The Longitudinal Early-onset Alzheimer's Disease Study (LEADS): Framework and Methodology

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This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/alz.12350.

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Word count: 5,987

Abstract: 159

Title: 89 characters (with spaces)

References: 87

Figures: 3

Tables: 2

Acknowledgments: This study is generously supported by R56 AG057195, U01AG6057195, U24AG021886, Alzheimer Association LEADS GENETICS-19-639372, U01 AG016976, P30 AG010133, P50 AG008702, P50 AG025688, P50 AG005146, P30 AG062421, P30 AG062422, P50 AG023501, P30 AG010124, P30 AG013854 and P50 AG005681.

Keywords: Alzheimer's disease, early-onset, young onset, EOAD, YOAD, LEADS

Declarations of interest: none

DISCLOSURES:

Dr. Apostolova served as a paid consultant for Biogen and Two Labs, serves on a DSMB for IQVIA, and receives research support from the NIH, the Alzheimer's Association, Roche, Life Molecular Imaging and Eli Lilly.

Dr. Aisen reports grants from NIA, FNIH, the Alzheimer's Association, Janssen, Lilly and Eisai, and personal fees from Merck, Roche, Biogen, Lundbeck, ImmunoBrain Checkpoint and Samus.

Dr. Eloyan has nothing to disclose.

Dr. Fagan has received research support from Biogen, Fujirebio, and Roche Diagnostics. She is a member of the scientific advisory boards for Roche Diagnostics, Genentech and AbbVie and also consults for Araclon/Griffols, DiademRes, and Otsuka Pharmaceuticals. There are no conflicts of interest.

Dr. Fargo has nothing to disclose.

Dr. Foroud has nothing to disclose.

Dr. Gatsonis has nothing to disclose.

Dr. Grinberg has nothing to disclose.

Dr. Jack serves on an independent data monitoring board for Roche and has consulted for and served as a speaker for Eisai, but he receives no personal compensation from any commercial entity. He receives research support from NIH and the Alexander Family Alzheimer's Disease Research Professorship of the Mayo Clinic.

Dr. Kramer has nothing to disclose.

Dr. Koeppe has nothing to disclose.

Dr. Kukull is supported by the NIA.

Dr. Murray served as a paid consultant for AVID Radiopharmaceuticals and receives research support from the NIH, the Alzheimer's Association, and the State of Florida.

Dr. Nudelman has nothing to disclose.

Mrs. Rumbaugh has nothing to disclose.

Dr. Toga has nothing to disclose.

Dr. Vemuri has nothing to disclose.

Mrs. Trullinger has nothing to disclose.

Dr. Iaccarino has nothing to disclose.

Dr. Day is supported by the NIA. He serves as a topic editor on dementia for DynaMed Plus (EBSCO Industries, Inc), is the clinical director for the Anti-NMDA Receptor Encephalitis Foundation and holds stocks in ANI Pharmaceuticals.

Dr. Graff-Radford receives research support from Lilly, AbbVie and Biogen.

Dr. Honig has nothing to disclose.

Dr. Jones has nothing to disclose.

Dr. Masdeu served on the speaker's bureau and consulted for Biogen. He received research support from the NIH, Avanir, Abbvie, Biogen, Eli Lilly, Esai, Novartis.

Dr. Mendez receives grant support from NIA and has received support from Biogen.

Dr. Musiek received research funding from Eisai Pharmaceuticals Inc.

Dr. Onyike has nothing to disclose.

Dr. Rogalski has nothing to disclose.

Dr. Salloway received consultation fees and research support from Biogen, Eisai, Lilly, Genentech and Roche.

Dr. Wolk received grants from Eli Lilly/Avid Radiopharmaceuticals, Merck, and Biogen, and consulted for Merck, Janssen and GE Healthcare.
Dr. Wingo has nothing to disclose.

Dr. Carrillo has nothing to disclose.

Dr. Dickerson receives research support from NIH and the Alzheimer's Drug Discovery Foundation. He consulted for Arkuda, Axovant, Lilly, Biogen, Merck, Novartis and Wave LifeSciences. He is editor for Neuroimage: Clinical and Cortex. He receives royalties from Oxford University Press and Cambridge University Press.

Dr. Rabinovici receives research support from NIH, Alzheimer's Association, American College of Radiology, Rainwater Charitable Foundation, Avid Radiopharmaceuticals, Eli Lilly, GE Healthcare, Life Molecular Imaging, Genentech and Roche. He has served as a paid consultant for Axon Neurosciences, Eisai, GE Healthcare, Genentech, Roche, Johnson & Johnson, and is an Associate Editor for JAMA Neurology.

Abstract:

Early-onset Alzheimer's disease (EOAD) patients are commonly excluded from large-scale observational and therapeutic studies due to their young age, atypical presentation or absence of pathogenic mutations. The goals of the Longitudinal EOAD Study (LEADS) are to: 1) Define the clinical, imaging and fluid biomarker characteristics of EOAD, 2) Develop sensitive cognitive and biomarker measures for future clinical and research use, and 3) Establish a trial-ready network. LEADS will follow 400 amyloid ß-positive EOAD, 200 amyloid ß-negative EOnonAD that meet NIA-AA criteria for mild cognitive impairment (MCI) or AD dementia, and 100 age-matched controls. Participants will undergo clinical and cognitive assessments, MRI, [^{18}F]Florbetaben and [^{18}F]Flortaucipir PET, lumbar puncture and blood draw for DNA, RNA, plasma, serum and peripheral blood mononuclear cells, and postmortem assessment. To develop more effective AD treatments, scientists need to understand the genetic, biological and clinical processes involved in EOAD. LEADS will develop a public resource that will enable future planning and implementation of EOAD clinical trials.

Introduction:

Approximately 5% of the 5.8 million victims of Alzheimer's disease (AD) in the US (~200,000 people) develop symptoms at age 64 or younger, and are classified as having early-onset AD (EOAD)[1 2]. The onset of dementia at such a young productive age has disproportionately devastating consequences for patients, families, and society [3 4]. Individuals with EOAD often face significant delays to diagnosis, or are misdiagnosed with non-degenerative conditions (e.g. other psychiatric or neurologic disorders, hormonal imbalance such as menopause, etc.) [5-10]. Diagnostic delay postpones access to disease education and therapy, as well as social and financial support for people who are in the peak earning years of their life. These delays also result in loss of employment, health insurance, and lasting emotional and financial strain on caregivers.

Although EOAD and late-onset AD (LOAD, age of onset 65 years or older), share the same pathologic substrate, there are notable differences in their clinical and biological phenotypes [11]. Compared to LOAD, individuals with sporadic EOAD show more rapid clinical decline [11-16], lower prevalence of amnestic versus non-amnestic predominant clinical presentations with greater impairment in non-memory domains [11 13 14 17-27]. For the same level of impairment, EOAD is associated with greater baseline cortical atrophy and hypometabolism, less hippocampal atrophy and more severe tau pathology than LOAD [21 22 25 28-39 40-48]. Pedigree analyses provide evidence for increased heritability in EOAD compared to LOAD. Yet, only a small minority (~3-10%) of EOAD carry a known autosomal dominant mutation in *APP* or *PSEN1/2* [49-51], suggesting that this population may be enriched for novel genetic risk factors [52 53].

