Neurofilament Light Chain (NfL) as a Potential Biomarker in Hereditary Transthyretin-Mediated (hATTR) Amyloidosis

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Introduction

Hereditary Transthyretin-Mediated (hATTR) Amyloidosis and Patisiran

- Rare, progressively debilitating, and fatal disease caused by a mutation in the transthyretin (*TTR*) gene that results in multisystem accumulation of amyloid fibrils (e.g., in nerves, heart, and gastrointestinal tract) and subsequent dysfunction across these multiple organs[1–5]
- Affects ~50,000 people worldwide[4]; median survival of 4.7 years following diagnosis, with a reduced survival of 3.4 years for patients presenting with cardiomyopathy[6–9]
- Patisiran, a lipid nanoparticle-delivered RNA interference (RNAi) therapeutic, reduces serum TTR levels by inhibiting hepatic synthesis of the disease-causing mutant and wild-type (wt) TTR proteins[10,11]
  - In the Phase 3 APOLLO study, patisiran demonstrated improvements in the primary endpoint of modified Neuropathy Impairment Score+7 (mNIS+7) and all secondary endpoints with an acceptable benefit:risk profile[12]
  - mNIS+7 is a composite measure of polyneuropathy; higher score indicates worsening of neuropathy (range: 0–304)
  - Patisiran is approved in select countries globally for the treatment of hATTR amyloidosis with polyneuropathy[13–16]

Neurofilament Light Chain (NfL): Well-Characterized Marker of Neuroaxonal Injury

- Neurofilament light chain (NfL) has been described extensively as a biomarker of neuroaxonal injury across central nervous system diseases (e.g., multiple sclerosis,[17] Alzheimer's,[18,19] and Huntington's[20]) and peripheral nervous system diseases (e.g., vasculitis,[21] chronic inflammatory demyelinating polyneuropathy,[22] Guillain–Barré syndrome,[23] and Charcot–Marie–Tooth disease[24])
- Elevation of NfL has been identified in blood and thought to be released into the circulation from damaged neurons, thus making it a proximal biomarker for early nerve damage[25,26]

Objective

- Evaluate impact on circulating protein biomarkers in response to patisiran treatment in patients with hATTR amyloidosis with polyneuropathy

Methods

Study Design and Plasma Measurements

- Plasma samples were collected from consenting patients in the APOLLO study
  - APOLLO (NCT01960348) was a Phase 3, randomized, placebo-controlled study of patisiran 0.3 mg/kg intravenously every 3 weeks in patients with hATTR amyloidosis with polyneuropathy[12]
- Proteomic analysis (by proximity extension) measured 1196 proteins within the plasma samples for potential biomarkers
- Healthy control samples (n=57) were matched to the baseline demographics of the APOLLO patients (Dx Biosamples, LLC, San Diego, CA)

Data Analysis

- A linear mixed model regression analysis for each protein determined if there was a significant differential time profile based on treatment
- Principal component analysis was performed using Python's sklearn package
  - For each APOLLO patient, a vector (v_1) from the mean healthy control to baseline of the APOLLO patients and a second vector (v_2) from baseline to 18 months were used to compute two metrics
    - ϕ is the ratio of the magnitude of v_2 to that of v_1
    - Θ is the angle between v_2 and v_1 and measures the direction of the proteome shift for the individual patient

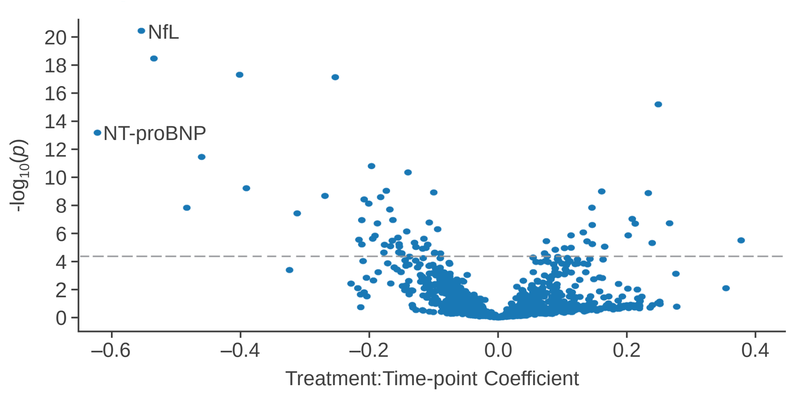
Results

Multiple Plasma Proteins Differ Significantly Between Placebo- and Patisiran-Treated Patients (Figure 1)

- A significant change in levels over time was observed for 66 proteins following patisiran treatment ($p<4.18\times10^{-5}$)
- NfL was identified as having the greatest significance ($p=3.95\times10^{-21}$)
  - A significant decrease in *N*-terminal prohormone of brain-type natriuretic peptide (NT-proBNP) levels with patisiran treatment was also seen ($p=7.02\times10^{-14}$), consistent with the decrease in NT-proBNP observed in the APOLLO study

