Mount Sinai Beth Israel Mount Sinai St. Luke's Mount Sinai Roosevelt	Protocol Name:	**Genetics of Parkinson's Disease Consortium Study**
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HRP-503 PROTOCOL TEMPLATE

- *Note that, depending on the nature of your research, certain questions, directions, or entire sections below may not be applicable. Provide information if and when applicable, and in cases where an entire section is not applicable, indicate this by marking the section "N/A". Do not delete any sections.*
- *This document refers to Checklists: these can be found here: http://icahn.mssm.edu/research/resources/program-for-the-protection-of-human-subjects/researchers-palette/pphs-form-and-document-kiosk*
- *This protocol template may be used for submissions to the Mount Sinai Beth Israel IRB or the Mount Sinai SLRHC IRB. If the research is only occurring at one site within the Mount Sinai Health Care System, you may delete references to the other sites as appropriate.*
- ***For any items below that are already described in the sponsor's protocol, the investigator's protocol, the grant application, or other source documents, you may simply reference the title and page numbers of these documents in the sections below, rather than cutting and pasting into this document.. Do NOT refer to any derived documents, such as the Sample Consent document, or other internal documents required with the submission.***
- *When you write a protocol, keep an electronic copy. You will need to modify this copy when making changes.*

Brief Summary of Research (250-400 words):

Briefly describe the research study in a short summary that can be understood by someone without scientific expertise in your field of research.

This is a multi-site systematic study of individuals with Parkinson's disease (PD) and parkinsonism and their family members. The main study focus is to characterize PD due to the LRRK2 gene, as well as other known and yet unknown genetic contributors to PD and parkinsonism, and to identify markers of disease progression as well as risk markers for developing disease. The testing battery consists of a general screening phase in which minimal exam, medical and family history, and blood samples are obtained, and an in depth phase consisting of neurological exam, analysis of gait, spiral drawing, neuroimaging using ultrasound (and DaTScan in a subset of unaffected family members), blood and urine sampling, neuropsychological testing, additional questionnaires about medical and family history, a phone interview about mental health, and lumbar puncture and skin biopsy in a small subset. In order to achieve the study goals we will recruit approximately 500 individuals and families with PD and parkinsonism at MSBI. An additional 300 and 600 individuals will be recruited at Columbia University and Tel Aviv Sourasky Medical Center respectively. In our genetic arm, we will explore, through new technologies such as genome wide association, exome and genome sequencing, and expression analyses, potential genetic modifiers of LRRK2, molecular expression of LRRK2 pathways, and also test for additional variants that increase risk for PD and parkinsonism.

1) Objectives

Research Question: *Begin this section by stating your research question, and the hypotheses to be tested.*

Then, describe the purpose, specific aims, or objectives of the Human Research.

Specific Aims

In this multi-institutional proposal (Beth Israel-BI, Columbia University-CU and Tel Aviv University-TA), we pursue two arms as outlined in detail below. In our clinical arm**, we will characterize the clinical expression of the LRRK2 G2019S mutation, including the evaluation of posited pre-motor, early markers of PD. We will also determine penetrance of diagnosed PD, and examine attitudes toward genetic testing**. A collaborative aim of this arm (with Caroline Tanner) will be an examination of environmental exposures and LRRK2 expression. In our genetic arm**, we explore, through genome wide association and expression analyses, potential genetic modifiers of LRRK2, molecular expression of LRRK2 pathways, and also test for additional PD variants that increase risk for PD.**

Clinical Research Questions

Question 1

What are the characteristics of the LRRK2 G2019S PD phenotype and do they differ from other AJ PD?

We will answer this question at two levels: **a)** using the core data, that is compatible with PD DOC, we will compare our larger group of approximately 250 affected carriers and approximately 1500 PD non-carriers and; **b)** using our in-depth assessment we will compare 150 PD carriers and 150 PD matched non-carriers.

A) Our first-level analyses will focus on comparisons of gender effects, family history, age-onset, onset symptoms, levodopa and DBS response, and PD assessed by the UPDRS and Hoehn and Yahr. These data should be available on most of the 2000 genotyped subjects; because we anticipate a subset of Tel Aviv subjects will be missing full clinical data we estimate that our groups will consist of 250 mutation carriers and 1500 non-carriers. For our newly recruited 583 subjects we also will have MOCA and GDS assessments.

We will specifically pursue differences suggested in previous work; these differences include an increased risk for the G2019S mutation in women and possible gender differences in phenotype (Orr-Urtreger 2008) (Clark 2007), an increased frequency of the PIGD clinical subtype (Alcalay, submitted) , and a more benign motor and non-motor phenotype in the mutation carrier compared to non-carrier groups (Healy 2008).

Statistical analysis:

With a fixed sample size of 250 PD mutation carriers and 1500 PD non-carriers we calculated the minimum detectable OR with an alpha of 0.05, and power of 0.8. Using the frequency of PIGD phenotype we detected in the non-carriers, 0.59, we can detect an OR of 1.49. If the prevalence of a specific finding

in non-carriers is .2, we can detect an OR of 1.66 and if it is .1, the minimum OR is 1.7. For continuous measures such as the specific cognitive or mood measures, we can detect a small effect size of 0.19.

Demographic and clinical characteristics will be compared between mutation carriers and non-carriers. Fisher's exact test for categorical characteristics and Student's t-test for continuous characteristics will be used to assess statistical significance.

B) We will compare a smaller group of 150 PD mutation carriers (probands) to 150 PD non-carriers matched for age, sex, and duration disease. Because these 150 affected carriers will be the probands for recruited families, we will prioritize recruitment to individuals with living parents and siblings; this should enrich our family study (see question #2) for mutation carriers at greatest risk (i.e. older relatives) for phenoconversion. To capture the different stages of disease, we will try to ensure a broad distribution of age of onset and disease duration. Further, in each group we will include 50 with PD disease duration of < 5 years. This group of relatively newly diagnosed PD will provide an additional clinical sample for our pre-clinical markers question (question #2); that is, features or markers which discriminate the recently phenoconverted group from controls are likely to have better operating characteristics to detect pre-clinical disease. Also, it is from this group we will sample patients for our expression study of unmedicated PD (see genetics arm below).

In addition to the core assessment, we will evaluate cognition (MOCA), mood (BDI, GDS, CIDI), sleep (RBDQ), dysautonomia (SCOPA-AUT), gait dynamics (using a tri-axial accelerometer), olfaction (UPSIT), heart rate variability (using ECG), and interval timing, and perform ultrasound and spiral analyses. Exposures (including collaborative screen of environmental exposures), general health and detailed family histories will be obtained; urine and blood will be collected for RNA studies and for future biomarker studies, and CSF will be collected on a subset. Skin biopsies will be obtained on a subset of Columbia cases through separate NIH funding (UDALL Center).

We will repeat the assessments at 15 month intervals (4 visits total) in order to add a longitudinal component to our data.

This more detailed assessment will allow us to pursue preliminary findings of an increased frequency of affective disorders and lower frequency of other non-motor features in mutation carriers compared to non-carriers. Potential pleiotropic manifestations, which are unexamined, will be explored in our general medical and family history questionnaires.

Statistical Analysis:

For the comparison of 150 PD carriers to 150 PD non-carriers we have 80% power to detect a minimum OR of 2.0 if the prevalence in non-carriers of dichotomous variables is 0.59 (as it was in our analysis of PIGD). We can detect an OR of 1.92 if the prevalence in non-carriers is 0.5, and 2.08 if the prevalence in non-carriers is 0.2. For continuous variables e.g. UPSIT we can detect a small to medium effect size of 0.32.

<u>Question 2</u>

Can we identify early, pre- diagnosis, markers of LRRK2 G2019S pathology?

Previous studies suggest that alterations in sleep, olfaction, brain imaging, and autonomic and motor function are present before the onset of diagnosed PD. Further, our preliminary data suggests

diagnosed carriers have affective disorder many years before the onset of clinical PD. We will assess for these alterations in 300-350 first - degree relatives and 100 spouse controls. As stated above we are targeting older relatives (parents and siblings) and based on previous studies (Marder 2003) we anticipate we will be able to recruit a large group of older relatives (280-300) (see Appendix). Also, based on our previous penetrance estimates, about 20% of older carrier relatives may have PD; our target is to identify at least 150 mutation carriers without PD and 150 non-carrier relatives without PD; we may therefore also include older children (>30 years) to reach this target recruitment. We will compare carrier relatives separately to non-carrier relatives and to controls.

We will perform "in-depth" examinations described above (MOCA, BDI, GDS, CIDI, RBDQ, UPSIT, SCOPA-AUT, accelerometry, interval timing, ultrasound, spiral analysis, heart rate variability analysis, neurological examination, and exposures and general health information) and obtain urine and blood for RNA and future biomarker studies. CSF will be collected on a subset. As with the PD group, relatives will be evaluated at baseline and at 15 month intervals for a total of 4 visits.

Statistical Analysis:

For the comparison of 150 carriers vs. 150 non-carriers, power estimates and statistical methods are the same as in 1B.

Question 3

What is the penetrance of the G2019S mutation for diagnosed PD? Are there sex differences?

We will assess penetrance using two approaches. First, we will use information on each first-degree relative from the detailed interview screen of first degree relatives of 250 carrier probands (Marder 2003) and 400 non-carrier probands and apply the kin cohort method (Wacholder 1998). In this method, the genotypes of the relatives are first estimated using Mendelian principles and the relationship of the relatives to the proband. Then the observed disease occurrence in the relatives is evaluated in relation to these estimated genotypes. This method assumes that, although the PD case probands are sampled through their AJ background, thereby increasing the proportion of carriers among cases and their first-degree relatives, the relatives of these PD cases are representative of randomly chosen individuals with certain genotypes. Hence familial influences on the relatives' PD risk other than the LRRK2 genotype are assumed to be negligible. We have successfully used this method to examine the penetrance of parkin mutations (Wang 2008).

As a second approach we will have direct examination and genotype data in 150 families and at least 300 first - degree relatives and will assess penetrance in gene carriers using Kaplan - Meier life table analyses. Penetrance in men and women will be compared.

Question 4

What is the level of knowledge about the heritability of PD in AJ PD families and what are their attitudes toward genetic testing? Do Israeli and US families differ?

Remarkably little is known about Parkinson's disease patients' understanding of heritability of Parkinson's disease and principles of genetics in general. With the recent thrust towards personalized medicine, and the invitation from 23 and me for 10,000 PD cases to submit their DNA for analysis and

receive the results of genetic testing, this is an opportune time to poll individuals. We have a unique opportunity to compare knowledge and attitudes about genetic testing in the US and Israeli populations, which may be different. We will use a questionnaire we have developed as well as a brief survey of privacy and insurance that is currently being used in a study of individuals at risk for Huntington's disease, the RESPOND study (Ray Dorsey PI) and will administer this to the 150 mutation positive and 150 negative probands and their families. There is only one published article comparing knowledge and attitudes toward genetic testing in PD in American and Asian populations. That study of 515 patients reported that attitudes toward potential benefit of testing were more positive and knowledge higher in American than Asian patients. However the US group was more worried about compromise in terms of insurance and job (Tan 2007). This survey was performed pre–GINA.

Statistical Analysis:

We will determine the frequency of PD cases that have undergone genetic testing in the US vs. Israel and the frequency of presymptomatic carriers who have undergone genetic testing in the US vs. Israel. If they differ, all analyses will be stratified based on residence. All analyses will control for family membership and relationship to the proband. We will explore whether attitudes toward genetic testing differ based on family history of PD, demographic characteristics, and perceived heritability of PD. Power analyses are provided for dichotomous variables in 1.b.

Genetics Arm Questions

Question 5

Can we identify genes that interact with LRRK2 or new PD genes?

We will perform a **whole-genome association study** (GWAS) that includes a first (screening) phase, a second (replication) stage with expansion of the study cohort, and a potential fine-mapping stage to identify genes that interact with LRRK2 and new PD genes.