Despite being highly motivated and having fewer age-related comorbidities compared to LOAD, EOAD are commonly excluded from clinical research and therapeutic trials – an oversight that has been increasingly criticized as being marginalizing and unethical[54]. Of the two major North American multicenter consortia, the Alzheimer's Disease Neuroimaging Initiative (ADNI) includes only a few EOAD cases all with a "typical" amnestic presentation and the Dominantly Inherited Alzheimer Network (DIAN) is focused solely on autosomal dominant EOAD.

The Longitudinal Early-onset AD Study (LEADS, NIA R56057195, NIA U016057195) is a prospective longitudinal multi-site, observational clinical and biomarker study of EOAD, conducted at key AD research hubs and clinical sites

across the U.S. The scientific goals of LEADS are to: 1) collect clinical, genetic and biomarker data in this under-studied AD population; 2) explore the unique features of EOAD yielding novel insights into the mechanisms, heterogeneity and heritability of AD; 3) develop clinical trial outcome measures sensitive to detect baseline deficits and track longitudinal changes in EOAD; and 4) establish a network of sites that will enable future planning and implementation of clinical trials in EOAD. Herein, we describe the study design and methodology.

Methods:

1. *Study Design:*

LEADS (www.leads-study.org) is a prospective multisite observational clinical and biomarker study registered in clinicaltrails.gov (NCT03507257). LEADS seeks to accomplish the following aims: **1)** Compare the baseline and longitudinal cognitive and functional characteristics (aim 1) as well as magnetic resonance imaging (MRI), amyloid positron emission tomography (PET), tau PET and cerebrospinal fluid (CSF) measures (aim 2) of EOAD vs. LOAD and identify optimal outcome measures for clinical trials; **2)** Investigate the influence of APOE genotype in EOAD (aim 3); and **3)** Characterize genetic contributions to EOAD (exploratory aim 4).

LEADS leverages existing infrastructure and processes applied in the National Alzheimer Coordinating Center (NACC) Alzheimer Disease Center (ADC) Network and the ADNI study. Biomarker collection closely follows the study design employed by ADNI along with the informatics systems provided by the Laboratory of Neuro Imaging (LONI - http://loni.usc.edu/). Similar to ADNI, LEADS links longitudinal clinical and cognitive assessments with multiple imaging and biofluid markers that capture different elements of the AD pathophysiological cascade. The study infrastructure is comprised of eight cores (Administrative, Clinical, MRI, PET, Genetics and Biorepository, Biostatistics, Informatics and Neuropathology Cores) and, at present, 19 clinical sites. LEADS sites were carefully selected for their research expertise in EOAD as well as the availability of both FBB and FTP tracer delivery at the site. The study employs an innovative partnership model between federal (NIA), academic (cores and sites), non-profit (Alzheimer's Association; www.alz.org) and private stakeholders (Life Molecular Imaging and AVID who provide amyloid and tau PET ligands, respectively, at research cost).

Recruitment for LEADS follows the ADNI model of competitive recruitment. EOAD is a rare AD variant and thus sites enrollment will not be capped.

The coordinating center for LEADS is located at the University of Southern California Alzheimer's Therapeutic Research Institute (ATRI). ATRI also serves as the coordinating center for ADNI, the Alzheimer's Clinical Trials Consortium (ACTC) and other observational and therapeutic studies in the field. ATRI provides electronic data capture, regulatory and operational support, data management, central and on-site clinical monitoring, safety oversight and reporting to the Data and Safety Monitoring Board.

LEADS is one of the first studies of its size and scope in the US to employ an academic institution (Indiana University [IU]), as the central Institutional Review Board (IRB). The IU central IRB reliance process utilizes the SMART IRB agreement. Determinations to rely on the IU IRB as the single IRB for the study are made by the local Human Research Protection Programs (HRPPs) at all sites and cores locations. The local HRPPs convey all state and local policies that govern the research at their site to the Regulatory Team at ATRI, and the central IRB at IU. Site-specific documents, such as Informed Consent Forms, HIPAA authorizations, etc. are generated for each site by making all necessary changes to the IU IRB approved study-wide template in response to local policies. The IU IRB reviews all site-specific regulatory documents before approving the site and fully executing the reliance agreements. All LEADS participants provide informed consent according to the Declaration of Helsinki, U.S. federal regulations, local state laws and regulations, and policies of the IU IRB. Throughout the study the IU IRB receives reportable event reports from all sites, reviews study-wide and site-specific amendments, and facilitates renewals for LEADS. Local site HRPPs maintain responsibility for all other ancillary reviews per the SMART IRB agreement. Storage and distribution of IRB approved documents is managed through the online portal IRB Reliance Exchange (IREx).

2. Clinical Procedures:

The original grant application proposed to enroll 400 subjects meeting National Institute on Aging – Alzheimer Association (NIA-AA) criteria for MCI or mild dementia [55 56], ages 40-64, referred from memory clinics or existing research cohorts across our clinical sites, and 100 cognitively normal age-matched participants. Additional funding was secured via an administrative supplement to enroll and characterize up to 200 amyloid ß-negative cognitively impaired participants who were presumed to have EOAD (EOnonAD) (3U01 AG057195-02S1), increasing our target enrollment to 700. The Alzheimer's Association facilitates recruitment both through its nationwide chapter network and through the TrialMatch initiative by identifying EOAD participants and connecting them with our enrolling sites. All LEADS participants are offered co-enrollment in the federally funded Alzheimer's Disease Research Centers (ADRCs) where available.

In 2021 we successfully competed for a Competitive Revision supplement and added the following additional research activities:

1) Addition of mo36 visit with clinical, cognitive and biomarker assessment for all EOAD and EOnonAD participants
2) Addition of mo48 visits with clinical, cognitive and peripheral blood collection for those EOAD and EOnonAD
3) Addition of mo24 visit for our CN participants
4) Addition of plasma Aβ40 and 42, P-tau217 and neurofilament light (NfL) assessments
5) Addition of ^{18}F-Fluorodeoxyglucose (FDG) PET for all CN and EOnonAD participants to further characterize neurodegenerative patterns in EOnonAD.
6) Addition of social worker support at our sites.

2.1. *Inclusion/Exclusion Criteria*

EOAD and EOnonAD participants must meet NIA-AA criteria for dementia or MCI and have a global Clinical Dementia Rating (CDR) score ≤ 1. Unlike ADNI and the vast majority of clinical trials, LEADS does not exclude individuals with predominantly non-amnestic presentations. Individuals meeting criteria for the dysexecutive, logopenic primary progressive aphasia or posterior cortical atrophy variants are eligible to enroll.

