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BIOMARKERS FOR THE EARLY-DETECTION AND MONITORING OF HUNTINGTON'S DISEASE

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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 sensitive, specific, 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.

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 and PD subjects; n=124) (**Table 1**) and identified sRNAs that are uniquely expressed in the frontal cortex of Huntington's brains.

Variable	Control[10]	PD[11]	Pre-HD[10]	HD (Grade 2-4)[10]
N	95	29	2	26
Age at Death	68.6 ±14.3	77.5 ±9.0	67.5 ±26.1	59.5 ±10.7
CAG Repeat Size			42.0 ±0	44.6 ±2.9
Age of Onset		66.8 ±9.6		44.5 ±11.8
Disease Duration		10.5 ±6.5		15.0 ±6.1
Striatal Score				2.70 ±0.65
Cortical Score				1.25 ±0.50

Table 1: Description of samples used in discovery cohort

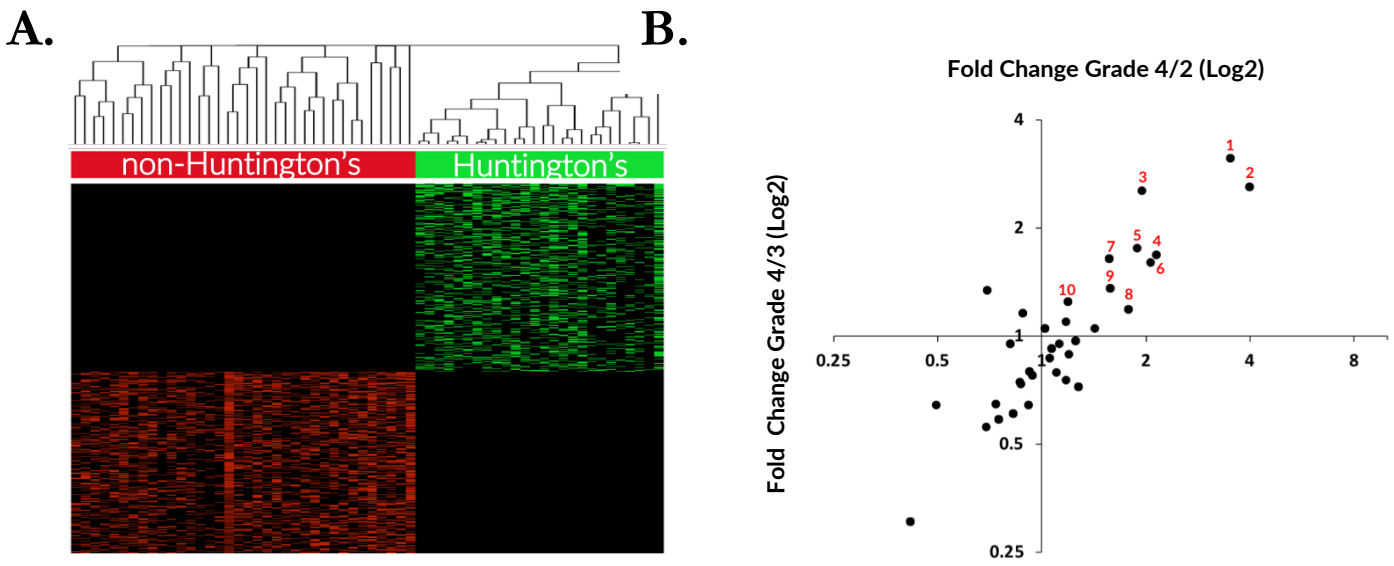

Figure 1: Discovery of Huntington's Disease specific biomarkers that classify and correlate with disease progression. A. Small RNA reads from sRNA-TRIM and sRNA-MAP were analyzed by sRNA-FIND. Semi-supervised hierarchical clustering showed the presence of Huntington's Disease (green) and non-Huntington's Disease (red) specific small RNAs in each group respectively. Correlation of small RNA abundance (RMP) and Vonsattel disease grade uncovered 10 biomarkers that increased exponentially with

These sRNAs were validated by targeted RT-qPCR in independently collected HD frontal cortex samples (n=64) (**Table 2** and **Figure 2**),

Variable	Control	Pre-HD	HD (Grade 2-4)
N	32	2	30
Age at Death	67.2 ±9.1	77.2 ±12.4	60.9 ±10.8
CAG Repeat Size		43.0 ±1.0	44.0 ±2.8
Age of Onset			45.9 ±12.2
Disease Duration			15.1 ±6.2
Striatal Score			2.67 ±0.63
Cortical Score			1.24 ±0.48

Table 2: Description of frontal cortex samples used in validation cohort 1

and cerebrospinal fluid (n=60) from the PREDICT HD natural history study (**Table 3** and **Figure 2**).

