MicroRNAs as Biomarkers for Parkinson's Disease

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Background

The diagnosis of Parkinson's disease (PD) currently relies on clinical recognition of cardinal motor symptoms. Yet the clinical manifestations of PD are highly heterogeneous and the underlying pathophysiologic mechanisms which lead to disease are poorly understood. miRNAs are short, noncoding RNAs that regulate genes, and in the brain their regulatory effects have profound effects on neuronal development[1]. Prior studies have shown deregulation of miRNAs in neurodegenerative diseases, including several studies that suggest a role for miRNAs in the pathogenesis of PD[2].

Our lab has previously identified miRNAs that are differentially expressed in PD post-mortem brain samples when compared to controls.[3,4] We believe that the microRNA (miRNA) profile of PD brains may offer insight into the molecular and pathological mechanisms that occur in the disease and prove to be a biomarker for identifying patients and clinical subtypes. To be a useful biomarker, however, miRNAs need to be identified in samples that are easily obtained from patients, such as serum or CSF. We started with evaluation of spinal fluid as we hoped that it would provide an accurate reflection of transcriptional changes we had seen in previously studied brain specimens.

Objectives

To identify potential biomarkers for Parkinson's disease by evaluating miRNA in cerebrospinal fluid samples collected from PD and healthy control subjects.

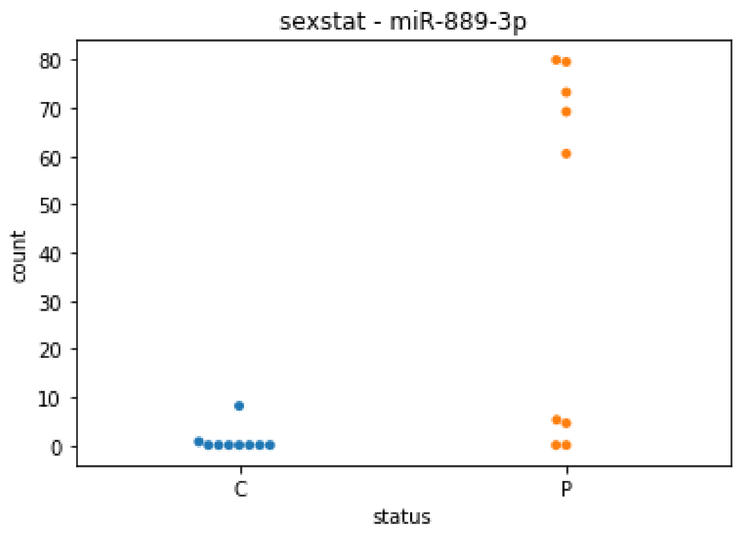
miRNA Preparation

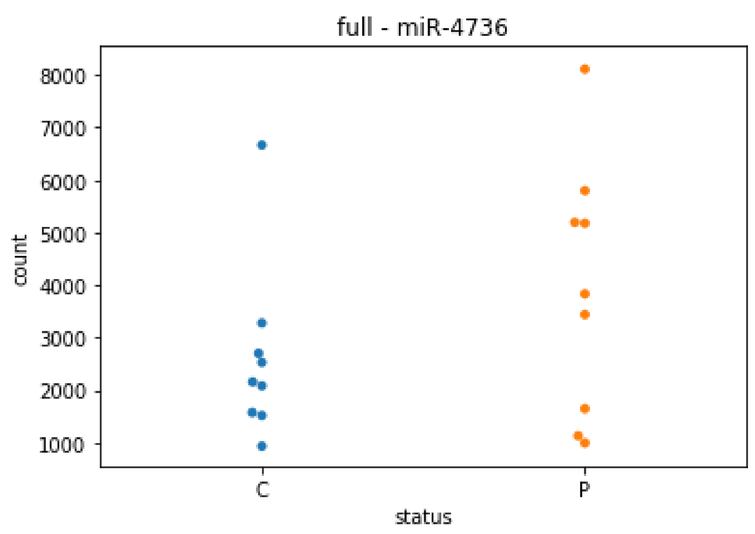
14 patients with clinically diagnosed Parkinson's disease and 10 healthy controls were recruited from the Parkinson's Disease Center at Boston University Medical Campus. Demographic data, a Unified Parkinson's Disease Rating Scale, and a Montreal Cognitive Assessment were obtained for each participant. A lumbar puncture using a Sprotte lumbar puncture kit. RNA was then extracted using standard protocol. miRNA levels were measured using the HTG Molecular Diagnostics miRNA whole transcriptome protocol HTG EdgeSeq system.

