UNIVERSITY OF CALIFORNIA SAN DIEGO

Clinicopathologic Studies of Alzheimer's Disease and Related Dementias

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in

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by

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The dissertation of Denis S. Smirnov is approved, and it is acceptable in quality and form for publication on microfilm and electronically.

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ABSTRACT OF THE DISSERTATION

Clinicopathologic Studies of Alzheimer's Disease and Related Dementias

by

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Alzheimer's disease (AD) is the single most common cause of dementia, but AD alone accounts for less than half of all cases. A variety of other brain pathologies can cause cognitive impairment either alone or in combination with AD. A clinical diagnosis often fails to capture this complexity, so evaluation of brain pathology at autopsy remains the gold standard for establishing the underlying causes of dementia. As disease-modifying therapeutics targeting the underlying mechanisms of neurodegenerative diseases are developed, there is a pressing need for clinicopathologic studies to identify distinct clinical presentations that rise from these various pathologies in order to simplify differential diagnosis, improve prognosis of future decline, and help better target interventions.

Here, I present a series of clinicopathologic studies that characterize and compare the dementia syndromes associated with neuropathologically-verified AD, Hippocampal Sclerosis, Lewy body disease, and their interactions. The first study demonstrates that Hippocampal Sclerosis, alone or in combination with AD, produces a dementia syndrome that is virtually indistinguishable from AD. The generally slower longitudinal trajectory of cognitive decline in patients with Hippocampal Sclerosis, however, may help clinically distinguish the disorder from AD. The second study revealed double-dissociations in patterns of cognitive deficits and longitudinal declines between Dementia with Lewy Bodies (DLB) and Parkinson's Disease Dementia (PDD) that likely reflect subtle differences in pathology. These results suggest that pooling DLB and PDD patients in clinical trials targeting Lewy body pathology may reduce the power to see an effect of treatment unless the appropriate cognitive domain for each is targeted by the trial outcome measures. The final two studies demonstrate considerable variability in clinical and cognitive presentation across age of onset within those with severe AD at autopsy, and show that this variability is (at least partly) mediated by the distribution of neurofibrillary tangle (NFT) pathology. Those with younger onset AD have disproportionately greater neocortical NFT pathology relative to their degree of hippocampal NFT pathology. These findings help explain the paradox that those patients with younger onset of symptoms tend to have higher likelihood of atypical clinical presentations of AD, even though they tend to have less concomitant non-AD neuropathology.

Chapter 1

General Introduction

Dementia is clinical syndrome of acquired global cognitive impairment that is severe enough to significantly interfere with one's usual daily functioning. It is estimated that dementia is currently present in over 50 million individuals worldwide, with an estimated prevalence of 15% in individuals over age 68 in the United States[1]. Alzheimer's disease (AD) is the single most common cause of dementia in the elderly with a usual age of onset between 60 and 90 years of age[2]. There is an exponential increase in the prevalence of AD across this age range, beginning at 1-2% prevalence in those 65 years old, and doubling approximately every 5 years to over 30% prevalence among those 85 years of age or older[3,4]. The occurrence of AD in those under the age of 60 is relatively rare – estimated at around 41 cases per 100,000 and making up approximately 6% of all diagnosed AD[5].

While the biological cause of AD remains debated, there are known genetic factors that confer increased risk of disease at an early age. While they only account for less than 0.1% of all AD cases, there are extremely rare instances of autosomal-dominant genetic inheritance of AD within families that confer near-guaranteed incidence of the disease with an early age of onset (e.g., in the third, fourth or fifth decade of life)[6,7]. For the remaining sporadic (non-familial) cases of AD, a major genetic risk factor is the ε4 allele of Apolipoprotein E (APOE) – a protein involved in the transport of cholesterol in the blood[8]. The ε4 allele, with a worldwide frequency of approximately 14%, confers an approximately three-fold risk of AD in those who are heterozygous and an approximately ten-fold risk in those who are homozygous[9,10]. Recently, additional genetic variations with small effects have been combined with APOE to create a Polygenic Hazzard Score for AD, which further improves the prediction of the age-specific risk for AD beyond the effects of APOE genotype[11].