Variable	Control	Pre-Low	Pre-Medium	Pre-High	HD
N	15	10	10	10	15
Gender (M:F)	7:8	3:3	3:4	4:3	4:6
CAG Repeat Size	20.5 ±4.1	42.5 ±1.5	42.7 ±2.0	43.1 ±4.6	42.0 ±1.5
Age at Visit	45.9 ±14.0	26.2 ±4.1	37.8 ±9.5	51.3 ±15.8	53.7 ±9.8
CAP_D		230.4 ±44.9	327.3 ±20.1	428.5 ±54.3	436.5 ±30.3
Onset Probability		20-12yr	11-6yr	5-1yr	

Table 3: Description of cerebrospinal fluid samples used in validation cohort 2

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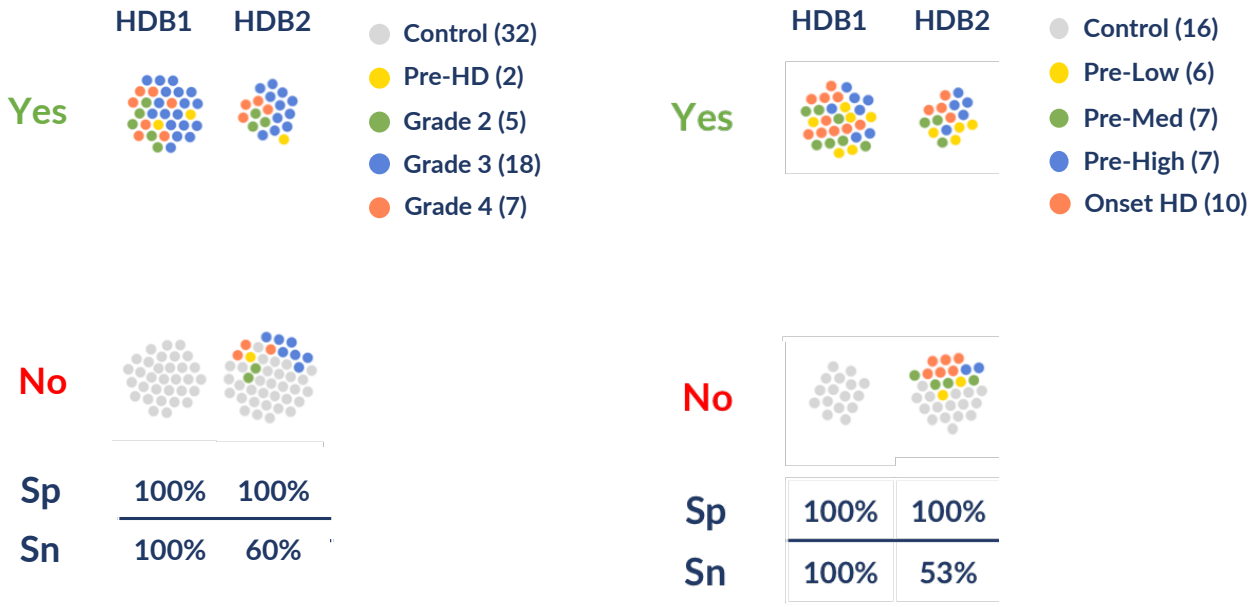

Figure 2: RT-qPCR validation of Huntington's disease specific small RNAs using total RNA from 0.5ug of frontal cortex (left) or 200uL of cerebrospinal fluid (right). In the graphs above each bubble represents a patient sample, the color depicts the type of sample and the yes/no score indicated if the Huntington's disease biomarker (HDB) scored positive or negative. No control samples scored positive for either HDB1 or HDB2 giving them 100% Specificity in frontal cortex and cerebrospinal fluid. All patient samples scored positive for HDB1 giving it 100% Sensitivity, making it a perfect predictor. Whereas HDB2 showed 60% and 53% Sensitivity in frontal cortex and cerebrospinal fluid samples, respectively.

Our results showed that these biomarkers correlated significantly with disease grade (p=0.005) and degeneration of striatal neurons (ρ= -0.650, p= <0.0001) in postmortem frontal cortex (n=64), and importantly were uniquely expressed only in individuals carrying a CAG repeat mutation.

