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Observational Study of Individual or Group Template: Tracing the Origin and Progression of Parkinson's Disease through the Neuro-Immune Interactome

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This study was initiated in 2020 and is continuing to a total 10-year follow-up

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Tracing the origin and progression of Parkinson's disease through the neuro-immune interactome

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Abstract

Parkinson's disease (PD) is among the most common neurodegenerative diseases. While neuroinflammation has been implicated in PD, the immune system's role in the initiation and progression of the disease process remains unknown. Recent reports have provided early, provocative evidence that in addition to the immune system's potential for amplifying degeneration, a T cell-mediated autoimmune process may be involved in specifically initiating PD pathology. The strong association of PD with HLA-DR haplotype that is commonly seen in autoimmune disease, along with recent findings of autoimmunity to alpha-synuclein and the role of the microbiome in disease models, leads to the hypothesis that PD is initiated by an autoimmune event, at least in a subset of patients. Specifically, we hypothesize that progression of PD pathology is initiated and/or abetted by an autoimmune process involving alpha-synuclein-specific T cell activation triggered by gut microbiome dysbiosis, followed by neuro-immune interactions that establish PD in the brain. We propose to address this hypothesis by integrating neuroimmunology, single cell genomics, mouse models, and microbiome approaches.

Background/Literature Review

- 1 Parkinson's disease (PD) is among the most common neurodegenerative diseases, affecting 1% of the population over 60 years of age. PD is pathologically characterized by the loss of dopaminergic neurons in the brain and by intracellular alpha-synuclein aggregates. Braak staging of PD pathology posits that alpha-synuclein aggregation initiates in the gut and propagates via the vagus nerve to the brain in a prion-like fashion. While neuroinflammation has been implicated in PD, the immune system's role in the initiation and progression of the disease process remains unknown. Recent reports have provided early, provocative evidence that in addition to the immune system's potential for amplifying degeneration, a T cell-mediated autoimmune process may be involved in specifically initiating PD pathology. The strong association of PD with HLA-DR haplotype that is commonly seen in autoimmune disease, along with recent findings of autoimmunity to alpha-synuclein and the role of the microbiome in disease models, leads to the hypothesis that PD is initiated by an autoimmune event, at least in a subset of patients. Specifically, we hypothesize that progression of PD pathology is initiated and/or abetted by an autoimmune process involving alpha-synuclein-specific T cell activation triggered by gut microbiome dysbiosis, followed by neuro-immune interactions that establish PD in the brain. We propose to address this hypothesis by integrating neuroimmunology, single cell genomics, mouse models, and microbiome approaches. Our proposal will examine whether T cell-mediated autoimmunity initiates the neurodegeneration process in PD, and if these early immunological processes converge on classic archetypes of neurodegeneration. This work will produce an unprecedented map (the "interactome") of the neuro-immune interactions that are perturbed in PD, identifying rational targets for clinical trials and paving the way for the development of new treatments.

Rationale/Significance

- 2 The pathological hallmark of PD is alpha-synuclein aggregation in the brain. The inciting event leading to this aggregation is unclear. This study aims to determine whether or not the inciting event(s) leading to this signature pathology is autoimmunity directed toward antigens in the gut microbiome, and whether or not similar autoimmunity occurs in the brain. This work will provide a rationale for why early treatment with immunomodulatory therapy may lead to a reduction in the development of PD in at-risk populations and will provide basic mechanistic data which will pave the way for the design of a clinical trial to test the efficacy of immunomodulatory agents in the prevention of PD in high-risk populations. It is critical to determine whether or not the autoimmune process occurs early on in individuals who have yet, to but are at high risk of developing PD (those with RBD). This will help to determine the likelihood that a clinical trial testing immunomodulatory agents in individuals with RBD for the prevention of PD will be

successful. This work has great potential to benefit society at large as PD is among the most prevalent neurodegenerative diseases in the US, affecting about 1% of the adult population over the age of 60.

Study Purpose and Objectives

- 3 We hypothesize that progression of PD pathology is initiated and/or abetted by an autoimmune process involving alpha-synuclein-specific T cell activation triggered by gut microbiome dysbiosis, followed by neuro-immune interactions that establish PD in the brain. We propose to address this hypothesis by integrating neuroimmunology, single cell genomics, mouse models, and microbiome approaches.
- 4 The primary objective of this study is to determine whether T cell-mediated autoimmunity initiates the neurodegenerative process in preclinical PD and PD, and if these early immunological processes converge on classic archetypes of neurodegeneration.
- 5 Secondary objectives of this study are to determine to what extent the T cell-mediated autoimmune process initiates in the gut microbiome; and whether or not different PD-related genes or HLA type are associated with the presence of T cell-mediated autoimmunity.