While the term *dementia* (from the Latin *de mens*, without mind) dates back to Roman physicians, it was not until the early 20th century that advances is microscopy, anatomy, and histology allowed clinicopathologic studies to tie clinical syndromes to pathology of the brain. In

one such study in 1907[12], Alois Alzheimer, a German psychiatrist and pathologist, published a case study of a 55-year-old woman with severe dementia and a 5-year history of progressive problems with memory, language, and behavioral disturbances. At autopsy, using the new silver staining method developed by Bielschowsky[13], he observed miliary foci (which we now recognize as amyloid plaques) and fibrils (neurofibrillary tangles) throughout the cerebral cortex. However, “Alzheimer’s disease” (as it was first termed in a 1910 textbook by his colleague Kraepelin[14]) did not garner much research interest and was categorized as a rare presenile (classically, before age 65) dementia.

A watershed event in the study of AD was the realization that widely prevalent “Senile Dementia” in those over age 65 was not a normal part of aging, but was for the most part caused by the same neurodegenerative disease that had been described decades earlier by Alzheimer. In a series of papers Blessed, Roth, and Tomlinson observed plaque and tangle pathology in more than 50% of their cases of senile dementia that was indistinguishable from that found in presenile AD[15–17]. In 1976 Robert Katzman summarized these findings in a landmark editorial[18], and suggested that based on epidemiological data, AD was the 4th leading cause of death in the elderly. Thus, presenile and senile forms of AD were united as a single entity with common clinical features and identical pathology.

AD is still formally characterized by the same two abnormal protein depositions of extracellular plaques composed largely of aggregated β-Amyloid (Aβ) protein and intracellular neurofibrillary tangles (NFTs) composed of hyperphosphorylated Tau[19]. Efforts to directly relate the severity of these pathologies to objective measures of cognition date back to 1968 and the same work of Blessed and colleagues[15], who for the first time showed a strong direct correlation between number of neocortical amyloid plaques and performance on a standardized test of mental status. Decades of clinicopathologic studies since then have shown associations between pathologic substrates of AD and severity of cognitive impairment and decline, though it

has now been established that the associations are much stronger with NFT pathology (than Aβ) that appears to relate more closely to neuronal death[20].

The NFT pathology of AD is believed to usually begin in limbic regions and then spread in a stereotyped pattern to associated neocortical regions as described by Braak and Braak[21]. This results in the insidious onset of a typical amnestic presentation, consistent with disruption of medial temporal lobe (MTL) structures critical for memory. Subsequent spread of NFT pathology beyond the MTL to temporal, parietal, and frontal cortices is accompanied by progressive deterioration of the associated cognitive abilities (e.g., language, visuospatial, and executive functions). Although the distinction between senile and presenile dementia is no longer thought to have a biological basis, there is substantial variability in clinical presentations of AD across the age of onset spectrum. Atypical presentations of AD are substantially more common in patients with earlier ages of onset and include Posterior Cortical Atrophy (PCA) with prominent visuospatial impairment[22], Primary Progressive Aphasia (PPA) with predominant language deficits[23], and a frontal variant with pronounced behavioral and dysexecutive features[24]. Each of these syndromes can be caused exclusively by AD pathology[25,26], but the distribution of NFT pathology is distinct in each and directly related to their clinical features[27,28]. A relationship between NFT distribution and age of onset was shown in a study that pathologically categorized AD patients into those with “Hippocampal Sparing”, “Limbic Predominant” or typical AD subtypes based on the densities of NFTs in limbic vs neocortical regions[29]. Those with “Hippocampal Sparing” (i.e. greater neocortical than limbic NFT burden) had the youngest average age of onset and the highest number of patients with an atypical clinical presentation (e.g. non-memory). These results suggest that age remains an important but poorly understood determinant of the clinicopathologic relationship in AD.

While AD is clearly the most common cause of dementia, over 60 distinct etiologies have been described[30]. Many of these additional causes are other neurodegenerative diseases that

also have an increased risk with advancing age. Indeed, it has been suggested that most cases of dementia developing after age 85 are caused by multiple distinct abnormalities[31,32], and a clinical diagnosis of dementia typically fails to reflect this pathologic complexity in the brain. Since pathologic assessment at autopsy remains the "gold standard" or only approach for the diagnosis of many of these dementia causing diseases, there is a pressing need for clinicopathologic studies to identify distinct clinical presentations that rise from these distinct pathologies or their combinations. This information could simplify differential diagnosis, improve prognosis of future decline, and help to better understand brain-behavior relationships. Among the most common neurodegenerative diseases that can cause dementia, either alone or in concert with AD, are Lewy body disease, hippocampal sclerosis, cerebrovascular disease, and fronto-temporal lobar degeneration.