A) **Screening:** This stage utilizes *previously* genotyped, independently supported whole-genome (WG) typed samples (including foundations, philanthropic, and NIH support). These yet-to-be-published samples will be made available for meta-analysis that will be completed within the first year. These include 825 AJ PD cases (n=225 Beth Israel; n=300 Columbia, and n=300 Tel Aviv) and a consolidated panel of > 680 AJ genotyped controls; these include 430 WG typed healthy controls from within the consortium, 250 with Crohn's and dystonia (BI) and the likely potential to expand utilizing other disease and healthy elderly cohorts (MSSM, AECOM, Yale, LIJMC, Hebrew U). With standard assumptions, this meta-analysis has 34% power to detect a 1.5-fold effect as genomewide significant, and 96% power for it to be part of the top 1000 SNPs (nominal significance 0.001; only 28% power for a 1.25-fold effect). We will test for new variants associated with PD as well as for alleles that interact with LRRK2. Each variant will be examined for association not only to disease status, but also phenotypic differences (including age at onset) between PD cases that carry a *LRRK2* G2019S mutation and modifier allele (s) and PD cases that carry a *LRRK2* G2019S mutation only, or a modifier allele only, or PD cases that carry neither *LRRK2* G2019S or modifier allele.

B) **Expansion/replication:** We will significantly increase our power to detect a small (1.25-fold) genetic effect if we expand the GWAS to an additional AJ PD population of 500 samples, having 49% power to raise the causal SNP with 1.25-fold effect to the top 1,000.

During the 2nd year the GWAS will further expand to first degree relatives of LRRK2 positive patients. This study will include 200 asymptomatic first degree relatives of *LRRK2* G2019S carriers, 100 carriers and 100 non-carriers that will be recruited until the end of the 1st year. Although phenotyping efforts by the clinical arm will continue into subsequent years, the subjects to be genotyped will already have been identified, facilitating GWAS using these samples. This analysis will increase our power to detect modifiers.

C) **Fine mapping:** For the LRRK2 region as well as any genomewide significant or top-10 region we will sequence representative individuals for all AJ haplotypes observed in that region. We estimate sequencing 100 individuals, but the actual number will be based on the SNP data. Costs and technologies for this task are in flux, but currently capture probes for the significant regions followed by SOLiD or Solexa sequencing offer the best solution.

Question 6

What transcription biomarkers and pathways characterize LRRK2, including those at risk but not manifesting PD? Are they different from other AJ PD?

We will perform **transcription profiling** to characterize classes of patients. Following the promising preliminary results on 120 samples (done on the TA cohort), we intend to conduct a genomewide scan for transcription biomarkers of PD, alternative splice forms, full-length genes, and entire pathways whose expression levels may characterize genotype classes. We will measure transcription profiles from whole blood in 400 newly collected samples, using Affymetrix Exon Array 1.0. The samples equally represent 4 groups of cases and controls according to their PD-relevant carrier status and the analysis will leverage the combination of genotype, expression and phenotype. We will interrogate 4 groups of samples, 100 each (96 + 4 replicates). **Cases**: 1. affected LRRK2 G2019S mutation carriers; 2. affected non-carriers matched to the mutation positive group for age, disease duration and gender. Also, an effort will be made to include about 25-30% of drug-naive newly diagnosed patients among the two groups above. **Controls**: 3. 1st degree relatives of affected LRRK2 G20199S mutation carriers; 4. Unrelated unaffected AJ matched to the previous group of first degree relatives for age and gender. All samples will have been whole-genome genotyped, passing quality control before expression profiling. The samples will be processed in TA and CU. We will conduct extensive QC and normalization steps to accommodate differences between centers in terms of sample preparation, and to detect any additional artifact signal between batches of samples.

While our GWAS aim focuses on connections between **SNPs vs. PD**, transcription profiling adds the following components:

1. **Transcripts vs. PD**: We will search for genes that change in expression between all groups of samples, or distinguish a specific group. This will be enhanced by **pathway analysis**, searching for significant enrichment for transcripts of particular annotation amongst differentially expressed genes.
2. **SNPs vs. Transcripts**: We will conduct a GWAS separately for each transcript level. We will search for associations that are significant enough to accommodate the testing burden of entire genome times the entire transcriptome, or that are in cis, therefore make exceptional candidates.

3. **SNPs vs. Transcripts vs. PD**: We will conduct 3-way analysis of SNPs, expression levels and affection status, searching for interactions whose significance exceeds pairwise effects. This will be further expanded to **pathway analysis**, searching for co-expressed sets of transcripts that together, mediate effects of the same SNPs on disease.
This combined analysis will be developed particularly for the unique dataset proposed here.
A natural extension of this work, planned for year 3, is validation experiments by the individual labs.

Question 7

Can we confirm and further characterize findings related to our GWAS and expression studies?

The third year genetics AJ PD LRRK2 project will be devoted for follow-up studies and analysis of candidate chromosomal regions, genetic variations (SNPs and CNVs) that will be detected in the genomic and transcripotomic genome-wide analyses in year 1-2. Our goal will be to confirm and identify significant genetic changes associated with PD pathogenesis or modification of disease course. The following studies are planned:

1) Validation studies will be performed to confirm the microarray results.
2) Replication studies will further be performed to complete the analysis of the entire cohort of PD cases (additional ~700-800 samples) and controls that will not be included in the microarrays studies.
3) In each candidate chromosomal region identified in the GWAS study, a list of candidate genes will be generated and systematic mutation screening will be done.
4) Copy number variations (CNVs) are currently considered as potential important genetic factors in complex diseases. Candidate CNVs will have to be confirmed and replicated in our extended cohort of PD cases and controls.
5) Validation of significant expression changes detected in the Expression microarrays analysis.

A wide variety of methods will be used for these studies, including:

1) TaqMan SNP analysis
2) GoldenGate Custom panel system
3) Sequenom
4) Real-Time RT PCR analysis
5) Custom sequencing, re-sequencing and deep sequencing

2) Background

Provide the scientific or scholarly background for, rationale for, and significance of the Human Research based on the existing literature.

Describe the relevant prior experience and gaps in current knowledge.

Describe any relevant preliminary data.

Explain the significance of the Human Research in terms of why this Human Research important and how will it add to existing knowledge.

Describe the importance of the knowledge expected to result.

Background and significance:

Mutations in LRRK2 constitute the most common genetic etiology of "typical" or "classical" PD identified to date, with most studies finding established mutations in 1-6% of unselected patients (Bonifati, 2007). However, there are ethnic differences in LRRK2 mutation frequency thought to be due to founder effects. One mutation, G2019S, is especially common in Ashkenazi Jews (about 15% of unselected cases and up to 30% of those with an affected relative) and Arab Berbers (about 40% regardless of family history) (Ozelius 2006, Lesage 2006). Much remains unknown about this important cause of PD. Gene penetrance for clinical PD is disputed. Many LRRK2 studies have been family - based (Latourelle 2008, Healy 2008); such ascertainment may distort penetrance estimates and also bias characterization of LRRK2 phenotypic range. Further, there are very few systematic studies of LRRK2 populations exploring gene expression, including clinical markers of early (pre-motor) disease and molecular pathways, or interacting genetic and environmental factors (Silveira-Moriyama 2008, Nandhagopal 2008, Golub 2008). Addressing these research needs will not only open new avenues for novel LRRK2 therapeutics but will provide insight into the disease mechanisms of all PD, especially because the LRRK2 phenotype and pathology appear to mimic "typical" PD (Papapetropoulos 2006). Finally, the G2019S mutation contributes to only a minority of PD, even among (GBA negative) affected Ashkenazi Jews (AJ) with a positive family history; thus, further interrogation of this population is warranted not only to identify LRRK2 gene modifiers but also to uncover new disease genes.

Rationale for using SPECT scanning:
Parkinson disease (PD) is a progressive, disabling neurodegenerative disorder characterized clinically by a resting tremor, bradykinesia, rigidity and postural instability. The disorder is characterized pathologically by degeneration of dopaminergic neurons in the substantia nigra, resulting in a decrease in striatal dopamine. Dopamine replacement with dopamine replacement therapies effectively reverses the motor symptoms early in the disease. However, as the disease progresses, patients develop a continual decline in motor function related to progressive loss of dopaminergic nerve terminals. Research in the past decade has focused on the development of neuroprotective medications to prevent or slow the rate of progression by interrupting pathways leading to cell death and/or restoring neuronal function.[i,ii,iii,iv] However, during the past several years, several neuroprotective drug trials for PD have failed. There is growing consensus that strategies to develop validated PD biomarkers that would allow early and potentially pre-motor longitudinal assessment of research subjects would substantially accelerate ongoing research to identify and test neuroprotective and neuropreventive medications in future studies.

Imaging tracers targeting presynaptic nigrostriatal function are the most widely developed biomarkers to identify PD early in its course and monitor its progression. Most PD imaging studies have used either F-Dopa and/or dopamine transporter (DAT) tracers to monitor dopaminergic degeneration.[v,vi,vii,viii,ix,x,xi,xii] Dopaminergic ligands are useful to assess PD in so far as they reflect the ongoing dopaminergic degeneration in PD. Numerous studies have shown that DAT density is reduced in striatum in postmortem brain from PD patients.[xiii,xiv,xv] In addition, several clinical imaging studies have shown

reductions in DAT uptake in PD patients and aging healthy subjects consistent with the expected pathology of PD and of normal aging. Specifically these imaging studies demonstrate asymmetric, putamen greater than caudate loss of dopaminergic uptake and the imaging loss correlates with worsening clinical symptoms cross-sectionally.[ix,xvi,xvii,xviii,xix,xx] In addition DAT ligands demonstrate reductions in activity with normal aging.[xxi,xxii,xxiii]

In longitudinal studies of PD progression, F-Dopa, VMAT2, DAT (ß-CIT and CFT), using both PET and SPECT have demonstrated an annualized striatal rate of reduction of about 4% to 13% in PD patients compared with 0% to 2.5% change in healthy controls.[xxiv,xxv,xxvi,xxvii,xxviii,xxix,xxx,xxxi,xxxii] Evidence from studies of hemi-PD subjects provides further insight into the rate of progression of disease. In early hemi-PD there is a reduction in DAT uptake of about 50% in the effected putamen and of 25-35% in the unaffected putamen. Since most patients will progress clinically from unilateral to bilateral in 3-6 years it is therefore likely that the loss of these *in vivo* imaging markers of dopaminergic degeneration in the previously unaffected putamen will progress at about 4-10% per annum.[x,xix,xxxiii,xxxiv]

While dopaminergic imaging has provided an extraordinarily useful biologic phenotype for PD, DAT imaging has not yet been fully validated as a PD biomarker. This study is proposed to demonstrate that DAT imaging at baseline is a biomarker for early neurodegeneration occurring in subjects at high risk to develop symptoms of PD.

Several lines of evidence strongly suggest that dopaminergic imaging can identify subjects during the pre-symptomatic phase of their disease. In this study, it is planned to assess individuals at high risk to develop PD based on genetic and/or clinical assessments to evaluate whether DAT imaging at baseline and/or longitudinal assessment will predict individuals likely to develop a clinical diagnosis of PD. The most extensive pre-clinical data are from studies imaging patients with hemi-PD. Typically patients begin disease with PD symptoms on one side of the body and progress to bilateral disease within 3-6 years.[xxxv] In several imaging studies there was a significant reduction in putamenal dopamine transporter uptake, ranging from 25-40% in the 'pre-symptomatic' striatum (contralateral to the side without symptoms) demonstrating pre-clinical dopaminergic loss in these patients who are known to progress to bilateral disease.[viii,xvii,xxxiii,xxxiv,xxxvi] Other attempts to identify pre-clinical disease using imaging methods have focused on at-risk populations such as family members or unaffected twins of PD patients. Studies using FDOPA have demonstrated that in several well characterized kindreds 11 of 32 asymptomatic relatives were found to have reduced FDOPA uptake, and three of these subjects subsequently developed symptomatic PD.[xxxvii] Several asymptomatic co-twins who also showed a reduction in FDOPA activity later developed symptoms of PD, although the concordance rate for monozygotic and dyzygotic twins remains uncertain.[xxxviii] In kindreds of genetically defined PD families, mild loss of imaging markers has been identified in gene positive but symptom negative individuals suggesting a pre-clinical period of neurodegeneration.[xxxix,xl,xli,xlii,xliii] While these studies must be confirmed with larger sample size and longer duration of follow-up, the current evidence strongly suggests that imaging can effectively identify pre-clinical dopaminergic degeneration. The proposed study with family members of LRRK2 gene positive subjects will directly examine subjects at high genetic risk for PD.