Cognitively impaired individuals with two or more first degree relatives with EOAD unless mutations in *APP*, *PSEN1*, *PSEN2, MAPT, C9ORF72* and *GRN* have been excluded. Those with a known mutation in *APP*, *PSEN1* and *PSEN2* are ineligible. Cognitively normal (CN) LEADS participants must have a Mini-Mental State Examination score of ≥ 24, a global CDR=0 and score within the cognitively normal range on neuropsychological testing.

Additionally, all LEADS participants must be ages 40-64 at the time of consent, have capacity to consent or, if cognitively impaired, have a legally authorized representative who can provide consent, have a study partner that knows them well, have no contraindications to MRI, be willing and able to complete all study procedures aside from lumbar puncture (optional), not be pregnant or lactating, not have lifetime history of other brain disorder (both neurologic and psychiatric except for seizures thought to be related to EOAD or headaches), no previous enrollment in therapeutic trials targeting amyloid ß and/or tau, moderate or severe substance abuse, or suicidal behaviors or ideations in the past 12 months. Individuals with MRI evidence of infection, focal lesions such as strokes, multiple or strategic lacunes, and/or space occupying lesions are also exclusionary. Our current enrollment statistics as well as the demographic, clinical and biomarker characteristics for our participants by diagnostic group can be seen in **Table 1**.

Recruitment for LEADS follows the ADNI model of competitive recruitment. EOAD is a rare AD variant and thus sites enrollment will not be capped.

2.3 Clinical and Cognitive Assessments

The originally funded application EOAD and EOnonAD individuals were to be followed for 24 months with a screening/baseline, month 12 and month 24 clinical and cognitive assessments, while CN participants were to undergo to screening/baseline and month 12 clinical and cognitive assessments. With the soon to be funded Competitive Revision we were able to add month 36 and 48 visits for all cognitively impaired participants and month 24 visit for CN. LEADS' schedule of events is shown in **Figure 1**.

The LEADS' clinical assessments include standardized history of present illness, past medical history, family history, concurrent medication, detailed general medical and neurological examinations. We also routinely obtain Autoimmune and Early Developmental History questionnaires. LEADS employs the NACC Uniform Data Set cognitive battery, the NACC Frontotemporal Lobar Degeneration module, the Alzheimer's Disease Cooperative Studies - cognitive behavior subscale (ADAS Cog) and several additional cognitive tests tapping into cognitive functions that are commonly impaired in rare AD variants (see **Figure 1** and **Table 2**). Clinical diagnosis is established in a multidisciplinary consensus conference at each clinical site following the NIA-AA diagnostic criteria for dementia and MCI. Diagnosis of logopenic aphasia and posterior cortical atrophy follow previously published criteria [57 58]

2.4.Genetic Screening and Counseling

The LEADS Genetics Core oversees genetic screening and genetic counseling for the study. During the consenting process, cognitively impaired participants are given the option to learn the results of their genetic screening performed as part of the LEADS. The participant watches a seven-minute video which discusses the potential benefits, risks and limitations of genetic testing for mutations in genes known to be important in the risk of EOAD (*PSEN1*, *PSEN2*, *APP*) and EOnonAD (*GRN*, *MAPT*, *C9ORF72*) and given the option of speaking to a genetic counselor to address any questions or concerns prior to consenting to receive the genetic screening results. The participants are given a link to the video so that they may review it at a later time or discuss with other family members if they wish to do so. Participants can opt in or out of genetic disclosure and can withdraw consent at any point prior to disclosure.

DNA samples are collected from all LEADS participants for research purposes. The DNA samples from all cognitively impaired LEADS participants are screened for genetic mutations in early-onset dementia-related genes *APP, PSEN1*, *PSEN2*, *GRN*, and *MAPT*. Data are processed and quality controlled following the GATK best practices workflow [59]. Data are annotated using Annovar, and then filtered using a list of known pathogenic mutations for EOAD and Frontotemporal Dementia.

These mutations were collated and curated from the Human Gene Mutation Database[60], ClinVar[61], the Leiden Open Variation Database[62], and the pathogenic mutations list curated by the DIAN study[63], which is used to determine if DIAN participants are eligible for clinical trials. Participants with known pathogenic mutations in *APP*, *PSEN1*, *PSEN2*, *GRN,* and *MAPT* are identified. Screening is also conducted to identify repeat expansions of the *C9ORF72* gene. DNA is screened with repeat primed PCR to identify samples showing evidence of pathogenic repeat expansion. A separate 6 mL vial of blood is also collected and stored for cognitively impaired participants, for the purpose of DNA extraction and genetic testing by a Clinical Laboratory Improvement Amendments (CLIA)-certified genetic laboratory.

Participants who do not show evidence of pathogenic mutations in the six screened genes are considered research negative. Negative mutation status is not verified by CLIA testing. Those who elect to receive their genetic results and are found to not carry a pathogenic variant in the research screen are informed of this by the site PI, study physician or genetic counselor in person or by phone. They also receive a letter explaining the results and disclosing that this testing was conducted in a research laboratory and the limitations of that testing.

For participants in whom a known pathogenic mutation is identified and who indicated that they wished to learn the results of genetic testing, the separate blood sample collected specifically for verification of research screening is sent by the National Centralized Repository for Alzheimer's Disease and Related Dementias (NCRAD) to a CLIA-certified laboratory for confirmatory testing. Once CLIA results are returned to the LEADS Genetics Core, consent status for participants is reviewed to verify which participants wish to receive genetic results. The participants who are positive for pathogenic mutations meet with the site's genetic counselor for disclosure. They are given a copy of the test report describing CLIA confirmation of the variant identified in research testing. They are no longer eligible to continue in LEADS but they are referred to other studies [i.e. Dominantly Inherited Alzheimer Network (DIAN), ARTFL-LEFFTDS Longitudinal Frontotemporal Lobar Degeneration (ALLFTD)]. To avoid inadvertent disclosure, those who decline result disclosure or withdraw consent prior to disclosure continue through the study per protocol but are excluded from analyses.