Lewy body pathology was first described in Alzheimer's laboratory by Fritz Jakob Heinrich Lewy in 1912[33] while studying Parkinson's disease (PD). For decades after its discovery, Lewy body pathology was only observed in brain stem nuclei (e.g., substantia nigra, dorsal vagal nucleus, nucleus basalis of Meynert) and thought to be associated only with motor symptoms of PD. However, in a seminal clinicopathologic study by John Woodard in 1962[34], these eosinophilic intraneuronal inclusion bodies (now termed the Lewy bodies) were found in the brainstem of 27 patients with psychiatric symptoms and cognitive decline, only a quarter of whom had motor PD. Lewy bodies were first observed in the neocortex by Kosaka in 1978[35] and soon after found to be associated with dementia[36,37]. In the 1990s, aggregated α-synuclein protein was discovered as the primary constituent of Lewy bodies[38]. The first diagnostic criteria for "Dementia with Lewy bodies" (DLB) were published in 1995 with core criteria of dementia, visual hallucinations, fluctuations in attention or consciousness, and motor symptoms of parkinsonism[39]. As Lewy bodies appeared to sometimes be associated with motor PD, sometimes with dementia without parkinsonism, and sometimes both, in 2005 the criteria were

revised so that DLB would refer to cases in which cognitive impairment preceded or occurred concurrently with motor parkinsonism, while Parkinson's Disease Dementia (PDD) would refer to cases in which dementia developed in the context of long-standing PD[40]. Clinicopathologic studies of both DLB and PDD have unequivocally shown that cognitive impairment is associated with the presence of LBs in limbic and neocortical regions, however the picture is complicated by the commonly concomitant pathology of AD, which occurs more often in DLB than PDD[41–43]. There is considerable debate if DLB and PDD should be considered a single entity or as distinct disorders based on various differences in the clinical and cognitive presentation[44–46].

Hippocampal Sclerosis (HS), a prevalent cause of dementia in the oldest-old, is characterized by severe neuronal loss and gliosis in the CA-1 and subiculum of the hippocampal formation, which occurs in the presence or absence of concomitant AD[47,48]. A number of clinicopathologic studies of patients with HS suggested that this condition may be an "AD mimic" due to its considerable similarities to AD in clinical and cognitive presentation[49,50]. Indeed, most patients with HS are misdiagnosed as AD clinically due to the current lack of clinical diagnostic criteria for HS[49,51]. Some have suggested that medial temporal FDG-PET hypometabolism in presence of negative biomarkers for AD may be indicative of HS, but this has yet to be replicated, and does not allow for diagnosis of co-occurring AD and HS, which is common based on pathologic studies[52]. It has been recently discovered that HS is almost exclusively associated with abnormal TDP-43 pathology. After proposal of several staging and diagnostic schema for TDP-43 pathology[53–56], most recent guidelines refer to this proteinopathy as Limbic-Associated TDP-43 Encephalopathy (LATE)[57]. LATE is staged by the spread of TDP-43 and may or may not be associated with HS. However, the clinical impact of TDP-43 in the presence or absence of HS or concomitant AD has yet to be well characterized.

Vascular dementia is believed to be a major cause of cognitive impairment in the elderly and is a common clinical diagnosis[58,59]. However, the relationships between vascular lesions at

autopsy and clinical presentation of dementia are often weak and variable[60,61], partially due to the limitations in the tools used to assess subtle vascular disease pathologically, and partly to the broad variability of the location and extent of vascular lesions across individuals. Furthermore, at autopsy, AD is often discovered as a contributing or even sole cause of cognitive impairment attributed to vascular disease clinically[62]. Nonetheless, those with both AD and vascular pathology exhibit more rapid cognitive deterioration than those with AD alone[63].

A constellation of rare conditions collectively known as Fronto-Temporal Lobar Degeneration (FTLD) can also result in dementia with unique clinical features reflective of the range of possible underlying pathologies – which may include aggregates of Tau, TDP-43, FUS, or other proteins[64]. While FTLD cases most often have onset between the ages of 40 to 65, and age is used as a major factor in establishing a probable FTLD etiology clinically, there is considerable overlap between the older cases of FTLD and the younger cases of AD, posing significant challenges for differential diagnosis[65,66].

