Advancement in Parkinson's Diagnosis: MicroRNAs as Parkinson's Disease Biomarkers

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What is Parkinson's Disease (PD)?

- Disease of a nervous system (slow but progressive)
- Initially affects the region of a brain, basal ganglia, a region that affects movement, posture, and balance
- In time, PD may affect the cortex – the thinking and remembering part of the brain
- More common in older people but not exclusive to them
- Average age of PD – 60 years
  - 15% of PD – developed at the age below 50
  - 10% of PD -- developed at the age below 40

What Causes PD?

- Molecular cause for PD is unknown
- Pathologically, dwindling supply of a chemical substance called dopamine (a neuro-transmitter) in substantia nigra and/or buildup of protein clumps called Lewy bodies
- Hereditary or familial PD – 15 -25% of population

Current Diagnosis and Treatments

Current diagnosis depends on clinical signs:

- Resting tremor (an involuntary shaking movement)
- Rigidity/ stiffness
- Bradykinesia (slowness or incompleteness of the movement)
- Postural instability (loss of balance and difficulty in walking)
- Stooped posture (up to 60% of the PD patients)

Current treatments:

- PD is incurable
- Medications are given to relief symptoms
- Levodopa/carbidopa – nerve cells use levodopa to make and replenish dopamine
- Drugs that mimic role of dopamine
- Deep brain stimulation – implantation of electrodes to produce electrical impulses and block signals that cause PD symptoms

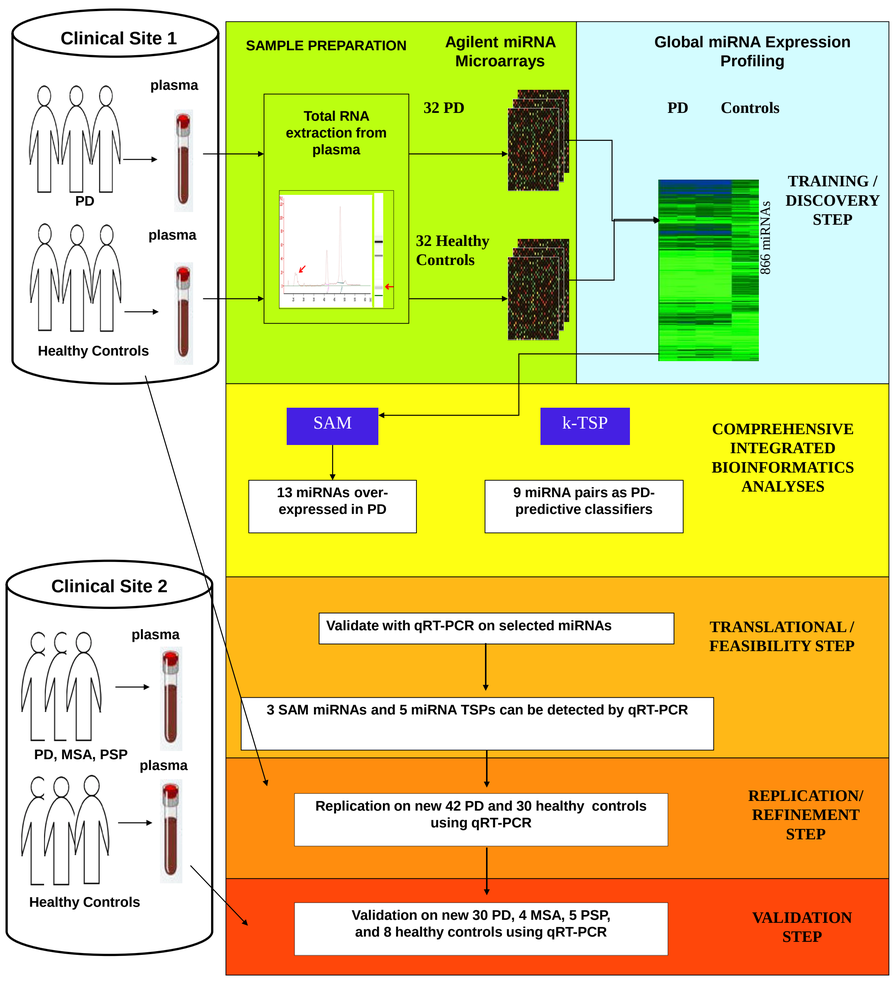
Why do we need to detect PD early ?

