Biomarkers for the early-detection and monitoring of Huntington's Disease

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Abstract

Huntington's Disease (HD) is a genetic disease caused by a CAG trinucleotide repeat in Exon1 of the huntingtin gene. Neurodegeneration results in the loss of cognitive and motor functions, and is caused by aggregation of mutant huntingtin protein in striatal neurons. Volumetric changes in the striatum can be detected decades before the manifestation of clinical phenotypes, indicating that therapeutic intervention would need to occur long before symptomatic presentation. In clinical practice and research settings, the Unified Huntington's Disease Rating Scale (UHDRS) is utilized to evaluate a patients overall physical and neurological health. UHDRS is also the most widely used outcome measure for establishing drug efficacy. However, symptoms such as movement difficulties and cognitive impairment can vary in severity from day-to-day and can reflect mood and other subjective factors, not only the underlying disease process. The lack of objective molecular biomarkers to monitor onset and progression of disease currently impedes Huntington's Disease drug development.

Small RNAs (sRNAs) are a class of 17-36 nucleotide non-coding RNAs that regulate gene expression at the posttranscriptional level. These master regulators play a critical role in every biological process and pathway, in every cell of our body. Several reports have identified that neuronally-derived exosomes contain sRNA cargo.

Therefore, we tested two hypotheses (i) that sRNAs discovered in postmortem Huntington's brain tissue would correlate with neuropathological and clinical features, and (ii) that these same markers could be identified in CSF so that they could be used to longitudinally monitor disease progression.

Methods and Results

Using the sRNA-FIND discovery platform, we analyzed small RNA sequencing data derived from stage-verified Huntington's patients (n=28) and non-Huntington's controls (including Healthy, AD, and PD) subjects; n=80), and identified sRNAs that are uniquely expressed in the frontal cortex of Huntington's brains. These sRNAs were validated using targeted RT-qPCR in independently collected frontal cortex samples (n=64) and cerebrospinal fluid samples (n=60)

Figure 1: Computational discovery of HD-specific small RNAs using sRNA-FIND that show binary classification (left) or exponential increase in abundance from Grade 2-4 (right).

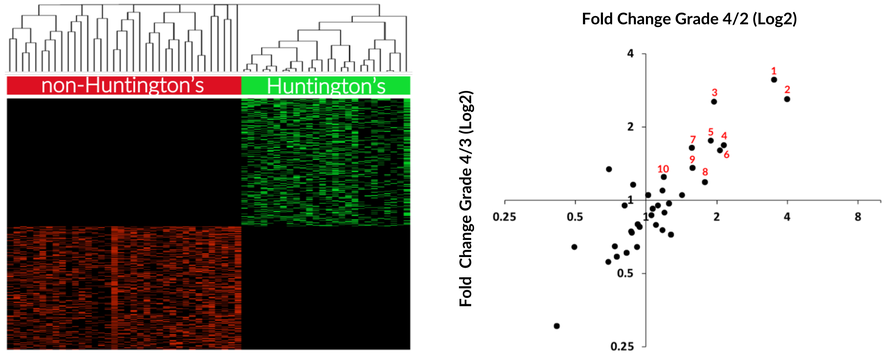


Figure 2: RT-qPCR validation of HD-specific small RNAs using total RNA from 0.5ng of frontal cortex (left) or 200uL of cerebrospinal fluid (right)

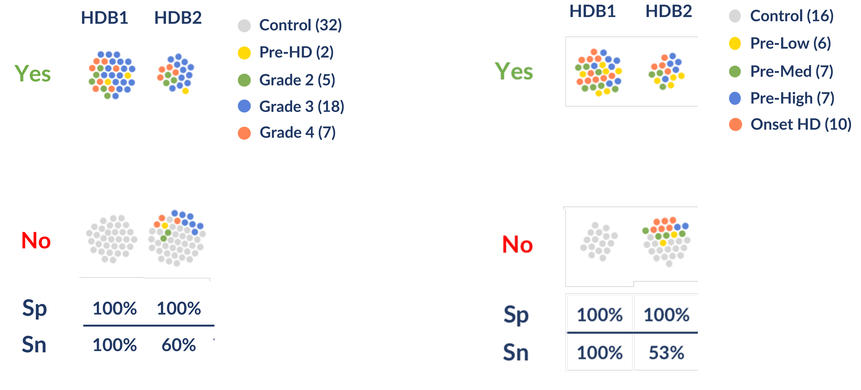


Figure 3: HD-specific small RNAs correlate with disease progression in frontal cortex (left) and cerebrospinal fluid (right) and are detected 20 year prior to symptomatic onset.

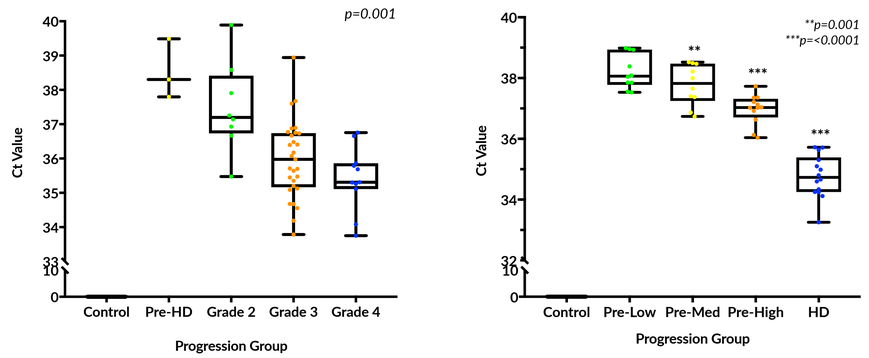
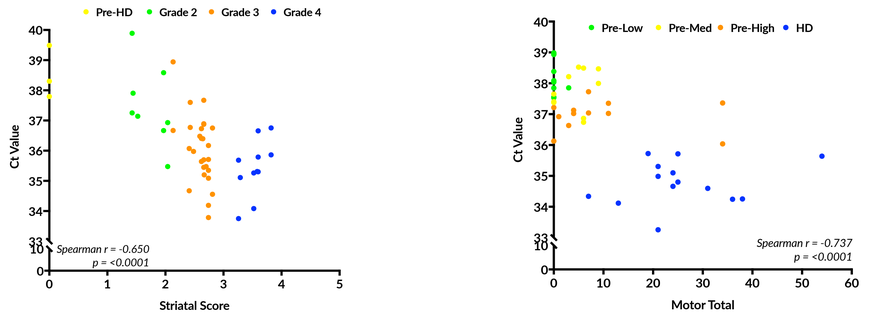


Figure 4: HD-specific small RNAs correlate with degeneration of striatal neurons (left) and loss of motor function (right)



Conclusion

The FDA's Center for Drug Evaluation and Research (CDER) issued a Letter of Support to sRNAlytics in January of 2019 encouraging the use and development of these sRNA biomarkers to monitor Huntington's Disease progression prior to symptom onset, and stated that these markers could be used to provide supporting evidence for and even serve as the basis for accelerated drug approval.

We are currently expanding our validation work in 2 additional Huntington's Disease studies utilizing CSF (n=126), and matched CSF and serum (n=50) samples. We believe that these sRNA-based diagnostic biomarkers will be critical to the development of new HD therapeutics, as they provide an objective, molecular marker to test drug efficacy in pre-symptomatic patients.