Abbreviated Title: TSPO PET in neurodegeneration
NIH IRB #: 19M0095
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Title: PET imaging of neuroinflammation in neurodegenerative diseases via a novel TSPO radioligand

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Total requested accrual
100 Adults with a diagnosis or with an increased risk of adult-onset neurodegenerative diseases
100 Healthy Volunteers

Project Uses Ionizing Radiation: ☐ No ☒ Yes

- ☐ Medically-indicated only
- ☒ Research-related only
- ☐ Both

IND/IDE ☐ No ☐ Yes
Drug/Device/# [^{11}C]PIB/#108861, [^{11}C]ER176/#122236_____
Sponsor: _NIMH IRP_____

Durable Power of Attorney ☐ No ☒ Yes

Multi-institutional Project ☒ No ☐ Yes
Institution#1_____ FWA #_____
Date of IRB approval _____

Institution#2_____ FWA #_____
Date of IRB approval _____

Data and Safety Monitoring Board ☒ No ☐ Yes

Technology Transfer Agreement ☒ No ☐ Yes

Samples are being stored ☐ No ☒ Yes

Covered Protocol Requiring DEC Clearance ☐ No ☒ Yes

Approved for Short Form Consent Process for Non-English Speakers ☐ No ☒ Yes

Flesch-Kincaid reading level of consent form:

- For Healthy adult volunteer: 8.5
- For Patient of neurodegenerative diseases: 8.5
- For Subject with increased risk of neurodegenerative diseases: 8.5
- Assent for an adult without consent capacity: 7.7

PRÉCIS:

Objectives

The primary objective is to explore if human subjects with neurodegenerative diseases exhibit different level of neuroinflammation, as measured by brain uptake of a 3rd generation [^{11}C]ER176 TSPO ligand, compared to control subjects. The secondary objectives are to determine, 1) if [^{11}C]ER176 TSPO brain uptake shows disease-specific patterns across different neurodegenerative diseases and/or genetic mutations, and 2) if longitudinal imaging of individual patients shows a correlation between interval change of tracer uptake and disease progression.

Study population

Adults referred with a clinical diagnosis or with an increased risk of frontotemporal dementia, amyotrophic lateral sclerosis, Alzheimer's disease, other related adult-onset neurodegenerative disorders, or healthy control subjects.

Design

Participants will undergo a general and neurological exam, a standard battery of neuropsychological tests to measure cognitive function, blood tests for analysis of TSPO polymorphisms, MRI of the brain, and PET imaging with the [^{11}C]ER176 TSPO radioligand and [^{11}C]PIB amyloid radioligand. Participants will be invited to return for repeat evaluations approximately 1, 2, and 3-5 years after their initial evaluation.

Outcome measures

Brain PET and MRI scans will be co-registered for anatomic definition of regions of interest, and SUV will be calculated in various brain regions. [^{11}C]ER176 PET data will be analyzed with compartmental modeling. [^{11}C]PIB PET and MRI data will be adjunctly used for segregating the collected data by disease subtype. For the primary objective, we will compare TSPO radioligand uptake of healthy controls compared to subjects with neurodegenerative diseases. For secondary objectives, we will determine if neuroanatomical regions of tracer uptake differ across different neurodegenerative disease subtypes, and if interval change of tracer uptake correlates with disease progression in longitudinal imaging of individual subjects.

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LIST OF ABBREVIATIONS

AD	Alzheimer's disease
ALS	amyotrophic lateral sclerosis
C9ORF72	chromosome 9 open reading frame 72
DLB	dementia with Lewy bodies
FTD	frontotemporal dementia
GRN	progranulin gene
HAB	high-affinity binder
IL	interleukin
LAB	low-affinity binder
MAB	mixed-affinity binder
MND	motor neuron disease
MRI	magnetic resonance imaging
PBR	peripheral benzodiazepine receptor
PD	Parkinson's disease
PGRN	progranulin protein
PET	positron emission tomography
PIB	Pittsburgh compound B
SUV	standardized uptake value
TBK1	TANK-binding kinase 1
TNF	tumor necrosis factor
TNFR	tumor necrosis factor receptor
TREM2	triggering receptor expressed on myeloid cells 2
TSPO	18-kDa translocator protein
V_T	total (specific plus nondisplaceable) distribution volume
f_P	free fraction in plasma

1. INTRODUCTION AND BACKGROUND

The rising incidence of Alzheimer's disease (AD) and other age-related neurodegenerative disorders in our aging population is a looming crisis in medicine (Reitz *et al*, 2011). Neurodegenerative diseases are common, dehumanizing, and usually untreatable. Today, approximately 5 million adults in the US have AD or another adult-onset neurodegenerative disorder, or nearly 1 in 8 individuals over the age of 65. This number is expected to increase to 11-16 million by 2050 as our aged population and life-expectancy increase. The most common neurodegenerative disease in those > 60 years old is AD. Other causes of dementia in aging adults include dementia with Lewy bodies (DLB), Parkinson's disease (PD), vascular dementia, frontotemporal dementia (FTD), and frontotemporal dementia with motor neuron disease (FTD-MND). Unfortunately, we currently lack effective disease-modifying therapies for the majority of these neurodegenerative diseases. It is now widely established that pathologic changes begin to occur in the brain decades before changes in cognition. Though it is increasingly clear that future treatments need to be started in pre-symptomatic patients, we currently lack imaging-based biomarkers to track disease progression in such individuals. This has proven to be a significant barrier in development of new disease-modifying therapeutics. By using a third-generation 18-kDa translocator protein (TSPO) PET radioligand, we propose to determine if human subjects with neurodegenerative diseases have increased levels of neuroinflammation compared to control subjects, and if disease-specific patterns of neuroinflammation occur in different neurodegenerative diseases (e.g. FTD vs AD).

Dysregulation of the immune system is a common observation in many neurodegenerative diseases, including AD, PD, and ALS (Lucin and Wyss-Coray, 2009). Microglia are central mediators of the immune response in the CNS, can have either neurotrophic or neurotoxic effects, and are morphologically activated in neurodegenerative diseases (Streit *et al*, 2008; Tambuyzer *et al*, 2008). Recent genome-wide association studies have provided additional genetic evidence for the importance of neuroinflammation as a causal factor in neurodegenerative diseases. For example, polymorphisms in TREM2, a microglia receptor involved in phagocytosis, were associated with an increased risk of AD (Guerreiro *et al*, 2013; Kleinberger *et al*, 2014; Nalls *et al*, 2015). Polymorphisms in IL1-beta, TNF-alpha are associated with an increased risk of DLB (Surendranathan *et al*, 2015). Double knockout of TNFR1 and TNFR2 results in a neuroprotective effect in an animal model of PD, accompanied by a simultaneous decrease in the level of microglial activation (Sriram *et al*, 2006).

Substantial evidence has also linked dysregulated microglial activity with FTD, in particular certain genetic subtypes of FTD. Mutations in the GRN gene are a common cause of familial FTD, and reduce expression of the secreted protein progranulin. Mouse Grn-/- macrophages exhibit a pro-inflammatory phenotype (Yin *et al*, 2010). Also, Grn-/- macrophages are neurotoxic in a brain slice model, and display accelerated clearance of dying cells (Kao *et al*, 2011; Yin *et al*, 2010). PGRN binds both TNFR1 (which is broadly expressed) and TNFR2 (which is primarily expressed by leukocytes) in a dose-dependent manner (Tang *et al*, 2011). Binding of PGRN to TNFR blocked the association of TNFR with its native ligand, TNFα. These results explain prior observations that PGRN inhibits TNF-induced neutrophil activation (Zhu *et al*, 2002). In addition, Tang et al. (2011) found that PGRN blocked TNFα mediated signaling in human regulatory T cells, and downregulated interferon (IFN) gamma secretion in effector T cells. These results suggest that PGRN might directly regulate intracellular signaling by directly binding to TNFR and by inhibiting TNFα-mediated pathways. Other genetic subtypes of FTD (and FTD with motor neuron disease; FTD-MND) may also involve dysregulated microglial function. Hexanucleotide repeats in C9ORF72 are the most common causes of familial FTD and ALS (Freibaum *et al*, 2015). Recently, A C9ORF72 KO mouse was described, which was found to have strikingly abnormal and hyperactive macrophages and microglia (O'Rourke *et al*, 2016). Less common mutations in the gene TBK1, which are associated with both FTD and ALS, may also play a role in microglial regulation (Pottier *et al*, 2015). Together, both genetic and animal model data suggest an important role of neuroinflammation in FTD and ALS pathophysiology.

Non-invasive imaging of neuroinflammation in living human subjects can be accomplished through the use of PET radioligands that target the TSPO. TSPO is a mitochondrial protein that is highly expressed in inflammatory cells including activated microglia in the brain. Previous PET studies from our laboratory using a TSPO radioligand, [^{11}C]PBR28, found significant correlations between brain uptake and clinical severity/progression of AD (Kreisl *et al*, 2016; Kreisl *et al*, 2013). Moreover, distribution of TSPO uptake in brain showed high correspondence with different clinical subtypes of AD that have distinct spatial patterns in their pathology (Kreisl *et al*, 2017). Small cross-sectional PET neuroinflammation imaging studies in FTD patients have been performed using 1st and 2nd generation TSPO radioligands (reviewed by Zhang, 2015). Overall, these studies saw increased TSPO radioligand uptake in FTD patients compared to control, with anatomical correlation between regional radioligand uptake and brain atrophy (Zhang, 2015).

