Overall: Specific Aims

"Antecedent Biomarkers for Alzheimer Disease: The Adult Children Study" (ACS) will determine when during the lifespan molecular markers of Alzheimer disease (AD) appear in the brain of cognitively normal, largely middle-age individuals and also will characterize the course of preclinical AD. With maturation of the original ACS cohort, we now will identify the factors that mediate the transition from preclinical AD to symptomatic AD (the latter term in this application is used to encompass both mild cognitive impairment due to AD and AD dementia). This renewal application examines the **hypothesis that disrupted neural integrity predicts the transition from preclinical to symptomatic AD** and thus proposes four Cores (Administration, Clinical, Fluid Biomarker, and Data Management and Biostatistics) to support four Projects: Project 1 (JC Morris and T Benzinger, Co-Project Leaders), "Tau burden and spatial spread in preclinical Alzheimer disease"; Project 2 (AM Fagan, PL), "Plasma and cerebrospinal fluid (CSF) biomarkers that predict risk for symptomatic Alzheimer disease"; Project 3 (B Ances, PL), "Alzheimer disease progression, host gut microbiome, and enteric dysfunction"; and Project 4 (D Head, PL), "Mechanisms and moderators of the effects of physical activity in preclinical AD". Although these Cores and Projects each address unique Specific Aims, they will generate a wealth of cross-sectional and longitudinal data from ACS participants to permit a cohesive and comprehensive examination of the overarching Aims of this renewal application. Hence, the ACS as a whole is far greater than the sum of its Cores and Projects.

Overall Specific Aim 1: **Determine factors that increase risk for preclinical AD**

The premise of the ACS is that offspring of a parent affected by late onset symptomatic AD are at increased genetic risk for developing preclinical AD and ultimately symptomatic AD. Our present understanding is that the cerebral accumulation of aggregated amyloid-beta$_{42}$ (Aβ_{42}), as assessed with positron emission tomography (PET) with the radioligand, PIB, in Project 1 and by CSF Aβ_{42} concentrations in Project 2, marks the onset of preclinical AD and that subsequent tauopathy, measured in Project 1 with the PET tracer, flortaucipir (FTP), predicts cognitive decline. The ACS will explore other possible factors that contribute to preclinical AD, including: 1) systemic and intestinal inflammatory changes and the gut microbiome in Project 3 and CSF measures of gliosis (e.g., sTREM2) in Project 2; and 2) influences such as cerebrovascular disease (CVD; assessed in Projects 1 and 4 and in the Clinical Core) and social determinants of health (SDOH) and self-reported race as assessed in the Clinical Core. The ACS also will explore in Project 4 whether physical activity moderates the risk for preclinical AD.

Hypothesis: The onset of preclinical AD, detected by CSF markers of Aβ_{42}, prior to cerebral Aβ accumulation as depicted by PET PIB, may be influenced by inflammation, physical activity, CVD, SDOH, and race.

Overall Specific Aim 2: Characterize the course of preclinical AD

Data from all four Projects, as well as from the Clinical and Fluid Biomarker Cores, will accomplish this Aim. Throughout the duration of preclinical AD, Project 1 will contribute longitudinal information on cerebral tau accumulation and spatial spread as assessed by tau PET. The Fluid Biomarker Core and Project 2 will provide longitudinal CSF concentrations of Aβ, total tau, and tau phosphorylated at position 181, as well as CSF and/or plasma measures that reflect synaptic dysfunction and neuronal injury, astrocytosis/microgliosis, and plasma and CSF markers of inflammation. Project 3 will obtain stool samples over time to examine the composition and function of the gut microbiome, accompanied by measures of gut permeability and inflammation. Project 4 will evaluate the effects of physical activity on cognitive performance and medial temporal lobe structure as assessed by magnetic resonance imaging (MRI) and diffusion tensor imaging.

Hypothesis: Although preclinical AD begins with the cerebral accumulation of aggregated Aβ, tauopathy and other factors (e.g., inflammation) drive the progression to neurodegeneration that triggers symptomatic onset.

Overall Specific Aim 3: Identify factors that accelerate or prevent the transition from asymptomatic to symptomatic AD.

All Projects will contribute to this critical Aim, as will the Clinical and Fluid Biomarker Cores. Project 1 will provide imaging data on CVD, white matter hyperintensities, and network patterns. Project 2 will evaluate novel plasma and CSF biomarkers that confer risk for the transition to symptomatic AD. Project 3 will conduct metagenomics (DNA) and metatranscriptomic (RNA) sequencing from stools from ACS participants to generate bacterial community composition and functional profiles of the gut microbiome. In addition to measures of physical activity, Project 4 will calculate the Framingham Heart Study cardiovascular risk composite, measure trophic factors (e.g., BDNF), and determine plasma cortisol concentration levels. In addition to obtaining demographic features, longitudinal clinical and cognitive measures, blood for *apolipoprotein E* genotype and polygenic risk score, and objective measures of CVD risk factors (blood pressure, body mass index, hemoglobin A1c), the Clinical Core will implement in the next funding cycle a longitudinally administered battery that assesses SDOH in all ACS participants.