Several clinical signs and symptoms associated with PD such as loss of olfactory function, sleep disturbances like RBD, autonomic dysfunction including constipation, and behavioral and cognitive changes may occur prior to the typical motor symptoms of PD.[xliv,xlv,xlvi,xlvii] These clinical features may be more accurately considered early features of PD rather than biomarkers. An emerging strategy to identify pre-motor parkinsonism has been to combine early clinical manifestations of PD with dopaminergic imaging. For example in studies of PD relatives tested for olfactory function and then undergoing dopamine transporter imaging, combining the loss of olfaction and dopamine transporter imaging density identifies a sub-group with increased risk of developing PD.[xlvii] In the proposed study, individuals at-risk for PD will be evaluated based on their olfactory dysfunction or other subtle non-motor symptoms (such as RBD) with DAT imaging in addition to other biomarker assessments. These biomarker assessments in pre-motor subjects aim to identify individuals at risk for phenoconversion to symptomatic PD.

Identification of pre-diagnostic PD offers the opportunity to provide treatment at an earlier stage with the goal of delaying or ultimately preventing the onset of symptoms. DaTSCAN™, the dopamine transporter ligand we plan to test in these studies, was approved in Europe in 2000 for the differentiation of PD from essential tremor. It targets the DAT allowing a quantitatitive assessment of DAT density on the presynaptic dopaminergic neurons projecting from the substantia nigra to the corpus striatum.[xlviii,xlix] DaTSCAN™ has been widely used as diagnostic tool and now we plan to evaluate DAT imaging as biomarker of for early and pre-motor PD onset and progression.

The recent and ongoing explosion of molecular genetic information uncovering several genes for PD has provided a rational strategy to identify and evaluate an at risk PD population.[l,li] While identified mutations generally account for a very small number of PD patients, in some populations like the Askenazi Jewish population or in regions of Spain or North African LRKK2 mutations may identify approximately 20- 35% of the PD population.[lii,liii,liv]

Pilot studies have already demonstrated that dopaminergic imaging may be abnormal in unaffected family member of PD patients gene positive for LRKK2 and other Park gene abnormalities.[xli,lv] We propose to combine genetic assessment with dopamine transporter imaging to study a large subset of the Askenazi Jewish subjects enrolled in the AJ LRRK2 Fox initiative.

Imaging individuals at increased risk for PD due to known genetic mutations as proposed below provides the most straightforward strategy to assess pre-diagnostic PD. The discovery of the LRRK2 genetic mutations in PD patients in general and in the Askenazi Jewish population in particular provide the specific rationale for this proposal.

The specific hypotheses of this study, which will be assessed by the use of DaTSCAN™ imaging, are therefore:

1) Among first degree relatives of LRRK2 subjects, gene carriers are more likely to demonstrate a striatal DAT deficit (compared to a healthy subject database) than non-carriers.

2) Among first degree relatives of LRRK2 subjects, gene carriers will demonstrate a reduction in striatal DAT uptake compared to non-carriers.

3) Among first degree relatives of LRRK2 subjects who are gene carriers the striatal DAT uptake will predict onset of PD symptoms.

In addition to these specific hypotheses, the striatal DAT uptake phenotype for both carriers and non-carriers will be correlated with clinical, genetic and biomic data acquired in this study to investigate potential LRRK2 disease modifiers. We will also image a small group of GBA gene mutation carriers who will provide pilot data to examine DAT imaging in another gene mutation and will provide a disease control for LRRK2.

3) Setting of the Human Research

Describe the sites at which your research team will conduct the research. If applicable, describe:

- *At which institutions or sites the research procedures will be performed by your research team (MSBI, MSSL, MSR; if offsite, please specify).*
- *The location(s) where potential subjects may be identified and recruited (if different than the above).*
- *Composition and involvement of any community advisory board for research conducted outside of MSBI, MSSL, MSR.*
- *For research conducted outside MSBI, MSSL, MSR and its research affiliates:*
  - *Site-specific regulations or customs affecting research.*
  - *Local scientific and ethical review structure.*

Patients and their family members will be recruited from the clinical practices of The Movement Disorder Divisions of MSBI, and assessments will also be completed at this site with the following exceptions: some subjects will participate remotely by phone or mail, and others who are unable to travel to a study site will be seen in their homes by the researchers. We will also advertise the study in foundation newsletters and local or specialty newspapers, journals, and through e-mails to our colleagues, and will obtain referrals from our current list of collaborators as well as other physicians who have heard about our study.

All SPECT imaging will occur at St. Luke's-Roosevelt Hospital in New York, NY - Nuclear Medicine Physicians –Munir Ghesani, M.D. and E. Gordon DePuey, M.D. All scans will be sent and analyzed at

Institute for Neurodegenerative Disorders (IND)/Molecular NeuroImaging (MNI) (New Haven, CT) – Imaging Medical Monitor – John Seibyl, M.D.

4) Resources Available to Conduct the Human Research

Explain the feasibility of meeting the recruitment goals of this project, and demonstrate (e.g., based on retrospective data) a potential for recruiting the required number of suitable subjects within the agreed recruitment period. (For example, how many potential subjects do you have access to? What percentage of those potential subjects do you need to recruit? If this has been reviewed by a committee for recruitment feasibility [e.g. Department of Medicine CTO], please indicate so.)

Describe the number and qualifications of your staff, their experience in conducting research, their knowledge of the local study sites, culture, and society.

For research conducted outside MSBI, MSSL, MSR and its research affiliates, describe the facilities used for conducting the research.

Describe your process to ensure that all persons assisting with the trial are adequately informed about the protocol, the investigational product(s), and their trial-related duties and functions.

Mount Sinai Beth Israel's Movement Disorders Center (MSBI) is an important force in Parkinson's Disease research. The clinical investigators in collaboration with basic science investigators at Mount Sinai College of Medicine, Albert Einstein College of Medicine at Yeshiva University and with institutions throughout the US, Canada, Europe and Israel have been engaged in scientific research to understand Parkinson's Disease (PD) and find new treatments that will contribute to developing a cure for PD. Beth Israel is a leader in providing comprehensive, compassionate and state of the art treatment for people with movement disorders. Our Movement Disorders Division includes 4 neurologists and 1 pediatric neurologist, all fellowship trained in movement disorders; in addition there are 3 fellows, a fellowship trained psychiatrist, a neuro-psychologist, nurse practitioner and genetic counselor. We provide treatment for over 3,800 patient visits yearly. We have been a National Parkinson's Foundation (NPF) Center of Excellence since 1996 and strive to provide a multidisciplinary humanistic approach to providing care. The Center is unique in that two of the four clinical movement disorder researchers, Drs. Susan Bressman and Rachel Saunders-Pullman, have led NIH funded projects in the study of Parkinson's Disease and movement disorders. They have extensive collaborations both In New York, California and worldwide, and are at the forefront in translational medicine, a research model with significant interface between the care and evaluation of patients and the research laboratory. This allows for approaches that go from bedside to bench that are not facilitated in centers where basic research is isolated.

5) Study Design

a) Recruitment Methods

Describe the source of potential subjects.

Describe the methods that will be used to identify potential subjects (e.g. ResearchMatch.org).

Describe materials that will be used to recruit subjects. Include copies of these documents with the application. For advertisements, submit the final copy of printed advertisements. When advertisements are taped for broadcast, provide the final audio/video tape. You may submit the wording of the advertisement prior to taping to preclude re-taping because of inappropriate wording, provided the IRB reviews the final audio/video tape.

The sources of potential subjects is described in section 3. Physicians and research coordinators will discuss possible participation with patients who qualify and their family members. Materials used in recruitment are attached.

b) Inclusion and Exclusion Criteria

Describe how you will screen for eligibility.

Describe the criteria that define who will be included or excluded in your final study sample.

(NOTE: You may not include members of vulnerable populations as subjects in your research unless you indicate this in your inclusion criteria.).

Subjects are eligible if they are Ashkenazi Jewish and have PD or parkinsonism, or they do not have PD, but a family member does. In addition, individuals who are not Jewish are eligible if a genetic mutation for PD has been identified in them or in a family member. We will not include individuals less than 18 years of age. We may include pregnant woman and adults lacking capacity, though this will be a minority of participants.

To participate in the SPECT imaging addendum, subjects will provide a separate signed SPECT informed consent and must have consented to participate in the main clinical trial. Ethical review board approval of this addendum and the related informed consent document will be sought prior to any SPECT imaging procedures being implemented. Up to 60 subjects will be entered into the protocol addendum.

Subjects will be eligible to participate if:

- The subject is aged 30 years or older.

- Written informed consent is obtained.

- Negative history of neurological or psychiatric illness based on evaluation by a research physician.

- For females, non-child bearing potential or negative serum pregnancy test within 5 days of DaTSCAN™ injection.

Subjects will be excluded if:

- The subject has a clinically significant clinical laboratory values, and/or medical or psychiatric illness.

- The subject has evidence of clinically significant gastrointestinal, cardiovascular, hepatic, renal, hematological, neoplastic, endocrine, neurological, immunodeficiency, pulmonary, or other disorder or disease.

- The subject has any condition that could, in the opinion o f the investigator, affect his or her response to the radiopharmaceutical and related testing procedures.

- The subject is pregnant.

c) Number of Subjects

Indicate the total number of subjects to be accrued locally. If applicable, distinguish between the number of subjects who are expected to be pre-screened, enrolled (consent obtained), randomized, and complete the research procedures (i.e., numbers of subjects excluding screen failures) and between subgroups of subjects (e.g. healthy volunteer, disease cohort).

If this is a multicenter study, indicate the total number of subjects to be accrued across all sites.

We plan to recruit approximately 500 individuals to this study at MSBI.

DaTSCAN™ will be assessed in 70 subjects (60 LRRK2 positive, 10 LRRK2 negative) who are first-degree relatives of LRRK2- positive PD subjects who are enrolled in the Genetics of Parkinson's Disease

Consortium Study being conducted at Beth Israel Medical Center (New York, NY) and Columbia Presbyterian Medical Center (New York, NY).

d) Study Timelines

Describe:

- *The duration of an individual subject's participation in the study (including follow-up).*
- *The duration anticipated to enroll all study subjects.*
- *The estimated date for the investigators to complete this study (complete primary analyses)*

The study is currently funded through 2015, but may be extended beyond this period depending on continuation of funding and study progress. A longitudinal cohort of individuals as been studied on a yearly basis for as many as 4 visits. Other subjects finish their participation in one study visit, but may be re-contacted during the course of the study to obtain follow-up information or to help with contacting relatives.

e) Endpoints

[Note: Endpoints are results, conditions or events associated with individual study subjects that are used to assess study treatments. Not all study designs involve them.]

Describe the primary and secondary study endpoints (i.e. outcomes used to judge the effectiveness of a treatment).

Describe any primary or secondary safety endpoints (i.e. events/results that would cause a study subject's participation to end due to safety).

No treatments will be administered. Severe adverse event resulting from lumbar puncture, skin biopsy, or DaTSCAN are unlikely, and would also be unlikely to preclude participation in other aspects of the study. However, the participant might refuse to continue in such a circumstance.

f) Procedures Involved in the Human Research

Describe and explain the study design.

Describe the procedures being performed, and <u>when</u> they are performed, including procedures being performed to monitor subjects for safety or minimize risks. Include

procedures being performed already for diagnostic or treatment purposes and differentiate between these and the procedures performed solely for the research.

Describe procedures taken to lessen the probability or magnitude of risks.

Describe the source records that will be used to collect data about subjects.

Describe what data will be collected including long-term follow-up.

Screening phase:
After obtaining informed consent, systematic demographic, family, medical history and environmental exposure information will be obtained for all patients. Patients who are recruited from the Beth Israel Dept of Neurology will have a sticker placed on the inside cover of their charts noting only their participation in the study. This will be done in order to better coordinate this study with the other studies we conduct in the department.