We also performed a clinical [^{11}C]PBR28 PET study in patients with FTD. All patients met revised criteria for frontotemporal dementia (Neary *et al*, 1998). Five patients were included (age 53.4 ± 5.9 years). Four patients met clinical criteria for behavioral variant FTD, while one patient met criteria for progressive nonfluent aphasia. Thirteen older controls (age 64.0 ± 5.0 years) were included for comparison. Patients in general showed increased [^{11}C]PBR28 binding in frontal cortex, which in many cases was evident on visual inspection (Fig 1). Using a region-of-interest analysis and correcting for age, sex, education, and TSPO genotype, FTD patients had 42% greater [^{11}C]PBR28 binding (distribution volume corrected for free fraction of radioligand in plasma, V_T/f_P) than controls in prefrontal cortex ($p = 0.034$) and 45% greater binding in striatum ($p = 0.025$). These results suggest that FTD is associated with activation of microglia, similar to that seen in Alzheimer's disease (Kreisl *et al*, 2013); albeit with different regional distribution. Regions of increased [^{11}C]PBR28 binding in FTD patients correspond to those with expected neurodegeneration.

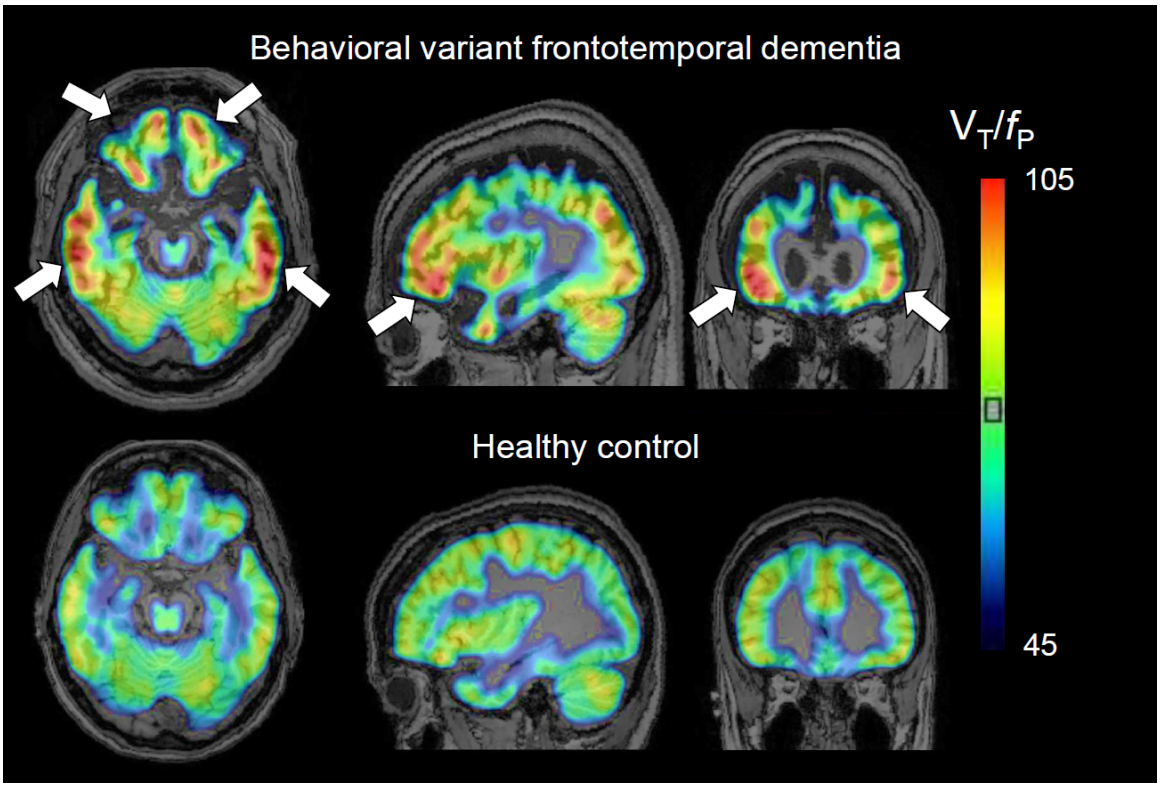

Fig 1. Representative [^{11}C]PBR28 parametric images (V_T/f_P) for a patient with behavioral variant FTD and a healthy control. Both subjects are high affinity binders (non-carriers of the rs6971 TSPO polymorphism). Arrows show increased binding in frontal and temporal cortices.

However, 1st and 2nd generation suffer from reduced uptake in a significant fraction of the human population that carry a single nucleotide polymorphism (rs6791) in the *TSPO* gene, making it difficult if not impossible to analyze scans obtained from such patients. Second, the sensitivity and specificity of 1st and 2nd TSPO radioligands is relatively poor compared to third generation radioligands. Finally, few longitudinal studies of TSPO PET imaging in patients with neurodegeneration have been performed, especially rare forms of neurodegeneration such as

FTD. Such studies are needed to determine if PET imaging of neuroinflammation may be a suitable biomarker for future treatment trials.

We recently developed a new 3rd-generation radioligand binding for TSPO, [^{11}C]ER176, which showed excellent brain uptake in eight healthy subjects as shown in Fig 2 (Ikawa *et al*, 2017). An apparently unique advantage of [^{11}C]ER176 compared to conventional TSPO radioligands is that it provides quantifiable distribution volume (V_T) regardless of single nucleotide polymorphism rs6971 in the *TSPO* gene. Specifically, with other TSPO radioligands, all subjects must be pre-screened by genetic tests, and low-affinity binders (LABs) must be excluded from PET scans because the brain has too little uptake to quantify. In contrast, a similar pre-screening and exclusion process is unnecessary for [^{11}C]ER176 because even LABs have substantial V_T values in their brain (Fig 3). It should be noted that although subjects need not be excluded a priori, the results must be corrected for genotype a posteriori. This provide particularly significant advantage in the present study, since our potential participants are those having rare disorders with severe disability, additional screening for exclusion of TSPO LABs may result in too limited number of subjects for meaningful interpretation, especially in rare disease subtypes such as FTD or in genetically-defined familial cases.

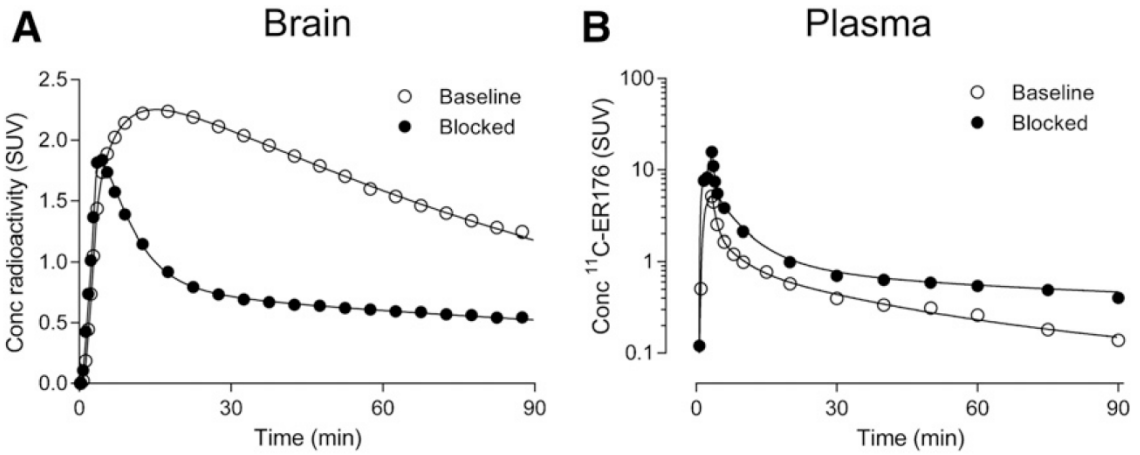

Fig 2. Brain uptake and plasma radioactivity concentrations (conc) of [^{11}C]ER176 in a representative HAB at baseline and after blockade with 90 mg of XBD173. (A) Brain time-activity curves from gray matter with unconstrained two-tissue compartment model fitting. (B) Time courses of parent concentration in arterial plasma fitted by multiplying triexponential-fitted total plasma radioactivity and sigmoid-fitted plasma parent fraction.

