CA3 levels in plasma associate strongly with site of onset in Amyotrophic Lateral Sclerosis (ALS)

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BACKGROUND

- Amyotrophic lateral sclerosis (ALS) is a progressive motor neuron disease with variable onset and unclear pathological mechanisms. Biological and clinical heterogeneity contribute to its long median time-to-diagnosis of 11.5 months[1], impacting patient care, clinical trial eligibility, and outcomes. Several factors influence time-to-diagnosis, including site of onset (limb, bulbar, others).
- With limb-onset associated with longer time-to-diagnosis compared to bulbar-onset[2] and bulbar-onset reported to have a faster rate of progression than limb-onset[3], there is a clinical need to explore diagnostic and prognostic biomarkers that are specific to site of onset.
- Proteomics methods are being increasingly applied to investigate biomarkers of interest to understand disease-related mechanisms. In neuromuscular diseases, like ALS, the skeletal muscle specific enzyme, carbonic anhydrase III (CA3), has been reported to be highly expressed[4].
- In this analysis, we explore biomarker associations by incorporating proteomic signatures with clinical variables and highlight CA3, and its association with site of onset (limb- vs. bulbar- onset) in ALS. Greater understanding of proteomic biomarkers associated with survival and function may better inform the diagnosis of ALS.

HYPOTHESIS

- We hypothesize that blood plasma proteomic signatures of early and suspected ALS patients may include biomarkers which can better inform the diagnosis of ALS.

METHODS

- Quantification of 553 plasma analytes was performed using the Somalogic platform on archived EDTA plasma samples taken from ALS patients and appropriate control populations. Samples were acquired from one or more of the ongoing studies at Massachusetts General Hospital. The samples were collected at the Neurology Clinical Trials Unit (NCTU) between 2003 and 2007.
- Patients were enrolled in any one of the drug efficacy studies undertaken by NCTU and plasma samples were obtained at baseline (before treatment) for the purposes of biomarker discovery (N=90).
- In total, data from 338 patients were analyzed (Table 1).
- Protein levels were log transformed and truncated at the 99th percentile.

Table 1. Patient Clinical Characteristics

Patient Characteristics		(TOTAL N=338) N
Early ALS	Patients within 2 years of confirmed ALS diagnosis	186
Suspected ALS	No confirmed ALS diagnosis, but ALS not ruled out	19
Control disease	ALS disease mimics including Multiple Sclerosis, Alzheimer's disease, other	43
Control healthy	Age/gender matched healthy controls	90

- Association analyses were performed between plasma protein levels and disease characteristics including diagnosis, site of onset, and duration (since diagnosis). Patient age, gender, and BMI were included as covariates, and the residuals after fitting age, gender, and BMI with a linear model were used for plotting.
- The Bonferroni method was used to adjust significance across all protein markers.

References:

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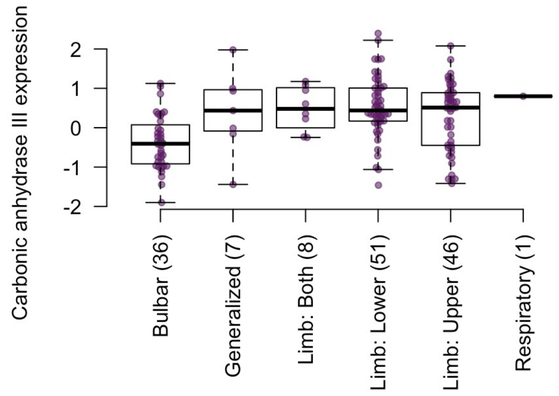
RESULTS

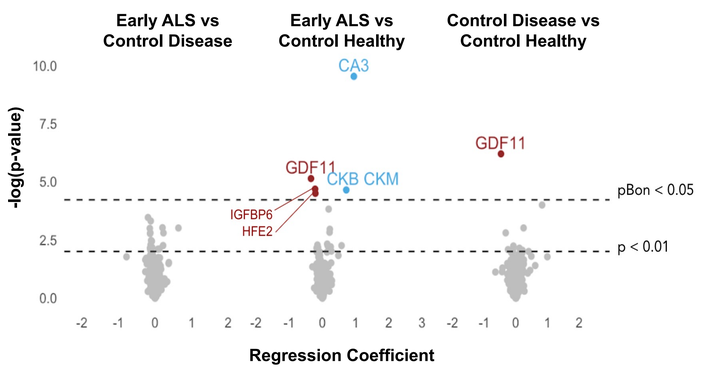
- Carbonic anhydrase 3 (CA3), a skeletal muscle-specific enzyme highly expressed in neuromuscular diseases, was the only blood plasma marker detected that differentiates among sites of onset. CA3 elevation is more pronounced in limb and other non-bulbar onset types in comparison to bulbar-onset ALS (**Figure 1**).

Figure 1. CA3 heterogeneity by ALS site of onset



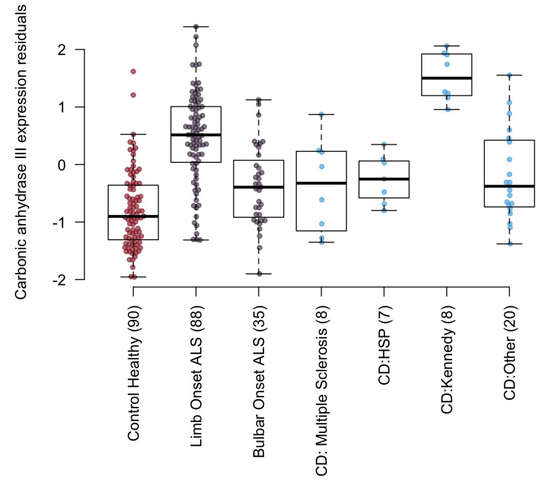
- Among five analytes that differentiate between ALS and healthy controls, other markers were CKB (elevated); GDF11, IGFBP6, and HFE2 (lowered); (**Figure 2**).

Figure 2. Proteomic biomarker analysis



- CA3 was elevated in patients with limb-onset ALS (N=105) relative to both healthy controls (N=90) and bulbar-onset ALS (N=36) [both adjusted p<.0001]. Elevated CA3 levels also differed in magnitude across the neuromuscular disorders, with limb-onset exhibiting similar elevation to multiple sclerosis and hereditary spastic paraplegia, but not as high as Kennedy's disease [p<.0001] (**Figure 3**).

Figure 3. Heterogeneity of CA3 elevation among related diseases



CONCLUSIONS

- Elevated levels of CA3 in plasma is characteristic of limb-onset ALS vs. bulbar-onset ALS and healthy controls. This finding suggests that a combination of clinical and -omic factors may be more informative diagnostic criteria than either as standalones. Elevated plasma CA3 levels appear to be a common finding across various neuromuscular disorders related to ALS, but especially in Kennedy's disease with highly elevated levels.
- These findings illustrate, first, that a greater understanding of omics-based diagnostics focusing on the limb/non-bulbar subtype may better inform ALS diagnosis, and that plasma CA3 analysis may be a useful addition to the diagnostic armamentarium for limb-onset ALS.
- In addition to limb- vs. bulbar-onset, CA3 exhibits varying degrees of elevation in different neuromuscular disorders that merit further investigation.
- These results highlight the clinical utility of using proteomics to assist patients, families, and clinicians in planning for care, in addition to helping to inform eligibility requirements for those interested in enrolling in clinical trials.

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