3. *Biomarker Data Collection:*

3.1.MRI methods

The administrative organization of the LEADS MRI Core includes two teams, one based at the Mayo Clinic and one based at Massachusetts General Hospital. The LEADS MRI Core is charged with supporting each of the sites in acquiring high-quality MRI scans with standardized procedures at the timepoints described above. We have employed the 3 Tesla ADNI-3 acquisition protocol, using the advanced protocol at as many sites as possible (http://adni.loni.usc.edu/methods/documents/mri-protocols/). While provided below are the typical scan parameters, these vary slightly by vendor and system type. The sequences acquired using 3 Tesla MRI scanners at all LEADS sites are: 1) Three plane/Tri-planar auto-alignment scout scan, yielding orthogonal orientation and AC-PC alignment, 2) Sagittal 3D accelerated MPRAGE/IRSPGR T1-weighted sequence: TR/TE/TI = 2300/3/900 ms, flip angle of 9 degrees, sagittal orientation, FOV = 256 x 240 mm with 208 slices, 1x1x1mm resolution, 2x acceleration, 3) Sagittal 3D Fluid-attenuated Inversion Recovery (FLAIR) TR/TE/TI = 4800/119/1650 ms, FOV = 256 x 256mm with 160 slices, 1.2x1x1mm resolution, 4) Axial T2*/gradient echo (GRE) for cerebral microbleed assessment with TR/TE = 650/20ms, FOV = 220x220mm, 176 slides, 5) Diffusion Tensor imaging (DTI) TR/TE = 3300/71 ms, FOV = 232 x 232x160mm, three shells b = 500, 1000, 2000 s/mm^2, 112 directions, 2x2x2mm resolution, 6) 3D Pseudo Continuous Arterial Spin Labeled (pCASL) perfusion imaging TR/TE 4885/10.5, FOV = 240x240x160mm, 1.9x1.9x4mm resolution, 7) Task-free functional MRI, T2*-weighted gradient echo–echo planar sequence with TR/TE = 600/30 ms, flip angle 53°; FOV = 220x220x160mm, 2.5x2.5x2.5mm resolution, 64 slices, SMS = 8, CAIPI shift=4. Subjects are instructed to remain awake with their eyes closed. 8) High resolution hippocampal sequence acquired obliquely to the long axis of the hippocampi, TR/TE 8020/50, FOV 175x60x175mm, 0.39x3x0.39mm resolution. Total exam duration is under 1 hour.

Each site receives electronic files from the LEADS MRI Core with the protocol to be loaded on their system. For site qualification, each site scans the ADNI phantom or other similar local phantom using the electronically loaded LEADS Phantom QC protocols and LEADS Human Scan protocols. Achieving a reproducible phantom placement position is a key element to the system performance analysis that is done at initial site certification, when there are software/hardware upgrades, or when substantial maintenance is performed. The MRI core has developed a scanning procedures manual that supports sites in performing the procedures as similarly as possible.

Each site captures information about the scan session for the electronic data capture record and uploads MRI DICOM images to LONI. De-identified data are downloaded from LONI by the LEADS MRI Core Mayo site, which employs the ADNI QC pipeline, including 1) visual inspection for artifacts (e.g., subject motion), and evaluation of image quality; 2) verification of adherence to all sequence protocols (protocol consistency check) based on DICOM header fields; 3) verification of hardware

and software versions; 4) immediate site contact in the case of a deviation; 5) logging of MR QC with the Informatics Core and the coordinating center at ATRI. The LEADS MRI Core MGH site performs FreeSurfer reconstruction and processing for regional estimates of subcortical gray matter (GM) tissue volume and cortical GM morphometrics including volume and thickness measures. The reconstructed MRI images are also used by the PET core for co-registration. The LEADS MRI Core Mayo site performs micro hemorrhage assessments, infarct grading, white matter hyperintensity volumes, and also computes longitudinal MRI change assessment using Symmetric Diffeomorphic Image Normalization tensor-based morphometry method (TBM-SyN)[64]. The MRI Core labs have monthly phone conferences and interact closely to ensure efficiency of data flow and to resolve issues that may arise with MRI scanning or processing.

3.2.PET methods

The LEADS PET Core teams are based at the University of California San Francisco (UCSF) and University of Michigan. The UCSF team is responsible for overall PET Core activities, amyloid, tau and FDG PET central reads for cohort assignment (impaired participants only) and PET quantification and analyses. The University of Michigan team is responsible for scanner qualification, image quality control and standardization. PET scans are stored at the Laboratory of Neuroimaging (LONI) at the University of Southern California. PET procedures in LEADS, including choice of radiotracers, image standardization and quantification are by design aligned with ADNI-3 to enhance comparability (http://adni.loni.usc.edu/wp-content/uploads/2012/10/ADNI3_PET-Tech-Manual_V2.0_20161206.pdf).

All LEADS participants undergo amyloid PET with ^{18}F-Florbetaben (FBB-PET) and tau PET with ^{18}F-Flortaucipir (FTP-PET, formerly known as ^{18}F-AV1451 and ^{18}F-T807). These radiotracers have been validated as sensitive and specific for detecting moderate-frequent neuritic plaques and Braak V-VI neurofibrillary tangle pathology respectively [65] and are approved for clinical use by the United States Food & Drug Administration.

In anticipation that the EOnonAD group includes a variety of nonAD etiologies, with the soon to be funded Competitive Revision we are adding FDG PET assessments to further characterize the EOnonAD participants. Some EOnonAD participants will likely harbor other neurodegenerative pathologies, while others may have psychiatric or medical etiologies for cognitive impairment. While MRI may offer insights into the specific nonAD etiology through regional atrophy patterns, FDG PET provides complimentary data to MRI regarding both the extent and pattern of neurodegeneration, as demonstrated in FTLD [66] and in amyloid-negative clinical AD [67]. LEADS CN will also receive one FDG PET assessments and will serve as age-matched comparison group.

All PET scanners are standardized with a ^{18}F-filled Hoffman brain phantom [68]. FBB-PET is acquired via i.v. injection of ~8 mCi of FBB, followed by acquisition of 4 x 5 min PET frames at t=90-110 min post-injection. FTP-PET is acquired via i.v. injection of ~10 mCi of FTP followed by acquisition of 6 x 5 min PET frames at 75-105 min post-injection. FDG PET will be acquired via i.v. injection of ~5 mCi, followed by acquisition of 6x5 min PET frames at 30-60 min post-injection [68]. Attenuation correction for each scan is performed using either CT or PET transmission data, and reconstruction uses site-specific iterative algorithms developed for ADNI. Raw PET images undergo quality control procedures, which include statistical noise check, motion assessment across temporal frames, checking for full coverage of the brain, visual check to look for common PET artifacts as well as visual and image header check to assure that the study protocol has been followed.

PET data flow in LEADS is shown in **Figure 2.** Raw images are set to a standard orientation, intensity-normalized and smoothed to standard resolution using procedures developed for ADNI[68]. Fully pre-processed FBB-PET, FTP-PET and FDG PET images are co-registered to the participants' respective T1 structural MRIs. Reference regions for FBB-PET (whole cerebellum) and FTP-PET (inferior cerebellar gray matter) are obtained via Freesurfer (FBB-PET) and Freesurfer plus the SUIT template (FTP-PET) [69 70]. FDG PET SUVR images will be created using mean activity in FreeSurfer parcellated pons as reference region. Images are scaled to the average binding in the reference regions to obtain Standardized Uptake Value Ratio (SUVR) images utilizing the full acquisition time: FBB-PET $SUVR_{90\text{-}110}$ and FTP-PET $SUVR_{75\text{-}105}$. Regional average PET SUVR values are extracted in native MRI space from regions defined in the Desikan-Killiani Atlas labeled by Freesurfer [71]. For FBB, a composite neocortical SUVR is computed and converted to Centiloid units[72] using the ADNI formula. For FTP-PET, average SUVR values from an AD meta-region of interest [73] and composite Braak stage-like regions [69] are computed. Summary and regional PET measures are shared via LONI.

