

# **$\alpha$ -Synuclein Reduced-expression Genotypes and Survival in Parkinson's Disease**

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## BACKGROUND

Several therapies are being developed to reduce  $\alpha$ -synuclein (SNCA) in Parkinson's disease (PD). This is justified because SNCA gene multiplication mutations and resultant over-expression are a cause of PD in some families, and because all PD cases (sporadic as well as familial) are characterized neuropathologically by synucleinopathy. It is our *hypothesis* that observational studies of the association of common functional SNCA variants with prognostic outcomes will predict the long-term benefits of therapies to reduce SNCA in PD. For example, we postulate that reduced SNCA expression, as defined by REP1 259 bp alleles, is associated with survival free of death in PD. Three references are attached (Elbaz 2003, Maraganore 2006, Lewis 2008).

## SPECIFIC AIMS

Consistent with our hypothesis, we propose within the Genetic Epidemiology of Parkinson's Disease (GEO-PD) Consortium a collaborative study with the following *specific aim*:

1. To determine whether genotypes defined by the SNCA reduced expression allele (REP1 259 bp) are associated with a reduced risk of death in PD.

## PRELIMINARY STUDIES

Several GEO-PD sites have participated in collaborative-pooled analyses of SNCA REP1 variability and PD susceptibility. Dr. Maraganore's team has since expanded the sample for the Mayo site and genotyped 1,103 PD cases for SNCA REP1 variability. It is anticipated that other sites that participated in the published collaborative study have since expanded their sample and genotyped additional PD cases, and that other GEO-PD sites either have SNCA REP1 data available or are able to produce these data for their PD samples. Furthermore, Dr. Maraganore's team has collected follow-up data for multiple outcomes (including vital status) for all but 404 subjects as of September 28, 2009 (projected completion date March 1, 2010). It is anticipated that other sites have available data regarding vital status in their PD cases for a defined follow-up date, or that these data can be collected (via telephone or mail contacts, medical records, death registries, other sources).

## STUDY DESIGN AND METHODS

We will fulfill these aims by performing an *observational study* using a *cases-only design*. Our *methods* will include demographic, clinical, environmental exposure, and SNCA REP1 genotype measures made at baseline, and vital status measures made at follow-up. Participating GEO-PD sites will describe site-specific study characteristics (sources of PD cases, clinical diagnostic criteria employed), and share de-identified baseline data for at least 100 PD cases, including: date of birth, date or age at onset of first motor symptom or sign; date at baseline (blood drawing for DNA extraction and storage), gender, race and ethnicity, family history of PD (at least one affected first degree relative, ye/no), levodopa therapy (yes/no), education (years), smoking (ever/never), and SNCA REP1 genotypes. The vital status can be defined by each site at follow-up as they prefer (via telephone or mail contacts, medical records, death registries, death certificates, other sources), provided that the lost to follow-up rate is low (less than 10%). The difference in time between the baseline and follow-up dates must be greater than one year for each case. Participating sites will share follow-up data for all subjects regarding death (yes/no/don't know), age at death (if applicable), date of follow-up (telephone interview, or mail receipt, or query of death registry, etc), and method of follow-up (telephone or mail contacts, medical records, death registries, death certificates, other sources).

The PI will be responsible for coordinating data sharing and statistical analyses. His team will standardize for all sites allele length calling, such that the most common allele will be defined as 261 bp ("0"), the common shorter allele as 259 bp ("-2"), and the common longer allele as 263 bp ("+2"); genotypes defined by rarer alleles will be excluded from the analyses. The primary outcome for these analyses will be time-to-event (if evidence of death) or time-to-censoring (if no evidence of death or unknown vital status). For cases with evidence of death, time-to-event will be defined as the time between baseline and death. For cases with no evidence of death, time-to-censoring will be defined as the time between baseline and follow-up. For cases with unknown vital status (not reachable for this study), the date of last contact alive will be used as the censoring date. We will consider the date at baseline as time 0 (or baseline) in primary analyses (to limit survival bias); however, we will also perform secondary analyses using approximated age at onset. For our dichotomous survival outcome, we will use standard statistical methods for survival analysis. We will use