Accurate diagnosis of the underlying cause of cognitive impairment and dementia has become increasingly important as disease-modifying therapies targeting the underlying protein aggregates continue to make progress in clinical trials. Extensive effort has resulted in the development of imaging, CSF, and even plasma biomarkers for AD, but unfortunately there is currently no definitive way to diagnose the non-AD related proteinopathies during life. Moreover, the high rate of co-occurrence of AD with these pathologies, especially with increasing age[32], means that a positive marker for AD does not rule out the presence of other pathologies that may influence progression and response to potential treatments. In this aged population, Occom's razor is replaced with Hickam's dictum: "patients can have as many diseases as they damn well please."

Given the lack of biomarkers for many of the non-AD causes of cognitive impairment, it is important to identify unique patterns of clinical and cognitive deficits that might provide clues

to the underlying etiology. The disruption of specific neuronal structures and circuits by each pathology might manifest in distinct and identifiable patterns of cognitive impairment which could help to distinguish among these similar dementia syndromes during life. Patterns of cognitive impairment can be revealed by examining performance on an extensive battery of cognitive tests that can be reduced into measures of specific cognitive domains (e.g. Memory, Language, Visuospatial ability, etc.) via statistical approaches such as principal component analysis or by expert judgement[67]. Patterns of cognitive impairment across these domains may be an effective method of differentiating which pathology is likely responsible for the impairment.

The identification of patterns of clinical and cognitive deficits that might be useful for differential diagnosis requires large-scale retrospective clinicopathologic studies of cases with known pathology. It is essential to tie the clinical presentations to pathologic ground truth since describing the features of a clinically diagnosed group will simply identify many of the features that were used to make the diagnosis in a circular fashion. Furthermore, certain pathologic entities that clearly result in cognitive impairment (e.g., HS and TDP-43) do not currently have criteria for clinical diagnosis and are nearly universally misdiagnosed as AD. Clinicopathologic studies that define pathologies independently of clinical context allow unbiased descriptions of the clinical phenotypes that could be used to devise more accurate clinical criteria.

Here, I present a series of studies in which we characterize the clinical and cognitive differences between a number of pathologies that cause or contribute to dementia. First, we examine the profiles of cognitive trajectories of patients with Hippocampal Sclerosis compared to those with AD, or those with both pathologies, and note differences that may aid in a clinical differential diagnosis during life. Next, we examine the clinical profiles and cognitive trajectories of two groups of patients with Lewy body pathology – those with DLB and those with PDD – and those with pathologically-confirmed pure AD, and demonstrate differences between the three groups that may contribute to differential clinical diagnosis and are essential to consider in the

design of clinical trials targeting these populations. Finally, we present a pair of studies that examine age-related heterogeneity in clinical and cognitive presentations of autopsy-confirmed AD and show that AD itself may present with atypical clinical and cognitive features in those with early onset. We further show that these atypical presentations occur in the absence of concomitant pathologies, and appear related to the relative distribution of limbic-to-neocortical NFT pathology.

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Chapter 2

Trajectories of Cognitive Decline Differ in Hippocampal Sclerosis and Alzheimer's Disease

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Abstract

Hippocampal Sclerosis (HS) is a prevalent cause of dementia in the oldest-old, but is generally misdiagnosed as Alzheimer's disease (AD) due to similarities in clinical presentation. To determine if clinical and cognitive features diverge over time, we compared results from longitudinal evaluations of participants in the UCSD Alzheimer's Disease Research Center with autopsy-confirmed AD (n=195), HS (n=21), or both HS+AD (n=18). Each group exhibited decline on all cognitive measures, with HS declining at a slower rate than AD on the Mini-Mental State Exam, immediate recall condition of a word-list learning test, and Dementia Rating Scale total and subtest scores (except Memory). Five years prior to the final evaluation, more prominent semantic and visuospatial deficits were apparent in AD than in HS despite comparable global cognitive impairment. Groups did not differ on any measure of Executive Function. HS+AD differed from AD only on the Boston Naming Test. Overall, results suggests that HS dementia is associated with cognitive deficits that progress more slowly than, but generally mimic, those observed in AD.