Central reads of baseline FBB-PET scans are performed in cognitively impaired participants using a hybrid approach that includes visual reads and global SUVR quantification. First, scans are visually read as "amyloid-positive or amyloid-negative" using validated criteria [65] by a PET Core physician who has completed the manufacturer online training program and is certified to read FBB-PET for clinical purposes. Visual reads are performed blinded to a participant's clinical information and scan quantification. SUVR quantification is performed using a PET-only processing pipeline that closely mirrors the MRI-based pipeline described above. A PET only pipeline is used for this step since study MRI scans are not always available at the time of central read. A global SUVR ≥ 1.18 (corresponding to 39.2 Centiloids) is used as a quantitative threshold for amyloid PET positivity. If the visual read and quantitative assessment agree that a scan is amyloid ß -positive, the participant is assigned to the EOAD cohort. If visual read and quantification agree that a scan is amyloid-negative, the participant is assigned to the EOnonAD cohort. If there is discordance between the visual read and

quantification, a "tie breaker" visual read is provided by an additional reader (also blinded to quantification results), and this is considered the consensus read for cohort assignment. Central amyloid PET reads are returned to the site principal investigators and disclosed to the cognitively impaired participants by the study physicians following best practices [74]. The central read report includes: a binary read outcome, a narrative description of tracer uptake intensity and distribution, the global neocortical SUVR and the results of consensus review (if applicable).

An analogous approach will be adapted to provide central clinical reads of FTP-PET scans. Scans will be visually interpreted as negative or positive for an AD pattern using the autopsy-validated method introduced by Fleisher and colleagues [75]. Scans will be quantitatively classified as tau-positive or tau-negative based on an SUVR threshold of 1.27 [76] using a temporal "meta region-of-interest" [73]. In instances when the visual read and quantification are discordant, a "tie breaker" visual read will be performed. The central read report will include the binary read outcome, a narrative description of tracer uptake intensity and distribution, the Temporal meta region-of-interest SUVR and the results of consensus review (if applicable).

Central reads of FDG PET will also be provided to the sites and the LEADS participants. Using the CN group and a voxelwise W-score approach [77] we will generate single-subject statistical FDG PET hypometabolism maps (W-maps), corrected for age. Individual W-maps and FDG PET SUVR images will be visually rated by the PET core blind to FTP-PET and clinical/neuropsychological data.

3.3.Genetics methods

The LEADS Genetics and Biofluids Core includes a genetics and biorepository team at IU and the biofluids team at Washington University. The Genetics and Biofluids Core oversees the genetic and counseling for the study as already discussed in the Genetic Screening and Counseling section.

As part of LEADS biospecimen collection protocol, blood collected at baseline for all participants is shipped to NCRAD, which extracts and stores DNA for genetic screening and genomic analyses. Following DNA extraction at NCRAD, in-house genotyping is done with a custom 96-SNP fingerprint panel, to check DNA quality and to verify that reported and genetic sex match. This panel is also used to generate genotypes for Apolipoprotein E (*APOE*), as it includes assays for rs429358 and rs7412. *APOE* genotypes for all participants are collected, and de-identified *APOE* genotypes are uploaded to LONI. *APOE* genotype results are not returned to participants.

3.4.Fluid Biomarkers

All LEADS participants are asked to consent for lumbar puncture (LP) for the evaluation of CSF biomarkers, although refusal is not exclusionary for the study. We anticipate that 75% of participants will agree to LP. CSF acquisition, processing and analysis conform to standardized protocols applied in ADNI [78 79]. CSF samples (15-20mL) are collected at baseline for all participants as well as longitudinally (at 12 and 24 months) in EOAD and EOnonAD participants. All CSF samples are sent directly to the NCRAD at IU for processing and storage according to standard operating procedures (SOPs). At defined study time points, NCRAD will provide aliquots of baseline and follow-up CSF samples to the Fagan Fluid Biomarker Laboratory at Washington University in St. Louis for the evaluation of established and emerging biomarkers of several AD pathologies including: 1) Aß40 and Aß42 (ß-amyloid plaques), total tau (neuronal injury) and ptau181 (neurofibrillary tangles) using the high-performance, automated LUMIPULSE® assay platform (Fujirebio, Malverne, PA); 2) neurofilament light chain (NfL) (axonal damage) via commercial enzyme linked immunosorbent assay (ELISA) (Uman Diagnostics Umeå, Sweden); 3) visinin-like protein 1 (VILIP-1) (tau-independent neuronal injury/death) and neurogranin (Ng) (post-synaptic dysfunction/injury) by microparticle-based immunoassays using Single Molecule Counting (SMC) technology employing antibodies developed in the laboratory of Dr. Jack Ladenson at Washington University in St. Louis; and 4) YKL-40 (astrogliosis/neuroinflammation) via commercial ELISA (Quidel, San Diego, CA). In a separately funded study, CSF samples will also be assayed for Aß40, Aß42, total tau and ptau181 using the high-performance, automated Elecsys® assay platform (Roche Diagnostics, Basel, Switzerland) for method evaluation purposes. Baseline CSF samples are expected to be analyzed in year 4 and again with longitudinal follow-up samples in year 5 of the grant or order to minimize potential assay lot-to-lot variability. All assays are performed with strict adherence to SOPs so to maximize rigor and reproducibility. Remaining sample aliquots are banked at NCRAD for future analyses of novel biomarkers.

In addition to CSF, blood is obtained and processed for analysis of a variety of blood products: 1) red top for serum biomarkers; 2) EDTA for plasma biomarkers and buffy coat for DNA; 3) PAXgene™ for RNA; and 4) sodium heparin for peripheral blood mononuclear cells (PBMCs). Samples are shipped to NCRAD for banking and eventual distribution to qualified investigators for future analyses.

Plasma biomarkers have in recent years shown tremendous promise as diagnostic biomarkers for AD [80-84]. Serial measures from blood are easier to obtain, safer and more affordable than either amyloid PET or lumbar punctures. With the soon to be funded Competitive Revision in collaboration with Dr. Randall Bateman's group from Washington University and Eli Lilly we will be able to add longitudinal plasma Aβ40 and Aβ42 (liquid chromatography–mass spectrometry approach [84 85]), as well as P-tau217 (P-tau217 Meso

Scale Discovery optimized Lilly protocol) and NfL (Quanterix single-molecule array assay - SiMOA) to LEADS.

3.5.Neuropathology Core

The LEADS Neuropathology core (NPC) coordinates autopsies at participating sites and performs clinicopathologic analyses on EOAD and EOnonAD brain tissue. The NPC will fulfill the following goals: 1) Facilitate brain procurement at the time of death from all LEADS participants; 2) Conduct thorough, uniform post-mortem neuropathologic examinations and assign all appropriate diagnoses for each case and contribute neuropathologic data to the LEADS database (including diagnoses, accurate staging, and semiquantitative assessments of distribution and density of neuropathologic lesions); 3) Maintain a resource of formalin-fixed brain tissue blocks for LEADS investigators and outside investigators with an updated database of frozen tissue location and availability; and, 4) Review tissue requests by committee and provide samples and data to qualified investigators.