Study Design

- 6 This study will be observational and have a prospective case-control longitudinal design. Eligible subjects with idiopathic REM behavior disorder (RBD), Parkinson's disease with RBD, and healthy controls will be recruited. Subjects will undergo clinical assessments, including cognitive, sensory and motor clinical assessment, dopamine transporter scanning, smell testing, and a sleep study to determine eligibility for the study. Eligible subjects will undergo phlebotomy, lumbar puncture, stool, nasal swab and gingival crevicular fluid collection, and genome-wide association scans at baseline and then undergo yearly sensory and motor clinical assessment to assess for phenoconversion to neurodegenerative disease.

Outcome Variables

- 7 The primary outcome will be the number and types of T cells which autoreact to alpha-synuclein in the blood and CSF of study subjects, as related to their 1) presence or absence of RBD and 2) clinical classification of having Parkinson's disease, prodromal Parkinson's disease features with or without evidence of motor symptoms, or absence of features suggesting PD and no evidence of RBD (controls).
- 8 Secondary outcomes will be the presence of increased clonality of T cells reflecting increased immune cell activation and the presence of cross reactivity of anti-alpha-synuclein T cells with microbial agents from subject gut stool samples. Additional

outcomes of interest will include: Relationship of differentially expressed single-cell genotypes of blood and CSFPBMCs to level of disease severity, including Motor Function (MDS-UPDRS), Non-motor PD symptoms (MDS-NMSS), Cognitive function (MoCA), RBD Severity (Yale RBD Questionnaire), Gastrointestinal Symptoms (GIDS-PD), MDS Prodromal PD Likelihood Ratio (LR), Dopamine Transporter Imaging Status, Differences in stool, nasal and oral microbiomes between RBD and PD subjects and between these groups and controls.

Study Population

- 9 Subjects 40 years or older will be included in the study. There will be five groups. Group 1: 20 subjects with RBD without motor or cognitive dysfunction, normal DaT Scan, no complaints of constipation, normosmic (not or at remote at risk for PD); Group 2: 20 subjects with RBD and asymmetric, reduced signal on DaT scan, symptomatic constipation, or hyposmia (at-risk for PD); Group 3: 20 subjects with RBD and with PD for less than 10 years (10 years from PD diagnosis at the time subject was first identified); Group 4: 20 subjects with PD but no RBD; Group 5: 25 healthy controls (without RBD, or PD). Subjects will be recruited from outpatient neurology and sleep medicine clinics and from the community using flyers and social media postings. Subjects in general will be healthy adults seen in an outpatient research setting. There will be no limitation in ability to move or walk as most subjects will be pre-clinical except for Group 4 which will include persons with only minor motor symptoms (which never includes gait disturbance) of PD.

Study Methods/Procedures

- 10 This research study is a prospective observational case-control study. The majority of assessments, including biospecimen retrieval and neuroimaging will occur in the first two months. Follow up visits done yearly thereafter will include collection of blood samples (10 lavender top tubes, 10 cc each) clinical evaluations, assessing symptoms by history.
- 11 Location: The research will may take place in nine locations: (1) the Church Street Research Unit (CSRU), (2) Hospital Research Unit (HRU) at YNNH, (3) School of Nursing biobehavioral room, (4) Neurology clinic space (800 Howard Ave), (5) Smilow Cancer Hospital (Nuclear Imaging Center), (6) Yale sleep laboratory at North Haven, (7) 2 Church street, suite 115, (8) Yale Physician Building (800 Howard Ave, Lower Level), (9) North Haven Neurology Movement Disorders Clinic (8 Devine Street). In these locations, written informed consent will be obtained by research staff in a private room or by telephone or Zoom. There will be attempts to complete in-person assessments, collect specimens, and neuroimaging on one night and the following day, but given the duration of these procedures and limited availability of neuroimaging, procedures may have to be broken up into two or three days.

