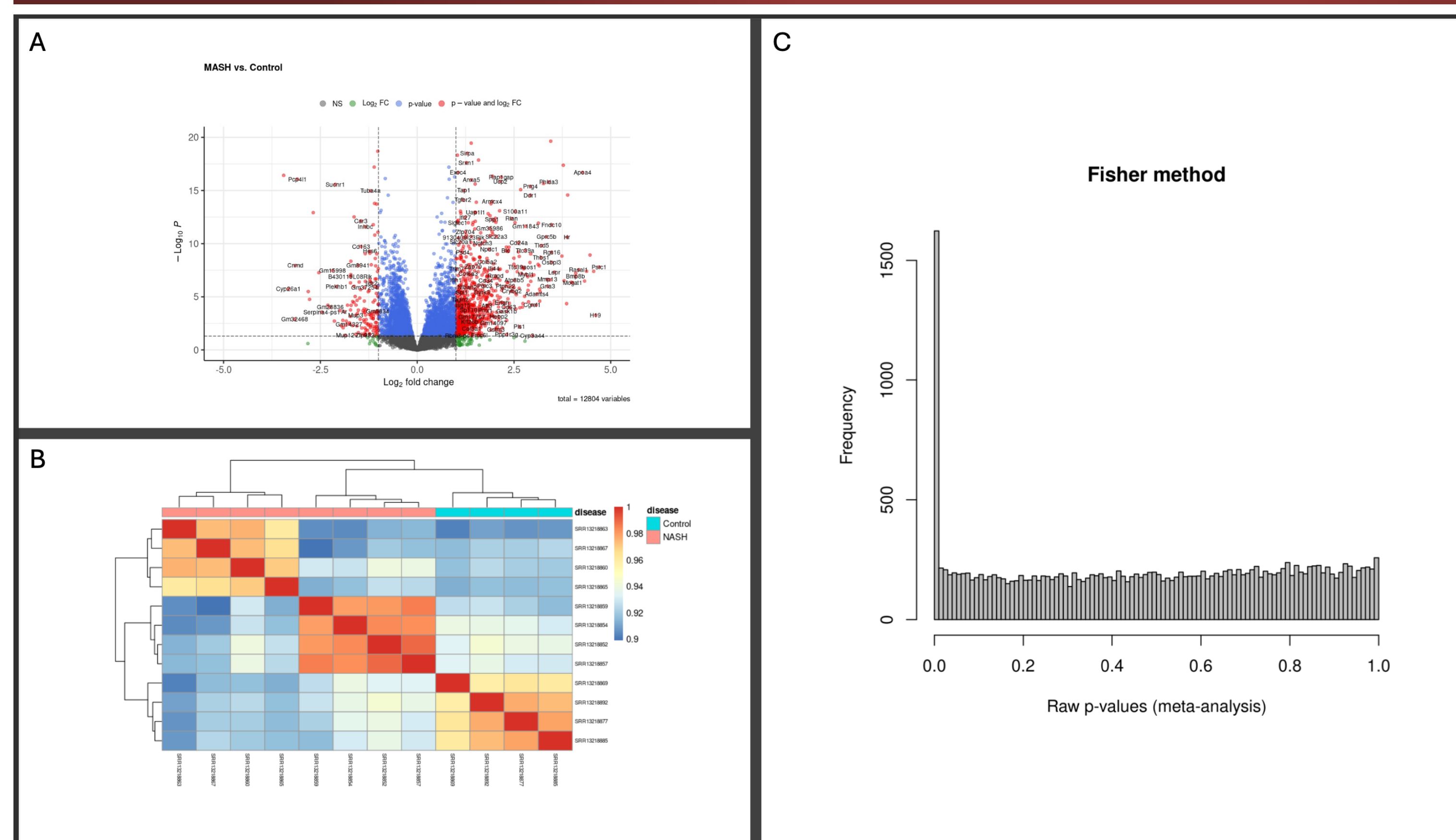


Figure 2. Statistical results for each study (A-B) and (C) meta-analysis. A) Volcano plot for SRP294364. B) Correlation heatmap for SRP294364. C) Fisher's method p-value histogram for combined studies. The histogram shows anti-conservative distribution.

Identification of Genes Differentially Expressed in Murine MASH

UPREGULATED GENES IDENTIFIED		
ENSEMBL ID	GENE NAME	POSSIBLE ROLE IN MASH
ENSMUSG00000030091	Nup210	Nup210 correlated with progression towards end-stage liver disease
ENSMUSG00000062190	Lancl2	Regulator of the Akt pathway, involved in glycemic control and liver regeneration
ENSMUSG00000033948	Zswim5	N/A
ENSMUSG00000037235	Mxd4	Myc-Max-Mad network involved in glucose intolerance and liver cancer progression
DOWNREGULATED GENES IDENTIFIED		
ENSEMBL ID	GENE NAME	POSSIBLE ROLE IN MASH
ENSMUSG00000039105	Atp6v1g1	Possible association with the development of MASH in murine models through RORα-induced lysosomal acidity and autophagic activity
ENSMUSG00000046785	Epm2aip1	Associated with glycogen synthase (GS). Downregulation causes hepatic insulin resistance.
ENSMUSG00000040471	Ggt6	Associated with risk factors for MAFLD. Involved in the glutathione metabolic pathway
ENSMUSG0000006958	Chrd	Kielin/Chordin involvement that promotes liver pathology in mice similar to MASH
ENSMUSG00000098702	1500015A07Rik	N/A
ENSMUSG00000017057	Il13ra1	Extracellular expression of IL3RA1 has been linked to the JAK/STAT pathway induced by IL13 and IL4
ENSMUSG00000034120	Srsf2	Srsf2 triggers development of HCC, which also involved inflammation and fibrosis

Table 2. Identified genes of differential expression in murine MASH as compared to normal murine liver through meta-analysis. Key genes involved in the clinical development of the disease show a relationship to cytokine activation, lysozyme acidification, glycemic control, autophagy, and insulin resistance.

Conclusions and Future Directions

- The meta-analysis method proved to be valuable in identifying significantly altered genes implicated in MASH, enhancing statistical power through the integration of diverse independent MASH studies
- The involvement of multiple genes across various pathways implies the synergistic contribution of these pathways to liver damage.
- Pathways consistent with clinical manifestations of the disease include cytokine activation, autophagy, insulin resistance, and glycemic control.
- Utilizing Fisher's method for Meta-Analysis of RNA-seq data could be employed in human studies to discern clinical biomarkers and potentially uncover novel pathways across complex patient populations.

References

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