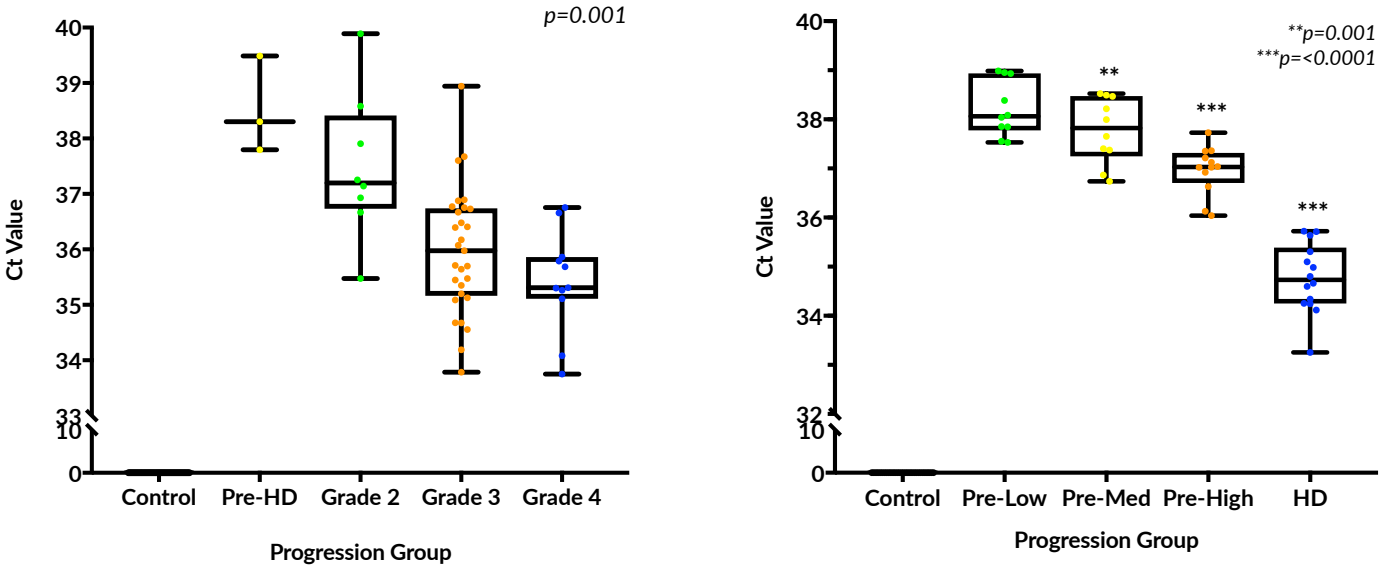

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Figure 3: HDB1 and HDB2 correlate with disease progression in frontal cortex (left) and cerebrospinal fluid (right) and can be detected 20 years prior to symptomatic onset (Pre-Low progression group, right).

Validation in CSF samples from the PREDICT-HD study showed that these sRNA biomarkers could be detected in patients who were predicted to be 20 years prior to onset. Additionally, these biomarkers correlated significantly with disease progression (p= 0.0001), CAPD Onset Probability Scores (ρ= -0.780, p= <0.0001) and Loss of Motor Function (ρ= -0.737, p= <0.0001).

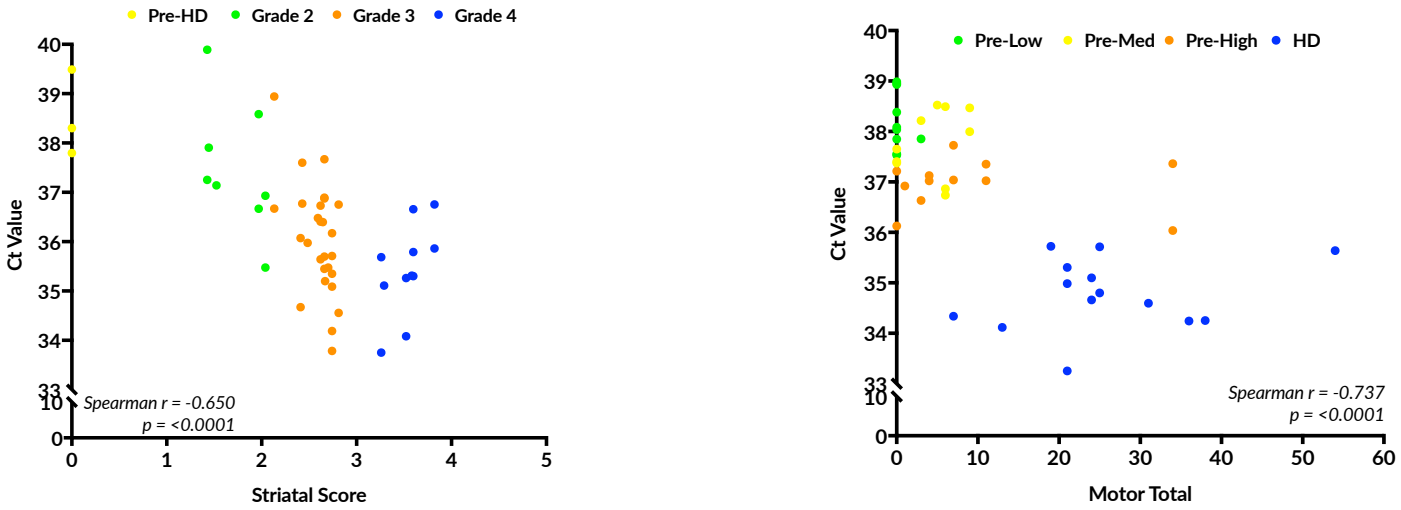

Figure 4: HDB1 and HDB2 correlate with degeneration of striatal neuron in frontal cortex samples (left) and loss of motor function in cerebrospinal fluid samples (right).

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.