2.1 Introduction

Hippocampal Sclerosis (HS) is characterized by severe neuronal loss and gliosis in the CA-1 and subiculum of the hippocampal formation[1,2]. While predominantly studied in temporal lobe epilepsy, HS is increasingly recognized as the cause of dementia in up to 25% of the "oldest-old"[3–6]. Despite clear pathologic differences at autopsy, HS dementia presents with prominent memory-loss[1] as well as impairment in language, executive function, attention, visuospatial abilities, and perceptual speed[6–8], and is typically misdiagnosed as Alzheimer's disease (AD) in the clinic[3,4,9]. While patients with HS tend to be older and less functionally impaired than those with AD[9], no clinically differentiating features have been identified.

Comparisons of cognitive profiles between AD and HS have yielded inconsistent results: greater visuospatial, executive, and attention impairments in AD versus HS[7], only greater executive impairment in AD[3], or no differences in cognition between the two[10]. Results indicating that patients with HS exhibit slower decline on the Mini Mental State Exam (MMSE) than those with AD[11] suggest that these variable findings may be attributable to differences in trajectories of decline across various cognitive domains which are obscured in cross-sectional comparisons. In a single longitudinal study, Nelson *et al.*[4] identified relatively preserved verbal fluency with similarly impaired word-list recall in HS compared to AD at baseline and 5.5-6.5 years before death. A modest group-level difference in the ratio of these measures was replicated in the National Alzheimer's Coordinating Center database[9], but with too much overlap for individual discrimination. We now extend this work by comparing trajectories of decline in HS, AD, or HS+AD on a comprehensive panel of cognitive measures to determine if profiles of decline can assist in clinical differentiation.

2.2 Materials and Methods

2.2.1 Standard Protocol Approvals, Registrations, and Patient Consents

The research protocol was reviewed and approved by the human subject's review board at the University of California, San Diego. Informed consent to participate in the study was

obtained at the point of entry into the ADRC longitudinal study from all patients or their caregivers consistent with California State law. Informed consent for autopsy was obtained at the time of death from the next of kin.

2.2.2 Participants

Cases for this study were selected from the brain bank of the Shiley-Marcos Alzheimer's Disease Research Center (ADRC) at the University of California, San Diego (UCSD). Cases were included if they had completed at least two longitudinal (approximately annual) clinical and neuropsychological evaluations between 1985 and 2018, received a pathologic diagnosis of hippocampal sclerosis (HS), Alzheimer's disease (AD), or both (HS+AD), and did not have another neurodegenerative pathology that could account for cognitive decline. We excluded cases with the following concomitant pathologies: Fronto-Temporal Lobar Degeneration, Parkinson's disease, Multiple Sclerosis, and Tangle-only Dementia. The presence of any Lewy body pathology also resulted in exclusion from the sample. Participants were also excluded if pathological data were incomplete, their last ADRC evaluation was more than 4 years from the date of death, or they died prior to age 65 (**Figure 1**). In contrast, presence of vascular pathologies did not warrant exclusion as there may be a causal relationship with HS[1,3,12,13]. The prevalence of these vascular pathologies in each group is summarized in **Table 1**.

2.2.3 Clinical Evaluation

Participants had annual standardized and detailed clinical, neurological, and neuropsychological assessments as previously described[14,15]. Global cognitive function was assessed with the MMSE[16] and the Dementia Rating Scale (DRS)[17] including its subscales for Attention, Initiation, Conceptualization, Construction, and Memory. Memory was further assessed with the Wechsler Memory Scale (WMS) Visual Reproduction Test immediate and delayed recall (adaptation)[18], the WMS-R Logical Memory Test, the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) Word List Learning Test[19], and the California Verbal Learning Test (CVLT)[20]. Language was assessed with the Boston Naming Test-30 item version

(BNT)[21], the Letter Fluency Test (F-A-S) and the Category Fluency Test ("animals", "fruits", and "vegetables")[22]. Executive Function and Attention was assessed with the Modified Wisconsin Card Sorting Test[23], Wechsler Adult Intelligence Scale-Revised (WAIS-R) Digit Symbol Substitution Test[24], Trail Making Test Parts A and B (measured as seconds/circle)[25], WAIS-R Digit Span Test[26]. Visuospatial Abilities were assessed with the Wechsler Intelligence Scale for Children-Revised (WISC-R) Block Design Test and the copy condition of the WMS Visual Reproduction Test. Functional impairment was assessed using the Pfeffer Outpatient Disability (POD)[27] scale or the Functional Assessment Questionnaire (in which case, scores were converted to appropriate corresponding POD scores).

