Evaluation of leucine-rich repeat kinase 2 (*LRRK2*)-related microRNAs as biomarkers for Parkinson's disease

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Introduction

Parkinson's Disease (PD)

- Neurodegenerative disorder primarily affecting dopaminergic neurons in the substantia nigra within the basal ganglia of the midbrain (1)
- Symptoms: tremors, rigidity, bradykinesia, gastrointestinal problems, depression and anxiety, insomnia, and dementia (2)
- Slow progressors: develop mild to moderate symptoms 10-20 years after diagnosis
- Fast progressors: develop severe symptoms less than 10 years after diagnosis (3)

Leucine-Rich Repeat Kinase 2 (*LRRK2*)

- "Rosetta Stone" of PD due to its multiple functions and association with many genetic and sporadic cases of PD (4,5)

Diagnosis and Treatment

- Currently no cure
- Current clinical diagnosis is based on motor symptom onset and patient medical history
- Treatment options:
  - Medication (Benztropine, Carbidopa/Levodopa)
  - Physical therapy and exercise to improve balance and range of motion
  - Deep brain stimulation surgery (DBS)

By the time PD is diagnosed, patients have usually lost ~50-80% of dopaminergic neurons (7).

MicroRNA as Biomarkers

- Biomarkers: any natural biological product in the body in which its presence or absence is indicative of onset of a specific disease (6)
- MicroRNAs (miRNAs): small single-stranded RNAs (~20 nucleotides in length) (7)
- Bind to a complementary sequence on the 3' UTR of a specific messenger RNA to regulate gene expression (7,8) (Figure 1)
- miR-29a and miR-29c: regulatory miRNA of LRRK2; shown to be downregulated in the blood of PD patients compared to healthy controls (8)

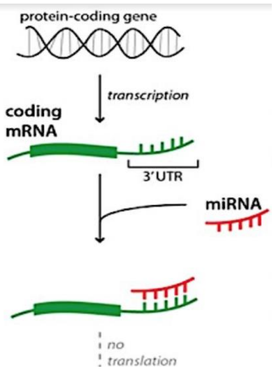

Figure 1. miRNA mechanism of regulating gene expression.

Objective

To determine if LRRK2-targeting miR-29a and miR-29c can reliably predict PD progression to develop non-invasive blood-based tests for disease progression.

Hypothesis

miR-29a/c will be able to differentiate slow from fast PD progression, and will be more highly expressed in fast progressors than slow progressors.

Methods

Sample Selection

- n=30 DATATOP serum samples: 15 slow progressors/15 fast progressors at time of diagnosis

RNA extraction and quantification

- Qiagen miRNeasy Serum/Plasma kit for isolation and purification of miRNA

Reverse Transcriptase PCR and preamplification of miRNA

- MultiScribe reverse transcriptase and TaqMan PreAmp master mix

Quantitative Real-time PCR

- TaqMan gene expression assay

Data Normalization and Statistical Analysis

- Cycle threshold (CT) values
- Markov Chain Monte Carlo algorithm, R (v.9.4) (8)
- Logistic regression tests, SAS JMP Pro 13 (8)

Results

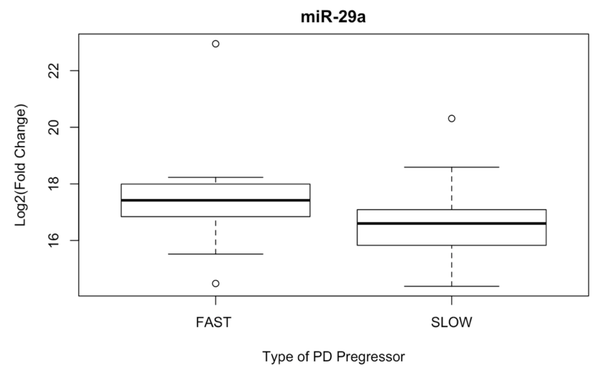

Figure 2: Boxplot representing average foldchange in miR-29a expression between fast and slow progressors.

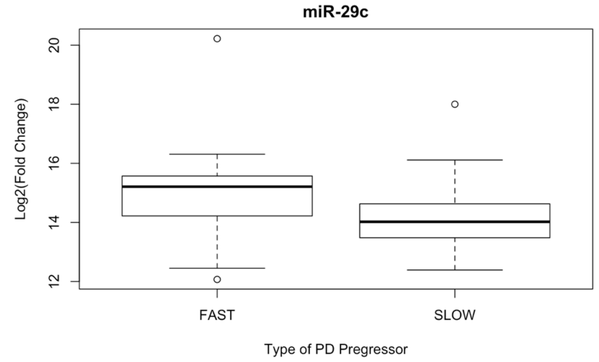

Figure 3: Boxplot representing average foldchange in miR-29c expression between fast and slow progressors.

Table 1: P-values from logistic regression tests.

	P-value
Age & Sex as Predictors	0.4058
miR-29a/c	0.2145
miR-29a	0.1220
miR-29c	0.2042

Age & Sex as Predictors

- Logistic regression was performed with age, sex and miR-29a/c variables to predict PD progression
- Age and sex were not significant predictors and were removed from the logistic regression model

miR-29a and miR-29c

- Logistic regression determined if expression of both miR-29a/c could accurately predict fast or slow progression of PD
- Expression of combined miR-29a/c cannot significantly predict PD progression

miR-29a or miR-29c

- MiR-29a and miR-29c were tested to determine if they can significantly predict PD progression independently
- Neither miR-29a nor miR-29c can significantly predict PD progression

Conclusion & Future Directions

Conclusion

- MiR-29a/c cannot significantly predict fast or slow PD progression either together or separately (Table 1)
- However, both target miRNAs are shown to be expressed higher in fast progressors compared to slow progressors (Figures 2,3)
- This result could indicate that LRRK2 plays a crucial role in progression rate of PD
- This supports our original hypothesis that miR-29a/c would be more highly expressed in fast progressors

Future Directions

- Use a larger, randomly selected PD patient sample to confirm this pilot study

References

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