Kaplan-Meier plots to visualize the overall survival function in the entire set of PD cases, and stratified by genetic (e.g., separate curves for 0, 1 or 2 copies of the 259 bp reduced expression allele), demographic (e.g. sex, education at baseline), clinical (e.g., disease duration, L-DOPA therapy at baseline), and environmental (e.g., smoking, pesticides use at baseline) variables. The associations between the outcomes and genetic, demographic, clinical, and environmental factors will be described using hazard ratios (HR) with 95% confidence intervals. Log rank tests will be used to determine significance. We will use Cox proportional hazards regression models to assess and describe the association of the outcomes with the genes, adjusting for demographic (e.g., age and sex), clinical, and environmental confounding variables, as well as for the method of follow-up (e.g., telephone or mail contacts, etc.) and the contributing GEO-PD site. An important assumption in Cox models is that the hazards are proportional over time. We will check the validity of this assumption using log cumulative hazard plots for groups over time and by examination of Schoenfeld residuals. We will also perform sensitivity analyses where the entire cohort will be split into two sub-cohorts based on the median date of the clinical assessment at baseline (older and newer cohorts), and on the median disease duration at baseline (to assess for survival bias). The analyses will be repeated in these (or other) strata, and we will compare the results.

### SIGNIFICANCE AND INNOVATION

This proposal represents the first large-scale, multicenter study of genetic variability and survival in PD. The observation that *SNCA* reduced-expression genotypes are associated with a reduced risk of death will provide proof of concept for therapies targeting *SNCA* in PD (*significance*). As such, our studies represent “virtual clinical trials” (*innovation*). As additional disease-modifying therapies for PD are developed, we envision performing similar virtual clinical trials, leveraging DNA, baseline, and follow-up data collected for this study.

### TIMELINE

1. Interested sites will email Dr. Maraganore ([dmaraganore@northshore.org](mailto:dmaraganore@northshore.org)) an email declaring their intent to participate by *January 15, 2010*. Interested sites provide site-specific information, to include the name and email address of the global site PI; the names of global site co-investigators; the estimated number of PD cases at least one year post-baseline and with available baseline data for each variable; the proposed method of follow-up; and a statement of agreement to share baseline data and follow-up data quid pro quo in compliance with the timeline (below). The PIs of interested sites must also be registered as members of the GEO-PD LinkedIn group. Dr. Maraganore will reply by email to notify sites of preliminary approval.
2. Preliminarily approved sites will provide Dr. Maraganore with de-identified, individual-level baseline data (using a standardized Excel spreadsheet) by *February 28, 2009*. Upon satisfactory receipt of the data and data cleaning, Dr. Maraganore’s team will perform HWE assessments and genotype standardization. Only sites with a minimum of 100 PD cases at least one year post-baseline, with complete baseline data, and fulfilling genotyping quality measures will be fully approved for further participation in the study.
3. At the 2010 GEO-PD meeting (Toronto, Canada, April 17-18), Dr. Maraganore will present a summary of the baseline data for the fully approved sites separately and combined, as well as estimates of statistical power based upon observed genotypes frequencies and projected person-years follow-up.
4. The fully approved sites will collect follow-up data for all cases with baseline data between April 2010 and December, 2010. The participating sites will provide Dr. Maraganore with de-identified, individual-level follow-up data (using a standardized Excel spreadsheet) for all cases by *December 31, 2010*. Upon satisfactory receipt of the data and data cleaning, Dr. Maraganore will send an email thanking participating sites for their completed participation.
5. Dr. Maraganore will have the data analyzed and a manuscript drafted for publication by the 2011 GEO-PD meeting (Chicago, USA; summer). He will present the final results of the study at the 2011 meeting.
6. When the paper is accepted for publication, Dr. Maraganore will share site-specific data with their PIs.

### CONTACT INFORMATION

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