2.2.4 Neuropathological Evaluation

UCSD ADRC procedures at autopsy were as follows: the brain was divided sagittally, and the left hemibrain was fixed in 10% buffered formalin, while the right hemibrain was sectioned coronally and then frozen at −70°C in sealed plastic bags. Routinely, tissue blocks from the right hemibrain of the midfrontal, inferior parietal, and superior temporal cortices, primary visual cortex in the occipital cortex, hippocampus, basal ganglia, substantia nigra, and cerebellum were removed and placed in 2% paraformaldehyde for subsequent thick sectioning by vibratome. Tissue blocks adjacent to the ones described above were stored at −70°C for subsequent immunoblot analysis for synaptic proteins and Aβ species (soluble and oligomers). Vibratome sections (40 μm thick) were stored in cryoprotective medium at −20°C for subsequent immunochemical studies. The formalin-fixed left hemibrain was serially sectioned in 1 cm slices, and tissue blocks from the regions described above were processed for histopathological examination by H&E and Thioflavin-S (Thio-S) to detect tau and β-amyloid deposits.

Brains were staged for degree of neurofibrillary tangle pathology by one pathologist (L.A.H) using a modification of the Braak staging scheme[28]. Estimates of neuritic plaque density were calculated using methods recommended by CERAD[29]. To match previously published

analyses comparing HS and AD[4,9], Alzheimer's disease was operationalized using the NIA-Reagan consensus criteria for the postmortem diagnosis of AD, wherein Braak stage V-VI with moderately to severely dense neuritic plaques corresponds to "high likelihood" that dementia is due to AD. Hippocampal Sclerosis was pathologically defined as cell loss and gliosis in CA1 and the subiculum of the hippocampus, out of proportion to AD pathology.

Participant brains were assessed for cerebral amyloid angiopathy and cerebrovascular disease. The severity of cerebral amyloid angiopathy was assessed semiquantitatively on thioflavin-S–stained preparations of the midfrontal cortex, superior temporal gyrus, inferior parietal cortex, and posterior hippocampus using a four-point scale ranging from 0 to 3 (absent, mild, moderate, and severe), using a method previously described[30]. The severity of arteriolosclerosis was scored separately on a similar semiquantitative four-point scale. Both were dichotomized as moderate/severe versus absent/mild for the purposes of comparison. Other pathologies were grouped as (1) large arterial and lacunar infarcts, (2) cortical microinfarcts, and (3) hemorrhages and microbleeds, and were simply dichotomized as present or absent.

2.2.5 Reference Values

Reference values for each cognitive measure (presented as green shading on figures) were derived from a group of "robust" normal controls who were diagnosed as normal on their first evaluation and remained normal for the duration of their participation in the ADRC longitudinal study. There were 241 individual "robust" normal participants who completed a total of 1109 visits after age 65. Based on all of these visits, the "normal" reference range was defined as being within 1.5 standard deviations of the mean score for each measure. The "robust" normal participants were not used in any calculation, model, or statistics, but purely served as a visual reference to guide interpretation of results.

2.2.6 Statistical Methods

Demographics, clinical characteristics, and final cognitive performance were compared using a 3-group ANOVA for continuous variables, followed up by Tukey's HSD post-hoc analysis for significant results, and a 3-group Fisher Exact Test for categorical variables, followed up by post-hoc pairwise Fisher Exact comparisons for significant results.

Trajectories of cognitive decline associated with AD and HS neuropathology were analyzed using data from the final visit and up to five prior annual evaluations covering a period of up to six years. This approach was chosen because starting at the first evaluation and moving forward is confounded by the variance in the clinical status of participants at entry into the study. With this approach, 100% of participants had data available at the last visit (by definition), 94% 1 year prior, 82% 2 years prior, 67% 3 years prior, 52% 4 years prior, and 38% 5 years prior. Data from a total of 1015 visits were available in this time window, with AD participants averaging 4.3 ± 1.4 visits, HS+AD averaging 4.8 ± 1.4, and HS averaging 4.0 ± 1.8 visits ($p = 0.24$).

