PARKINSON'S PROGRESSION MARKERS INITIATIVE

Play a Part in Parkinson's Research

Title: Whole Blood RNA Biomarkers of Parkinson's Disease

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Summary

We have previously identified RNA biomarkers in whole blood that can be used to distinguish Parkinson's disease (PD) patients from healthy individuals. These biomarkers have been evaluated in samples from two independent clinical studies, the Diagnostic and Prognostic Biomarkers for Parkinson's Disease (PROBE) and the Harvard Biomarker Study (HBS) (1-5). Here we tested whether this group of validated whole blood RNA biomarkers can be useful for distinguishing de novo PD patients from healthy controls. A total of nine biomarkers were tested in 200 RNA samples from patients enrolled in the Parkinson's Progression Markers Initiative (PPMI) study by quantitative PCR assays. This study is expected to provide a basis for testing the markers in individuals at risk for PD and to identify biomarkers of disease progression.

Method

Quantitative PCR assays: Samples with RNA integrity values >7.0 and absorbance 260/280 between 1.2 and 3.0 were used in this study. One microgram of RNA was reverse transcribed into cDNA using a mix of random hexamer primers (High Capacity cDNA Synthesis Kit, Life Technologies). Quantitative polymerase chain reaction assays (qRT-PCR) were performed using the DNA engine Opticon 2 Analyzer (Bio-Rad Life Sciences, Hercules, CA, USA). Each 25 microliters reaction contained Power SYBR Green (Life Technologies, USA) and primers at a concentration of 0.05 mM. The amplification conditions used were as follows: denature at 95 ^{0}C for 15 sec, annealing at 56 ^{0}C for 1 min, extension at 72 ^{0}C for 30 sec for 50 cycles of amplification. Melting curves were run in each qPCR experiment to ensure product specificity. PCR products were sent for sequencing analysis to confirm that the expected product was amplified. Using these amplification conditions we have observed primer efficiencies higher than 90%. Each sample was run in triplicates and no cDNA template and plate-to-plate controls were included. A complete list of the primers used in this study is shown in Table 1.

Expression data will be analyzed using the comparative ΔΔCt method. In this method the amount of target is normalized to an endogenous reference and relative to a calibrator. GAPDH was used in our studies as a reference transcript. For this method to be valid, the efficiency of the reference transcript amplification and the efficiency of the target transcript must be approximately equal. From the plot, a threshold cycle value, which represents the PCR cycle number that produced fluorescence detectable above an arbitrary threshold, is calculated. The amount of template at the start of the reaction determines the threshold cycle.

Statistical analysis: For each biomarker, a student t test will be used to compare patients and controls. Multivariate regression analysis will be used to determine if gene expression is dependent on age, gender, medication, body mass index, Hoehn &Yahr scale, and other covariates. Linear discriminant analysis will be used to identify the best group of biomarkers that can distinguish PD from HC with the highest predictive accuracy.

Table 1. Primer sequences for the biomarkers tested.

Biomarker	5' (Forward)	3' (Reverse)	References
GAPDH	CAACGGATTTGGTCGTATTGG	TGATGGCAACAATATCCACTTTACC	(1, 2)
SOD2	GTTCAATGGTGGTGGTCATATCA	GCAACTCCCCTTTGGGTTCT	(3)
APP	TTTTCTAGAGCCTCAGCGTCCTA	CCCTGGGCTTCGTGAACA	(4)
HNF4A	CAGAATGAGCGGGACCGGATC	CAGCAGCTGCTCCTTCATGGAC	(5)
COPZ1	GATTTTGTGGTGGGAAAGAGT	TGACAGCTCCCCTAGATCTTTG	(1, 2)
C5ORF4	GACATGGTGGATCCTGTGAAACT	GAAAGATATCATGCACTGGTTGAAA	(1, 2)
WLS	CAAGCTAAACAACCAAATCAGAGAAA	ATCACGGTAAGCCAGGGAAA	(1, 2)
EFTUD2	AGCAGGCGAGAGATGGATGA	CGGCTGTTGGGTAGTACTTCTTG	(1, 2)
ZNF160	GGGTCGCTACGGACTTAAAATC	CGGATCCAGGAACGTTTCTG	(1, 2)
PTBP1	GCTCAGGATCATCGTGGAGAA	ATCTTCAACACTGTGCCGAACTT	(5)

References

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