Hypothesis: Physical activity, inflammation, CVD, and stress (plasma cortisol, adverse childhood events, perceived stress, and neighborhood disadvantage) will moderate the transition to symptomatic AD.

Overall: Research Strategy

I. ACS 2020 renewal glossary: Glossary of abbreviations and terms used in this application

Alzheimer-centric Programs at Washington University School of Medicine (WUSM)

ADRC: Alzheimer Disease Research Center (P30 AG066444); JC Morris, PI; 5/1/20-4/30/25); endowed in 2010 by Charles F. and Joanne Knight (**Knight ADRC**)

PPGs: Program Project Grants; the Knight ADRC provides intellectual, infrastructure, and philanthropic support for two biomarker-centric PPGs, **HASD**: Healthy Aging and Senile Dementia (P01 AG03991; JC Morris, PI; 5/1/19-4/30/24) and **ACS**: Antecedent Biomarkers for Alzheimer Disease: The Adult Children Study (P01 AG026276; JC Morris, PI; 9/30/16-5/31/21). The current reporting period for ACS is 4/1/15-1/31/20.

Total Registry (TR): The 65y and older cohorts of HASD and ADRC are recruited, enrolled, and assessed identically with a uniform protocol. They are combined as the TR for clinical and analytic purposes.

DIAN: Dominantly Inherited Alzheimer Network (U19 AG032438; RJ Bateman, PI); the **Trial Unit** (**TU**) for DIAN conducts secondary prevention clinical trials.

MAP: The Memory and Aging Project, the clinical research office conducting the clinical and cognitive assessments for **participants** at WUSM who are enrolled in the ADRC, HASD, ACS, DIAN, and DIAN-TU studies. MAP is not a clinic and all assessments are for research purposes only. Participants are community living volunteers and 95% are non-Hispanic Whites (**NHW**) or African American (**AA**).

Funding Agency

NIA: National Institute on Aging; the repository for ADRC data is the NIA-funded National Alzheimer's Coordinating Center (**NACC**).

Definitions

AD: Alzheimer disease, the brain disorder regardless of clinical status*;* **LOAD** is "sporadic" late onset AD

Preclinical AD: asymptomatic AD, identified with molecular biomarkers for AD; individuals with preclinical AD are cognitively normal (**CN**).

Symptomatic AD: Clinically expressed AD, with symptom severity ranging from very mild (prodromal AD; mild cognitive impairment (**MCI**) due to AD) to severe; **AAO**: Age at symptomatic onset

CVD: Cerebrovascular disease

APP, ***PSEN1***, ***PSEN2***: *amyloid precursor protein*, *presenilin1*, and *presenilin2* genes; mutations in these genes cause autosomal dominant AD.

Aβ: Amyloid-beta protein, generated by cleavage of the amyloid precursor protein (**APP**)

APOE*/APOE**: the gene and protein, apolipoprotein E; the ***ε4 allele confers susceptibility for AD.

CL: Core Leader; **PL**: Project Leader

Prevention trials: In CN persons, **secondary prevention** denotes investigational treatment strategies for preclinical AD (i.e., cerebral Aβ accumulation has occurred) and **primary prevention** involves these interventions prior to the onset of preclinical AD (i.e., before cerebral Aβ accumulation).

Procedures/Instruments

CDR®: Clinical Dementia Rating, a trademarked global dementia staging instrument with scores from 0 (CN) to 0.5, 1, 2, and 3 (very mild, mild, moderate, and severe dementia).

CSF: Cerebrospinal fluid, obtained by lumbar puncture (**LP**).

MRI: Magnetic resonance imaging performed at 3 Tesla (**3T**).

PET: Positron emission tomography

- **PIB**: Pittsburgh Compound B, [^{11}C] amyloid radioligand
- **FTP**: Flortaucipir; [^{18}F] tau radioligand (aka AV-1451, T807)
- **SUVR**: standardized uptake value ratio; **MCSUVR**: mean cortical SUVR

UDS: Uniform Data Set, the standard clinical and cognitive assessment protocol for ADCs.

LIAD: Lifecourse Influencing Aging and Dementia battery, assessing social determinants of health (**SDOH**).