- 12 Polysomnography (PSG): Sleep testing may not have to be done in all subjects. Many will have had testing or may choose to get testing as part of standard of care, in which case, Dr. Koo will be given access to review and score raw data to determine if REM sleep without atonia is present. Sleep testing which has been done within 5 years of the subject's enrollment in the study may be considered. Some persons may choose not to have a PSG on account of insurance (having a co-pay) or (if not receiving care at YNHH), ordering a PSG may be so onerous (take a long time) that investigators may choose to perform the PSG as part of the research study. In this case, the PSG will be performed in the School of Nursing or the CSRU and will take place before participation in the other portions of the study (below) to help determine eligibility. PSG will include video monitoring, electroencephalography, leg and arm electromyography, nasal pressure transduction, oxygen saturation, respiratory effort belts, and EKG. Persons having clinical RBD by history but not having REM sleep without atonia may not be included in the study without polysomnographic confirmation. Potential subjects may be screened out based upon review of a previously performed PSG. If potential subjects choose to undergo PSG by the research team, then it is possible that if REM sleep without atonia is absent, then the person will be deemed ineligible to participate in the research and all additional procedures will be cancelled.
- 13 Anthropomorphic measurements and vital signs: Height and body weight will be measured by nursing staff and BMI will be calculated. Blood pressure and heart rate will be taken with the subject lying down for 5 minutes immediately on standing and then after standing for 2 minutes. Values for height and weight, as well as pulse and blood pressure in all 3 positions will be recorded in the nurse's notes in the medical record and in the subject's study record.
- 14 Questionnaires/Clinical Assessments: Subjects will give information regarding medical history, medications, and family history. Neurological examination and Physical examination will be done by a study doctor. Clinical assessment questionnaires, including Movement Disorder Society (MDS) Unified Parkinson's Disease Rating Scale (MDS-UPDRS), Scales for Outcomes in Parkinson's disease Autonomic Dysfunction (SCOPA- AUT) will be completed for all enrolled subjects. Parts 1, 2 and 4 by research staff and the motor examination (Part III) by physician or research staff who have been trained and qualified in proper administration of the scale. Subjects with RBD will complete the Paris Arousal Disorders Severity Scale and the Yale RBD Questionnaire. The REM Sleep Disorder Screening Questionnaire (RBDSQ) will be completed by all subjects. The MDS-Non-Motor Symptom Scale (NMSS) will be administered by research staff. Questionnaires will be completed online using the Yale Qualtrics/REDCap Survey tool. Epworth Sleepiness Scale (ESS), Gastrointestinal Dysfunction Scale-Parkinson's Disease (GIDS-PD), Brief Motor Questionnaire, and PD and RBD History Review will be done. Montreal Cognitive Assessment (MOCA) will be administered, and this data will be entered into Qualtrics or REDCap. The motor part of the MDS-UPDRS and MOCA (at times virtual) will be completed in-person and will be done by research staff and will take

about 45 minutes. Constipation will be assessed using the Bristol and Rome constipation scales. The Rome diagnostic criteria for constipation includes (a) fewer than 3 spontaneous bowel movements per week; (b) straining for more than 25% of defecation attempts; (c) lumpy or hard stools for $\geq 25\%$ of defecation attempts; (d) sensation of anorectal obstruction or blockage for $\geq 25\%$ of defecation attempts; (e) sensation of incomplete defecation for $\geq 25\%$ of defecation attempts; (f) manual maneuvering required to defecate for $\geq 25\%$ of defecation attempts. There will also be a dietary survey given. Questionnaires will take 60-90 minutes to complete. For each non-PD subject, prodromal PD risk will be estimated using the Online Prodromal PD Calculator (<https://www.movementdisorders.org/MDS/Members-Only/Prodromal-PD-Calculator.htm>) MDS clinical diagnosis criteria will be done on all participants.

- 15 **Neuroimaging: Dopamine Transporter (DAT) scan:** A DaT scan is a nuclear medicine test that detects the loss of dopamine nerve terminals in the brain associated with PD. DaT is an approved imaging test used in the differentiation of PD from mimetic syndromes, and where reasonable will be performed as standard of care. A radiotracer, Ioflupane I-123 is injected intravenously. The radiotracer binds to the protein, dopamine transporter, in the brain. The subject then undergoes a nuclear scan to image the tracer in the brain. First, potassium iodide or Lugol's solution is given orally 1-4 hours before radiotracer to prevent radiation exposure to the thyroid gland from the nuclear scan. Subjects allergic to iodine will not undergo DAT scanning but are permitted to take part in other parts of the study. 5 mCi of I-123 is administered intravenously. The patient is placed supine on the imaging table and the head is placed on the headrest. The SPECT scan is run over about 60 minutes. The procedure will take about 4-5 hours and is associated with minimal risk. This procedure will only be done on RBD subjects/participants who have not had a positive Dat scan in the past (i.e. Group 1 and Group 2). If it was negative and more than 6 months ago participants may need a new DaT scan.
- 16 **Smell Testing (self-administered):** Subjects will undergo smell testing. Smell identification Test, also known as the University of Pennsylvania Smell identification Test, will be used to assess olfactory threshold, discrimination, and identification. Testing uses four enveloped-sized booklets, each containing ten "scratch & sniff" odorants. Smell testing duration is approximately 45 minutes.
- 17 **Medical and Neurologic Examination:** General medical exam will be performed by a qualified physician. Examination will include heart, lung, and extremity examination. Neurologic exam will include assessment of cranial nerves, motor strength and tone, sensation and reflexes.
- 18 **Screening for medication allergy:** Medications used in the study include iodine for the DaT scan and 1% or 2% lidocaine for the LP. Persons allergic to iodine will not undergo DaT scanning but may participate in other parts of the study. Persons allergic to lidocaine will be given 0.25% or 0.5% bupivacaine as an analgesic for the LP.