A detailed neurological examination will be performed and rated using the Unified Parkinson's Disease Rating Scale (UPDRS) and Modified Hoehn and Yahr Staging Scale (accepted and validated measures of symptomatology and motor function in PD) and the Schwab-England activities of daily living scale (a measure of disability from PD). In addition, we will obtain information about autonomic dysfunction using the PD Non-Motor Symptom Questionnaire and a blood (15 ml) or saliva sample will be obtained for DNA extraction.

Examination of mood and cognition will be done using the Geriatric Depression Scale/GDS and Montreal Cognitive Assessment/MoCA.

Bloods collected in Israel will remain in Israel. Coded blood/saliva samples from Columbia and Beth Israel will be sent to the tissue culture facility at Albert Einstein College of Medicine for DNA extraction. The extracted BI DNA samples will then be sent to Dr. Ozelius' lab at Mt. Sinai College of Medicine, while the Columbia DNA samples will be sent to Dr Lorraine Clarke at Columbia. The samples will be analyzed for the presence of the G2019S mutation in LRRK2 and used for genome wide association and follow-up analyses as listed in research questions 5-7 above.

Study participants will be told that they may be re-contacted in the future for additional testing.

In Depth Phase:
We will re-contact patients from the initial study group to invite them to come back for an additional visit and 3 subsequent follow-up visits at 15 month intervals. This group of subjects will include 150 LRRK2 mutation positive and 150 LRRK mutation negative individuals. Patients will also be asked to contact their relatives to determine if they are interested in participating. Our goal is to enroll about 500 first degree relatives from this group of patients (350 from the LRRK+ group and 150 from the LRRK- group),

as well as 100 of their spouses for this phase of the study. Consenting family members will have the same work-up as the patients (a total of 4 study visits).

In depth visit:
This visit will take approximately 4 hours and will consist of the following testing:

1. Examination: The examination will be repeated on all patients and videotaped for future review. Patients and all newly recruited relatives and spouses will have the videotaped exam with UPDRS, Hoehn and Yahr, and Schwab-England ADL scales completed.
2. General health and detailed family histories will be obtained/updated (30 min), and an in depth cancer/medical health screen will be administered (20-30 min).
3. Samples: Urine will be collected for assessment of metabolites and biomarkers. Blood will be collected for RNA studies (7.5 ml), current and future biomarker studies (8-15 ml), DNA extraction if not already done (15 ml). An additional 8.5-15ml of blood may be obtained on a subset of patients in order to isolate white cells (PBMC) and establish cell lines, and skin biopsies (separate funding), cerebrospinal fluid, and tumor samples will be obtained on a subset of cases. Skin biopsies are obtained via skin punch by a trained dermatologist and used for immunohistochemical studies aimed at examining endogenous mutant proteins. The skin punch is done using a local anesthetic, and may leave a tiny scar smaller than the tip of a pen. To obtain cerebrospinal fluid a local anesthetic is given and a needle is inserted at the base of the spine. About 30 ccs of fluid (2 tablespoons) is withdrawn. Tumor samples and accompanying pathologic reports will be obtained from tumor tissue previously removed as part of the participant's regular cancer care. Participants will be asked to sign a form authorizing the release of stored tumor tissue. The tumor samples will undergo genetic analysis in order to examine mutations and shared pathways in movement disorders and tumorigenesis.
4. Cognition scales (about 45 min): Letter Number Sequencing, Hopkins Verbal Learning Test-Revised, Form 1 (HVLT-R): Immediate Recall Trials, Judgment of Line Orientation Test (JLO), Finger Tapping Test, Color Trails Test, Stroop, HVLT-R: Delayed Recall Trial and Recognition, Digit Span Test, Letter Fluency (FAS), Category Fluency (Animals), Montreal Cognitive Assessment/MoCA (5-10 min) if not previously completed.
5. Mood (1 hour on the phone): *Composite International Diagnostic Interview/CIDI - The following 8 sections of the CIDI will be used: Screening, Depression, Mania, Panic Disorder, Social Phobia, Generalized Anxiety Disorder, Demographics, 30-Day Functioning (WHO-DAS). This interview will only be done at baseline.
   Mood (10 min): Beck's Depression Inventory (BDI), Geriatric Depression Scale (GDS) if not previously completed, on participants over 50, State Trait Anxiety Inventory. A subset will complete the Hamilton Anxiety Rating Scale (HAM-A) and the Hamilton Depression Rating Scale (HDRS)

6. Sleep (10 min): REM Sleep Behavior Disorder Questionnaire/RBDA, Epworth
7. Dysautonomia (10 min): Scales for Outcomes in Parkinson disease/SCOPA-AUT

8. Olfaction (10 min): University of Pennsylvania Smell Identification Test/UPSIT
9. Interval timing (25 min): (see Appendix for details of methods). This test will be done only at baseline.
10. Exposures: (10 min) including collaborative screen of environmental exposures
11. Genetic testing knowledge and attitudes questionnaire (15 min). This questionnaire will be administered only at baseline.
12. Transcrainal ultrasound (15 min): a risk free procedure in which an ultrasound transducer is placed at the temple, and sound wave frequencies are recorded electronically.
13. Spiral analysis (10 min): subjects draw spirals on a digitized tablet
14. ECG (see Appendix for details of methods) (15 min).
15. Gait and armswing analysis (see Appendix for details of methods) (15 min)

A subset of 70 first degree relatives (60 LRRK2 positive and 10 LRRK2 negative) will also be included in a neuroimaging sub-study (see DAT scan sub-study protocol and consent) at St. Luke's Roosevelt Hospital.

*A separate appointment will be made to complete the CIDI phone interview.

Participants will be asked whether they are interested in receiving information about brain donation to the Columbia University MJFF supported PD Brain Bank. Those consenting to receive the information will also be asked to consent to link their information obtained in this study to their brain donation, should a donation be made upon their death. Consent will be given by initialing the appropriate spot on the study consent. Since participants cannot consent to brain donation while alive (New York State law), potential donors will be instructed to fill out a letter of intent and share this letter with the Brain Bank and their next of kin. (A copy will also be kept in the research chart at BI). The donation will be arranged with the written consent of the next of kin at the time of death. Information from the brain sample and linked clinical information from the MJFF Rochester database will be de-identified and kept in a database at Columbia University which will have the same security protections listed below for the Columbia Consortium Database.Study participants from Columbia University will be asked whether they are will to go to Beth Israel for ultrasound and spiral analysis with Dr. Saunders-Pullman. Participants will indicate their willingness to have their contact and study data shared with Dr. Saunders-Pullman on the Columbia study consent. They will then also sign the Beth Israel consent when they go for the testing at Beth Israel. Both CU and BI consents have similar provisions for agreeing to share information with the following outside collaborators: Dr. David Eidelberg at North Shore hospital and Dr. Kenneth Marek at Yale University for neuroimaging studies.

Analysis:
For this study we will utilize many of the methodological tools used in our prior studies. This includes standardized and validated in-person and telephone interviews to obtain clinical and environmental data and videotaped standardized examination protocols. For analyses assessing phenotypic features among the family members we will initially compare the frequency of discrete trait outcomes between carriers and

non-carriers (e.g., presence or absence) by chi-square tests. Continuous outcomes (e.g., age at onset) will be contrasted between groups by t-tests. To allow for possible confounding in these comparisons due to age or gender effects, we will follow these initial analyses by logistic regression (for discrete outcomes) including age and gender as covariates, or linear regression (for continuous outcomes) with age and gender as covariates. Alternately for those outcomes with age-dependent expression, it may be the age-onset that is itself of interest. Thus we also propose to perform survival analysis, such as proportional hazards analysis, to compare time-to-onset between carriers and non-carriers. This would be particularly important when our subjects have not experienced the full risk period and hence are right censored. As in the regression analyses, we can also include gender as a covariate in the hazards analysis, while "group" (e.g., carrier group vs. non-carrier group) is the main effect under examination. Other significant co-variates, including clinical features and putative environmental modifiers will also be modeled in the logistic regression and proportional hazards analyses. Further information on statistical analyses is given at the end of each research question above.

Co-investigators:
The study co-investigators working with Dr. Susan Bressman at Beth Israel are: Rachel Saunders-Pullman, MD, Vicki Shanker, MD, Mark Groves, MD, Deborah Raymond, MS,

The study co-investigators outside Beth Israel are: Karen Marder, MD and the research team at Columbia University, Nir Giladi, MD and the research team at Tel Aviv University, Laurie Ozelius, PhD, Zhenyu Yue, PhD, and Joel Duddley, PhD and the research teams at Mt. Sinai College of Medicine, Neil Risch, PhD and the research team at UCSF, Seth Pullman, MD and the research team at the Clinical Motor Physiology Laboratory, Johann Hagenah and the research team at the University of Luebeck, Carly Tanner and the research team at UCLA, Gary Heiman, PhD and Cora DeLeone at Rutgers/Columbia Universities, David Eidelberg, MD and research team at North Shore University Hospital, Kenneth Marek, MD and the research team at The Institute for Neurodegenerative Disorders (IND), Barry Smith, MD, at Beth Israel Medical Center, Eduardo Tolosa, MD, University of Barcelona Hospital, Jan Aasly, MD, St. Olav's Hospital, Norway, Rivka Inselberg, MD, The Chaim Sheba Medical Center, Israel, Jose-Felix Martin-Masso, MD, San Sebastian, Spain, Ilir Agalliu, MD, PhD and Dr. Cuiling Wang, MD, Albert Einstein College of Medicine, MartaSan Luciano,MD, UCSF, Christopher Coffey, PhD, The Michael J. Fox Foundation for Parkinson's Research, Tatiana Faroud, MD, Indiana University, Kathryn Brockmann, MD, University of Tubingen, Germany, Saskia Biksup, University of Tubingen, Germany, Olaf Bodamer, MD, Boston Children's Hospital, Hardy Rideout, PhD, University of Athens, Dimitri Krainc, MD, Northwestern University, Pramod Mistry, MD, Yale University, Sylvain Chouinard, MD and Guy Rouleau, MD at Montreal Neurological Institute and Hospital, Ysuf Hannun, MD and Lina Obeid, MD at SUNY Stonybrook, Jacek Bielawski, PhD and Ala Beilawska, PhD, at Medical University of South Carolina.

The co-investigators listed above who are not members of this study's Beth Israel research team have been listed under "others" on part B of the HIPAA form. Also listed here is Barry Smith, MD, at Beth Israel Medical Center who will do the skin punch procedure for obtaining fibroblasts on a subset of research subjects.

Funding:
This study is funded by The Michael J. Fox Foundation for Parkinson's Research, by the National Institutes of Health K02NS073836, The Genzyme Gaucher Generations Project, and by the Bigglesworth Foundation.

Subjects having SPECT scan will undergo the following procedures:

General Consenting Procedures
The investigator is responsible for ensuring that the subject understands the risks and benefits of participating in the study, including answering any questions the subject may have throughout the study and sharing any new information that may be relevant to the subject's willingness to continue his or her participation in the trial in a timely fashion. The informed consent document will be used to explain the risks and benefits of study participation to the subject in simple terms before the subject is entered into the study, and to document that the subject is satisfied with his or her understanding of the risks and benefits of participating in the study and desires to participate in the study. The investigator is responsible for ensuring that informed consent is given by each subject or legal representative. This includes obtaining the appropriate signatures and dates on the informed consent document prior to the performance of any protocol procedures. Participants will be informed that they may withdraw from the study at any time without penalty or consequence**.**

Screening Procedures
Subjects will be seen at the clinical referring site to complete screening requirements of the main study protocol. At the very minimum, subjects will:

- Be asked about their medical history and any medicines that they are taking.
- If they are a female of child-bearing potential, they will be given a serum pregnancy test. The pregnancy test must be administered within 5 days of the subject receiving their DaTSCAN and must be negative. The clinical site will provide the results of the pregnancy test to the imaging center.

If judged eligible by the study investigator and, if a female of child-bearing potential, determined not to be pregnant, they will be referred to St. Luke's-Roosevelt Hospital for SPECT imaging with DaTSCAN™.

IMAGE ACQUISITION, PROCESSING, AND ANALYSIS

Detailed SPECT imaging procedures are described in a Technical Operations Manual provided to the imaging site during a Technical Site Visit conducted by the imaging Core Lab and are described briefly below.