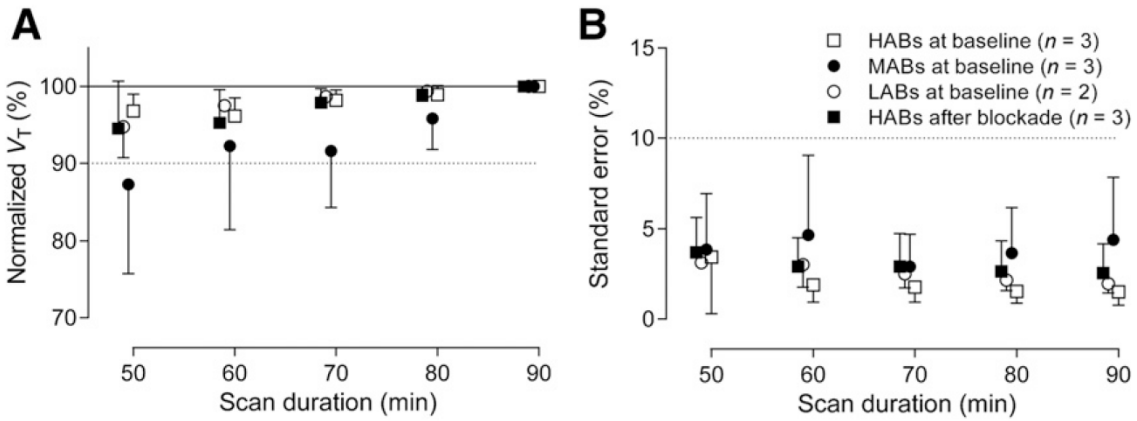

Fig 3. Time-stability analysis: V_T obtained from both baseline scans for each genotype and blocked scans for HABs, as well as its identifiability, is plotted as function of duration of image acquisition. (A) V_T was calculated for putamen using an unconstrained two-tissue compartment model with increasingly truncated acquisition times. Values are normalized as percentage of terminal value attained from 90 min of imaging. (B) Corresponding percentage SE, which is inversely proportional to identifiability, is plotted. Data are mean ± SD.

2. STUDY OBJECTIVES

a. Primary objectives

We will explore if human subjects with neurodegenerative diseases exhibit different brain uptake levels of the [^{11}C]ER176 TSPO ligand compared to control subjects.

b. Secondary objectives

We will explore if,

1) [^{11}C]ER176 TSPO brain uptake shows disease-specific patterns across different neurodegenerative diseases

2) Longitudinal imaging of individual patients shows that interval changes in tracer uptake correlate with disease progression.

3. SUBJECTS

a. Description of study populations

- Adults with a diagnosis or with an increased risk of FTD, ALS, AD, or a related adult-onset neurodegenerative disease
- Accrual ceiling of 100 patients with age-related neurodegenerative diseases or subjects with genetically increased risk of such diseases, and 100 healthy volunteers who are matched for age (± 5 years) and sex
- Dropouts will not be replaced
- NIH employees may participate if they meet the eligibility criteria, but NIMH employees/staffs or NIH employees who are subordinates/relatives/co-workers of investigators may not participate.

b. Inclusion criteria

1) Patients will be included if they

- Are age 18 or older

- Have the ability to understand and sign an informed consent, or have a DPA or a court-appointed guardian (or be able to understand the DPA process to appoint a DPA) to provide consent for adults without consent capacity
- Have been given a diagnosis by a neurologist of frontotemporal dementia, frontotemporal lobar degeneration, primary progressive aphasia, semantic dementia, motor neuron disorder, amyotrophic lateral sclerosis, primary lateral sclerosis, progressive bulbar palsy, corticobasal syndrome, Huntington disease, Alzheimer's disease, or other related adult-onset neurodegenerative disease

2) Subjects with an increased risk of neurodegenerative diseases will be included if they
   - Are age 18 or older
   - Are able to give written informed consent
   - Have known family history or other risk of an adult-onset genetic neurodegenerative disease, and/or mutation in a gene known to cause an adult-onset neurodegenerative disease
3) Healthy subjects will be included if they
   - Are age 18 or older
   - Are willing and able to complete all study procedures
   - Are able to give written informed consent
   - Are medically healthy
   - Are enrolled in 01-M-0254 "The Evaluation of Participants with Mood and Anxiety Disorders and Healthy Volunteers" (PI: Dr. Carlos Zarate) or 17-M-0181, "Recruitment and Characterization of Healthy Research Volunteers for NIMH Intramural Studies" (PI: Dr. Joyce Chung)

c. Exclusion criteria

1) Patients or subjects with an increased risk of neurodegenerative diseases will be excluded if they
   - Have other major neurological or medical diseases that may cause progressive weakness or cognitive dysfunction, such as structural brain or spinal cord disease, metabolic diseases, paraneoplastic syndromes, infectious diseases, peripheral neuropathy or radiculopathy or other significant neurological abnormalities
   - Have an unstable medical condition that, in the opinion of the investigators, makes participation unsafe (e.g., active infection or untreated malignancy)
   - Require daytime ventilator support at the time of study entry
   - Are unable to travel to NIH
   - Have recent exposure to radiation related to research (e.g., PET from other research) that, when combined with this study, would be above the allowable limits
   - Have inability to lie flat and/or lie still on camera bed for at least two hours, including claustrophobia, overweight greater than the maximum for the scanner, and uncontrollable behavioral symptoms, which will be screened by an interview with patient and/or caregiver during the screening visit
   - Are pregnant or breastfeeding

- Participants must not have substance use disorder or alcohol use disorder. However, alcohol or cannabis use by themselves are not exclusion criteria, unless that use impairs function
- Are unable to have an MRI scan (e.g., pacemakers or other implanted electrical devices, brain stimulators, dental implants, aneurysm clips (metal clips on the wall of a large artery), metallic prostheses (including metal pins and rods, heart valves, and cochlear implants), permanent eyeliner, implanted delivery pumps, or shrapnel fragments, metal fragments in the eye)
- NIMH employees/staffs or NIH employees who are subordinates/relatives/co-workers of investigators

2) Healthy subjects will be excluded if they
   - Have any history of medical illness or injury with the potential to affect study data interpretation or to be any medical contraindication to the procedures performed in the study, including active infection and untreated malignancy.
   - Have clinically significant laboratory abnormalities based on tests performed under screening protocol 01-M-0254 or 17-M-0181 and specified in Section 4.c "Screening"
   - Have recent exposure to radiation related to research (e.g., PET from other research) that, when combined with this study, would be above the allowable limits
   - Have inability to lie flat on camera bed for at least two hours, including claustrophobia and overweight greater than the maximum for the scanner
   - Are pregnant or breastfeeding
   - Participants must not have substance use disorder or alcohol use disorder. However, alcohol or cannabis use by themselves are not exclusion criteria, unless that use impairs function
   - Are unable to have an MRI scan (e.g., pacemakers or other implanted electrical devices, brain stimulators, dental implants, aneurysm clips (metal clips on the wall of a large artery), metallic prostheses (including metal pins and rods, heart valves, and cochlear implants), permanent eyeliner, implanted delivery pumps, or shrapnel fragments, metal fragments in the eye)
   - NIMH employees/staffs or NIH employees who are subordinates/relatives/co-workers of investigators

4. STUDY DESIGN AND METHODS

a. Study overview

This is a single center cross-sectional and longitudinal study in three groups of participants: patients with FTD, ALS, AD, or a related adult-onset neurodegenerative disease, subjects with an increased risk of such diseases due to disease-associated mutations, and healthy volunteers as a control group. We plan to conduct interim analysis on the cross-sectional part of this study once approximately 10-15 subjects' data each in the patient group and the control group are collected, and determine if there is significant difference of [^{11}C]ER176 brain uptake between the two groups. This will help us reviewing the initial pool of cross-sectional data and accordingly modifying plans for further cross-sectional/longitudinal data collection if necessary.

As noted above, participants must meet inclusion and exclusion criteria and sign an informed consent document. All participants will undergo brain PET scan with [^{11}C]ER176 and brain MRI. Brain PET scan with [^{11}C]PIB will be done in all participants except for those whose diagnosis has been confirmed by genetic testing. [^{11}C]PIB PET will be obtained for differentiation of preclinical or atypical AD from normal aging or FTD/ALS-related neurodegenerative diseases, since AD-spectrum disorders have shown to be significantly overlapped in both populations (Rabinovici *et al*, 2011; Vlassenko *et al*, 2011). Brain MRI will be obtained for co-registration of the PET images to identify the anatomical structures, and the results could provide additional information such as atrophy pattern which is useful in differential diagnosis.

Brain PET scans will be performed with vital sign monitoring. The [^{11}C]ER176 PET scan will be associated with arterial sampling for the input function, but in patients who are unable to or refuse to undergo arterial sampling, the scan may be done with venous sampling or without any blood sampling. To develop a substitutional analysis method for those PET data without arterial sampling, a few venous samples will be additionally obtained in some subjects who undergo arterial sampling during [^{11}C]ER176 PET scan. No arterial input function will be taken for [^{11}C]PIB PET scan. The brain MRI will be obtained before or after the PET scan, and can be performed on the same day. The completion of each PET and MRI will take approximately two and one hours, respectively.

Number of visits and time commitment of participants

The initial visit will include the general medical history and examination, neurological examination, neuropsychological evaluation, and blood sampling for rs6971 TSPO polymorphism genotyping. In the event that the subject has had a recent general medical history and examination, neurological examination, neuropsychological evaluations, or adequate brain MRI as part of enrollment in other protocols, these portions of the evaluation will not be duplicated. Brain MRI, [^{11}C]ER176 PET, [^{11}C]PIB PET, and neuropsychological evaluation may be scheduled on separate days from the initial visit, depending on the schedule of the examiner and the radiology and PET departments. Two PET scans may occur on the same day and, if so, be separated by at least 2.5 hours to allow for decay of the first injection ($T_{1/2}$ = 20 min). All participants including healthy subjects may be asked to return to NIH after an interval of at least six months (but no more than 5 years) to repeat the clinical evaluations, MRI, and PET scans as shown in Table 1. Therefore, this protocol initially requires two to five visits for the first evaluation, and additional visits for the 2nd, 3rd, and 4th evaluations are helpful for the study but not necessary for initial enrollment. For patients and newly diagnosed patient participants returning for each follow-up evaluation, the capacity assessment by study team investigators will be repeated and the consent will be re-signed.

