PARKINSON'S PROGRESSION MARKERS INITIATIVE

Play a Part in Parkinson's Research

Clinical, imaging, plasma and CSF correlates of alpha-synuclein (*SNCA*) genomic variability

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Abstract

Genetic studies have consistently shown *SNCA* is associated with susceptibility to idiopathic Parkinson's disease albeit largely based on a motor diagnosis, limited *SNCA* genotyping (Rep1 (D4S3481) or specific SNPs). The PPMI cohort provides longitudinal clinical and biomarker assessment enabling prior cross-sectional studies to be replicated and extended. Despite the relatively small sample size and limited years of follow up, we hypothesize *SNCA* genomic variability may: 1) influence motor, cognitive (i.e. executive or amnestic subtypes) and neuropsychiatric disturbances (e.g. the conversion of PD without cognitive disturbances to PD with mild cognitive impairment (MCI), or PD with dementia), and/or the slopes of those progressive changes; 2) may be a covariate and/or explain outliers in the measurement of alpha-synuclein gene and/or protein expression in leucocytes, plasma and, cerebrospinal fluid (CSF), and; 3) contribute to the loss of DAT receptors (SPECT imaging).

Methods

Our team has employed massively parallel paired-end sequencing to interrogate genetic variability in 0.5Mb DNA for every subject enrolled in the PPMI cohort. Our effort has focused on the 135kb *SNCA* genomic locus, we have also sequenced the coding exons and 5' and 3' untranslated regions of ~most genes linked/associated with neurodegeneration and/or of biological relevance to dopamine synthesis or metabolism (n=171 genes).

In brief, human DNA provided by Coriell Repositories was fragmented to 150bp using a Covaris® E220 sonicator, and enriched for regions of interest using a TargetSeqTM Custom Enrichment Kit protocol (Life Technologies, Carlsbad, CA, USA); bead emulsion PCR with the EZ Bead system (Life Technologies, Carlsbad, CA, USA). DNA fragment libraries from individual samples were individually barcoded with unique adapters, custom captured, pooled (n=96 subjects/run) and assessed using SOLiD 5500xls re-sequencing (Life Technologies, Carlsbad, CA, USA). Paired-end 75bp forward and 35bp reverse reads were generated and mapped to the reference human genome (hg19). Bioinformatic analysis includes paired-end sequence alignment, duplicate removal, single nucleotide variant (SNV) calling and indel detection using Lifescope version 2.5.1 softwares (Life Technologies, Carlsbad CA, USA). All information regarding SNVs and indels are output by Lifescope in variant call format (VCF) version 4.1 (http://www.1000genomes.org/wiki/Analysis/Variant%20Call%20Format/vcfvariant-call-format-version-41). Annotation of SNVs for conservation and protein damage prediction was performed with ANNOVAR1. For a subset of variants Sanger sequencing was performed on

ABI 3730xl capillary array and results analyzed on SeqScape (Life Technologies (formerly Applied Biosystems, Inc.(ABI)). *SNCA* copy number analysis was performed on all patient samples using Taqman probes and real-time PCR on an ABI7900.

The *SNCA* locus is the most important genetic locus implicated in Parkinson's disease, it is the only gene robustly implicated in progression and unequivocally in Lewy body disease. While there is an argument for whole-human genome sequencing on all PPMI subjects, at the time of our application the budget required was prohibitive, the coverage and depth of nucleotide sequencing would have been inadequate in regions of interest, and incidental findings (unrelated to neurodegeneration) were of concern.

Data summary and preliminary findings

Of the 0.5Mb of sequence targeted we successfully captured ~97.4% with a minimal read depth >50-fold (Figure 1). We have also performed *SNCA* copy number analysis but have not identified multiplications. All raw and annotated sequence data, and summary/descriptive data, have been packaged for distribution, and are available via the MJFF PPMI Consortium/LONI. Our team would be pleased to directly address any problems or ambiguities in the archive.

Thus far our analysis has included an assessment of ancestry informative markers, intrinsic to the data, which refine self-declared ethnicities and provide eigenvectors to serve as covariates in association testing. Although a pilot study (given PPMI's sample size), we have completed a study of *SNCA* variability with readily available clinical and biological variables, using appropriate softwares (PLINK, R archive and BEAGLE) and adjusting for potential covariates (age, gender, ethnicity and education).

Remarkably, *SNCA* genetic variability in the PPMI cohort is significantly and robustly associated with an overall diagnosis of Parkinson's disease and age at onset. Provocative but non-significant trends were observed in trait components, including the trajectory of cognitive decline. *SNCA* genetic variability was not associated with quantitative measures of alpha-synuclein or tau protein in body fluids.

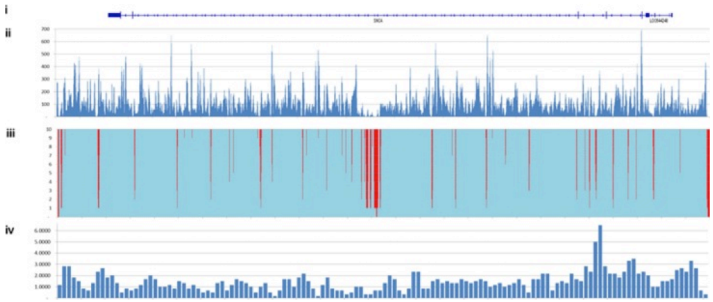

Figure 1. (i) *SNCA* gene exons and introns; (ii) average read depth; (iii) areas of read depth <10 due to repeat sequence; (iv) % nucleotide variants in 2kb bins.

Future plans are to work with Dr. Ken Merek's team to assess whether there may be *SNCA* associations with imaging, as per the original objectives of our Michael J. Fox award. We are also collaborating with Dr. Clemens Scherzer's team to look at the influence of *SNCA* genomic variability on gene expression in blood leucocytes. We plan reiterative clinical and biomarker

analyses, as patients within the PPMI cohort are followed longitudinally, and as their symptoms evolve.

References

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Document creation date: March 27, 2015