II. Responses to Summary Statement (Release Date: 11/30/15)

1. "Concerns about clinical cohort: 1) focus; 2) number of subjects; 3) heterogeneity". 1) The ACS focus is to characterize the risk for development of preclinical AD and to understand the factors mediating the transition from asymptomatic to symptomatic AD. 2) The TR can be utilized for some ACS Aims because the clinical and cognitive protocols for ACS and HASD/ADRC are intentionally identical. The Clinical Core of this proposal reports that in the next grant cycle there will be 178 originally CN participants who progressed to CDR>0 (47 ACS, 131 HASD/ADRC). As recommended by previous reviewers, we will continue to leverage the TR as appropriate to provide ample statistical power to address all ACS Aims. 3) We appreciate that not all symptomatic ACS participants have an amnestic presentation of AD or even AD itself; some symptomatic ACS participants have non-AD clinical diagnoses. The reviewers misperceived that ACS is a study of early onset symptomatic AD; it is not. By design, the ACS cohort is young (at entry, two thirds of ACS participants are 45-64y) so that the onset of preclinical AD can be ascertained and followed. As adult children of a parent with LOAD, ACS individuals who develop symptomatic AD have a mean (+/-SD) age of onset of 73.6y (+/- 5.2y).

2. "Aims of many projects not clearly delineated"; "overlapping aims" of Projects 1 and 4: Poorly described Aims particularly characterized Project 3 in the 2015 application, which was eliminated from ACS. Projects 1 and 4 have been integrated as Project 1 in this renewal application.
3. "Aims cannot be readily achieved": Table 1 in this Overall details the extraordinary completion rates of biomarker studies by ACS participants. We began tau PET imaging in the current grant cycle and have completed 235 baseline and 66 follow-up scans in ACS participants.
4. "Strengthen the EAC": Keith Johnson, MD (Massachusetts General Hospital, tau PET expertise) and Lisa Barnes, PhD (Rush Medical College, SDOH expertise) have joined the EAC.

Note: This renewal application was prepared in March-May, 2020 during the COVID-19 pandemic. The clinical and cognitive ACS assessment instruments are modified for remote administration in accordance with the recommendations of the Clinical Task Force of the ADRCs. ACS leadership cautiously anticipates that by the start of the next budget period (June, 2021), the pandemic will have abated and pre-pandemic protocols will have been re-instated for clinical and cognitive assessments, blood/CSF collection, neuroimaging, and measures of physical activity. This renewal application is written and budgeted with that expectation. However, the "new normal" will require that both participants and staff be protected and methods of data collection increasingly will involve new technologies to meet participant and staff preferences. We recognize that the pandemic particularly challenges persons age 65 and older and thus we monitor them carefully.[1]

III. Significance

The ACS is a longitudinal molecular biomarker study of preclinical AD in CN individuals who are at increased genetic risk for AD because of a parental history of AD dementia.[2] The overarching goal of this renewal application is to fully characterize preclinical AD, defined as the detectable presence of AD pathology in the brain of living individuals who remain CN. This characterization includes clarifying the factors that increase (or decrease) risk for the onset of AD, elucidating the ordering of biomarker changes in preclinical AD from onset to symptomatic AD, and identifying factors that mediate or moderate this transition (we do not assume that all persons with preclinical AD inevitably will become symptomatic, and appreciate factors that may confer brain and cognitive resilience).[3]

ACS participants comprise two groups of individuals who are age 45-74y at initial assessment: one group has a parent affected with symptomatic AD with AAO before age 80y and the other (control) group has both parents unaffected by symptomatic AD. Individuals from families with a known pathogenic mutation in the *APP, PSEN1, or PSEN2* genes are excluded from ACS. Consequently, ACS identifies the preclinical antecedents of "sporadic" LOAD. The mean AAO for ACS participants who progress to symptomatic AD is 73.6y (+/- 5.2y).

Table 1. ACS Participation in Study Procedures as of 1/31/2020

Assessment	Initial n (%)	1st follow-up (3 y), n (%)	2nd follow-up (6 y), n (%)	3rd follow-up (9 y), n (%)	4th follow-up (12 y), n (%)
MRI	432 (93)	327 (100)	226 (100)	150 (91)	81 (76%)
Amyloid PET	408 (87)	282 (96)	210 (100)	135 (84)	77 (73%)
CSF	414 (88)	246 (84)	175 (85)	107 (67)	52 (48%)

Note: Eligibility for follow-up may vary by procedure due to complicating factors (e.g., pacemaker for imaging studies), or previous refusal to complete a procedure. Follow-up completion rates are adjusted for eligible active participants; e.g., a participant enrolled and completing the baseline assessment in 2016 is eligible only for their first follow-up at 3y after baseline (i.e., in 2019).