All procedures and their associated exposure to radiation are for research purposes only.

Table 1. Number of visits/evaluations and time commitment of participants

	1st evaluation (0-6 months)		2nd evaluation (6-18 months)		3rd evaluation (18-30 months)		4th evaluation (30-60 months)	
	1st Visit Screen	2nd Visit	3rd Visit	4th Visit	5th Visit	6th Visit	7th Visit	8th Visit
Capacity assessment in patients' group and informed consent	X		X		X		X	
History and physical exam	X		X		X		X	
Lab tests*	X	X	X	X	X	X	X	X
Neuropsych testing**	X		X		X		X	
MRI **	X		X		X		X	
Brain PET**		X		X		X		X

* Blood sampling for the rs6971 polymorphism genotyping will be done during the 1st evaluation. For women of child-bearing potential, a urine pregnancy test will also be done within 24 hours before PET ligand administration and MRI scan.
** Neuropsychological testing this may require an additional visit depending on the schedule of the examiner or condition of the subject. Depending on the availability of PET and MRI scanners, an MRI scan may be scheduled at any point in time for this protocol, either before or after the PET procedures, which may require an additional visit. If [^{11}C]ER176 PET and [^{11}C]PIB PET are conducted on different days, this may require an additional visit. Therefore, as a maximum five visits may be required in each evaluation period, and up to twenty visits may be required in 5 years (not shown on above table).

b. Recruitment

Letters will be sent to clinicians who see patients with dementia or motor neuron disease and to Dementia or ALS clinics with information for referring patients to the study. A brochure with a lay description of the protocol and contact information for the study team will be provided to physicians to give to their patients. The brochure may be sent electronically to those requesting study information. The printed brochure will be used in color as submitted or may be printed in black and white. The color of the brochure may vary. Color changes will not be used to change the emphasis of the brochure. The size of the brochure may vary, but all parts of the brochure, including fonts and pictures, will be changed proportionately to the rest of the brochure. Disproportionate changes in size will not be used to change the emphasis.

The protocol and the contact for further information will be listed on www.clinicaltrials.gov and a description of the protocol will be included on the NINDS intramural clinic page. Links to the clinical trials listing or protocol description page may be posted electronically on websites such as those of the ALS Association, the Muscular Dystrophy Association, The ALS Therapy Development Initiative, ALS worldwide, the Northeast ALS consortium, the Association for FTD, and CurePSP.

An advertisement describing the protocol will be posted on ResearchMatch.org, a national electronic, web-based recruitment tool that was created through the Clinical & Translational Science Awards Consortium in 2009 and is maintained at Vanderbilt University.

An advertisement will be provided to the Agency for Toxic Substances and Disease Registry (ATSDR) who will use a notification tool that will allow persons with ALS (PALS) enrolled in the National ALS Registry to find out about this study (through www.CDC.gov).

Flyers (with tabs) for healthy volunteers may be posted electronically on websites, such as NIH or the other sites' hospital websites, libraries, nursing homes and other advocacy groups and may be sent electronically to those requesting study information.

Recruitment strategies will also include use of Listservs, Craig's List, advocacy websites, resource listings or other website postings and Study-specific page on the NIMH-IRP: Join A Study Website with URL (placeholder). Short text descriptions of this study will also appear on Facebook. Facebook posts will be made only via official NIMH accounts. Associate Investigators and members of the NIMH Marketing & Community Relations Unit will distribute recruitment materials.

All recruitment materials, including listserv notices, social media, letters to physicians, recruitment website content, and other venues will be approved by the IRB prior to use.

Some patients may be recruited under 17-N-0131, "Investigating Complex Neurodegenerative Disorders related to amyotrophic lateral sclerosis and frontotemporal dementia" (PI: Dr.Justin Kwan), or 00-N-0043, "Clinical and molecular manifestations of inherited neurological disorders" (PI: Dr. Kenneth Fischbeck). In this case, the similar pre-screening/screening processes or study procedures such as neurological examinations and neuropsychological testing that have been recently done will not be duplicated in this study.

Healthy volunteers will be recruited under 01-M-0254, "The Evaluation of Participants with Mood and Anxiety Disorders and Healthy Volunteers" (PI: Dr. Carlos Zarate) or 17-M-0181, "Recruitment and Characterization of Healthy Research Volunteers for NIMH Intramural Studies" (PI: Dr. Joyce Chung), and not under the current protocol. NIH Employees/staff will not be directly recruited by or through their supervisors or co-workers to participate in this study.

c. Screening

Consent will be obtained before any study procedures, including screening procedures, are done.

In patients with neurodegenerative diseases or subjects with an increased risk of neurodegenerative diseases, demographics, history of present illness, family history of neurological disorders, history of medications or surgeries/implants, general medical examination, neurological examination, and laboratory tests will be performed by a study clinician. Patients will be evaluated with their potential tolerability and willingness for study procedures such as arterial line and lying still in PET scanner, and their caregivers will be also interviewed to provide information about tolerability. If patients have had overlapping recent evaluations from other NIH protocols or from outside hospitals and the records are available, duplicate evaluations will not be performed as part of this study.

Healthy volunteers will be screened under protocol 01-M-0254, "The Evaluation of Participants with Mood and Anxiety Disorders and Healthy Volunteers" (PI: Dr. Carlos Zarate) or 17-M-0181, "Recruitment and Characterization of Healthy Research Volunteers for NIMH Intramural Studies" (PI: Dr. Joyce Chung). Basic screening in healthy volunteers will include physical exam, medical and psychiatric history, and laboratory tests.

Laboratory tests for screening include CBC; acute care panel; hepatic panel; mineral panel; urinalysis; urine drug screen; urine pregnancy test (females); vitamin B12, folate, and lipid panel; hepatitis panel (A, B, C); HIV & syphilis screening test; total protein; uric acid; creatine kinase; cholesterol; thyroid panel; prothrombin and partial prothrombin tests; and EKG. The radial artery pulse is checked for the presence of adequate ulnar collateral flow and the absence of any metals or foreign objects in bilateral wrists. Screening results will be reviewed by a clinically credentialed investigator before the subject undergoes any specific study procedures.

d. Study procedures

1) Neuropsychological testing

All participants will undergo neuropsychological testing to evaluate their cognitive or behavioral functionalities. Such testing may include mini-mental status examination, Montreal Cognitive Assessment, and Clinical Dementia Rating Scale, as well as more comprehensive neuropsychological testing.

If patients have had the similar recent neuropsychological testing from other NIH protocols or from outside hospitals and the records are available, duplicate evaluations will not be performed as part of this study.

2) Genetic testing

Blood tests for genotyping the rs6971 polymorphism within the TSPO gene on chromosome 22q13.2 will be performed in all participants, and the results will be used for analyzing each individual's [^{11}C]ER176 PET data.

3) Brain MRI

A brain MRI will be obtained for anatomic localization and will be performed on a 3 Tesla scanner located at the NIH Clinical Center (Bethesda, Maryland) and will take about one hour. Subjects will undergo safety screening prior to the MRI to rule out contraindications such as cardiac pacemaker. If an adequate brain MRI has been

performed within the last 6 months at the NIH, we will not perform a duplicate MRI scan and their earlier MRI will be used for anatomical localization.

Pregnancy tests: For women able to become pregnant, urine pregnancy testing will be done within the 24 hours prior to any MRI or PET scan. If the pregnancy test is positive, PET and MRI will not be done, and the subject will be taken off the protocol.

4) PET Procedures

i. Radioligands

[^{11}C]ER176 and [^{11}C]PIB will be prepared according to the IND and administered via an indwelling intravenous catheter over approximately one to three minutes.

ii. Insertion of the intravenous line

An intravenous line is placed in the arm. The venous line is used for the injection of radioligand and is removed at the end of the day. In some subjects, additional intravenous line may be placed and used for venous sampling during the PET scan. This venous samples will be used for developing substitutional analysis method for PET data obtained without arterial sampling. We will evaluate during the screening visit whether a subject could tolerate a second venous line or not, and the option is given as a checkbox in consent forms.

iii. Arterial Line Placement

Arterial line may be placed for [^{11}C]ER176 scan, but not for [^{11}C]PIB scan. After the presence of adequate ulnar collateral flow has been confirmed, a radial artery catheter will be inserted by the Anesthesiology Department or the Vascular Access Department. Before catheter insertion, the skin at the puncture site will be locally anesthetized. If there is difficulty in placing arterial line due to patient's poor tolerability, medical contraindication, or other reasons, the [^{11}C]ER176 scan may be done with venous sampling or without any blood sampling. We will evaluate during the screening visit whether a subject could tolerate an arterial line or not, and the option is given as a checkbox in consent forms.

iv. PET scan

One [^{11}C]ER176 PET and one [^{11}C]PIB PET will be performed in each evaluation period of each subject. The two scans may occur on the same day or on different days. Brain PET imaging will be performed using a PET or PET/CT scanner for up to two hours. Participants will be placed on the scanner bed with their head held firmly in place fixed to the bed. A 68Ge, 137Cs, or CT transmission scan will be performed to measure and correct for attenuation. Tracer infusions will be performed when the subject is already on the scanner bed. After an intravenous bolus of up to 20 mCi, blood samples may be drawn from the arterial and/or venous catheter during the [^{11}C]ER176 PET scan. We will collect about 15-25 arterial samples and/or about 4-6 venous samples. The arterial sampling will be initially performed continuously at earlier time points and discretely at later time points, but this plan can be modified if required for achieving better quality of data. The total amount of blood sampling volume will be about 150 mL in each brain scan. [^{11}C]PIB PET scan will be done without arterial sampling. PET images will be acquired in three-dimensional mode with increased length of frame for a total of approximately two hours. If the scan lasts more than two hours, the subject may

be offered a break out of the camera for approximately 15 minutes. Vital signs (blood pressure, pulse, and respiratory rate) and EKG (either 3- or 12-lead) will be recorded no more than three hours before injection, and again about 15, 30, and 120 minutes after tracer injection. After the scan, the arterial and/or venous lines will be removed and the subject will be instructed to void frequently to minimize radiation exposure.