LEADS NPC is comprised of three hub sites that will oversee participating clinical sites. The three hub sites are located at IU, the UCSF, the Mayo Clinic Jacksonville (MCJ) and are led by Dr. Bernardino Ghetti, Dr. Lea T. Grinberg, Dr. Melissa E. Murray, respectively. The NPC leaders at IU, UCSF, and MCJ hubs will support the clinical sites, collaborate with LEADS investigators, organize and participate in consensus-building activities, and lead tissue-sharing efforts.

Brain procurement and processing will follow site-specific protocols to allow each site to maintain methodological continuity within cohorts. While these protocols differ slightly between sites, all sites will follow NIA-AA guidelines for regional sampling and staining [86]. These guidelines focus on regions relevant to AD, Lewy body disease, TDP-43 proteinopathies, and vascular brain injury. To harmonize methods and data elements across centers, each site will complete the NACC Neuropathology Form, following guidelines provided in the NACC Neuropathology Guidebook.

Sites will be supported and encouraged to donate intact fixed hemibrains from each case. If a site elects to retain brain tissue locally, a standard set of formalin-fixed tissue blocks will be shipped to the appropriate NPC hub. While we recognize that high quality frozen tissue is essential for research purposes, after careful consideration and thoughtful discussions with non-hub clinical sites, the NPC has devised an approach in which the sites will keep frozen tissue and the NPC will maintain a centralized inventory list to facilitate tissue location and distribution to investigators. This approach will avoid possible compromise of tissue integrity while in transfer from the site to the hub. Requests for LEADS samples can be made via an online request portal (see section Data Sharing

Procedures). Recipients of LEADS samples will be encouraged to make data from their tissue studies widely available to the research community to support studies utilizing LEADS tissue samples.

4. Statistical Methodology

The Biostatistics Core for LEADS is located at the Center for Statistical Sciences, Brown University School of Public Health, which also serves a similar role in the IDEAS studies. The data flow in LEADS is depicted in **Figure 3**.

The main goals of our study are to compare the baseline and longitudinal cognitive and functional characteristics as well as MRI, amyloid PET, tau PET and CSF biomarkers of EOAD vs. LOAD, and to identify optimal outcome measures for EOAD clinical trials. We plan to use ADNI LOAD subjects in our comparisons. To allow for this comparison, our clinical protocol, scanner qualification, imaging protocols, fluid biomarker acquisition and processing have been aligned to those in ADNI. Our Biostatistics Core will work with ADNI's Biostatistics Core to select a comparison group with LOAD that will allow us to achieve our scientific goals. We will select a matching cohort from ADNI LOAD of the same size as the LEADS EOAD participant group, matching for global CDR, MMSE ± 2 points, education ± 2 years, sex and *APOE* genotype. For our main comparison analyses we will use linear mixed models including subject-specific random intercepts and slopes controlling for covariates. We will also implement clustering analyses and machine learning techniques[87] to develop composite measures sensitive to cognitive change over time. In addition, we will implement unsupervised latent variable analysis techniques to identify latent features in the psychometric data predictive of cognitive and functional decline in EOAD. K-fold cross-validation will be used for tuning the parameters in the machine learning models. Additional analyses in the imaging space will be conducted by the LEADS PET and MRI cores.

Data Sharing and Publications

LEADS Data Sharing Policy follows the principles of Productivity, Transparency, Fairness, and Inclusiveness. The full policy can be reviewed at https://leads-study.medicine.iu.edu/researchers/. Analyses that are specified in the specific aims of the project from the original grant application and any subsequent revisions and renewals will be led by the LEADS Principal Investigators and Core Leaders. Analyses that are not proposed in the specific aims of the main grant and its subsequent revisions and renewals can be conducted by investigators within and outside of LEADS after their proposals are reviewed and approved by the Data Sharing Committee. Requestors will be asked to specify the principal hypotheses, the materials needed (variables, imaging data, biospecimens, etc.), the analytic plan, and assurance of non-overlap with planned LEADS analyses. Data requests will be reviewed based on scientific merit, feasibility and appropriateness of the investigator's qualifications

and resources to protect the data. An IRB approval will be required prior to releasing LEADS data. After a request is approved and IRB approval of the proposed analyses is verified, de-identified data will be made available through the LONI interactive data portal to investigators to conduct analyses. Analyses will be based on frozen data sets that have been quality controlled and cleaned. Investigators will be asked to return any leftover samples. New data generated through analyses of LEADS datasets will need to be provided to the Publications Committee prior to submission of the manuscript for publication for review for possible inclusion in the project database or into another NIH-approved government database such as dbGap or NIAGADS. A six-month embargo will be placed on returned data to allow publication of results. The Publications Committee will review all manuscripts ensuring proper description of informed consent, approach to confidentiality, acknowledgement of LEADS investigators and funding sources, and disclosure of potential and actual conflicts of interest. Acceptance of LEADS data obligates the recipient to cite/reference all LEADS funding sources in presentations or publications that may result from this research. No sharing of data with a third party will be allowed without the permission of the LEADS Executive Committee.

Public Private Partnership

LEADS is an innovative partnership between federal, academic and private stakeholders. LEADS investigators are partnering with Life Molecular Imaging and Eli Lily/AVID Radiopharmaceuticals, who provide the amyloid and tau PET tracers at reduced cost. LEADS investigators are working with industry partners such as Eli Lilly, Roche and Araclon Biotech, as well as investigators from Washington University to expand the fluid biomarker analyses to include CSF and plasma Aβ and tau biomarkers in addition to other promising disease-associated markers such as neurofilament light (NfL). Further collaborations with the private sector will be leveraged to expand LEADS research.

LEADS received a $1 million grant from the Alzheimer's Association to expand the genetic analyses of the study to include whole genome sequencing, DNA methylation and copy number variant studies. LEADS also received a $235,000 Diversity Recruitment Grant by the Alzheimer's Association to support minority recruitment.

In an ongoing collaboration with researchers from the School of Health Sciences and the Purdue Institute for Integrative Neuroscience at Purdue University we will produce the first induced pluripotent stem cell lines (iPSCs) from the PBMCs collected from LEADS participants.

Future Plans

The over-arching goals of LEADS are 1) to advance our knowledge about disease mechanisms and heterogeneity, 2) to develop sensitive composite clinical and biomarker tools that capture disease progression in this unique cohort, 3) to establish a network of sites that will enable future planning and implementation of clinical trials in EOAD. Additional efforts for international expansion and the development of a LEADS Trials Unit are ongoing. In collaboration with the Alzheimer's Association we are also planning to develop educational webinars and support groups for EOAD participants and their families at the local site level as well as a Site Ambassador Program that will liaise between study participants and LEADS researchers.

