Introduction

- Alpha-1 antitrypsin deficiency (AATD) is a rare genetic disease characterized by low levels of serum alpha-1 antitrypsin (AAT), which primarily affects the lungs.
- Patients with the protease inhibitor (Pi)*ZZ genotype express misfolded AAT.
- Differential expressed protein (DEP) and pathway analyses were also conducted.
- Currently no pharmacological treatments for AATD-associated liver disease (AATD-LD) exist.
- Proteomics data to identify biomarkers relevant for therapeutic targeting and to elucidate disease pathophysiology are limited.

Objective

To detect treatment-responsive protein biomarkers and cellular pathways by leveraging a novel proteomic platform for biomarker discovery using serum samples from patients with AATD-LD treated with fazirsiran.

Methods

- Omik-Explore 3072, a high-throughput dual-antibody-based proteomics platform, was utilized for protein biomarker discovery.
- Serum samples were assessed at baseline and at 14, 16, 24, 28, and 48 weeks post-treatment initiation from 16 adults with AATD-LD, a PiZZ genotype, and biopsy-proven liver fibrosis who participated in AROAAT-2002 (NCT03946449), a phase 2, open-label trial of fazirsiran (100 or 200 mg); treatment with fazirsiran resulted in >80% reduction in serum and liver Z-AAT.
- Additional study design information has been previously described.
- Omik data were integrated with single nucleic RNA-sequencing (Snucseq) data to map expressed proteins to potential source cells in the liver and improve data interpretability.
- The Snucseq data consisted of samples from four patients with non-alcoholic steatohepatitis (NASH)/metabolic dysfunction-associated steatohepatitis (MASH) and two healthy controls.
- A mixed effects model was applied to measure biomarker change from baseline over time and a false discovery rate (FDR)-adjusted p-value was used to select the top biomarkers (Figure 1).
- Differentially expressed protein (DEP) and pathway analyses were also conducted.

Results

- Integration of the Omik panel with the Snucseq data is shown in Figure 2.
- Fazirsiran treatment resulted in continuous and sustained reductions in DEPs in serum through 48 weeks (Figure 3A).
- DEPs were primarily mapped to hepatocytes, mesenchymal cells, immune cells (T- and macrophages), cholangiocytes, and endothelial cells (Figure 3B).
- DEPs revealed potential common mechanisms associated with liver fibrosis between AATD-LD and other liver diseases, including NASH/MASH and non-alcoholic fatty liver disease/metabolic dysfunction-associated fatty liver disease.
- Of the liver mesenchymal cell-enriched proteins, many were components of the extracellular matrix associated with hepatic steatosis cell activation and liver fibrosis.
- Downregulated proteins that mapped to hepatocytes were associated with cell stress and apoptosis.

Figure 2. Integration of the Omik panel with Snucseq data suggested liver cell sources for the circulating biomarkers

Figure 3. (A) Fazirsiran treatment led to continuous and sustained reductions of proteins in the serum; (B) mapping DEPs to Snucseq data helped to define liver cell origin and contribution of distinct liver cell types to the observed serum DEPs

Conclusions

This study leveraged a novel proteomic platform for biomarker discovery in patients with AATD-LD, identified candidate biomarkers reflecting disease progression, and demonstrated potential benefit of fazirsiran treatment in reducing cellular stress and damage, apoptosis, inflammation and extracellular matrix turnover/fibrosis.

Collectively, these findings provide molecular evidence to support potential clinical benefit of fazirsiran in patients with AATD-LD.

Biomarkers identified in these analyses may have clinical utility but require validation in larger studies.

- The study was limited by:
  - the small sample size (n = 16)
  - the lack of comparator data from healthy patients or patients who received placebo; future research should generate additional Omik proteomic analyses from these comparator populations.
  - Quantitative assays, such as enzyme-linked immunosorbent assays, would provide beneficial in validating findings from this study and bridging the gap to clinical practice.

Abbreviations

- AAT: alpha-1 antitrypsin
- AATD: alpha-1 antitrypsin deficiency
- AATD-LD: alpha-1 antitrypsin deficiency-associated liver disease
- AROAAT-2002: A Phase 2, Open-label Trial of Fazirsiran (100 or 200 mg) in Adults with Alpha-1 Antitrypsin Deficiency Liver Disease
- DEP: differentially expressed protein
- FDR: false discovery rate
- Fazirsiran: Intellia Pharmaceuticals and Takeda Pharmaceuticals.

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