DaTSCAN™ Injection Day Procedures

On injection day each subject will then undergo the following evaluation:

- Review of concomitant medications.
- Females of child bearing potential will have a urine-HCG pregnancy test.
- Review of adverse events since last visit.
- Subjects will be asked about their potential allergy to iodine or shellfish.
- Subjects who do not report an iodine or seafood allergy will be administered a thyroid blockade by mouth (7 drops of Lugol's solution in water).
- Subjects will be injected with 111-185 MBq (3.0-5.0 mCi) of DaTSCAN™
- Subjects will be imaged 4 + 0.5 hour after injection.

For female subjects of child-bearing potential, evidence of a confirmed negative serum-HCG pregnancy test obtained within 5 days of injection with DaTSCAN, must be provided to the imaging center.

Access into an antecubital vein will be established and each subject will receive a single i.v. injection of DaTSCAN™ with a total activity of 111-185 MBq (3-5 mCi), but not exceeding 185 MBq, and a maximum volume of 5 mL. DaTSCAN™ will be injected with the subject in a supine or recumbent position and aseptic technique using sterile syringes and needles should be used.

The DaTSCAN™ will be administered via slow (not less than 15-20 seconds) i.v. injection and will be followed by a saline flush of approximately 5 mL. The exact amount of activity will be assessed prior to injection. If for logistical reasons a subject receives the injection earlier than reference time, the volume of DaTSCAN™ administered should be reduced to ensure the subject does not receive >185 MBq. There have been no incidences of intentional overdose with DaTSCAN™ in clinical trials. However, in the event of overdose or misadministration, frequent micturition (by means of increased hydration) and defecation should be encouraged in order to minimize radiation dosage to the patient. Subjects will then wait 4 ± 0.5 hours before proceeding with SPECT scanning.

Imaging Procedures

As a general protocol, raw projection data will be acquired into a 128 x 128 matrix stepping each 3 degrees for a total of 120 (or 4 degrees for a total of 90) projections into two 20% symmetric photopeak windows centered on 159 KeV and 122 KeV with a total a scan duration of approximately 30 – 45 minutes. Specific scan parameters including collimation and acquisition mode will be selected for each site on the basis of an assessment during the technical site visit. Insofar as possible, it is recommended that acquisition be in step and shoot mode with each head rotating 360 degrees using a parallel hole collimator to permit the reconstruction of a viable image even if one head is faulty.

The acquisition parameters and reconstruction algorithm defined during the phantom measurements at each site should be used during this study. The imaging center will record these parameters on standard forms. For subject data, the time at which the image acquisition begins and ends, the total imaging time and the total counts acquired will be recorded.

Post-Imaging Procedures

Subjects will be contacted by phone by the clinical study coordinator, 7 ± 3 days following the injection/scan to assess any adverse events.

Image Processing

Transfer of imaging data is done by either direct DICOM push or secure file transfer protocol (sFTP) to the Core Lab, Institute for Neurodegenerative Disorders (IND)/Molecular NeuroImaging (MNI) New Haven, CT. The imaging site will be provided an sFTP address and login code, or instructions on how to set up the direct DICOM transfer. Using the file naming procedures detailed in the Technical Operations Manual, the site will record the files to be sent on the SPECT Scan Information Source Document and SPECT Imaging Data Transfer Information Source Document. These sheets will be faxed to IND/MNI. This fax serves to notify the Core Lab team that image files have been sent. The Core Lab will then review the file transfer document against shat was received on the sFTP server or DICOM server to check for the presence of all expected data files. Each image file will be reviewed for accuracy and completeness using a standard quality assurance procedure. Once the images are verified in this matter, an imaging data receipt form will be completed and faxed back to the sending site. This document must be filed in the study folder. If there is a problem with the data file sent, a contact will be made by the Core Lab to the site. Depending upon the nature of the problem, the resolution may involve simple provision of additional information, the resend of one or more image data files, or other action to ensure the completeness of the image data files.

Image files sent o the Core Lab will undergo a two step quality assurance check, including review of the file naming, ensuring image and headers are de-identified, check of total counts, energy window, movement artifacts, reconstruction artifacts, timing of the acquisition relative to DaTSCAN™ injection, among other checks. If queries arise in the course of these QC checks, the Core Lab will contact the designated site person for resolution.

The image raw data as well as the reconstructed image data will be provided to the imaging core lab in DICOM 3.0 or other compatible format to enable the technology department to supervise the image quality on an ongoing basis. In keeping with regulatory requirements, it is critical that no subject names or other identifying information be associated with the transferred image files or the associated paperwork. Scans should be identified on the basis of the site's two-digit site number, subject's three-digit number, and acquisition date.

Image Analysis

Image processing and analysis will be performed by specialists masked to the clinical diagnosis or other clinical data.

Both the imaging site and the core lab will reconstruct, attenuation correct, and filter the imaging data. Only the core lab will derive regional striatal uptake ratios. The imaging site may implement either filtered back-projection or an iterative reconstruction algorithm using standardized approaches. Strategy for filter selection will be reviewed to ensure that compromise of the quantitative validity of the analysis does not occur by use of enhancing filters which deteriorate the linearity of the signal response. Methods for homogeneous attenuation correction (Chang 0) will be reviewed during the technical site visit.

STATISTICAL ANALYSIS

The primary imaging outcome will be the mean striatal DaTSCAN™ uptake. This is a region-of–interest (ROI) based analysis (calculated as [(striatal uptake/non-displaceable uptake) – 1]). Non-displaceable (background) uptake in the brain will be calculated from an ROI drawn around the occipital lobe. The striatal ROI data will be analyzed to examine putamen, caudate and whole striatal uptake in each hemisphere.

Image processing and quantitative analysis will involve the following 4 steps:

- Attenuation correction: Attenuation correction ellipses will be fit to the transaxial data based on the transmission image and a Chang zero order (homogeneous) correction applied to the reconstructed data. Twenty-four hour uptake of DaTSCAN™ is low in cortical areas and placement of the ellipses is difficult. The linear attenuation coefficient (μ) is empirically based on a [^{123}I] distributed source phantom.

- Reorientation of axial slices along the canthomeatal line: Slice reorientation is performed to align images parallel to the canthomeatal plane. This is a user-operated, iterative process.

- Summation of striatal slices: on the reoriented image filet, the striatal slice with the most intense uptake is determined by thresholding the color scale (index slice). The 2 slices above this slice, the index slice, and 1 slice below are summed (total z dimension = 1.3 cm).

- Placement of ROI template and extraction of count density data: The standard template contains right and left caudate, right and left putamen, and the posterior portions of both occipital lobes. Using strict criteria the technologist will draw regions of interest and will calculate the outcome measures specified: specific striatal uptake – non-displaceable uptake; asymmetry index and caudate:putamen ratios and % injected dose. Specific-to-nondisplaceable activity ratios are determined by subtracting occipital densities (nondisplaceable uptake) from total caudate or putamen count densities (specific + nondisplaceable uptake) from 4 summed slices and dividing by occipital counts. The data collected at each stage of the analysis, including the raw projection data, reconstructed data, attenuation corrected data, reoriented data, summed striatal slice data, and ROI file data will be saved and will be available for further analysis.

All imaging data will be de-identified. All image analysis will be completed blind to any clinical or genetic information. The primary imaging outcome will be striatal DaTSCAN uptake.

If it is assumed that 50% of the first-degree relatives of LRRK2 PD will be gene carriers and it is hypothesized that 25% of the carriers will have a DAT deficit (based on the pilot data from Adams et al.[lv]) and 5% of the non-carriers will have a DAT deficit, then with 60 carriers and 60 non-carriers there would be 90% power to detect a difference between the groups with alpha 0.05.

It is hypothesized that if there is a reduction in 20% in mean striatal DAT uptake, then with 60 carriers and 60 non-carriers we would have 90% power to detect a difference between the groups with alpha .05.

RECORD RETENTION
The investigator must retain essential documents for the maximum period required by applicable regulations and guidelines or Institutional procedures.

g) Specimen Banking

If specimens will be banked for future use, describe where the specimens will be stored, how long they will be stored, how the specimens will be accessed, and who will have access to the specimens.

List the information to be stored or associated with each specimen (including how the specimens are labeled/coded).

Describe the procedures to release specimens, including: the process to request a release, approvals required for release, who can obtain specimens, and the information to be provided with specimens.

All specimens will be labeled with a unique identifier. A portion of blood will be sent to the tissue culture facility at Albert Einstein College of Medicine for DNA extraction as described above for the screening phase. In addition, blood collected at BI for RNA/expression studies will be sent to Dr. Clarke for analysis along with the Columbia samples, and a portion of urine, serum, and plasma will be stored at the facility where it was drawn for future study*. The remaining portions of blood and urine will be labeled with a unique identifier generated by the study biorepository and sent to the repository (Coriell Institute and/or Indiana University). The following information will accompany each sample we send to a repository: ID, Type of sample, date of draw, gender of participant, CTCC unique ID (see section on CTCC below), time and date of most recent dose of PD medication. The purpose of moving samples to central repositories is to allow sharing of samples with other researchers who must apply for permission to use samples and data. Samples and data stored at BI or with Dr. Ozelius may also be released by the study investigators to collaborators assisting with our study aims.

*As of September 2015 a portion of the de-identified extant samples and data, as well as de-identified samples and data on 1-10 newly ascertained individuals from MSBI will be transferred to the NIH biorepository at Coriell Institute.

All Israeli samples will be housed and analyzed in Israel.

Tumor samples and accompanying pathologic reports obtained from a small subset of subjects for the tumorigenesis sub-study will have any identifying information removed and will be sent to Dr. Saskia Biskup for analysis at the Center for Genomics and Transcriptomics in Tubingen, Germany. After analysis, any remaining tumor tissue will be returned to BIMC or to the hospital laboratory where the sample was originally stored.

h) Data Management and Confidentiality

Describe the data and specimens to be sent out or received. As applicable, describe:

- *What information will be included in that data or associated with the specimens?*
- *Where and how data and specimens will be stored.*
- *How long the data will be stored.*
- *Who will have access to the data?*
- *Who is responsible for receipt or transmission of the data and specimens?*

Describe the steps that will be taken secure the data (e.g., training, authorization of access, password protection, encryption, physical controls, certificates of confidentiality, and separation of identifiers and data) during storage, use, and transmission.

Describe any procedures that will be used for quality control of collected data.

Describe the data analysis plan, including any statistical procedures. Provide a power analysis, if applicable (e.g. pilot study).

All data collected and generated by this study will be kept in a limited access password protected database at Beth Israel that is only accessible by Dr. Bressman and her research team. In addition, **De-identified** data from the Beth Israel Medical Center and Columbia University will be maintained on a separate secure server at Columbia University and only accessible to the investigators from the consortium. Israeli data will be kept on a server in Israel. A subset of **anonymized** data will be kept on a separate secure server at the University of Rochester along with similar data from other Parkinson disease research studies funded by The Michael J. Fox Foundation (See Appendix for CTCC protocol and a list of data elements that will be sent to Rochester). Similarly, a different subset of **anonymized** data will be kept on a separate secure server at the NINDS PDBP (Parkinson's Disease Biomarkers Project). This data and will be shared with outside researchers after approval of a written request by a committee of study investigators and representatives of the associated funder (The Michael J Fox Foundation or NIH). This process has been set out in a Memorandum of Understanding between the relevant parties and a signed record of the agreement is on file with grants management.

Columbia Consortium Database:

Each participant will be assigned a unique identifier, and only the investigators at the site where the participant was seen will be able to identify the participant with this code. All clinical data collected in this study will be linked to the participant's unique identifier and entered into PDF forms. These forms will be submitted through a Password protected, RSA encrypted and IP Filtered connection to an FTP server on the Columbia University network. The data from the PDF forms will then be transferred to the Progeny Database Server and a backup copy of the original PDF file will be kept on the FTP server. This data transfer to the Progeny Database Server will be entirely automated and will occur behind the Columbia University Fire Wall through a password protected and IP filtered connection for increased security. The Progeny Database server is positioned inside the CUMC Datacenter. It is protected by the Columbia University Fire Wall and is only accessible through password authorized computers at each site. This ensures maximum security and reliability. Both the FTP and the Progeny servers will be managed by the CUMC IT Department.