Longitudinal linear mixed-effects models were used to assess how performance (expressed as raw scores) on each cognitive test declined with time to last evaluation. The participants' performance was modeled with fixed effects of pathologically-confirmed diagnostic group, years of education, presence of an APOE ε4 allele, age at death, and interval from last evaluation to death, as well as each term's interactions by time (expressed as inverse time in years from last visit). Participant specific intercepts and slopes were included as random effects, which are assumed to follow a normal distribution with unknown variance. To account for floor effects on some of the measures, data after the first score of 0 were dropped on a by-participant and by-test basis. Each continuous variable was centered. All contrasts were in reference to AD. This parameterization allows for the estimation of the predicted performance for an average participant (varying only the pathologic diagnosis) via the group term, as well as the longitudinal decline each year via the group by time interaction term. Each of the covariates (education, age

at death, APOE ε4, and interval from last evaluation to death) is able to influence both the final performance as well as slope of decline.

All analyses were performed in R version 3.4.2[31] using the *lme4* package[32] with restricted maximal likelihood (REML) estimation. Degrees of freedom for the fixed effects were estimated by the Satterwaithe approximation as implemented in the package *lmerTest*[33].

2.3 Results

A total of 195 cases with autopsy-confirmed AD, 18 with HS+AD, and 21 with HS alone were identified (**Table 2.1**), and clinical data for the last visit (approximately 1.6 years prior to death for all groups; p = 0.89) and up to 5 additional prior annual visits were selected for modeling. Groups did not differ in gender distribution (p = 0.60) or education (p = 0.55). An APOE4 allele was present in 43% of HS cases, 66% of AD cases, and 78% of HS + AD cases (p = < 0.05), with a significant post-hoc difference only between pure HS and pure AD (adjusted p < 0.05). HS and HS + AD participants were older than pure AD participants by approximately 7 years at symptom onset (p < 0.001), last visit (p < 0.001), and death (p < 0.001). The groups did not differ in the prevalence of self-reported history of hypertension (p = 0.86), diabetes (p = 0.13), or stroke (p = 0.99). Of the vascular pathologies assessed at autopsy, only prevalence of microinfarcts significantly differed between groups, with a greater proportion of HS than AD participants affected on pairwise *post-hoc* testing (adjusted p < 0.05). However, the proportion of participants with at least one vascular pathology present was near 70% in all three groups (p = 0.99).

The consensus clinical diagnosis for participants in each group was Possible or Probable AD in over 85% of cases at the final visit (**Table 2.2**) and over 70% at the first visit used for modeling (**Table 2.3**). There were no differences between groups (p = 0.49 and p = 0.44 respectively). At the first modeled visit, the groups did not differ in the use of antidepressant medications, antipsychotic medications, or NMDA antagonists, although a higher

proportion of the HS+AD than the AD group was taking acetylcholinesterase inhibitors (**Table 2.3**, adjusted p <0.05).

2.3.1 Decline in Global Cognitive Measures

Our primary analysis used the MMSE and the Mattis DRS, which were the most commonly administered tests with the most complete data. At the last visit AD and HS+AD participants were more impaired than pure HS on these measures (**Table 2.2**, $p < 0.01$). Tukey's post-hoc analysis revealed significant differences between HS and AD, as well as HS and HS+AD groups on every measure (adjusted $p < 0.05$), but no differences between AD and HS+AD.

Mixed effects linear regression was used to assess the trajectories of decline for the participants. All 3 groups exhibited decline over their last 5 years of evaluations (**Figure 2.2A**). For a demographically average individual with all else held constant, HS participants declined 3.3 ± 1.4 % of the maximal score per year slower than AD participants on the MMSE ($p = 0.02$), and 2.9 ± 1.2 % slower on the total Mattis DRS ($p = 0.02$).

The DRS includes 5 subdomains, that assess Attention, Initiation, Conceptualization, Construction, and Memory. HS participants declined 3.1 ± 1.4 % ($p = 0.03$) slower per year on Attention, 3.1 ± 1.3 % ($p = 0.02$) slower on Initiation, 5.4 ± 1.9% ($p = 0.004$) slower on Construction, and 3.5 ± 1.5 % ($p = 0.02$) slower on Conceptualization. HS did not differ from AD in rate of decline on the Memory subscale ($p = 0.51$). The mixed HS+AD group did not differ from pure AD on any DRS subscale.