Participants in AD research studies who consent to LP to obtain CSF and/or to have amyloid PET scans are not representative of the general population, so we maximize the strengths of the study by optimizing longitudinal imaging and fluid biomarker collection. A prospective ACS participant thus must be eligible for and, in principle, willing to undergo the full ACS protocol at baseline and at 3y intervals thereafter. This policy negatively affects AA recruitment due to the mistrust and fear of "invasive" medical research that stems in part from well-documented historical abuses.[4] This requirement for LP is waived for AA in the ADRC and HASD cohorts, which together are 19% AA, exceeding the percent of the greater metropolitan St. Louis population that is AA (18% of the total population and 15.3% for the population $\geq$ 45y of age).[5] In ACS, where LP is expected for all, 11% of the cohort is AA; although suboptimal, this racial diversity is far greater than other AD biomarker-intensive cohorts (e.g., the BIOCARD cohort is 98.6% Caucasian).[6]

Our biomarker completion rates do not reach 100%. A participant may discover that she cannot tolerate a brain MRI due to claustrophobia, and thus cannot have amyloid or tau PET scans as regions of interest cannot be generated. Similarly, well-intentioned participants change their minds about LP when it is time to schedule this procedure. Participants who are eligible at baseline may develop medical conditions that disqualify them. Even with these barriers, completion rates for ACS procedures are extraordinary (Table 1).

The ACS has broad and deep **significance** for the AD field:
1. Its overarching goal, to study AD prior to its symptomatic onset, enables and informs the design and implementation of secondary and primary prevention trials with investigational anti-AD therapies.
2. The ACS cohort is the world's largest longitudinal multimodal **preclinical** AD database and biorepository.

3. Highly impactful research publications are generated from ACS resources (see Progress in **Approach**).
4. Many investigators request its data and biospecimens to address research goals.
a. In the current cycle (2016-2021), the ACS already has fulfilled 200 investigator requests for the highly valued ACS resources (see Core A: Administration).
b. New research efforts that incorporate the ACS include: 1) at WUSM: C. Xiong: RF1 AG053550; Antecedent Biomarker Changes in AD: Ordering, Stages, and Implications for Trials; 9/1/16 – 3/31/21; C. Xiong: R01AG067505 (pending); Cross-sectional and Longitudinal Racial Disparity in Molecular Biomarkers of AD; 4/1/20 – 3/31/25; S. Hartz/J. Mozersky (co-PIs): R01 AG065234; Returning Research Results that Indicate Risk of Alzheimer Disease to Healthy Participants in Longitudinal Studies"; 2/1/20 - 1/31/25; 2) external investigators; B. Zlokovic/A. Toga: P01 AG052350; Vascular Contributions to Dementia and Genetic Risk Factors for Alzheimer's Disease; 9/30/16 – 5/31/21; M. Albert: RF1 AG059869; Preclinical AD Consortium; 9/1/18 – 3/31/23; M. Weiner: RF1 AG059009; Validation of Online Methods to Predict and Monitor Cognitive Decline; 9/1/18 – 3/31/23; C. Masters: R01 AG058676; Alzheimer's Dementia Onset and Progression in International Cohorts; 9/30/18 – 5/31/23.
5. This renewal application preserves the successful ACS leadership and structure developed in its first 15y of funding. At the same time, it incorporates novel scientific initiatives and, in Project 3, includes investigators who are new to AD research. The organization of this renewal application is shown in comparison with the current budget period (Table 2; 2 Associate Directors will now assist Morris).

Table 2. The Adult Children Study: Current and Proposed Organizational Structure

Current (Y11-15; 2016-2021)	Proposed (Y16-20; 2021-2026)
Core A: Administration (JC Morris, PI/CL)	Core A: Administration (JC Morris, PI/CL; T Benzinger, C Xiong, Associate Leaders)
Core B: Clinical (JC Morris, CL)	Core B: Clinical (JC Morris, CL)
Core C: Biomarker (AM Fagan, CL)	Core C: Fluid Biomarker (AM Fagan, Co-CL; S Schindler, Co-CL)
Core D: Data Management & Biostatistics (C Xiong, CL)	Core D: Data Management & Biostatistics (C Xiong, CL)
Project 1: Preclinical predictors of progression from cognitive normality to impairment (JC Morris, PL)	Project 1: Tau burden and spread in preclinical AD (JC Morris, T Benzinger, co-PL)
Project 2: CSF biomarkers of AD progression (AM Fagan, PL)	Project 2: Plasma and CSF biomarkers that predict risk for symptomatic AD (AM Fagan, PL)
(Project 3 was eliminated from the current budget period after discussion with NIA in light of critiques in the Summary Statement)	Project 3: Alzheimer disease progression, host gut microbiome, and enteric dysfunction (B Ances, PL)
Project 4: Dynamic ordering of imaging biomarkers in preclinical AD (T Benzinger, PL)	Project 4: Mechanisms and moderators of the effects of physical activity in preclinical AD (D Head, PL)