Pregnancy tests: For women able to become pregnant, urine pregnancy testing will be done within the 24 hours prior to any MRI or PET scan. If the pregnancy test is positive, PET and MRI will not be done, and the subject will be taken off the protocol.

v. Follow-up procedures

We will offer an opportunity to all participants to return for repeat examination, neuropsychological testing, MRI, and PET imaging. Such repeat evaluations may occur at 6-18 months, 18-30 months, and 30-60 months after initial examination.

vi. Relationship to other protocols

There is no specific relationship of this protocol to other protocols, although patients may be referred to other NIH protocols if they are eligible. Examples of current protocols that patients may be referred to include 13-N-0188: Natural History and Biomarkers of C9orf72 ALS and FTD and 15-N-0126: HERV-K Suppression Using Antiretroviral Therapy in Volunteers with Amyotrophic Lateral Sclerosis (ALS). If patients are willing, we will maintain their information in a registry to allow re-contact and referral to future protocols on adult-onset neurodegenerative disorders.

e. End of participation

Results of clinical testing, neurological examination, and brain MRI may be shared with patients and referring physicians through the CRIS portal.

Participants will not be told the result of [^{11}C]ER176 PET scan since it is difficult to interpret. But the result of [^{11}C]PIB PET scan may be given if the participant wanted and agreed to receive at the consenting process. None of participants will be informed of genotyping results of rs6971 TSPO polymorphism since the clinical significance is unknown in any population. Such practice is typical for participants undergoing PET studies at the NIMH, since these scans are performed for research only, and not routine medical care. Eligible subjects may be offered enrollment in other protocols at the NIH. No treatment will be offered.

Patients will return to the referring health care provider for treatment and long-term management between evaluations or after completion of this protocol. Patients who do not have an appropriate health care provider will be referred to one in the community.

5. MANAGEMENT OF DATA AND SAMPLES

a. Storage

We will follow NIH guidelines to prevent identification of study participants and other violations of subject confidentiality. Information will be stored using a confidential case number, and no identifiers (name, address, phone number, etc.) will be used that could allow direct linking of database information to individual subjects. Secure e-mail will be used for all electronic communications of subject information between

investigators. Demographic and clinical data will be archived on a password-protected server.

All diagnostic information collected from patients will be recorded in the medical record and CRIS system according to the policies of the NIH Clinical Center Medical Records Department. Research data will be stored on secure servers or in locked cabinets in a room that is kept locked when unoccupied. Blood samples will be stored in locked freezers in laboratories of protocol investigators. All specimens will be labeled with a code that does not include patient identifiers.

Protocol investigators will have access to all clinical data. Laboratory staff of the protocol investigators will have access to coded specimens. All imaging data will be stored on the NIMH server under password-protected accounts accessible only to the principal investigator and directly involved study personnel to preserve subject privacy. All data are regularly backed up, either by the NIMH system administrator or by NIMH CIT personnel.

Any loss or destruction of samples will be reported to the IRB.

Upon termination of the protocol, data will be deposited in a repository protocol to facilitate future data sharing according to the initial protocol consent. Unused samples will be destroyed.

b. Data and sample sharing plan

This protocol is not subject to the Genomic Data Sharing (GDS) policy. It does not generate "large-scale" human genomic data as defined in the NIH Supplemental Information to the GDS Policy. Image and other research data including genetic results will be shared with collaborating laboratories outside of NIH: Banner Alzheimer's Institute (Phoenix, AZ) under FWA #00002630, Memory and Aging Center, University of California San Francisco (San Francisco, CA) under FWA #00000068, and Georgetown University (Washington, DC) under FWA #IORG0000193.

Data may be shared with collaborating laboratories at the NIH or outside of the NIH and/or submitted to NIH-designated repositories and databases if consent for sharing was obtained. Repositories receiving data from this protocol may be open-access or restricted access.

Data will be stripped of identifiers and may be coded ("de-identified") or unlinked from an identifying code ("anonymized"). When coded data are shared, the key to the code will not be provided to collaborators, but will remain at the NIH. Data may be shared with investigators and institutions with an FWA or operating under the Declaration of Helsinki (DoH) and reported at the time of continuing review. Sharing with investigators without an FWA or not operating under the DoH will be submitted for prospective IRB approval. Submission to NIH-sponsored or supported databases and repositories will be reported at the time of the Continuing Review. Submission to non-NIH sponsored or supported databases and repositories will be submitted for prospective IRB approval.

Required approvals from the collaborating institution will be obtained and materials will be shipped in accordance with NIH and federal regulations.

6. ADDITIONAL CONSIDERATIONS

a. Research with investigational drugs or devices

[^{11}C]ER176 will be prepared as described previously under IND 122236 held by NIMH IRP. [^{11}C]PIB will be prepared under IND 108861 held by NIMH IRP. All these PET radioligands will be synthesized in recently re-established NIMH cGMP facility.

b. Gene therapy

Not applicable.

7. RISKS AND DISCOMFORTS

Risks include those associated with: a) neurological and neuropsychological examinations, b) genetic tests for rs6971 TSPO polymorphism, c) MRI, d) placement of a venous and an arterial line, e) arterial blood sampling, f) radiation exposure from [^{11}C]ER176, [^{11}C]PIB, and the transmission scan, and g) PET scanning.

a. Neurological and neuropsychological examinations

The clinical examinations and ratings may be tiring. The neuropsychological tests are not harmful, but may be frustrating or stressful. If patients do not wish to do a test, that test will not be administered.

b. Genetic test for rs6971 TSPO polymorphism

Venous blood sampling for the genetic test is associated with only minimal risk. There is usually some discomfort when the needle is inserted for phlebotomy.

The result of TSPO polymorphism will be used only for later PET data analysis, and not shared with study participants or their family. Since the clinical significance of TSPO polymorphism is unknown other than different level of binding affinity to PET radioligands, the risk would be low even if the genetic information happens to be reidentified.

c. MRI

People are at risk for injury from the MRI magnet if they have pacemakers or other implanted electrical devices, brain stimulators, some types of dental implants, aneurysm clips (metal clips on the wall of a large artery), metallic prostheses (including metal pins and rods, heart valves, and cochlear implants), permanent eyeliner, implanted delivery pump, or shrapnel fragments. Welders and metal workers are also at risk for injury because of possible small metal fragments in the eye of which they may be unaware. Subjects will be screened again for these conditions before having any scan, and if they have any, they will not receive an MRI scan.

It is not known if MRI is completely safe for a developing fetus. Therefore, all women of childbearing potential will have a urine pregnancy test performed no more than 24 hours before each MRI scan. The scan will not be done if the pregnancy test is positive.

People with fear of confined spaces may become anxious during an MRI. Those with back problems may have back pain or discomfort from lying in the scanner. The noise from the scanner is loud enough to damage hearing, especially in people who already have hearing loss. Everyone having a research MRI scan will be fitted with hearing protection. Subjects will be asked to complete an MRI screening form for each MRI scan they have. There are no known long-term risks associated with MRI scans.

d. Placement of a venous and/or an arterial line

Venous catheter insertion can be associated with discomfort, bruising, infection, or clot formation. Using proper placement techniques will minimize these risks. In case of tracer extravasation, we will stop the study, remove the venous line from the arm, and apply cold to the site.

Arterial catheterization has been shown to be a generally safe and reliable method of obtaining arterial blood samples (Lockwood, 1985). Placement of a radial arterial catheter may cause bruising or infection. There is also a risk of occlusion and microemboli. Over 3,000 arterial catheters have been placed to date in the PET Department. Of these, only two complications requiring physician's care arose. In the first case, a small radial artery aneurysm developed several months later, which was successfully repaired surgically. In the second case, a radial artery thrombosis developed 28 days later, which was also successfully repaired surgically.

e. Blood sampling

Participants may have arterial (or venous) blood sampling. The total amount of blood drawn will not exceed 400 mL. Blood sampling may lead to the formation of small subcutaneous hematomas caused by blood leaking from a punctured blood vessel. Such hematomas cause only minor discomfort. They are not dangerous and require no treatment other than reassuring the patient. There is also a small risk of infection at the site of the needle puncture, which can be readily treated with antibiotic therapy. We will ask participants not to donate blood within 8 weeks prior to the study or for 8 weeks following the study.

f. Radiation exposure risks

Radiation exposure in this protocol will be from [^{11}C]ER176, [^{11}C]PIB, and the associated transmission scans.