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Table 1. Demographic, clinical and biomarker characteristics of the currently enrolled participants.

Table 2. Clinical and Cognitive Assessments

Figure 1. Schedule of Events.

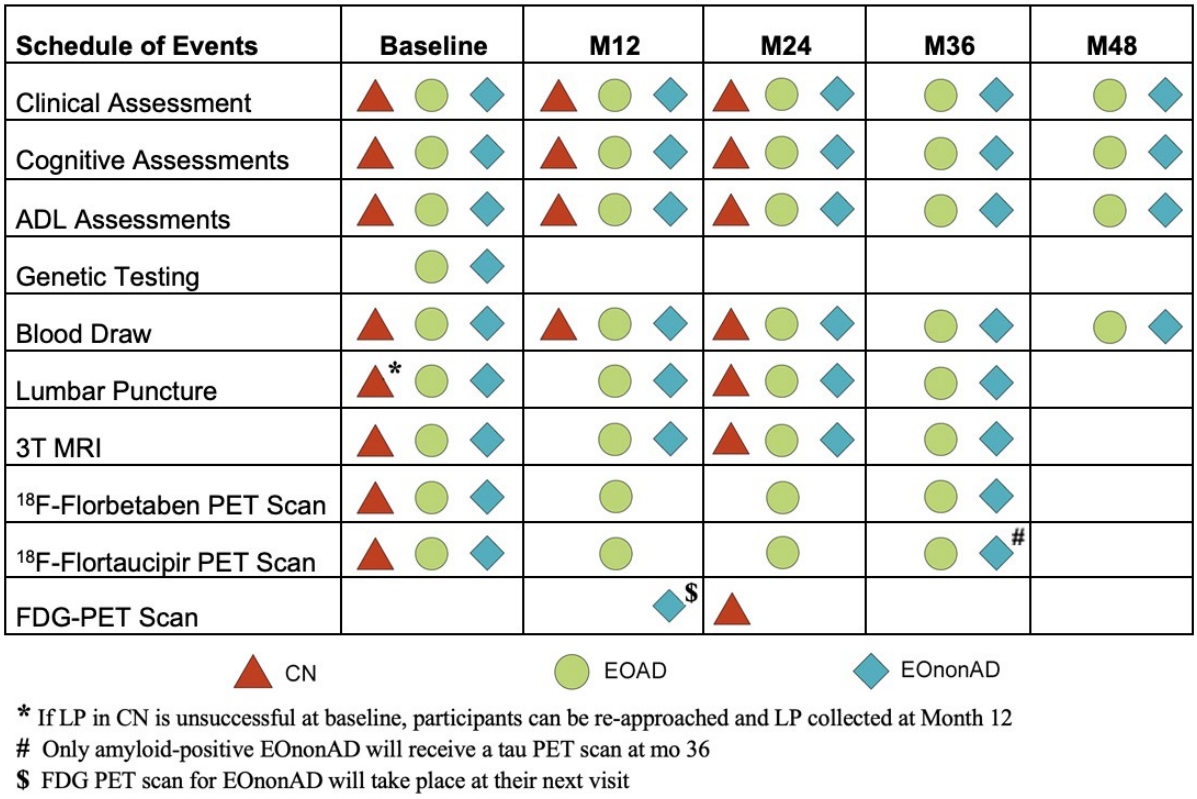

Figure 2. PET data processing

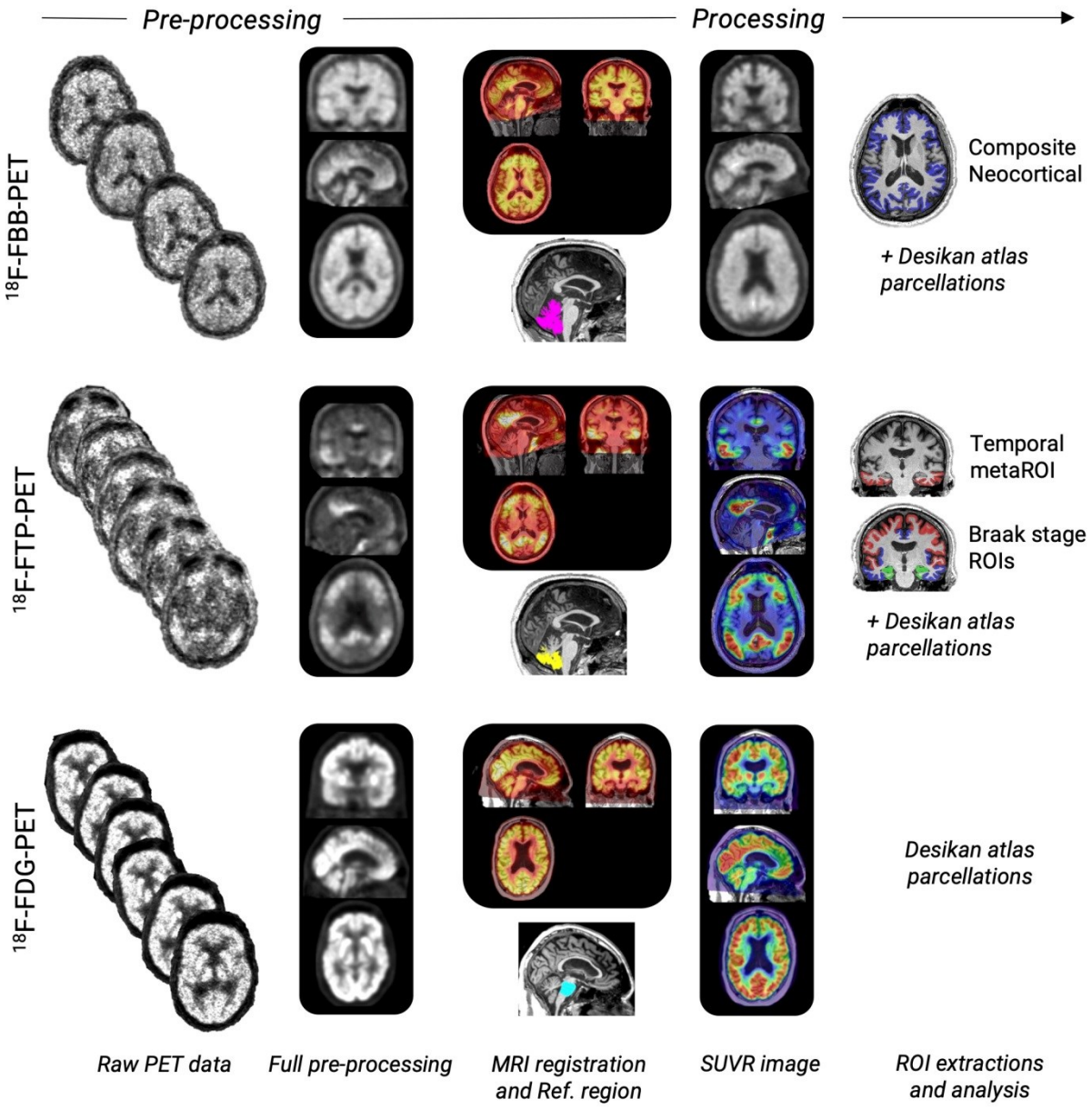

Figure 3. LEADS data flow

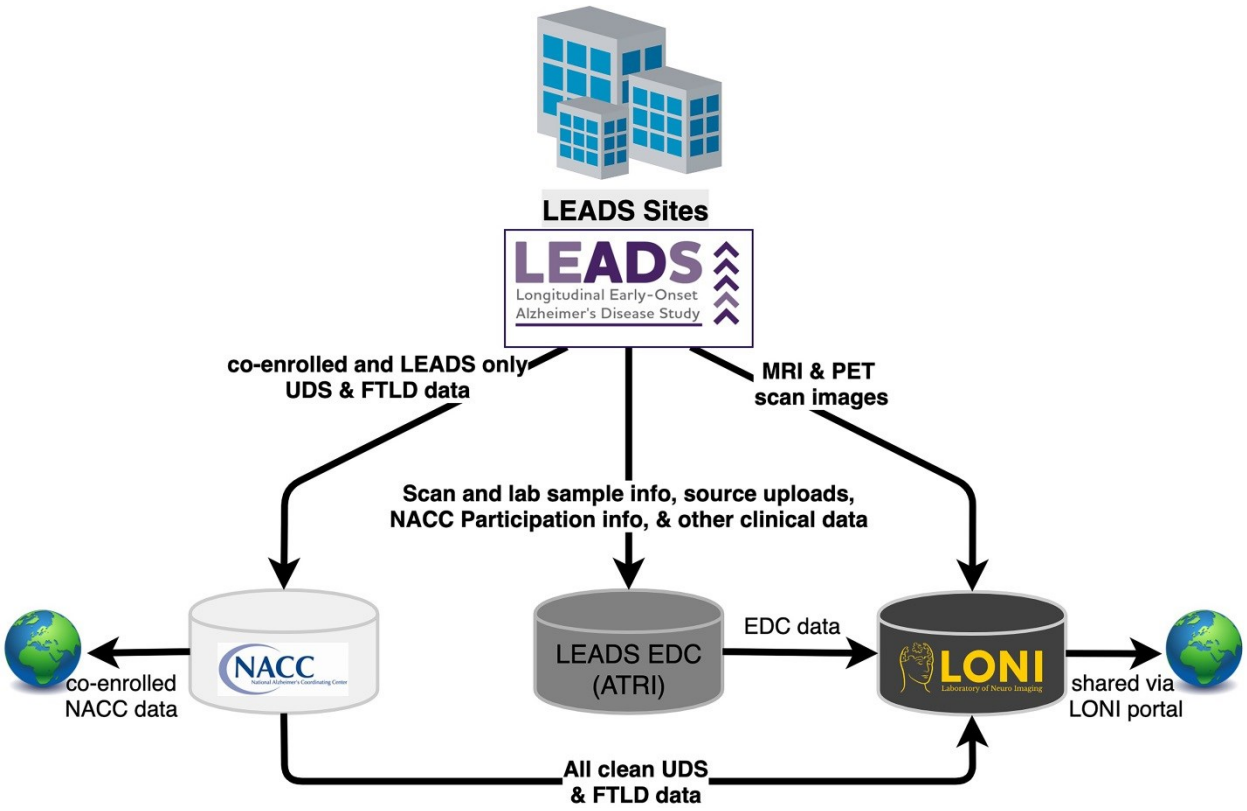

Table 1. Demographic, clinical and amyloid PET SUVR characteristics of the currently enrolled LEADS participants.

	CN (N = 114)	EOAD (N = 170)	EOnonAD (N = 65)	EOAD vs CN, *p-value*	EOnonAD vs CN, *p-value*	EOAD vs EOnonAD, *p-value*
Age, years, mean (SD)	55.93 (5.88)	59.54 (3.77)	57.91 (6.55)	**<0.0001**	0.08	**0.03**
Sex, % F	60.81%	50.75%	33.96%	0.1630	**0.003**	**0.04**
Education, years, mean (SD)	16.91 (2.12)	15.34 (2.45)	15.62 (2.52)	**<0.0001**	**0.002**	0.47
MMSE, mean (SD)	29.25 (0.84)	21.70 (4.68)	26.10 (3.39)	**<0.0001**	**<0.0001**	**<0.0001**
CDR=0.5, %	N/A	56	81	N/A	N/A	**<0.0001**
FBB SUVR, mean (SD)	1.01 (0.07)	1.54 (0.17)	0.99 (0.06)	**<0.0001**	0.11	**<0.0001**

Table 2. Clinical and Cognitive Assessments

	Cognitive Dementia Rating Scale (CDR)

LEADS specific cognitive measures	Mini Mental State Exam (MMSE)
	Rey Auditory Verbal Learning Test (RAVLT)
	Digit Symbol Substitution Test
	ADAS Cog
	TabCat (Flanker, Line Length, Line Orientation, Match)
NACC UDS MODULE	Montreal Cognitive Assessment (MoCA)
	Craft Story Immediate and Delayed Recall
	Benson Complex Figure Copy and Delayed Recall
	Number Span Test: Forward
	Number Span Test: Backward
	Category Fluency - Animals