IV. Innovation

1. The ACS protocol intentionally is identical to the uniform protocol that is used in the ADRC and HASD grants, which study individuals (both CN and those with symptomatic AD) who are age 65y and older. Each of the 3 grants is budgetarily distinct and does not overlap with the budgets of the other grants, and each investigates its unique Specific Aims. The overall focus on preclinical AD and AD biomarkers and the shared uniform protocol, however, provide tremendous synergy in achieving a combined dataset that not only is much larger than could be accomplished by any individual grant but also offers the opportunity to study AD biomarkers and their correlates across 7 decades as our active participants range from age 45 (ACS) to 104 (HASD). The synergy between ACS, HASD, and ADRC optimizes the use of our intellectual and financial resources by avoiding redundant labor and confusion that separate protocols for each grant would require and efficiently allows a single grant's cohort to be augmented by the cohorts of the other grants, thus optimizing statistical power. Because the ACS protocol includes the full UDS3, ACS data are entered into NACC.
2. The Knight ADRC's Genetics and High Throughput -Omics Core provides genetic analyses of ACS participants (charged to the ACS budget), including DNA and RNA extraction and banking, *APOE* and GWAS genotyping and individual level polygenic risk scores and stores samples and genetic and genomic data as a resource. The ADRC Neuropathology Core provides neuropathological assessments for ACS participants who come to autopsy (costs supported by Morris' discretionary funds) and maintains brain tissue as a biospecimen resource (see the Clinical Core Letters of Support from Drs. Cruchaga and Harari and from Dr. Perrin).
3. Proposed Projects 1 and 2 will continue the ACS longitudinal assessment of molecular biomarkers of AD (amyloid PET, tau PET, CSF $A\beta_{42}$, CSF tau and ptau181), as well as brain MRI, to examine their relationship to preclinical AD. For the remaining 27 active participants of 61 persons who enrolled in a 2002 ACS pilot study, the next budget period will more than encompass the estimated ≥20y duration of preclinical AD. Participants who enrolled when NIA funding began in 2005 will complete their 5th (in 2021) and 6th (in 2024)

every 3y biomarker assessments. In 2020, 170 (56%) of the 296 active ACS participants are 65y or older. In the next budget period, we estimate that 59 ACS participants will transition to CDR>0.

We propose studies of the following conditions that may mediate or modify the preclinical AD process.

3.a. Inflammation: Projects 1 (advanced diffusion sequences) and 2 (inflammatory markers in CSF and plasma) will measure aspects of inflammation in relation to preclinical AD. Project 3 introduces a novel study of preclinical AD as it addresses the gut's non-human residents, the trillions of bacteria that form microbial communities that perform specific and vital functions and affect human physiology, metabolism, and immune responses. Although gut microbiota have been sparsely studied in AD, evidence exists that persons with symptomatic AD differ from older adults with regard to bacteria associated with inflammation. Because inflammation is implicated in AD pathogenesis,[7,8] Project 3 will investigate the possible role of the gut microbiome in the initiation and progression to preclinical AD in the context of gut inflammation and intestinal permeability. The ACS cohort, supplemented with individuals enrolled in HASD and ADRC, will provide stool samples at baseline and every 3y thereafter for Project 3 studies. Serologic analyses (e.g., C-reactive protein, interleukin-6; α-1 acid glycoprotein) will measure systemic inflammation. Project 3 is the first integrated longitudinal study of the gut microbiome, intestinal dysfunction, and systemic inflammation in preclinical AD.

3.b. Cerebrovascular disease

AD and CVD frequently co-occur.[9,10] The factors responsible for CVD are readily determined and have established treatments that may serve as primary prevention strategies for AD. Indeed, improvements in controlling cardiovascular risk factors have been cited as a key element in the recent declines in age-specific risk of AD in high-income countries.[11] The influence of these factors for the development and progression of preclinical AD has been less well studied.[12] We propose in this application to calculate the Framingham Heart Study risk score and obtain clinical (e.g., systolic blood pressure, body mass index [BMI], HbA1c) and imaging (e.g., infarcts, white matter hyperintensities) variables to assess their influence on preclinical AD.

4. Physical activity and exercise

Physical activity, exercise, and cardiorespiratory fitness have been proposed to lessen risk of cognitive decline,[13] protect against dementing illnesses,[14-17] and modify AD biomarkers.[18-20] Earlier efforts to evaluate exercise effects in ACS participants[21,22] were limited. New Project 4 proposes to assess, both cross-sectionally and longitudinally, the effects of physical activity measured with actigraphy over 7 days and by cardiorespiratory fitness on cognitive function, medial temporal lobe structure, and AD molecular biomarkers. This Project also will assess effects of physical activity on trophic factors (e.g., BDNF) and stress exposure (adverse childhood experiences) and stress reactivity (plasma cortisol).

