

## GEO-PD - RVCD genotyping project

### Background

The hypothesis underlying the GWAS approach is that common variants underlie common diseases (CVCD). Most single nucleotide polymorphisms (SNPs) genotyped have minor allele frequencies at >5% frequency in the populations in which they were tested. Several genome-wide association studies (GWAS) have now been performed in Parkinson's disease. Primary results and subsequent meta-analyses show common genetic variability in alpha-synuclein (*SNCA*) and tau (*MAPT*) contributes to disease susceptibility, largely validating past discoveries. Although other gene loci have been nominated the majority have failed replication or have yet to be validated.

The overall objective of this study is to test the alternate hypothesis that multiple rare variants underlie common diseases (RVCD). It is important to appreciate that GWAS is not designed to address this issue, yet empirical genetic data strongly supports the RVCD hypothesis in Parkinson's disease. One example is ancestral founder mutations in *LRRK2* which have the highest population and allelic attributable risk. *LRRK2* has not been implicated in GWAS analyses. Nevertheless, our recent GEO-PD Consortium study "*LRRK2* variation in Parkinson's disease" that directly assesses multiple variants suggests a positive association in idiopathic disease (analysis in progress). Many of the most pathogenic *LRRK2* variants including R1441C/G/H, R1628P, G2019S and G2385R were discovered and subsequently implicated in Parkinson's disease by a combination of sequence analysis, with direct genotyping of the risk mutation across many populations and samples. In many instances we have found evidence for a common ancestral founder through subsequent microsatellite repeat (STR) genotyping, providing additional support for pathogenicity. Other examples of the success of this approach and the RVCD hypothesis include findings in *parkin*, *PINK1* and *DJ-1* mutations in early-onset disease.

Independent laboratories are not in a position to investigate the frequency, penetrance or clinical phenotypes associated with rare variants, either because of a lack of power/ appropriate samples or financial cost. The GEO-PD, because of its access to many samples of both familial and sporadic disease, and ethnically distinct patient samples and control groups, is uniquely positioned assess the frequency, disease prevalence and overall relevance of rare mutations for the global PD community.

### Specific Aims

- (A) To resolve the role of known *rare* coding variants in susceptibility to Parkinson's disease
- (B) To determine haplotypes within carriers (to assess evidence of an ancestral founder)

### Study design and methods

We anticipate genotyping  $\geq 100$  variants per sample in a minimum of 10 novel genes. Funding will be sought through charitable foundations. We propose to screen primarily non-synonymous substitutions (cSNPs) within the combined GEO-PD patient-controls series (~10,000 subjects) either nominated in the literature, to provide a definitive statement on pathogenicity, or 'private' cSNPs of which GEO-PD members contribute from resequencing studies in their specific populations. Cost permitting, we intend to complement the cSNP set with additional, informative haplotype-tagging short-tandem repeat markers to address whether 'common variants' may adequately tag multiple 'rare variants' that cause common disease. This is a fundamental issue in genome-wide association studies.

We require a 'minimal clinical/demographic dataset' and 250ng DNA from all samples to be genotyped for Consortium members. The minimal clinical data set will include whether a sample is a case (familial/sporadic) or a control, year of birth, and age at symptom onset.

Ideally, it would be good to know the first symptom, disease duration and PD sub-type (tremor predominant, akinetic-rigid or mixed), however this will be optional to include.

DNA should be good quality and diluted in water or low/minimal [TE] buffer. Ideally all samples will be provided or plated into a 96-well format, and will be transferred to a 384-well format for genetic analysis. Our laboratory has Beckman Biomek FX and Cartesian robots for liquid handling/pipetting, and subsequent genotyping will be performed using Sequenom iPLEX chemistry using MALDI-TOF mass spectrometry for which the genotyping call rate over all assays is >90%. iPLEX is best suited to the scale of this project given the number of samples and genetic variants that need to be assessed.

### **Significance and innovation**

The past few years has seen an increasing number of linkage, GWAS and candidate-gene assignments in Parkinson's disease that nominate specific gene mutations, published in some of the high-ranking genetics journals (e.g. Nurr1, GIGY2 and FGF20). Furthermore, journals have a positive publication bias and it is more difficult to publish negative studies. However, false positive assignments and the need for Consortia to address the RVCD hypothesis are likely to burgeon with the advent of "next-generation" sequence analysis. This proposal aims to pre-empt the problem, to make the most of available resources and data, and to provide definite direction for subsequent functional neuroscience that promises to deliver novel therapeutics based on molecular etiology.

### **Timeline**

- 1) Interested sites should initially contact Dr. Farrer ([farrer.matthew@mayo.edu](mailto:farrer.matthew@mayo.edu)) to provide more study details. They should indicate the general number of samples (cases and controls) and variants they might want to assess (deadline December 31<sup>st</sup> 2009)
- 2) Participating groups will be asked to sign non-disclosure and material transfer agreements (deadline for receipt January 15<sup>th</sup> 2010)
- 3) Participating groups will be asked to provide minimal clinical data and IRB/Ethical approvals, and list of specific variants to genotype (deadline for receipt February 15<sup>th</sup> 2010)
- 4) Deadline for sample receipt will be April 1<sup>st</sup> 2010
- 5) Genetic variant genotyping panels will be finalized at the Toronto April 2010 GEO-PD meeting.
- 6) Dr. Farrer and/or Dr. Reiss's teams will offer to perform the genotyping by September 1<sup>st</sup> 2010, with statistical analysis and data release to site investigators by October 1<sup>st</sup> 2010.
- 7) Sub-Consortia with "positive" carriers will be convened for more detailed evaluations (Oct 1<sup>st</sup> 2010)
- 8) All variants assessed will be disclosed in a joint GEO-PD publication for the 2011 GEO-PD meeting (sub-Consortia may publish limited findings prior)

### **Contact information**

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