	Category Fluency - Vegetables
	Trails Making Test Part A
	Trails Making Test Part B
	Multilingual Naming Test (MINT)
	Phonemic Fluency, F and L
NACC UDS FTLD MODULE	Regular and Irregular Word Reading
	Word Picture Matching
	Semantic Associates Test
	Northwestern Anagram
	Sentence Repetition
	Noun and Verb Naming
	Sentence Reading Test
	Social Norms Questionnaire

	Social Behavior Observer Checklist
	Behavioral Inhibition Scale
	Interpersonal Reactivity Index
	Revised Self-monitoring Scale
Behavioral Measures	Geriatric Depression Scale (GDS)
	Neuropsychiatric Inventory Questionnaire
ADL Measures	Functional Activities Questionnaire (FAQ)
	Amsterdam IADL measure*

***** Amsterdam IADL was added in February 2020

Research in context:

Systematic Review: We assessed all relevant literature by searching PubMed for papers on early-onset Alzheimer's disease (EOAD). Despite being highly motivated and having fewer age-related comorbidities compared to patients with late-onset AD, EOAD patients are commonly excluded from clinical research and therapeutic trials.

Interpretation: To fill this gap we launched a multi-site observational Longitudinal Early-onset AD Study (LEADS) that will collect longitudinal clinical and biomarker data from a large cohort of EOAD participants, as well as develop a clinical trials network.

Future Directions: Efforts towards international LEADS expansion and the launch of a LEADS Trials Unit are ongoing. In collaboration with the ALLFTD study, we are planning a registry for participants interested in enrolling in clinical trials for EOAD or FTD with cognitive screening and blood biomarker assessments.