5. Racial differences

The ACS is committed to studying preclinical AD in the two major racial groups in the St. Louis area, NHWs and AAs (together, Hispanics and Asian American represent only ~6% of the population). Concentrations of CSF tau and ptau181 are significantly lower in AAs than in NHWs;[23,24] whether differences involve other biomarkers (e.g., tau PET studies of tau burden and spread) or clinical expression is unknown. There are substantial challenges to the enrollment, participation, and retention of AAs in studies of preclinical AD.[4,25] Guided by the African American Advisory Board (AAAB) of the Knight ADRC, we address issues of trust, communication, education, incentives, and engagement with the St. Louis AA community to improve the recruitment and retention of AAs in ACS (see Letter of Support from AAAB Chair, Dr. Douglass Petty). AA participants indicate that they are motivated by the opportunity to learn their individual research results, which we now provide with the support of the R01 awarded to Drs. Hartz and Mozersky. Participants also indicate greater trust when the research team includes "people who look like me". Dr. Denise Head serves as Leader of Project 4; Joyce Balls-Berry, PhD, will join the Knight ADRC July 1, 2020, as Leader of the new Knight ADRC Community Engagement Core (see Letter of Support from Dr. Balls-Berry). We collaborate with the Emory ADRC to mutually share aliquots of plasma and CSF and UDS data for AAs assessed at each site. We will provide Emory with 220 baseline and 100 longitudinal CSF and matching plasma aliquots from our AA participants and receive from Emory 160 baseline and 100 longitudinal aliquots from their AA participants (each set will include an equal number of samples from matched NHW participants; see Letter of Support from Dr. Allan Levey).

Complex racial differences in AD[26] often are assumed to reflect genetic or other biological factors.[27] Life experiences seldom are addressed, although these experiences are known to contribute to biophysiological changes. [28-33] We developed the Lifecourse Influencing Aging and Dementia (LIAD) battery (see Clinical Core) as part of the clinical assessment for all ACS, HASD, and ADRC participants to assess social determinants of health (SDOH) that may affect AD risk.[34] Prior to the next budget period, the ACS will pilot the administration of the LIAD battery to determine its psychometric properties.

6. Innovative projects using ACS biospecimens to inform ACS research:

a. Suzanne Schindler, MD, PhD, and colleagues are extending their original study[35] regarding the clinical utility of a plasma $A\beta_{42}/A\beta_{40}$ biomarker for AD, as assayed with high precision liquid chromatography-mass spectrometry, for its correlation with cerebral amyloidosis and for its diagnostic value for preclinical AD.

b. The first Knight ADRC Developmental Project in its initial P30 budget period (5/1/20-4/30/25) funds Nico Barthelemy, PhD, who will extend to "sporadic" preclinical AD his finding in mutation carriers from families with autosomal dominant AD that tau phosphorylation state changes mark the progression of preclinical AD.[36]

V. Approach

A. Background

Pathological AD is defined by abnormal aggregates of the proteins amyloid-beta (Aβ), deposited in the extracellular cerebral parenchyma as plaques, and hyperphosphorylated tau, which accumulates intracellularly as neurofibrillary tangles (NFTs), neuritic plaques, and neuropil threads. The occurrence of symptomatic LOAD is strongly age-associated, such that over 90% of persons with AD dementia are age 70y or older.[37] In addition to Aβ and tau proteinopathies, the brains of most older adults with AD have age-associated co-pathologies that confer clinical and neuropathological heterogeneity to the disorder.[9] These co-pathologies include TDP-43[38] and α-synuclein proteinopathies, neuroinflammation, disruption of blood-brain barrier integrity,[12] the possibility of invading pathogens,[39] and other cellular and molecular mechanisms.[40] A large number of genes and multiple potential risk factors also contribute to the pathobiological complexity of AD.

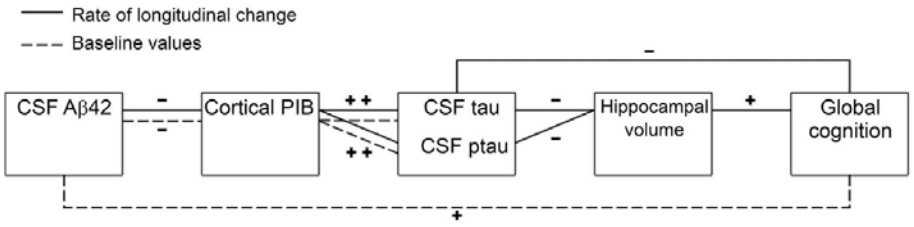

Figure 1. Longitudinal and cross-sectional biomarker correlations. + and – represent positive and negative correlations, respectively. Ref 46.