The data that is sent to the servers at Columbia University will include demographic, history, exam, blood ID #, date received, source physician, location of final sample, as well as the genetic data. No names or contact information will be sent to the servers at Columbia University, but this information will be kept in the database at the institution where the patient was studied (Beth Israel, Columbia, Tel Aviv University). At Beth Israel we will keep this information along with the clinical data on BI patients in a separate Progeny database server housed at BI. The database is site-limited and password protected and only genetic counselors, the database manager, research associates, and investigators have access to it. The genetic data is also exported to various programs for statistical analyses.

Rochester Database (CTCC):

A unique, 9 digit identification number will be assigned to each individual record as part of this study. In order to obtain this number, 9 pieces of information about the subject will be entered by the Beth Israel team onto a secure website at the clinical Trials Coordination Center (CTCC) at the University of Rochester. These 9 pieces of information are: first name, last name, gender, day of birth, month of birth, year of birth, city of birth, country of birth and mother's maiden name. Once these 9 pieces of information have been entered a 9 digit identification number will be generated and written in the research file. The information entered in order to obtain this identification number will then be automatically deleted from the website.

Data will initially be transferred to the CTCC via password protected zip file, sent via email. The data to be sent will include the core elements as agreed to in the MOU (i.e., demographic information, diagnostic information, environmental questionnaires, PD gene status, and other assessments such as UPDRS, Hoehn and Yahr, Scwhab and England, MoCA, GDS, BDI, SCOPA-AUT, UPSIT). The data will contain no names or contact information. Any questions regarding the data will be returned to the originating site for resolution.

About the CTCC network: The CTCC maintains a dedicated 100 Mb full duplex switched Ethernet LAN upon which approximately 15 servers reside and 84 PCs connect. The CTCC LAN is protected from outside and or public Internet intrusion via a CISCO firewall. Internet traffic is routed to the CTCC LAN via a 100MB fiber optic connection through the University of Rochester extended local area network (ELAN). External CTCC staff access to the CTCC LAN is available on an as required basis via Virtual Private Networking (VPN) which is integrated as a component of the CISCO firewall. Standard Operating Procedures, training and a "Computing Code of Conduct" govern the processes by which users of CTCC network services are provided accounts, granted access to various assets and guided in best practices for permissible use and maintenance of required security. High level administrative access to network resources is held solely by the members of the Clinical Informatics and IT Support groups within the CTCC (currently a combined total of six individuals including managers). These groups are managed by the Manager of Clinical Informatics and the Manager of IT Support, respectively, both under the direction of the Director of Data Management for the CTCC.

The CTCC maintains a data center which houses all of its production servers. The data center is a secured facility with keyed access available only to select IT staff and management. The data center is continually monitored by onsite personnel. Should equipment indicate an error, appropriate CTCC staff are contacted by data center personnel. All servers are maintained on conditioned power with redundant connections and backup generators that automatically come online in the event of power failure to the data center.

The entire CTCC environment is backed up nightly via an automated StorageTek Tape Library Robot. Full backup tapes are rotated offsite on both a weekly and monthly basis with retention periods lasting up to a year.

Comprehensive service and maintenance contracts are maintained (when available) on all hardware specified above. In the case of the Unix servers, a maintenance contract with 4 hour response is maintained with the expectation that in the event of a catastrophic failure, downtime of no more than 24 hours would be required to totally rebuild the environment from backup media. Windows servers are also maintained under service contract with same day on-site service stipulations.

PDBP at NINDS:

A unique identifier is created for PDBP samples similar to the CTCC ID above. The NINDS identifier is called the Global Unique Identifier Tool ("GUID"). Detailed information on the GUID can be found at the following website: https://pdbp.ninds.nih.gov/assets/PDBP%20GUID%20Tool%20Guide.pdf. Information on the PDBP, including data and sample protections can be found at: https://pdbp.ninds.nih.gov/jsp/how-pdbp-works.jsp.

Additional measures we are taking to ensure the confidentiality of study data include storage of written information in locked filing cabinets/offices at all times, communication with laboratory staff and collaborators using only ID numbers rather than names, and no use of names or other identifying information in publications or other publicly available documents. Information collected as part of this research will not be disclosed to people outside the study (except as required by law), and will not be made part of any subject's clinical records. In addition, we have obtained a certificate of confidentiality from the NIH.

Imaging data will be entered and maintained in a study specific database. These data will be merged with study clinical, genetic and biological sample data, as appropriate.
The confidentiality of SPECT scan results and related study results that could identify subjects must be protected, respecting the privacy and confidentiality rules in accordance with the applicable regulatory requirements.

Study information will be recorded on study forms. Participants will be identified by a number assigned at the time of the screening visit and participant names will be known only to the investigators. Representatives from the US Food and Drug Administration (FDA) will have access to the medical records connected to this study. Study records will be kept confidential to extent provided by law. Name or other identifying data will not be used in any report or publication of this study. The data from this study will be maintained at IND for a minimum of fifteen years.

Additional de-identified data that will be sent to BI:

1. **Data for pooled meta-analysis** will be received from the following physicians: Dr. Jan Aasly, Trondheim, Norway, Dr. Rivka Inselberg, Tel Hashomer, Israel, Dr. Jose-Felix Martin-Masso, San Sebastian, Spain, Dr. Eduardo Tolosa, Barcelona, Spain. With the exception of Dr. Tolosa, this

data will be related to cancer history in patients with and without a mutation in the LRRK2 gene and will be part of a Meta-analysis Sub-study which has received Privacy Board for approval. Data from Dr. Tolosa will include all of the same data we are collecting on patients and family members at BI, and subjects from Barcelona will sign consent for the data transfer to BI.

2. **Data for multi-site, systematic questionnaire will be received from the following:** Dr. Nir Giladi, Tel Aviv, Israel, Dr. Karen Marder, Columbia University, Dr. Eduardo Tolosa, Barcelona, Spain, Jan Aasly, MD, St. Oslav's Hospital, Norway, Tatiana Faroud, MD, PROGENI, Kathryn Brockmann, MD, University of Tubingen, Germany. This standardized data collection effort will be related to cancer and general medical history in patients with and without PD and/or a mutation in the LRRK2 gene and will be part of a multi-site sub analysis. Subjects at collaborating sites will sign consent for the data transfer to BI.

i) Provisions to Monitor the Data to Ensure the Safety of Subjects

This information is only required when Human Research involves more than minimal risk to subjects.

Part I *describes the safety monitoring activities that will be undertaken in during the study. This should be completed for all studies that require more than the basic minimum DSMP.*

Part II *describes Data and Safety Monitoring Committees or Boards and should be completed when one is needed for the DSMP*

Part I: Elements of a Data and Safety Monitoring Plan

1. List the name(s) of the individual(s) at MSSM who will be responsible for data and safety monitoring of this study. For each individual, indicate their role, name, title, and department information. The Principal Investigator may be the only monitor of a study.

If the qualifications of an individual to serve as a monitor are not contained in the IRB application, they must be added to the DSMP either as a narrative description or as a CV.

Because no there is no intervention in this study, there is no official safety monitor. However, there is a safety procedure as follows for the DaTScan, lumbar puncture, skin punch, which involve more than minimal risk:

Individuals having one of these procedures will receive information about possible symptoms to be aware of with phone numbers of study doctors and doctors' services, and instructions to go to the emergency room if they have bleeding, pain, or neurological signs. Study subjects will be contacted by the research staff one week after the procedure to ask about any sequelae, and research staff will note adverse events in the patient's research chart. Any SAEs would also be reported to the IRB.

ADDITIONAL SAFETY INFORMATION FOR SUBJCECT HAVING DATSCAN:

monitoring safety of the subjects that enter this trial. Safety will be evaluated by the following:

Adverse Events

A clinical adverse event is any untoward medical occurrence in a subject administered a pharmaceutical product, without regard to the possibility of a causal relationship. Study site personnel will record the occurrence and nature of each subject's pre-existing conditions. During the study, site personnel will record any change in the condition(s) and the occurrence and nature of any adverse events. For the purposes of this addendum, adverse events will be recorded for the period beginning with administration of the thyroid protection through 7 days (± 3 days) post DaTSCAN™ injection.

Investigators will document their assessment of the potential relatedness of each adverse event to study drug and/or drug delivery system via an Institute for Neurodegenerative Disorders (IND) adverse event form. Serious adverse events will be documented by the investigator and reported immediately to the Institute for Neurodegenerative Disorders and the IRB. A serious adverse event is defined by one of the following outcomes:

- Death
- Inpatient hospitalization
- A life-threatening experience
- Persistent or significant disability/incapacity
- Congenital anomaly/birth defect

Pregnancy Testing

Serum pregnancy testing will be performed within 5 days of injection with DaTSCAN™ for any females of child-bearing potential. Subjects must be confirmed as negative for preganancy.

DATSCAN™ INFORMATION

Product Information

DaTSCAN™ is a diagnostic radiopharmaceutical for central nervous system imaging. DaTSCAN™ is FDA approved and indicated for detecting loss of functional dopaminergic neuron terminals in the striatum of patients with clinically uncertain Parkinsonian Syndromes (PS).

DaTSCAN™ is manufactured by GE Healthcare, North Arlington Heights IL, USA. Information concerning the ordering process DaTSCAN™ will be provided to the imaging sites in a Technical Operations Manual for this study.

The information below refers to the standard pack size vial which contains, an isotonic solution of 185 MBq (5mCi) of [^{123}I]Ioflupane in 2.5 ml of solution. In addition, an extended pack size is available which contains, at reference time, an isotonic solution of 370 MBq and which will be needed for some sites depending on the transport time to the site.

Chemical name:	[^{123}I] labelled N-ω-fluoropropyl-2β-carbomethoxy-3β-(4-iodophenyl) nortropane
Product name:	[^{123}I] Ioflupane or DaTSCAN™ previously known as [^{123} I] FP-CIT injection
Radiolabel & Half Life ($t_{1/2}$):	^{123}I (gamma radiation): $t_{1/2}$ = 13.2 hours
Active ingredient:	[^{123}I] Ioflupane; 74 MBq (2mCi)/ml at reference time
Specific Activity:	2.5- 4.5 x 10^{14} Bq (6,800-12,200Ci)/mmol at calibration time
Other ingredients:	[^{127}I] ioflupane, approx. 0.1 µg/mL Glacial acetic acid, 5.7 mg/mL Sodium acetate trihydrate, 7.8 mg/mL Ethanol, 5% v/v Water for injection, 2.5 mL
pH:	4.0 – 6.0
Radionuclidic purity:	> 99.9%
Radiochemical purity:	≥ 96% [^{123}I]Ioflupane at release, ≥ 94% at expiry
Total radioactivity:	90-110% of the declared activity at calibration time
Expiry time:	7 hours post reference time
Dosage form:	Sterile isotonic solution for i.v. administration
Dose per subject:	Single i.v. injection of radioactivity in the range 111-185 MBq per subject in a maximum volume of 2.5 mL

DaTSCAN™ should be stored at room temperature (< 25°C, no freezing). During both storage and preparation, appropriate radiation precautions should be observed.

Drug Accountability

The Imaging site will be responsible to track drug accountability. Radiopharmaceutical agents should only be used by qualified personnel with the appropriate government authorization for use and manipulation of radionuclides within a designated clinical setting. All DaTSCAN™ containers (opened, unopened, or empty) must be destroyed on-site at the nuclear medicine facility according to the facility's standard operating procedures.

The Nuclear Medicine site is responsible for ensuring that delivery of DaTSCAN™ from the manufacturer are correctly received, recorded, handled, and stored safely and properly in accordance with all applicable regulatory guidelines, and used in accordance with this protocol. Details regarding this can be found in the Technical Operations Manual provided to the imaging center for this study.

DATA AND SAFETY MONITORING PLAN

The site investigators and coordinators will collect and document information regarding all adverse events that occur over the course of the study and will be responsible for conducting the subject call 7 ± 3 days following the injection/scan to assess any adverse events.

Serious adverse events (SAE) will be reported by the clinical investigator to the site's institutional review board (IRB) and sponsor of the study. The SAE's will be reviewed by the medical monitor for the study of DaTSCAN™. Information regarding the severity of the SAE and its relationship to the study medication will be reviewed to determine whether it was unexpected or unanticipated and thus warrants a Med Watch report. In the case that a Med Watch report is warranted, the investigators would be responsible for reporting this information to their IRB. For AE's and SAE's that occur during the imaging visit, it is the responsibility of the imaging site to document and report these incidents to the clinical investigator.

