PARKINSON'S PROGRESSION MARKERS INITIATIVE

Play a Part in Parkinson's Research

EVALUATION OF PD-LINKED TRANSCRIPTS IN PPMI

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Summary

Neuronal and peripheral transcriptional changes are linked to Parkinson's disease (PD) --- including the earliest stages of Lewy body neuropathology[1-5]. These transcriptional changes may provide leads for biomarkers, therapeutic targets, and elucidate the disease pathobiology.

The objective of this study was to evaluate associations between the expression of three susceptibility genes and their splice variants in peripheral blood and early-stage, *de novo* Parkinson's disease. A cross-sectional, case-control study of *de novo* cases with Parkinson's disease and age- and sex-matched controls enrolled in the Parkinson Progression Marker Initiative (PPMI) was performed. Transcript counts of the three PD-linked loci *PARK1 (SNCA)*, *PARK15 (FBXO7)*, and *PARK7 (DJ-1)* were analyzed. Five *SNCA* probes targeting distinct transcript isoforms, four probes targeting distinct FBXO7 transcript isoforms, and one probe targeting DJ-1 transcrips were employed. In addition, probes targeting the genes DHPR and ZNF746 were included. Transcript counts were assayed in human blood in a high-precision NanoString gene expression assay that directly measures RNA counts in total RNA without bias from reverse transcription into cDNA. This technology uses molecular "barcodes" and single molecule imaging to detect and digitally count transcripts.

Method

Sample collection. *PPMI was designed to minimize bias from sample processing by collecting, handling and analyzing specimens of cases and controls in a standardized and blinded manner* according to rules of evidence (Ref.[6,7] and "*Eleven good practices for RNA biomarker studies*", in Scherzer, *Biomarkers in Medicine*, 2008[8]). Phlebotomy and biospecimens processing was performed in a standardized manner. Cases and controls were processed in parallel to avoid bias due to "run order" of samples. Venous whole blood was collected in PAXgene tubes to preserve RNA quality.

RNA isolation and quality control was performed at Coriell according to the PPMI Biospecimens Manual. Briefly, from each subject venous blood was collected in PAXgene (Qiagen, Valencia, CA) tubes, incubated at room temperature for 24 hours, and frozen and shipped on dry ice to Coriell. RNA was extracted following the PAXgene procedure. RNA quality was determined by using the RNA Integrity Number package[9]. Only RNAs meeting three stringent Q/C parameters will be included in the analysis. These were (1) RIN ≥ 7.3 by Agilent Bioanalyzer (high mRNA integrity), (2) ratio of absorbances at 260 nm/280 nm ≥ 1.8 (no protein contamination).

Controlling assay performance. Probes were designed according to the manufacturer's design principles[10], including screening for inter- and intra-reporter and capture probe interactions, and selection for probes with optimal melting temperatures[10]. Direct counts of the target RNAs were measured by digital expression analysis based on NanoString technology (without reverse transcription into cDNA). Probes for the target and control RNAs were multiplexed and assayed according to the manufacturer's protocol on the nCounter Digital Analyzer. Our previous low-density array qPCR analysis of 246 human blood samples highlighted five RNAs with low variation in abundance (*GUSB*, *MON1B*, *SRCAP*, *GLT25D1, RPL13*) and these were included together with UBC to control for input RNA (as reference genes). The technician was blinded to the diagnosis. No-template (negative) controls containing water substituted for template were included (NTC). To control for plate-to-plate variation and drift equal amounts of RNA derived from Human Universal Reference RNA (UR) were spotted at the beginning, end, and throughout the entire experiment.

References

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