- Already at advanced stage when first diagnosed in clinic – lost 50-70% dopaminergic cells
- Early detection may help to stop or delay progression, besides misdiagnosis
- Biomarker – A molecule that can be objectively measured as an indicator of normal or pathogenic biological process

microRNAs as Biomarkers for PD

- microRNAs (miRNAs) are small (~22-23 nucleotide long) ribonucleic acids (RNA) that can be found naturally in cells
- Showed to reflect disease status in human cancers
- We showed proof-of-concept to use miRNAs to differentiate PD from healthy subjects[1]
- miRNAs can be potential biomarkers for PD[2,3]

Uncovering miRNA Biomarkers

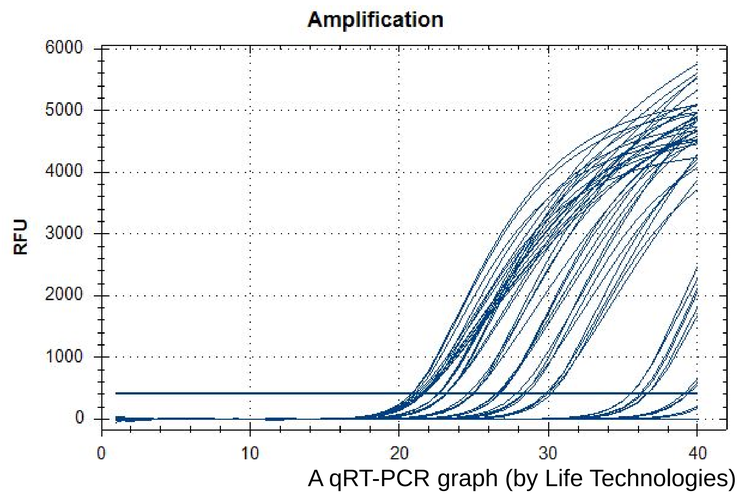


Step 1: Discovery Set

This Phase of experiment aimed to identify specific miRNA signatures for PD patients. Using a high-throughput technology called microarray, miRNA expression in the blood plasma can be compared between PD patients and healthy controls. We identified 13 single miRNAs and 9 pairs of miRNAs to be promising candidates to distinguish PD from healthy subjects.

Step 2: Replication Set

Quantitative real-time PCR (qRT-PCR) was used to quantitate the miRNA expression in a new set of samples (from the same clinic) called replication set. This study was performed to 1) ensure miRNA expression can be replicated to distinguish PD from healthy controls, 2) validate data obtained in the microarray analysis, and 3) calculate the biomarker performance. A panel of 4 miRNAs was demonstrated to be able to detect 91% of PD patients correctly (sensitivity) and detect 100% of healthy subjects correctly (specificity) as well[1].



A qRT-PCR graph (by Life Technologies)

Step 3: Validation Set

A new cohort of samples from a new clinical site was then used as a validation set. We found the panel of PD-related miRNA biomarkers has the predictive power of 83% sensitivity and 75% specificity[4]. Implementing vigorous validation studies will ensure development of reliable and robust disease biomarkers before translation into clinical applications.

Future Directions

Organize and cluster miRNA biomarkers found to be significant in PD

Other Researchers have been working with similar concepts and come up with different results depending on the methodology used. There is a need for us to determine what is relevant and what may not be reliable data for diagnosing PD effectively.

Determine the regulatory effects of PD-related miRNAs

It is great that we have detected these biomarkers, but what are they actually doing in the body? Determining the biological significance of these biomarkers may lead to insights in understanding the regulation of gene expression that characterizes PD. Little is know about these. A detail understanding of these mechanisms offers a chance for clinicians to better diagnosis and treat Parkinson's disease.

Develop reliable diagnostic qRT-PCR analysis to detect PD sooner

This is the main aim of our research. Ideally, we hope to relate miRNA expression levels in the blood of PD patients as early as possible. This will allow doctors and other experts to begin treating the disease before it has done its damage to the patients dopaminergic neurons. The ideal diagnostic method should carry the following characteristics:

- Non-Invasiveness collection of patient sample
- High Sensitivity and High Specificity to the disease
- Detectable in the pre-symptomatic stage of the disease
- Easily quantifiable using protocols such as qRT-PCR

References

[1]Khoo *et al.* (2012) Plasma-based circulating microRNA biomarkers for Parkinson's disease. *Journal of Parkinson's Disease* 2: 321-331.

[2]Khoo *et al.* (2013) Could miRNA expression changes be a reliable clinical biomarker for Parkinson's disease? *Neurodegenerative Disease Management* 3: 455-465.

[3]Shinde *et al.* (2015) Biofluid-based microRNA biomarkers for Parkinson's disease: an overview and update. *AIMS Medical Science* 2: 16-26.

[4]Petillo *et al.* (2015) Parkinson's disease-related circulating microRNA biomarkers – a validation study. *AIMS Medical Science* 2: 7-15.

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