MSSM Principal Monitor: (DaTScan)
Indicate whether this person is the PI, a Team Member, or is Independent:
Last Name:
First Name:
Academic Title:
Department:
Mailing Address:
Phone:
Fax:
E-mail:

MSSM Additional Monitor:
Indicate whether this person is the PI, a Team Member, or is Independent:

Last Name:
First Name:
Academic Title:
Department:
Mailing Address:
Phone:
Fax:
E-mail:

2. Justify your choice of principal monitor in terms of the assessed risk to the research subject's health and wellbeing. In high risk studies when the principal monitor is independent of the study staff, indicate the individual's credentials, relationship to the PI, and the rationale for selection.
3. List the specific items that will be monitored for safety (e.g., adverse events, subject compliance with the protocol, drop outs, etc.).
*4. Indicate the frequency at which **ACCUMULATED** safety and data information (items listed in number 3 above and interim analysis of efficacy outcomes) will be reviewed by the monitor(s) or the Data Monitoring Committee (DMC). Although this information must be reviewed at least annually, the higher the study risks, the more frequently reviews must be scheduled.*
5. Where applicable, describe rules which will guide interruption or alteration of the study design.
6. Where applicable, indicate dose selection procedures that will be used to minimize toxicity.
7. List any specialized grading system that will be used to evaluate adverse events (e.g., National Cancer Institute Common Toxicity Criteria).
8. Describe procedures that will be used to assure data accuracy and completeness.
9. Should a temporary or permanent suspension of your study occur, in addition to the IRB, indicate to whom (NIH, FDA, sponsor, IRB) will you report the occurrence.

Part II. Data Monitoring Committee/Data Safety Monitoring Board (DMC/DSMB)
When appropriate, attach a description of the DMC. Provide the number of members of the DMC, their names and area of professional expertise. DMC reports must be made available to the local PI and the IRB. The report need not contain specifics of the study or data, but there must be assurance that subject safety is not being compromised and that the results of treatment do not warrant early termination of the study.

j) Withdrawal of Subjects

Describe anticipated circumstances under which subjects will be withdrawn from the research without their consent.

Describe any procedures for orderly termination.

Describe procedures that will be followed when subjects withdraw from the research, including partial withdrawal from procedures with continued data collection.

Withdrawal without consent is unlikely since this is an observational study. However, subjects may choose to withdraw from the study or from continued participation or may refuse to do parts of the protocol. In such cases the subject's choice would be noted in the Progeny database and also in the research chart.

6) Risks to Subjects

Describe the reasonably foreseeable risks, discomforts, hazards, or inconveniences to the subjects related to the subjects' participation into the research. Do not only refer to the drug/device insert or investigational brochure. As relevant for the IRB's consideration, describe the probability, magnitude, duration, and reversibility of the risks. Consider physical, psychological, social, legal, and economic risks.

If applicable, indicate which procedures may have risks to the subjects that are currently unforeseeable.

If applicable, indicate which procedures may have risks to an embryo or fetus should the subject be or become pregnant. Include risks to others (e.g. sexual partners) if applicable.

Psychological: You may experience anxiety as a result of your participation in this study. If you desire, you may ask the investigator for a referral or make an appointment with one of the investigators to address this.

Blood: Persons submitting blood samples may experience transient pain and there may be a black and blue mark at the site at which blood is drawn.

Swab/Saliva: There is no anticipated risk to this procedure.

Skin punch: Persons having a skin punch may experience brief pain at the site at which the local anesthetic is injected and may be left with a tiny scar smaller than the tip of a pen at the site of the punch.

Tumor: There is no anticipated risk to this procedure.

Lumbar puncture:
The needle used for this procedure (the Sprotte 24 g spinal needle) has a smaller gauge than the conventional needle and has therefore been reported to be associated with a lower rate of complications. The lumbar puncture may cause pain at the site where the needle goes in and the spinal fluid is taken. There is a small risk of infection or bleeding. After the lumbar puncture, patients may experience get a headache. This risk is minimized with the smaller gauge needle and by getting 1 to 2 hours of bedrest following the procedure. If a headache occurs, it is usually mild and can be controlled by bedrest, drinking lots of fluids, and a pain pill, such as Tylenol. Rarely, the headache is severe and may require additional treatment with a "blood patch." In this procedure, a small amount of the patient's own blood is injected into the lumbar puncture site. This procedure is generally effective in stopping the headache.

Patients will be asked whether they are allergic to local anesthesia (lidocaine) or to Betadine. Although very rare, it is possible to have an allergic reaction to the local anesthetic used for the lumbar puncture. Signs of an allergic reaction include swelling and/or a rash on your skin where the anesthetic was injected. To minimize any possible risk, the lumbar puncture will be done by a staff person who is specifically trained in the procedure.

Ultrasound, ECG, Gait analysis: There are no anticipated risks to these procedures.

Loss of Privacy: There always exists the potential for loss of private information; however, there are procedures in place to minimize this risk.

DaTSCAN™ Safety

Radiation Risks

DaTSCAN™ is a radioactive material. The amount of radiation (569/432 mrem) is similar to that received during 1-2 CT scans of the brain. However, radiation is cumulative over a lifetime, so the exposure received in this protocol should be considered in light of your previous radiation history.

For research subjects participating in studies with radiopharmaceuticals the FDA has established guidelines for radiation exposures. Subjects may not receive more than 5 rads per target organ with the exception of the gonads, lens of the eye, and blood forming marrow where the single injection exposure limits are 3 rads. Over the course of one year the limits of exposure for research subjects is 15 rads to the dose limiting target organ or 5 rads to the gonads, lens of eye, or blood-forming marrow. Thus, the consent form asks study participants to discuss radiation exposures they have had in the past year with the study doctor.

DaTSCAN is provided as a radiotracer dose; hence, no mass effects have been noted in the over 200,000 clinical injections of the diagnostic radioligand in 10 years experience in Europe. The short physical half-life (13.1 h) and relatively low photopeak energy of 123-I (159 kEV), similarly poses no significant additional radiation burden in subjects with hepatobiliary or renal disease.

Acute Studies

The No Observable Adverse Effect Levels from acute toxicity studies of ioflupane (the active ingredient of DaTSCAN™) are described in more detail in the Investigator's Brochure for DaTSCAN™.[lvi]

Table 1: No Observable Adverse Effect Levels (NOAELs) from Acute Animal Toxicity Studies and Maximum Human Equivalent Dose Multiples for 2.5-mL DaTSCAN™ Formulation

Species	NOAEL (mg/kg)	Multiple of Maximum Human Equivalent Doses
Rats	1 mg/kg	27,000

Rabbits	0.06 mg/kg	3,200
Dogs	0.3	30,000
Cynomolgus monkeys	0.1 mg/kg	5,500

Repeated-Dose Studies

In 14-day repeat-dose studies, no evidence of toxicity was observed in rats or rabbits following daily doses of ioflupane of up to 0.6 mg/kg or in dogs up to doses of 0.1 mg/kg (between 9,000 and 32,000 times the maximum human single dose).[lvi] Behavioral effects due to pharmacological activity were observed in these studies. There was no evidence in *in vitro* or in *in vivo* mutagenicity studies of mutagenic potential.[lvi] Carcinogenicity or reproductive toxicity studies with ioflupane have not been performed.

Clinical Studies

Clinical studies of DaTSCAN™ in patients with movement disorders or dementia and in healthy volunteers have shown that DaTSCAN™ has radiation dosimetry that is favorable for SPECT imaging and has safe, high and stable brain uptake.[lvi] There is rapid striatal update, with 3 – 6 hours post-injection as the optimal time window for diagnostic imaging. Reduced striatal uptake is observed with PD patients when compared to healthy volunteers. GE recommends injected activity in the range of 111 to 185 MBq. Additionally, GE has data to indicate an excellent safety profile for DaTSCAN™ based on post-marketing experience in Europe with an estimated > 168,000 doses. More detailed information on the safety of DaTSCAN can be found in the Investigator's Brochure.[lvi]

7) Provisions for Research Related Harm/Injury

Describe the availability of medical or psychological resources that subjects might need as a result of any anticipated adverse events that may be known to be associated with the Human Research.

If the research involves more than minimal risk to subjects, explain any medical treatments that are available if research-related injury occurs, who will provide it, what will be provided, and who will pay for it.

Subjects who experience anxiety from participation are invited to discuss their concerns with one of the investigators. Only a small subset of patients will have skin punch, lumbar puncture, or DaTSCAN, and severe adverse events are unlikely. However, all patients are given the contact numbers of the office and answering services for Drs. Bressman and Saunders-Pullman (for LP or DaT) and Dr. Barry Smith (skin punch) in case they have questions or concerns, and are told to go

to the closest emergency room in cases of severe pain, bleeding, or other neurological symptoms. ER visits and treatment would be billed through the patient's insurance.

8) Potential Benefits to Subjects

Describe the potential benefits that individual subjects may experience from taking part in the research. Include, as may be useful for the IRB's consideration, the probability, magnitude, and duration of the potential benefits.

Indicate if there is no direct benefit. Do not include benefits to society or others (this is already described in the Study Objectives above).

Subjects may or may not benefit personally from this study, but will benefit by possibly helping future patients or family members by participating.

9) Provisions to Protect the Privacy Interests of Subjects

[Note: This section is soliciting <u>different information</u> than the confidentiality information solicited in section #5h.]
Describe the steps that will be taken to protect subjects' privacy interests, particularly a person's desire to control how, and with whom, they interact and communicate, especially on issues that prospective research participants may deem sensitive or private. Consider privacy interests that may arise from the time participants are identified for recruitment until they complete study participation. Consider privacy interests that may arise in communications with the study subjects (e.g. phone messages, mail, etc), including through long-term follow-up.

Describe what steps you will take to make the subjects feel at ease with the research situation in terms of the questions being asked and the procedures being performed. "At ease" does not refer to physical discomfort, but the sense of intrusiveness a subject might experience in response to questions, examinations, and procedures.

Describe why it is acceptable and appropriate for members of the research team to approach the prospective participant about the research.

Measures include removing identifiers from any data or sample that is shared with collaborators outside MSBI or in publication. Hard copies of data are kept in research charts and not made part of the patient's research record. Charts are kept in locked cabinets/offices. Electronic data is kept in a password protected database to which only the study staff has access. Patients are made aware that while it is unlikely that any identifying information would be shared, there are also

certain legal protections (GINA) that would protect them from health insurance discrimination. In addition, we have obtained a Certificate of Confidentiality for the study to help us protect patient information.

Members of the research team are highly trained and appropriate with patients, and patients are not pressed to participate if they choose not to. However, because they/their family members are afflicted with a movement disorder solicitation for research participation is both appropriate and welcomed in most cases.

10) Economic Impact on Subjects

Describe any foreseeable costs that subjects may incur through participation in the research (exclude billing for procedures that are part of clinical care e.g. copayments for studies that involve an overlap of clinical care & research).
In answering this question, the Office of Grants and Research Administration (OGARA) must be consulted in determining the appropriate responsible party for subject care costs incurred as part of the clinical research study.

There are no subject costs incurred from participating in this study.

11) Payments to Subjects

Describe the amount and timing of any payments to subjects. For research participant stipends and travel reimbursement, please consult IRB staff prior to submission if Greenphire's ClinCard and the related consent form language should be used.

Subjects are not paid for participation in the main study. However, they are reimbursed via ClinCard for parking up to $34 when they come to MSBI for their study visits, and up to $100 if they are traveling to New Haven or Long Island for neuroimaging. Subjects having SPECT scanning with DaTSCAN will be paid $75 for participating via Clincard. The study does not cover costs related to clinical visits for evaluation by an MSBI physician or genetic counselor.