- 19 **Blood and CSF Collection:** Subjects will be prepared for venipuncture by research nursing staff. A total of 150 mL of blood (15, 10-mL vials) will be taken from each subject during the study in EDTA (ethylenediaminetetraacetic acid) tubes (lavender top) for genetic analysis and separation of peripheral blood mononuclear cells (PBMCs). No more than 100mL of blood will be collected at any one visit. 50mL will be collected in the first draw, and an additional 100 mL will be collected at a second visit a minimum two weeks later. Research team members pick up the samples and transport them at room temperature to the Hafler lab at 300 George St., where the sample will be processed. PBMCs will be isolated and stored in liquid nitrogen for genetic and mononuclear cell analysis. PBMCs will be used for 10x scRNA-seq and scTCR-seq, and for tetramer sorting followed by Smart-seq2 scRNA-seq, as well as banking for future validation. The serum supernatant will be retained for future use.
- 20 **Lumbar Puncture:** LP will be performed as per Department of Neurology Standard Operating Procedure for Clinical and Research Lumbar Punctures by one of the following study investigators experienced in performing the procedure: Based upon availability, Dr. Brian Koo, Dr. Kathryn Zuchowski, or Dr. Erin Longbrake. For subjects who cannot undergo standard lumbar puncture, a qualified neuroradiologist from the Department of Radiology may perform a lumbar puncture under fluoroscopy.
- 21 **Gingival crevicular fluid Collection (self-administered):** A swab is used to gently brush the gingival mucosa of the subject along the line where the teeth meet the gums. The swabs will then be placed in transport medium and transported to the laboratory.
- 22 **Anterior nasal sample collection (self-administered):** Subjects will be given a cotton-tipped swab and asked to gently swab the interior surface of both nares, using a circular motion, and covering the entire surface. The swabs will then be placed in transport medium and transported to the laboratory.
- 23 **Stool Collection:** Stool samples are best collected while the subject is at home and sent in via mail. A stool collection kit which includes three capped specimen containers, stool collection bowl, and a postage-paid FedEx box is provided to the subject. The subject is asked to fill the specimen containers with about 1-2 teaspoons of stool. Tubes contain preservative which prevents sample breakdown and spoiling. Tubes are placed in a biohazards bag and then into the pre-paid box. Subjects are specifically asked to return boxes to FedEx during the week on or before a Wednesday so that samples are not sitting over the weekend. If a subject is taking antibiotics at the time of entry into the study, collection of stool samples will be delayed until at least 2 months following completion of the prescribed course of medication.
- 24 **Investigational Assay:** Using disease-relevant anti- α -synuclein TCRs identified by scRNA-seq, we will evaluate the potential for cross-reactivity against microbial antigens predicted with an algorithm developed and validated in healthy subjects. Then we will use a novel method we developed to identify both host-encoded and bacterial-encoded T cell epitopes to test the hypothesis that human gut bacteria in PD patients may initiate



pathogenic T cell responses in PD through molecular mimicry. In parallel, we will utilize novel high-throughput metabolite profiling approaches to screen PD associated bacterial strains from participants for the production of bioactive metabolites that enhance alpha-synuclein aggregation, alter aggregate clearance, or engage specific host receptors that modulate the host immune response.

- 25 Follow up visits: Follow up visits will occur yearly for up to 10 years for all subjects. The duration of the study is needed to capture time of phenoconversion to a neurodegenerative disease. Follow up visits will entail clinical assessments, including conduction of the NMSS, MOCA, Brief Motor Questionnaires, MDS-UPDRS, blood collection and a follow-up question enquiring whether or not the subject has received a diagnosis of a new neurological disorder during the preceding year and the nature of that diagnosis. Paris Arousal Questionnaire will be completed by controls only. Some of the questionnaires will be completed by the subject on redcap.