It may be simpler and more productive to consider the origins of AD **prior** to symptomatic onset. The symptomatic stage of AD is the end-stage of a decades-long disease process that develops and progresses without clinical expression. This asymptomatic preclinical stage of AD is detected and monitored with molecular CSF biomarkers and with PET imaging with radioligands to detect aggregated Aβ and tau.[41] Biomarker studies of preclinical AD suggest that it begins in middle age in the absence of the age-associated comorbid disorders that confound the study of "sporadic" LOAD.[42] Biomarkers related to Aβ first become abnormal, followed by abnormal tau biomarkers and "downstream" indicators of disease (e.g., cerebral hypometabolism and volume loss) until the continuous pathologic cascade ultimately culminates in cognitive decline (Figure 1).[41,43-45] Modeling of molecular biomarkers of preclinical AD suggests that concentrations of CSF $A\beta_{42}$ show accelerated age-related change at ~age 46y and amyloid PET accumulation begins at ~age 54y.[46]

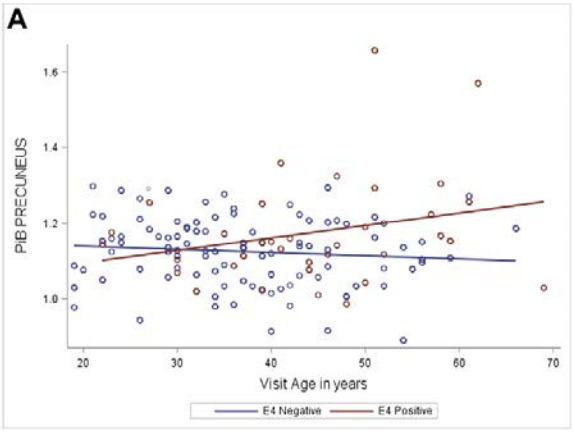

Figure 2. Interactions between age and *APOE* status for amyloid deposition. Ref. 51.

Although below the detection threshold of current biomarkers, it is possible that AD pathophysiology begins before the 5th decade of life.[47] Careful study of AD neuropathology in 2,332 brains found pretangle pathology in the locus ceruleus beginning in the 2nd and 3rd decades of life; NFTs begin to be detected in the tranentorhinal cortex in the 3rd decade.[48] Similar to other studies,[49,50] we found in DIAN mutation noncarriers age 19-66y an age-related increase in amyloid deposition in *APOE4* carriers beginning at age 30-40y (Figure 2), although this increase was not statistically significant when participants ≥ 50y were excluded.[51] We maintain a lower age limit of 45y for ACS, but will consider younger participants as we monitor the evidence.

Based on clinicopathologic[52-54] and stereologic[55,56] studies, we developed a model wherein preclinical AD that had yet to cause measurable synaptic or neuronal loss in regions highly vulnerable to the AD process permitted the affected individual to remain CN. Once synaptic and neuronal integrity is sufficiently breached, symptomatic AD ensues.[57] AD molecular biomarkers enable the detection of AD pathology in living persons.[58,59] We were the first to report the *in vivo* detection of preclinical AD[60] and to demonstrate that biomarker-defined preclinical AD is not benign.[61,62] These studies of older CN individuals, however, included too few participants under the age of 65y to identify when during adulthood preclinical AD might begin. To address this question, we inaugurated the ACS.

The ACS research team encourages the ACS individuals to contribute to the goals of the study (i.e., the cohort is made up of **participants**, rather than subjects). An annual ACS Participants Meeting for ACS participants and their family members reviews recent progress toward ACS research goals, discusses new initiatives, and ensures that all goals align with those of the participants. Our original informed consent documents indicated that we would not return individual research results to the participants. The large majority of ACS participants, however, have strongly expressed interest in knowing their results.[63] The participants

themselves engaged the ACS study team in discussions that led to a ADRC pilot project (S Hartz, PI; 5/1/17-4/30/18) and a 2019 NIA administrative supplement to explore returning results to ACS participants who wish to know them. A new R01 (see **Innovation**) awarded to S. Hartz and J. Mozersky (co-PIs), will evalaute methods to: 1) return and explain individual results (e.g., brain MRI, amyloid PET, *APOE* status); 2) inform participants of the limitations of the results regarding risk for AD; and 3) assess possible drawbacks to learning one's results (e.g., adverse psychological reactions; loss of confidentiality; effect on future ACS performance).