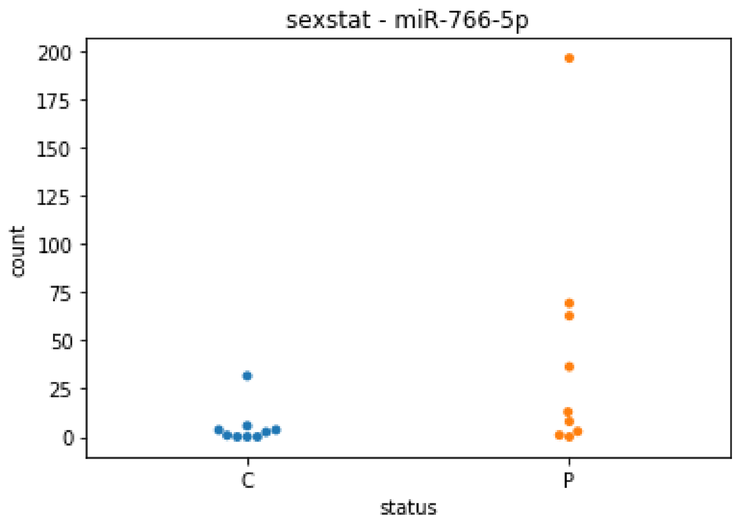
Statistical Analysis

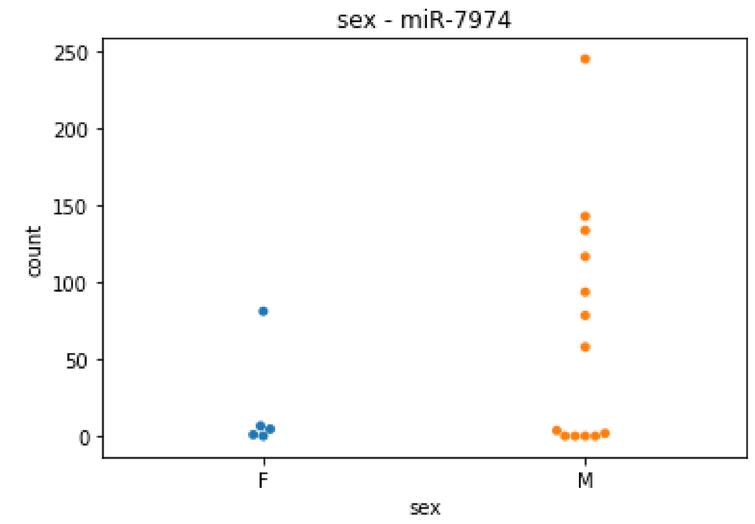
The raw counts matrix obtained from HTG was examined for quality and five samples were removed due to failed sequencing, per recommendation from HTG. Outlier sample counts in the resulting raw counts matrix were shrunk using the procedure described in (Labadorf 2015) to reduce false positive associations due to extreme counts.[5] This trimmed raw counts matrix was subject to normalization and differential expression analysis using the DESeq2 (Love 2014) R package.[6] Models including age, sex, and case status were run and examined for significantly associated miRNAs at FDR<0.2. Swarm plots of normalized counts for significantly associated miRNAs were created using jupyterlab, python, pandas, and seaborn python packages.

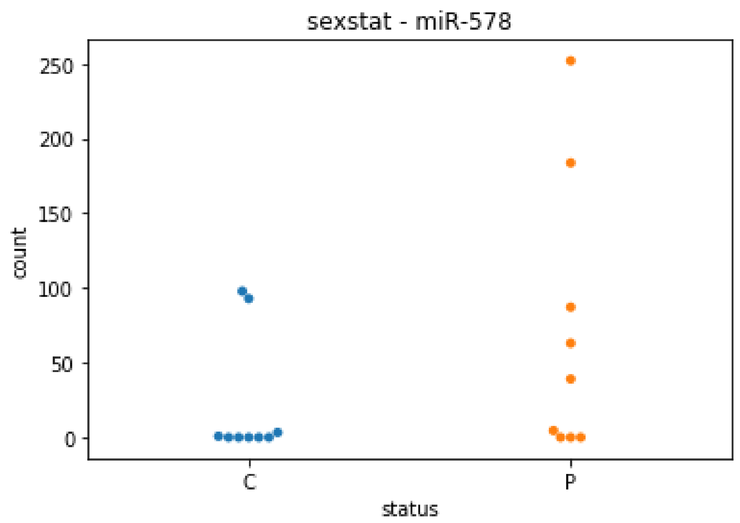
Results











Figured depict differential expression of control versus patient. Plot marked "sex" depict miRNA counts that are associated with sex alone. Those marked "sexstat" depict miRNA counts that are associated with case status after adjusting for sex. Plot marked "full" depict miRNA counts that are associated with case status after adjusting for sex and age.

C=healthy control, P=Parkinson's disease patient, M=male, F=female

Conclusions

- We were able to successfully identify miRNAs in CSF that are differentially expressed between PD patients and controls
- Mir-578, mir- 766-5p, mir- 889-3p, and mir-4736 show differential expression in PD patients versus controls and should be further evaluated as possible biomarkers for PD.
- Mir-7974 was differentially expressed in CSF between men and women regardless of PD status.
- Expression of these miRNA can serve as a starting point in analyzing if UPDRS score (i.e. disease severity) or MOCA score (i.e. cognitive status) determines the degree of expression for PD patients.

Future Directions

- We hope to obtain additional samples of CSF from PD patients enrolled in the PPMI study to further validate the findings of this study in a larger cohort.
- Using longitudinal data, we would like to identify miRNA that may be predictive for the development of PD.
- Identification of miRNA that correlate with progression of disease would allow for monitoring patients, especially in future trials of disease modifying therapy.
- Sequencing of the miRNA in these samples through RNASeq has begun. We hope to identify isoforms of miRNA which may be implicated in the pathogenesis of PD.

References

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