Results

Figure 1. Proteins Identified to Have a Change Corresponding to Patisiran Treatment (Relative to Placebo) over 18 Months



Proteins are shown here as a volcano plot, with the strength of the association on the y-axis ($-\log_{10}(p)$) and the effect size on the x-axis (shown as the treatment × time coefficient from the model)

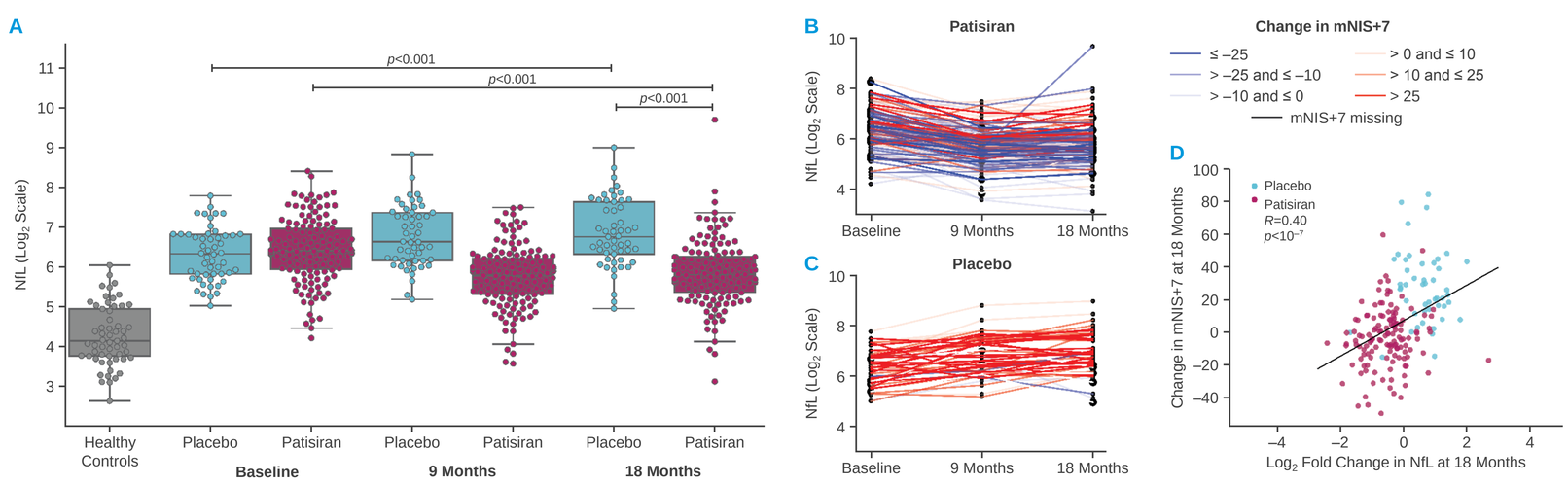
Plasma NfL Is Increased in Patients with hATTR Amyloidosis and Decreases with Patisiran Treatment

- Patients with hATTR amyloidosis with polyneuropathy had greater than 4-fold higher plasma NfL at baseline relative to healthy controls (**Figure 2a**; $\log_2$ scale)
- Changes in NfL over time largely correlated with whether patients had a decrease or increase in their corresponding mNIS+7 (**Figures 2b** and **2c**)
- Plotting each patient's change in mNIS+7 against the change in NfL at 18 months demonstrated a correlation of 0.40 (**Figure 2d**)