B. Variables Obtained at Baseline and Every 3Y (participants ≥65y clinically are assessed annually)

A. Clinical Core

1. Clinical measures (see Table 4, Clinical Core): History of COVID-19, demographics, health history, family history of AD, SDOH (LIAD; see Table 5, Clinical Core), the International Physical Activity Questionnaire (IPAQ), dyanometer handgrip, "up and go" gait speed, all UDS3 measures (including GDS, NPIQ, Functional Assessment Scale), CDR, neurological exam, blood pressure, BMI, HbA1c

2. Cognitive measures (see Table 6, Clinical Core): Benson Figure, Animal Naming, Vegetable Naming, Letter Fluency, Craft Story 21, Free and Cued Selective Reminding, Multi-lingual Naming Test, Number Span, Slosson Oral Reading Test, Trailmaking A & B, WAIS-R Digit Symbol, WAIS-III Letter-Number Sequencing, WMS Associate Memory , Simon Test, Stroop Switch, CVOE Switching, Tapping Task, MoCA, MMSE

B. Project 1

PET with radioligands for Aβ (PIB) and tau (FTP), brain MRI: volumetrics, resting state functional connectivity, DTI, white matter integrity and networks), advanced diffusion (neuroinflammation), cerebrovascular lesions (infarcts, microhemorrhages), white matter hyperintensities.

C. Fluid Biomarker Core/Project 2

1. CSF: $A\beta_{42}$, $A\beta_{40}$, ptau181, markers of synaptic dysfunction (Ng, SNAP-25), markers of neural injury/death (tau, VILIP-1, NfL), novel markers of disease progression (ptau217, ptau205) and inflammation.
2. Plasma: NfL, novel candidate markers of inflammation

D. Project 3

1. Gut microbiome sequencing from stool samples: metagenomics (derived microbial taxonomic composition; derived alpha [within sample] and beta [between sample] diversities); metatranscriptomic sequencing (derived microbial gene and pathway expression; derived alpha [within sample] and beta [between sample] diversities)
2. Enteric dysfunction and gut permeability: intestinal permeability measures (α-1-antitrypsin from stool; antibodies to lipopolysaccharide (LPS) core antigen and LPS binding protein in plasma or serum); intestinal inflammation measures in stool (calprotectin, lipcalin-2, myeloperoxidase, neopterin)
3. Plasma markers of systemic inflammation (CRP, IL-6, α-1-acid glycoprotein)

E. Project 4

7-day actigraphy to obtain metabolic equivalents (METs); cardiorespiratory fitness (Ekblom-Bak submaximal exercise test to generate estimated $V0_2$ max values); blood draw for trophic factors (BDNF, VEGF, IGF-1) and plasma cortisol; structural MRI and DTI from Project 1 for hippocampal and parahippocampal volumes and diffusivity; Framingham Heart Study risk score;[64] IPAQ from Clinical Core.

C. Progress

C.1. Cohort Description The ACS participants are community-living volunteers, recruited primarily by "word of mouth", and are age 45y-74y at baseline. The majority enroll because of a parental history of AD. At present, about 60% of the ~300 active ACS participants have a parental history and ~40% do not.

C. 2 Overall Progress

In addition to the progress[35,65-74] reported by Projects 1 and 2, AD biomarker changes were evaluated in 3284 CN persons ranging in age from 18y to 101y from the ACS, BIOCARD, WRAP, and AIBL cohorts. Changes in the CSF $A\beta_{42}/A\beta_{40}$ ratio began at 46y. Only the longitudinal rate of change in CSF tau and ptau181 correlated with cognitive change. Different stages of preclinical AD present different targets for prevention trials.[46]

D. Coherence and interrelationships. The multidisciplinary ACS team is well-integrated and highly collaborative. The ACS has been led since inception by Morris, who has co-authored multiple ACS publications with every Washington University investigator in this application except Drs. Tarr and Dantas in Project 3, who are new to AD research. With this application, Drs. Benzinger and Xiong join the ACS leadership as Associate Directors. Drs. Fagan, Grant, Head, and Xiong were investigators in the initial ACS grant from 2005 to 2010; Drs. Ances, Benzinger, Hassenstab, and Moulder joined in 2011-2016 and Drs. Gordon and Schindler in 2016-2021. The stable leadership and the experienced multidisciplinary scientific team contribute importantly to the success and productivity of ACS. Dr. Xiong reviews each ACS manuscript for rigor and reproducibility before it is submitted for publication. All Core and Project Leaders meet every two months to review progress toward goals, address problems, nominate ideas and analyses for manuscripts, and discuss new scientific initiatives. The Administration, Clinical, and DMBC interact regularly with all ACS components. Drs. Morris, Moulder, and Xiong have been directly involved in the preparation of each component of this application.

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