In our previous study with [^{11}C]ER176, we calculated the radiation exposure of the radioligand from whole-body imaging in nine healthy subjects (Ikawa *et al*, 2017). The radiation exposure (rem) from a 20 mCi injection was: effective dose (0.30), with three highest organs kidneys (1.06), spleen (1.00), and lungs (0.77).

According to a published data with [^{11}C]PIB, the radiation exposure (rem) from a 10 mCi injection is: effective dose (0.18), with three highest organs gallbladder wall (1.54), liver (0.70), and urinary bladder wall (0.61) (Scheinin *et al*, 2007).

With regard to exposure from the transmission scan, the PET Department recently implemented Dr. Innis's suggestion to decrease the current (amperage) and, thereby, the radiation from the CT. We do not need a high resolution (high current) image for attenuation correction; a low resolution, like that from a line source, is perfectly adequate to correct attenuation in the PET emission scan. With the lowered current, the exposure to the lens of the eye is now 0.26 rem, about 1/3 of the previous value.

We wish to maintain flexibility in the choice of PET cameras. Among the various PET cameras, PET/CT has the highest exposure. In addition, we routinely include the dose from two transmission scans in the event that it must be repeated in a subject. The effective doses for two head transmission scan from a PET/CT are ~0.04. Thus, the total effective dose in each [^{11}C]ER176 PET scan is 0.34 rem, and each [^{11}C]PIB PET scan is 0.22 rem. Each subject will undergo one [^{11}C]ER176 and one [^{11}C]PIB PET scans and the total effective dose in six months is 0.56 rem, and may be 1.12 rem in a year if the subject is re-evaluated

within a year after the first evaluation. If a subject is fully re-evaluated following the schedule in Table 1 after one year, two years, and three to five years from the first evaluation, the total effective dose is 2.24 rem in five years, which is well below the limit of 5 rem per year established by NIH's Radiation Safety Committee. All subjects will be asked about any prior research participation involving radiation exposure so that the total exposure, in combination with the present study, will not exceed an effective dose of 5 rem per year.

g. PET scan

PET scans, which detect injected radioactivity within the body, are not associated with any known physical hazards to the subject lying on the table. We routinely use a series of procedures to minimize the risk of discomfort during scanning sessions. Namely, the procedures are conducted in the presence of trained health professionals to whom participants will have ready access should they experience any problems. Participants can communicate with the trained health professionals while in the scanner and can be removed from the scanner and withdraw from the study at any time if they wish to do so. Participants can also request that the operator stop the scan.

8. SUBJECT SAFETY MONITORING

a. Parameters to be monitored

The subjects will be evaluated by a physician upon entry into the protocol and monitored by a physician or nurse practitioner throughout their participation in the protocol, including during the PET procedure. Pulse rate, blood pressure, respiratory rate, and EKG (either 3- or 12-lead) will be recorded within three hours before tracer injection and again at about 15, 30, and 120 minutes after injection. Laboratory tests including CBC, blood chemistry, and urinalysis will be done before and after tracer administration to monitor for changes.

A credentialed, licensed independent practitioner will be present during the neurological examination. Neuropsychological testing will be carried out by trained evaluators.

Testing will be performed in the NIH Clinical Center. It is anticipated that most patients will be evaluated as outpatients, but occasionally patients may be admitted to the inpatient ward for convenience. Testing will be done in outpatient clinic areas, Neurotesting Unit, day hospital, or NMR center with trained medical personnel and emergency equipment immediately available.

Procedures may be stopped for any of the following reasons:

1) The patient may request to have the procedure stopped.
2) In the investigator's judgment, continuing the procedure would be detrimental to the patient's health and well-being.

b. Toxicity criteria

Toxicity will be assessed according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) v.4.03 http://evs.nci.nih.gov/ftp1/CTCAE/About.html.

c. Criteria for individual subject withdrawal

A patient may be taken off the protocol for any of the following reasons:

1) The patient may request to withdraw from the protocol at any time they wish.
2) In the investigator's judgment, continued participation in the study would be detrimental to the patient's health and well-being.
3) The subject fails to comply with the requirements of the protocol.

4) The subject's performance on testing is unreliable or they cannot be validly tested, for example, if their neurological status changes in such a way as to make interpretation of the neurological cause of their impairment impossible.
5) The patient becomes too disabled to travel to NIH for the follow-up evaluation testing.
6) If a patient no longer has capacity to give consent and does not have a suitable DPA.

9. OUTCOME MEASURES

a. Primary outcome measures

Difference of [^{11}C]ER176 brain uptake between healthy controls and neurodegeneration subjects.

b. Secondary outcome measures

1) Disease-specific patterns of [^{11}C]ER176 TSPO brain uptake across different neurodegenerative diseases.

The clinical phenotype of individuals with neurodegenerative diseases differs based on disease subtype, which in turn reflects differences in regional neurodegeneration across disease subtypes. For example, patients with AD usually exhibit prominent degeneration in the parietal cortex and hippocampus, individuals with FTD usually exhibit degeneration in the frontal and/or temporal lobes, and individuals with ALS exhibit motor cortex degeneration. Because previous studies with 1st and 2nd generation TSPO ligands have shown anatomical correlation between areas of cortical neurodegeneration and increased TSPO ligand uptake, we anticipate that our 3rd generation TSPO ligand will reveal anatomical differences in ligand uptake across different neurodegenerative diseases. We will quantify TSPO signals in pre-selected brain regions (for example, parietal cortex, temporal cortex, frontal cortex, motor cortex) in each individual, and compare regional TSPO uptake across different brain regions as a function of disease subtype. Voxel-based morphometry analysis of cortical neurodegeneration will be used to determine if TSPO uptake correlates anatomically with regional neurodegeneration.

2) Longitudinal imaging of individual patients shows a correlation between interval change of tracer uptake and disease progression.

10. STATISTICAL ANALYSIS

a. Analysis of data/ study outcomes

PET and MRI scans will be coregistered for anatomic definition of regions of interest. SUV will be calculated in various brain regions. Parametric images will be created using PMOD software (PMOD Technologies Ltd., Zurich, Switzerland). Brain imaging data and/or blood data will be analyzed with compartmental modeling to achieve quantitation of each radioligand's target binding (e.g. V_T, V_T/f_P, SUV ratio) using PMOD software. Cross-sectional or longitudinal group comparison and correlation analysis with other clinical parameters will be conducted in each brain region using either regions-of-interest or voxel-based image analysis method. Statistical analyses will include ANCOVA for cross-sectional comparison among groups, and multilevel mixed model for longitudinal comparison among groups.

b. Power analysis

This study is exploratory and we do not know how much would [^{11}C]ER176 brain uptake be different between dementia patients and healthy subjects. Thus, a formal power calculation is impossible. We request accrual ceiling of 100 subjects with a diagnosis or with an increased risk of an age-related neurodegenerative diseases and 100 age and sex-matched healthy volunteers. Dropouts will not be replaced.

With regard to the interim analysis in cross-sectional part of this study, we plan to collect approximately 10-15 subjects in each of two groups: healthy controls and patients with neurodegenerative diseases. In our previous data from five FTD patients and control subjects scanned with [^{11}C]PBR28, which is an older generation TSPO radioligand, the estimated effect size was 0.50-0.65 in ANCOVA with TSPO genotype and demographic factors as covariates at a significance level of 0.05. If we assume [^{11}C]ER176 has the same effect size and variability with [^{11}C]PBR28, 10-15 subjects in two groups could achieve 79-93% power in the same statistical condition by G*Power program (v 3.0, University of Düsseldorf, Germany).

Power calculation for the longitudinal study is rather limited because we don't have any longitudinal preliminary data in FTD patients. If we assume that longitudinal [^{11}C]ER176 uptake change in FTD is almost similar to [^{11}C]PBR28 uptake change in AD (approximately 20% increase within 2.7 years) (Kreisl *et al*, 2016), a sample size of 100 could achieve 72-99% power at a significance level of 0.05 in a multilevel mixed model with 4 repeated measures in 5 groups (3 subgroups of FTD, AD, and healthy control group) by GLIMMPSE Software v 2.0.

11. HUMAN SUBJECTS PROTECTION

a. Subject selection

The selection of subjects will be based on the inclusion and exclusion criteria. Although we will attempt to recruit minority populations and women, ALS and FTD are slightly more prevalent in males than females. The frequency of familial neurodegenerative disorders occurs with different frequencies in some ethnic populations. For example in the US, ALS is more common in Caucasian populations (Garcia-Redondo *et al*, 2013; Jang *et al*, 2013; van der Zee *et al*, 2013). Thus, we anticipate inequities in the distribution of race and ethnicity compared to the general population because this reflects the disease predilection.

b. Justification for exclusion of children

Children will be excluded because this study focuses on adult onset neurodegenerative disorders. Although children under the age of 18 may be carriers of genes for neurodegenerative disorders, they are unlikely to be symptomatic.

c. Justification for inclusion of other vulnerable subjects and Justification for exclusion of other vulnerable subjects

Vulnerable subjects who exhibit or develop symptoms of cognitive, neurological, and/or behavioral dysfunction will be included in this study, because these are a hallmark of the diseases under study.

Protections per Policy 404 will be observed for NIH employees who meet the eligibility criteria. NIMH employees/staff and their immediate family members will be excluded from the study per NIMH policy.