Effect of Disease Progression and Treatment on Overall Plasma Proteome Signature

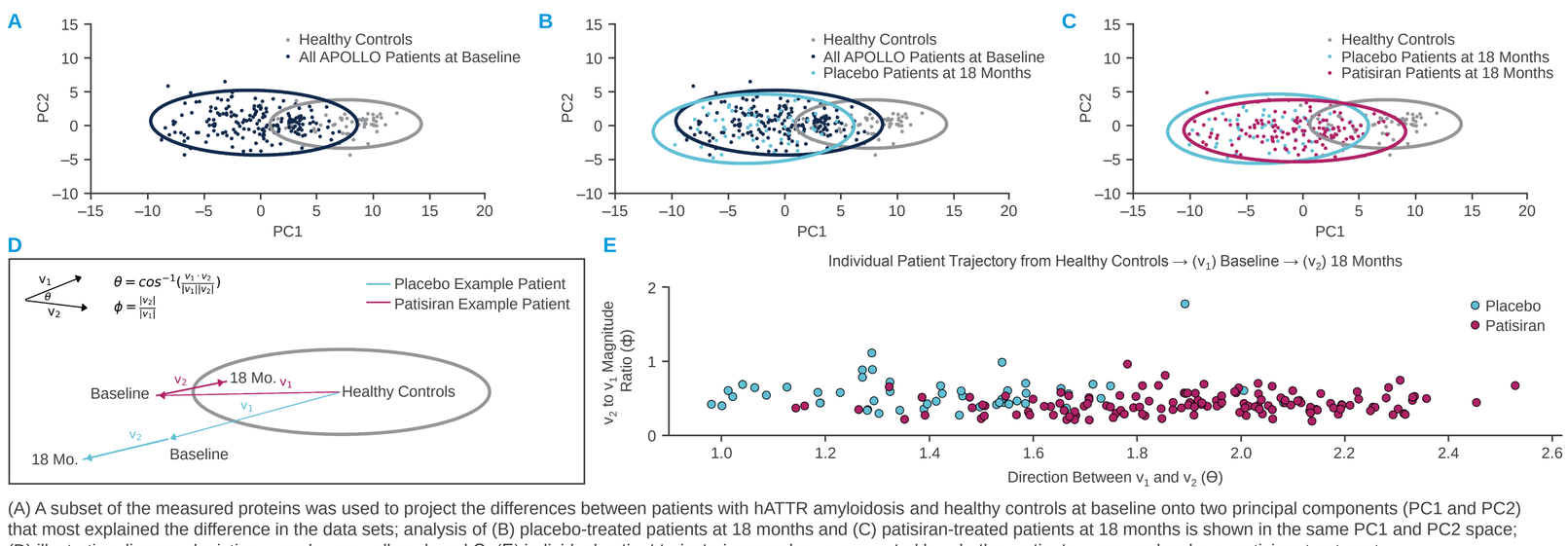
- Projecting each individual's data onto the first two principal components (PC1 and PC2) reveals a separation between healthy controls and patients with hATTR amyloidosis with polyneuropathy at APOLLO baseline (**Figure 3a**)
- At 18 months, the proteomes of placebo-treated patients separated from healthy controls (**Figure 3b**), mostly along the first primary component (PC1), while those of patisiran-treated patients did not separate as extensively from healthy controls (**Figure 3c**)
- Due to the significant heterogeneity in the proteome of patients at baseline, individual patient trajectories were captured
- Plotting ϕ against Θ separates placebo- and patisiran-treated patients (**Figure 3d** and **3e**)

Figure 2. Change over Time in NfL in Patients Treated with Placebo and Patisiran



(A) Levels of NfL in healthy controls and placebo- or patisiran-treated patients at baseline, 9 months, or 18 months; trajectories of individual patients on (B) patisiran or (C) placebo over time, color-coded by their corresponding change in mNIS+7 (from baseline to 18 months); (D) correlation between change in NfL levels from baseline to 18 months and the corresponding change in mNIS+7 colored by treatment

Figure 3. Global Changes in Plasma Proteomes Observed with the Proteome of Patisiran-Treated Patients Trending Toward That of Healthy Controls at 18 Months



(A) A subset of the measured proteins was used to project the differences between patients with hATTR amyloidosis and healthy controls at baseline onto two principal components (PC1 and PC2) that most explained the difference in the data sets; analysis of (B) placebo-treated patients at 18 months and (C) patisiran-treated patients at 18 months is shown in the same PC1 and PC2 space; (D) illustrative diagram depicting v_1 and v_2 as well as ϕ and Θ; (E) individual patient trajectories are shown, separated by whether patients were on placebo or patisiran treatment

Conclusion

- NfL is a well-described biomarker for neuroaxonal damage but limited research has been done thus far on its applicability in hATTR amyloidosis
- Across >1000 unique proteins that were assessed from samples collected in the APOLLO study, plasma levels of NfL had the most significant change in regards to patisiran treatment and were reduced following patisiran treatment at 9 and 18 months
  - NfL may serve as a biomarker of nerve damage and polyneuropathy due to TTR amyloid deposition
- A correlation was seen between each patient's change in polyneuropathy (as measured by mNIS+7) and change in NfL over 18 months, indicating that decreasing levels of NfL are associated with an improvement in polyneuropathy in the APOLLO study
- Patisiran treatment also demonstrated a general shift in the proteome of patients toward that of healthy controls relative to placebo at 18 months
- This study represents the most comprehensive plasma proteomics analysis in patients with hATTR amyloidosis to date and the first system-wide proteomics interrogation of response to an RNAi therapeutic in humans
  - NfL offers the potential for earlier diagnosis of polyneuropathy in patients with hATTR amyloidosis and for monitoring disease progression and/or regression over time, with or without treatment

Abbreviations: hATTR, hereditary transthyretin-mediated; mNIS+7, modified Neuropathy Impairment Score+7; NfL neurofilament light chain; NT-proBNP, *N*-terminal prohormone of brain-type natriuretic peptide; PC, primary component; RNAi, RNA interference; TTR, transthyretin; v, vector; wt, wild-type. **Acknowledgments:** Editorial assistance in the development of the poster was provided by Adelphi Communications Ltd, UK, funded by Alnylam Pharmaceuticals. **Funding:** This study was sponsored by Alnylam Pharmaceuticals.

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