12) Consent Process

*Indicate whether you will you be obtaining consent. (If not, proceed to the **Waiver or Alteration of the Consent Process** section below). If you will be obtaining consent, describe:*

- *The setting of the consent process.*
- *Describe any waiting period available between informing the prospective subject and obtaining the consent.*

- *Also describe:*
  - *The role of the individuals listed in the application as being involved in the consent process.*
  - *The time that will be devoted to the consent discussion.*
  - *Steps that will be taken to minimize the possibility of coercion or undue influence.*
  - *Steps that will be taken to ensure the subjects' understanding.*
  - *Describe any tools that will be utilized during the consent process*

This study has the following consent forms:

1. Main Study Consent
2. Main Study Consent-Surrogate for enrolling subjects who lack capacity
3. DAT Consent BI- for BI staff to consent subjects enrolled in the main study at MSBI for DATscan Sub-study
4. DAT Consent SLR- for SLR staff to consent subjects enrolled in the main study at the Columbia University site for DATscan Sub-study
5. Tumor Sub-study Consent- See description of tumor sub-study in Procedure section
6. Results Consent- for consenting subjects wishing to obtain neuropsychological testing results from the study

All subjects will be consented by one of the research coordinators or study physicians. This process will most often occur at MSBI, but may also occur at the subject's home in the case of a home visit. Regardless of the setting, the researcher will take the time necessary to discuss each section of the consent form with the subject to explain and answer any questions (20-30 min). Potential subjects may also take the form home for further review before deciding whether to participate. All subjects are told that they need not participate, and the consent form states that if they choose not to it will not affect their care at MSBI.

Children

Federal regulations define "children" as persons who have not attained the legal age for consent to treatments or procedures involved in the research [clinical investigation] under the applicable law of the jurisdiction in which the research [clinical investigation] will be conducted (45 CFR 46.402(a) and [21 CFR 50(o)]). If the Human Research involves children:

- *Describe the criteria that will be used to determine whether a prospective subject has not attained the <u>legal age for consent to treatments or procedures</u> involved in*

the Human Research under the applicable law of the jurisdiction in which the Human Research will be conducted (e.g., individuals under the age of 18 years).

- *NOTE: For research conducted <u>outside</u> of New York State, obtain consultation from Mount Sinai legal counsel as to the definition of "minor" in the jurisdiction(s) where you are performing your research, given the treatments and procedures involved in the Human Research. [Contact the IRB Office regarding how to obtain a Legal consultation.] After receiving consultation with Legal, provide an explanation in this section about whether you will be enrolling subjects who are defined as minors in other jurisdictions and the basis for your conclusion that they are not legally capable of consenting to the treatments or procedures involved in the research.*

- *Describe whether parental permission will be obtained from:*
  - *Both parents unless one parent is deceased, unknown, incompetent, or not reasonably available, or when only one parent has legal responsibility for the care and custody of the child.*
  - *One parent even if the other parent is alive, known, competent, reasonably available, and shares legal responsibility for the care and custody of the child.*
- *Describe whether permission will be obtained from individuals other than parents, and if so, who will be allowed to provide permission. Describe the process used to determine these individuals' authority to consent to each child's general medical care.*
- *Indicate whether assent will be obtained from all, some, or none of the children. If assent will be obtained from some children, indicate which children will be required to assent.*
- *When assent of children is obtained describe whether and how it will be documented.*
- *Describe whether child subjects may be expected to attain legal age to consent to the procedures of the research prior to the completion of their participation in the research (including storage of samples). If so, describe the process that will be used to obtain their legal consent to continue participation in the study. Describe the timing of this process, and what will occur if consent is not obtained from the now-adult subjects.*

Children will not be enrolled in this study.

Cognitively Impaired Adults

If the Human Research involves <u>adults who may be unable to consent</u>, describe the process to determine whether an individual is capable of consent.

Indicate who (must be either an attending physician, or independent consulting physician, with appropriate training, licensing and certification qualifications to make the determination regarding capacity) will make the assessment and how the assessment will be made. Pay special attention to the required qualifications regarding assessing incapacity due to mental illness, mental retardation or developmental disability.

Research involving incapacitated adults must also comply with Mount Sinai Beth Israel, Mount Sinai St. Luke's, and Mount Sinai Roosevelt policies regarding Adult Research Subjects who Lack Capacity.

The assessment of capacity must include the cause and extent of incapacity and the likelihood that the subject will regain capacity. The plan must also indicate that documentation of the determination and assessment will be placed in the medical record, when applicable, in addition to the research record.

If the Human Research involves cognitively impaired adults:

- *If permission of a legally authorized representative will be obtained:*
  - *List the individuals from whom permission will be obtained in order of priority. (E.g., durable power of attorney for health care, court appointed guardian for health care decisions, spouse, and adult child.)*
  - *For research conducted outside of New York State, obtain consultation from Mount Sinai legal counsel as to the definition of "legally authorized representative" in the jurisdiction(s) where you are performing your research. [Contact the IRB Office regarding how to obtain a Legal consultation.] After receiving consultation with Legal, provide an explanation in this section about which individuals are authorized under applicable law to consent on behalf of a prospective subject to their participation in the procedure(s) involved in this Human Research.*
- *Describe the process for assent of the subjects. Indicate whether:*
  - *Assent will be required of all, some, or none of the subjects. If some, indicated, which subjects will be required to assent and which will not.*
  - *If assent will not be obtained from some or all subjects, an explanation of why not.*
  - *Describe whether assent of the subjects will be documented and the process to document assent.*

Cognitively impaired adults will be recruited to the study. This was previously approved by the MSBI IRB.

Determination of capacity to consent to the study will be performed by a board certified or board eligible neurologist or psychiatrist who is not part of the study team, and aware of the risks and benefits of the study and the study protocol. It will involve systematically administering a series of statements and

questions which will allow the subject to express whether there is capacity. If there is capacity, then subjects will be enrolled with the main consent. If the subject lacks capacity, the surrogate consent will be obtained.

A participant deemed by a neurologist or psychiatrist, who is not part of the study team, not to have capacity to consent to this research study, at the time of or prior to the time of consent, upon detailed interview, will have a prospective surrogate identified to give consent on their behalf. Furthermore, given the degenerative nature of Parkinsonism, subjects will be assessed each time an additional study related assessment is performed. The prospective surrogate and participant will be notified by the research team of the assessment of incapacity prior to consent.

The hierarchy for consideration of surrogate is defined by:

1) A health care agent appointed under the New York Health Care Agents and Proxy Law;
2) An agent that fulfills the NYS Family Health Care Decisions Act:
    - a court appointed guardian;
    - the spouse or domestic partner;
    - an adult child;
    - a parent;
    - a brother or sister; or
    - a close friend.

Additional to surrogate consent to this study, verbal assent of the participant regarding inclusion into this study will be obtained by a researcher in the presence of the participant's surrogate. The surrogate and/or participant can at any point during the research decide to discontinue the participant's involvement in the study.

Non-English Speaking Subjects

Indicate what language(s) other than English are understood by prospective subjects or representatives. If subjects who do not speak English will be enrolled, describe the process to ensure that the oral and written information provided to those subjects will be in that language. If you intend to exclude potential participants who do not speak English, provide a justification for doing so.

While we are unlikely to need to enroll non-English speakers, potential subjects who do not speak English may be enrolled through an interpreter, and we have previously translated consent forms to both Spanish and French. However, due to the expense of obtaining and re-certifying expired consents in other languages, we do not always have a translated consent. In these rare instances,

we rely on the interpreter and carefully go through the entire consent to make sure the subject understands everything before signing. This is then also documented in the research chart.

1) Process to Document Consent in Writing

Describe whether and how consent of the subject will be documented in writing. If using the standard IRB consent template, simply indicate this.

If the consent process will not be documented in writing (consent will be obtained but the subject or representative will not sign a consent document) review the "CHECKLIST HRP-416 Criteria for Waiver of Written Documentation of Consent" and address each of the criteria for approval. Describe whether you will be using a consent document without a signature page, some other kind of script, etc.

Consent is obtained in writing using the standard IRB consent template.

2) Vulnerable Populations

Indicate specifically whether you will include or exclude each of the following populations:

Include	*Exclude*	*Vulnerable Population Type*
X		*Adults unable to consent*
	X	*Individuals who are not yet adults (e.g. infants, children, teenagers)*
	X	*Wards of the State (e.g. foster children)*
X		*Pregnant women*
	X	*Prisoners*

- *If the Human Research involves cognitively impaired adults, review your institution's policy to ensure that your protocol has sufficiently addressed these additional regulatory criteria for approval, including protocol specific information to support the determinations..*
- *If the Human Research involves persons who have not attained the legal age for consent to treatments or procedures involved in the research ("children"), review the "CHECKLIST HRP-421 Criteria for Research Involving Children" to ensure that your protocol has sufficiently addressed these additional regulatory criteria for approval, including protocol specific information to support the determinations.*

- *If the Human Research involves pregnant women, review the "CHECKLIST HRP-418 Criteria for Research Involving Pregnant Women" to ensure that your protocol has sufficiently addressed these additional regulatory criteria for approval, including protocol specific information to support the determinations.*
- *If the Human Research involves non-viable neonates or neonates of uncertain viability, review the "CHECKLIST HRP-419 Criteria for Research Involving Non-Viable Neonates" "CHECKLIST HRP-420 Criteria for Research Involving Neonates of Uncertain Viability" to ensure that your protocol has sufficiently addressed these additional regulatory criteria for approval, including protocol specific information to support the determinations.*
- *If the Human Research involves Prisoners, review the "CHECKLIST HRP-417 Research Involving Prisoners" to ensure that your protocol has sufficiently addressed these additional regulatory criteria for approval, including protocol specific information to support the determinations.*
- *Describe other aspects of the subject population that may increase their vulnerability (home/institution-bound individuals; students participating in their professor's research, cognitively-impaired minors, etc)*

If the Human Research involves individuals who are vulnerable to coercion or undue influence, describe additional safeguards included to protect their rights and welfare.

See other sections for a discussion of adults unable to consent. Pregnant woman will also be recruited if they fit the study criteria, but WILL NOT undergo DaTSCAN, lumbar puncture, or skin biopsy. There is no extra risk to pregnant woman from participating in other aspects of the study since no device, biologics or study drugs are being used.

3) Multi-Site Human Research (Coordinating Center)

If this is a multi-site study where you are the lead investigator, describe the management of information (e.g., results, new information, unanticipated problems involving risk to subjects or others, or protocol modifications) among sites to protect subjects.

As above, all data and specimens shared with other sites are de-identified. Each site is responsible for triaging problems or issues for its own subjects. However, the study sites have regular study calls together, and any safety issues or problems involving subjects would be discussed with other sites during these calls to ensure proper response at all sites.

4) Community-Based Participatory Research

(Note: "Community-based Participatory Research" is a collaborative approach to research that involves the community in all aspects of research process. Community-based Participatory Research begins with a research topic of importance to the community and has the aim of combining knowledge with action and achieving social change to improve health outcomes and eliminate health disparities. Simply recruiting participants from the community is not CBPR. If your research does not involve the community in all aspects of the research process, mark N/A)

Describe involvement of the community in the design and conduct of the research.

N/A

5) Sharing of Results with Subjects

Describe whether results will be shared with subjects or others (e.g., the subject's. their primary care physicians)), and if so, describe how it will be shared. As applicable, this may include individual patient results (genetic testing), incidental findings, or overall study findings.

Participants will not receive research results. They will, however, be apprised of major findings either directly, or through a newsletter or press release. They will also be notified directly as new clinical genetic tests for movement disorders become available, and genetic counseling and clinical testing will be facilitated for those who are interested in learning their genetic status.

6) External IRB Review History

If you have previously submitted this protocol for review by an external IRB (non-Mount Sinai Beth Israel IRB or Mount Sinai SLRHC IRB), provide the name of the reviewing IRB and the associated project identification number. Indicate whether this protocol was found to be "not approvable" by the external IRB. If so, provide details of the review including the date of review and the IRB contact information.

N/A

7) Control of Drugs, Biologics, or Devices

If the Human Research involves drugs, biologics, or devices, describe the plans to store, handle, and control those drugs, biologics or devices so that they will be used only on subjects and be used only by authorized investigators.

Note: If there are required departmental policies that regulate the control of drugs, biologics, or devices, provide that information here.

Note: For studies involving research drugs or biologics, you will need to obtain the approval of Investigational Drug Service (IDS) at your institution where the research will be performed, regardless of whether you will be utilizing the IDS to manage the control of research drugs and biologics.

N/A

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