Pregnant women will be excluded because this protocol involves exposure to ionizing radiation. Lactating women will be excluded because radioisotopes may be excreted in milk.

d. Safeguards for vulnerable populations and sensitive procedures

Some patients who will be enrolled in this protocol may be cognitively impaired and unable to give fully informed consent. The patient's ability to understand will be assessed by asking questions about the protocol. If the assessment indicates lack of capacity for consent, and the patient already has a DPA or a court-appointed guardian or wishes to designate a

DPA to continue in the protocol, consultation with the Human Subjects Protection Unit (HSPU)/Ability to Consent Assessment Team (ACAT) will be obtained to determine the suitability of the DPA or the court-appointed guardian. If the DPA/court-appointed guardian is determined to be acceptable, consent will be obtained from the DPA/court-appointed guardian and the patient will be asked to give assent to continue in the study. If the patient does not have capacity to consent and does not have an acceptable DPA/court-appointed guardian, they will be dropped from the study.

Patients will be assessed for their ability to tolerate scanning procedures by interviews with patients/caregivers during the screening process.

Pregnancy testing will be performed before PET and MRI scanning for any participants of child-bearing potential.

Protections per policy 404 will be followed for NIH employees and staff participating in this study.

12. BENEFIT

a. Benefit

This study offers no direct benefit to individual subjects but will lead to generalizable knowledge about RA and myositis, and development of novel inflammatory biomarkers.

13. CONSENT DOCUMENTS AND PROCESS

a. Designation of those obtaining consent

All study investigators obtaining informed consent have completed the NIMH HSPU 'Elements of Successful Informed Consent' training.

b. Consent procedures

All participants will receive a verbal explanation in terms suited to their comprehension of the purposes, procedures and potential risks of the study and of their rights as research participants. Participants will have the opportunity to carefully review the written consent form and ask questions regarding this study prior to signing.

Each subject, and their DPA/court-appointed guardian when applicable, will receive an oral and written explanation of the purposes and potential risks of participation in this protocol. Specifically, they will be told that (a) the information derived may eventually lead to better understanding of their illness; (b) some PET imaging as used in this study might offer diagnostic interpretation, which will not be disclosed; (c) a confidential code number will be used to ensure that information cannot be linked or traced to any person or family; (d) data will be used for research purposes only; and (e) subjects will be given ample opportunity to ask questions of the study investigators.

If, in the opinion of the study staff, PI, or subject, the study participation is adversely affecting the subject's emotional and or physical well-being, the individual circumstances will be reviewed to determine what additional steps should be taken, such as termination of the study and making appropriate referrals to address their underlying health problems. If the subject desires not to proceed further with testing, we will end these sessions at any time point.

FTLD, AD, and other related neurodegenerative diseases may impair cognition and/or behavior and therefore, as a function of their underlying illness, some patients may not have the ability to consent for research. We do not wish to exclude such patients from this study because they are representative of the patient population.

Study team investigators will review the informed consent document with the patient followed by a quiz to determine understanding, reasoning, and choice. If the patient does not pass the capacity quiz and he/she already has a Durable Power of Attorney (DPA) or a court-appointed guardian, appropriateness of the DPA as a Legally Authorized Representative (LAR) for research participation will be assessed by the Human Subjects Protection Unit (HSPU)/Ability to Consent Assessment Team (ACAT), while the appropriateness of a court-appointed guardian as a LAR would have been already established in a court of law. Once appropriateness is established, the LAR will sign the consent form and the patient will sign the assent form.

In the patients who does not pass the capacity quiz and who do not have a DPA or a court-appointed guardian, but who are capable of understanding the DPA process when assessed by HSPU/ACAT, he/she may assign a DPA to authorize the individual's research participation provided HSPU/ACAT finds the DPA is appropriate. In this case, the assigned DPA will sign the consent form and the patient will sign the assent form.

Some subjects who pass the capacity quiz during the first evaluation may be at high risk for losing the capacity to consent in future evaluations. These subjects will be asked to sign the consent form during the first evaluation. Additionally, during the first evaluation, we will also encourage these subjects to complete a Research Advance Directive so we have it available should they lose capacity to consent during future evaluations. Besides this, if any questions remain even after a subject has passed the capacity quiz, the HSPU/ACAT will be consulted.

The capacity assessment by study team investigators will be repeated and the consent will be re-signed for patients and newly diagnosed patient participants returning for the 2nd, 3rd, or 4th evaluation (see Table 1 under Study Design and Methods section).

In the case that the patient does not have a DPA or a court-appointed guardian, lacks the capacity to provide informed consent, and lacks the ability to fill out an NIH Advance Directive and assign a holder of a DPA, then the patient will not be included in the study.

In all cases, the HSPU/ACAT will monitor the consenting and/or assenting processes.

c. Consent documents

The consent forms contain all required elements. Four different consent forms are submitted with the present protocol: healthy volunteers, patients with neurodegenerative diseases, and subjects with increased risk of neurodegenerative diseases.
An assent document is appended for patients without capacity for consent.

14. DATA AND SAFETY MONITORING

a. Data and safety monitor

Data and safety will be monitored by an independent safety monitor (ISM) for this study: Dr. Bryan Smith, MD. Dr. Smith is a board-certified neurologist with full clinical privileges at the NIH Clinical Center.

b. Data and safety monitoring plan

The PI will prepare a report on data and safety parameters for the Independent Monitor approximately every 12 months. The Independent monitor will provide a written monitoring report to be submitted to the IRB at the time of continuing review.

c. Criteria for stopping the study or suspending enrollment or procedures

In the event of a serious adverse event related to the research, or if new data shed light on the danger of any procedures used, the study team, including the PI, will suspend further testing until the IRB and investigators have reviewed the safety information and determined whether to continue the study.

15. QUALITY ASSURANCE (QA)

As per ICH-GCP 5.18 and FDA 21 CFR 312.50 clinical protocols are required to be adequately monitored. Monitoring for the NIH site will be conducted according to the "NIMH Intramural Program Guidelines for Monitoring of Clinical Trials". Monitors under contract to the NIMH OCD ORO will visit the NIH site to monitor aspects of the study in accordance with the appropriate regulations and the approved protocol. The objectives of a monitoring visit will be: 1) to verify the existence of signed informed consent documents and documentation of the ICF process for each monitored subject; 2) to verify the prompt and accurate recording of all monitored data points, and prompt reporting of all SAEs; 3) to compare abstracted information from clinical databases (e.g. CTDB) with individual subjects' records and source documents (subjects' charts, laboratory analyses and test results, physicians' progress notes, nurses' notes, and any other relevant original subject information); and 4) to help ensure investigators are in compliance with the protocol. The monitors also will inspect the clinical site regulatory files to ensure that regulatory requirements (Office for Human Research Protections-OHRP), FDA, and applicable guidelines (ICH-GCP) are being followed. During the monitoring visits, the investigator (and/or designee) and other study personnel will be available to discuss the study progress and monitoring visit.

The investigator (and/or designee) will make study documents (e.g., consent forms, clinical database records and pertinent hospital/sources or clinical records readily available for inspection by the local IRB, FDA, the site monitors, and the NIMH staff for confirmation of the study data.

A specific protocol monitoring plan will be discussed with the Principal Investigator and study staff. The plan will outline the frequency of monitoring visits based on such factors as study enrollment, data collection status and regulatory obligations.

16. REPORTING OF UNANTICIPATED PROBLEMS, ADVERSE EVENTS AND PROTOCOL DEVIATIONS

Reportable events for this protocol will be tracked and reported in compliance with Policy 801.

a. For Research Radioligand Studied under IND

The PI will report SAEs according to the requirements of 21 CFR 312.64(b). The PI will record nonserious AEs.

17. ALTERNATIVES TO PARTICIPATION

Subjects do not receive any treatment in this study or forego any treatment in order to participate in this study. The alternative, therefore, is not to participate.

18. PRIVACY

All research activities will be conducted in as private a setting as possible.

19. CONFIDENTIALITY

a. For research data and investigator medical records

Data will be kept in password-protected computers. Only study investigators will have access to the data. This study collects sensitive medical information. The PI will train study staff regarding obtaining and handling potentially sensitive and private information about co-workers through staff discussions and written branch/section procedures.

b. For stored samples

Blood samples will be stored in secured freezers on the NIH campus. Subject name and identifying information will be removed and we will assign the samples a code. The key to the code will be kept in a separate, secure area. Only study investigators will have access to data.

c. Special precautions

Every necessary step will be taken to prevent identification of study participants and other violations of subject confidentiality. Information will be stored using a confidential case number, and no identifiers (name, address, phone number, etc.) will be used that could allow direct linking of database information to individual subjects. Where temporary linking of information with identifiers is needed, such identifiers will be temporarily attached to the data, and will be removed after information has been encoded. Secured e-mail will be used for all electronic communications of subject information between investigators.

Demographic and clinical data will be archived in EXCEL on a password protected PC server and the Clinical Trial Database (CTDB). Clinical Safety Monitoring data will be archived together with other data. Laboratory test results will be stored on the CRIS. Only study investigators and internal/external monitors will have access to the samples and data. De-identified results from this clinical trial will be posted on http://www.clinicaltrials.gov.

20. CONFLICT OF INTEREST

a. Distribution of NIH guidelines

NIH guidelines on conflict of interest have been distributed to all investigators.

b. Conflict of interest

There are no conflicts-of-interest to report.

21. RESEARCH AND TRAVEL COMPENSATION

All participants will be compensated for time and research-related inconveniences. Reimbursement is based on NIH standards for time devoted to the research project. Participants will be paid for each portion of the study they have completed whether or not they opt for early withdrawal from participation. Without any delay of study procedures or unanticipated inconvenience, the total possible compensation is $670 in healthy subjects and $860 in patients' group per each evaluation period. If the investigators need to delay study procedures or if additional time is need for completion, subjects may receive additional compensation in accordance with NIH guidelines.

Patients and their escort will receive support for travel, meals and lodging according to NIH travel policy guidelines. Lodging may be provided directly or reimbursed according to NIH guidelines.

Employees and staff who participate during work hours must have permission from their supervisor. NIH employees must either participate outside of work hours or take leave in order to receive compensation.

Healthy subjects

Visit 1 to NIH	
Neuropsychological testing	$90
Blood test for genetics	$10
Visit 2 to NIH	
PET scanning	$150
Arterial catheter	$60
Antecubital venous catheter(s)	$30
Pregnancy test	$10
Movement restriction	$10
Visit 3 to NIH	
PET scanning	$150
Antecubital venous catheter(s)	$30
Pregnancy test	$10
Movement restriction	$10
Visit 4 to NIH	
MRI	$100
Pregnancy test	$10
Total	$670

Patients or Subjects with increased risk of neurodegenerative diseases

Visit 1 to NIH	
Clinical evaluation and screening procedures	$90
Blood test for genetics	$10
Escort fee	$20

Visit 2 to NIH	
Neuropsychological testing	$90
Escort fee	$20
Visit 3 to NIH	
PET scanning	$150
Arterial catheter	$60
Antecubital venous catheter(s)	$30
Pregnancy test	$10
Movement restriction	$10
Escort fee	$20
Visit 4 to NIH	
PET scanning	$150
Antecubital venous catheter(s)	$30
Pregnancy test	$10
Movement restriction	$10
Escort fee	$20
Visit 5 to NIH	
MRI	$100
Pregnancy test	$10
Escort fee	$20
Total	$860

22. REFERENCES

Freibaum BD, Lu Y, Lopez-Gonzalez R, Kim NC, Almeida S, Lee KH*, et al* (2015). GGGGCC repeat expansion in C9orf72 compromises nucleocytoplasmic transport. *Nature* **525**(7567): 129-133.

Garcia-Redondo A, Dols-Icardo O, Rojas-Garcia R, Esteban-Perez J, Cordero-Vazquez P, Munoz-Blanco JL*, et al* (2013). Analysis of the C9orf72 gene in patients with amyotrophic lateral sclerosis in Spain and different populations worldwide. *Human mutation* **34**(1): 79-82.

Guerreiro R, Wojtas A, Bras J, Carrasquillo M, Rogaeva E, Majounie E*, et al* (2013). TREM2 variants in Alzheimer's disease. *The New England journal of medicine* **368**(2): 117-127.

Ikawa M, Lohith TG, Shrestha S, Telu S, Zoghbi SS, Castellano S*, et al* (2017). 11C-ER176, a Radioligand for 18-kDa Translocator Protein, Has Adequate Sensitivity to Robustly Image All

Three Affinity Genotypes in Human Brain. *Journal of nuclear medicine : official publication, Society of Nuclear Medicine* **58**(2): 320-325.

Jang JH, Kwon MJ, Choi WJ, Oh KW, Koh SH, Ki CS*, et al* (2013). Analysis of the C9orf72 hexanucleotide repeat expansion in Korean patients with familial and sporadic amyotrophic lateral sclerosis. *Neurobiology of aging* **34**(4): 1311.e1317-1319.

Kao AW, Eisenhut RJ, Martens LH, Nakamura A, Huang A, Bagley JA*, et al* (2011). A neurodegenerative disease mutation that accelerates the clearance of apoptotic cells. *Proc Natl Acad Sci U S A* **108**(11): 4441-4446.

Kleinberger G, Yamanishi Y, Suarez-Calvet M, Czirr E, Lohmann E, Cuyvers E*, et al* (2014). TREM2 mutations implicated in neurodegeneration impair cell surface transport and phagocytosis. *Science translational medicine* **6**(243): 243ra286.

Kreisl WC, Lyoo CH, Liow JS, Snow J, Page E, Jenko KJ*, et al* (2017). Distinct patterns of increased translocator protein in posterior cortical atrophy and amnestic Alzheimer's disease. *Neurobiology of aging* **51**: 132-140.

Kreisl WC, Lyoo CH, Liow JS, Wei M, Snow J, Page E*, et al* (2016). (11)C-PBR28 binding to translocator protein increases with progression of Alzheimer's disease. *Neurobiology of aging* **44**: 53-61.

Kreisl WC, Lyoo CH, McGwier M, Snow J, Jenko KJ, Kimura N*, et al* (2013). In vivo radioligand binding to translocator protein correlates with severity of Alzheimer's disease. *Brain* **136**(Pt 7): 2228-2238.

Lockwood AH (1985). Invasiveness in studies of brain function by positron emission tomography (PET). *Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism* **5**(4): 487-489.

Lucin KM, Wyss-Coray T (2009). Immune activation in brain aging and neurodegeneration: too much or too little? *Neuron* **64**(1): 110-122.

Nalls MA, Bras J, Hernandez DG, Keller MF, Majounie E, Renton AE*, et al* (2015). NeuroX, a fast and efficient genotyping platform for investigation of neurodegenerative diseases. *Neurobiology of aging* **36**(3): 1605.e1607-1612.

Neary D, Snowden JS, Gustafson L, Passant U, Stuss D, Black S*, et al* (1998). Frontotemporal lobar degeneration: a consensus on clinical diagnostic criteria. *Neurology* **51**(6): 1546-1554.

O'Rourke JG, Bogdanik L, Yanez A, Lall D, Wolf AJ, Muhammad AK*, et al* (2016). C9orf72 is required for proper macrophage and microglial function in mice. *Science* **351**(6279): 1324-1329.

Pottier C, Bieniek KF, Finch N, van de Vorst M, Baker M, Perkersen R*, et al* (2015). Whole-genome sequencing reveals important role for TBK1 and OPTN mutations in frontotemporal lobar degeneration without motor neuron disease. *Acta neuropathologica* **130**(1): 77-92.

Rabinovici GD, Rosen HJ, Alkalay A, Kornak J, Furst AJ, Agarwal N*, et al* (2011). Amyloid vs FDG-PET in the differential diagnosis of AD and FTLD. *Neurology* **77**(23): 2034-2042.

Reitz C, Brayne C, Mayeux R (2011). Epidemiology of Alzheimer disease. *Nature reviews Neurology* **7**(3): 137-152.

Scheinin NM, Tolvanen TK, Wilson IA, Arponen EM, Nagren KA, Rinne JO (2007). Biodistribution and radiation dosimetry of the amyloid imaging agent 11C-PIB in humans. *Journal of nuclear medicine : official publication, Society of Nuclear Medicine* **48**(1): 128-133.

Sriram K, Matheson JM, Benkovic SA, Miller DB, Luster MI, O'Callaghan JP (2006). Deficiency of TNF receptors suppresses microglial activation and alters the susceptibility of brain regions to MPTP-induced neurotoxicity: role of TNF-alpha. *The FASEB Journal* **20**(6): 670-682.

Streit WJ, Miller KR, Lopes KO, Njie E (2008). Microglial degeneration in the aging brain--bad news for neurons? *Front Biosci* **13**: 3423-3438.

Surendranathan A, Rowe JB, O'Brien JT (2015). Neuroinflammation in Lewy body dementia. *Parkinsonism & related disorders* **21**(12): 1398-1406.

Tambuyzer BR, Ponsaerts P, Nouwen EJ (2008). Microglia: gatekeepers of central nervous system immunology. *Journal of Leukocyte Biology* **85**(3): 352-370.

Tang W, Lu Y, Tian Q-Y, Zhang Y, Guo F-J, Liu G-Y*, et al* (2011). The Growth Factor Progranulin Binds to TNF Receptors and Is Therapeutic Against Inflammatory Arthritis in Mice. *Science*: 1-28.

van der Zee J, Gijselinck I, Dillen L, Van Langenhove T, Theuns J, Engelborghs S*, et al* (2013). A pan-European study of the C9orf72 repeat associated with FTLD: geographic prevalence, genomic instability, and intermediate repeats. *Human mutation* **34**(2): 363-373.

Vlassenko AG, Mintun MA, Xiong C, Sheline YI, Goate AM, Benzinger TL*, et al* (2011). Amyloid-beta plaque growth in cognitively normal adults: longitudinal [11C]Pittsburgh compound B data. *Annals of neurology* **70**(5): 857-861.

Yin F, Banerjee R, Thomas B, Zhou P, Qian L, Jia T*, et al* (2010). Exaggerated inflammation, impaired host defense, and neuropathology in progranulin-deficient mice. *Journal of Experimental Medicine* **207**(1): 117-128.

Zhang J (2015). Mapping neuroinflammation in frontotemporal dementia with molecular PET imaging. *Journal of neuroinflammation* **12**: 108.

Zhu J, Nathan C, Jin W, Sim D, Ashcroft GS, Wahl SM*, et al* (2002). Conversion of proepithelin to epithelins: roles of SLPI and elastase in host defense and wound repair. *Cell* **111**(6): 867-878.