The model predictions (**Figure 2.2B – 1D**), plotted over the raw data, indicate that at their final visit all groups were impaired relative to the reference range of scores obtained from cognitively intact control participants (green shading), with AD and HS+AD more severely impaired than HS on all measures (all $p < 0.05$). 5 years prior to their final visit, all 3 groups were near the normal range on all measures except DRS Memory, which was significantly more

impaired in AD than in HS ($p = 0.03$). A similar pattern is observed when actual scores from the first modelled visit were examined (Table 3; HS > AD on DRS Memory, $p < 0.03$).

2.3.2 Decline in Domain Specific Cognitive Tests

As a secondary analysis, we explored trajectories of decline on specific cognitive measures that had data available for at least 50% of visits, using the same model parameterizations. Because most of these measures had less complete data than the MMSE and DRS, models were underpowered to detect differences in slope - instead, inferences are drawn from model predictions at specific time points (**Figure 2.3**).

Memory was assessed using the CERAD Word List (available for 66% of visits), the California Verbal Learning Test (52% of visits), Visual Reproduction Test (56% of visits), and the Logical Memory Test (56% of visits). The CERAD Word List Immediate Recall had sufficient data to demonstrate a significant 2.8 ± 1.1 % slower decline in HS participants compared to AD participants ($p = 0.01$). At the final visit, HS participants were significantly less impaired than AD participants on all five memory measures (all $p < 0.05$). 5 years prior to final visit, HS were significantly less impaired than AD only on Logical Memory immediate recall ($p < 0.01$) and Visual Reproduction Test immediate recall ($p < 0.01$). These groups showed comparable performance on CERAD Word List immediate recall and recognition, as well as CVLT immediate recall, at this earlier time point. The delayed recall conditions of these tests were at floor for participants in all 3 groups and could not be modeled.

Attention was assessed using the Digit Span test (58% of visits). HS participants performed significantly better than AD participants at both the final visit ($p < 0.05$) and 5 years prior ($p < 0.01$).

Executive function was assessed using the Digit Symbol Substitution test (53% of visits), the Wisconsin Card Sorting Test (53% of visits), and the Trail Making Test Parts A and B (56% of visits). HS, AD, and HS+AD participants did not differ in performance on these measures at any time point.

Visuospatial ability, assessed via the Block Design test (82% of visits), was less impaired in HS than AD at the final visit ($p < 0.001$) and 5 years prior ($p < 0.05$).

Language was assessed using Verbal Fluency (85% of visits) and the Boston Naming Test (86% of visits). At the final visit, HS participants performed significantly better than AD participants on the Category Fluency test ($p < 0.001$), the F-A-S Letter Fluency test ($p < 0.05$), and the Boston Naming Test ($p < 0.01$); however, 5 years prior, they performed better only on the Category Fluency test ($p < 0.05$).

2.3.3 Trajectories of Functional Impairment

In addition to less severe cognitive impairment, HS participants were also less functionally impaired at their final evaluation than either AD or HS+AD as judged both by their global Clinical Dementia Rating (CDR) score ($p < 0.001$) and their Pfeffer Outpatient Disability (POD) scale score ($p < 0.001$) (**Table 2.2**). HS were also less impaired than HS+AD on the CDR and POD at the first modeled visit (**Table 2.3**, both $p < 0.05$). Our longitudinal modeling approach applied to POD scores (available for 86% of visits) demonstrated nearly identical slopes of increasing functional impairment across groups, with HS participants significantly less impaired than AD participants at both final visit ($p < 0.001$) and 5 years prior ($p < 0.05$). HS+AD participants were also more impaired than AD participants at the final visit ($p< 0.05$).

2.3.4 Age-Matched Validation

When we repeated the above analyses with a subset of AD participants matched for age to the HS and HS+AD participants, the pattern of findings was unchanged for all global cognitive and functional tests (data not shown). Results in domain-specific measures were also similar, although with fewer significant effects due to a reduced number of participants in the models.

2.4 Discussion

We examined the profiles of cognitive decline in 234 participants with longitudinal neuropsychological testing who had neuropathologically confirmed diagnoses of HS, AD, or both (HS+AD) and no other neurodegenerative pathologies that could account for cognitive

decline. Consistent with previously published work[3,9], participants with HS were less functionally and cognitively impaired than those with AD or HS+AD proximal to death (**Table 2.2**). Less cognitive impairment at the last visit apparently reflects slower decline in HS than AD since the groups showed comparable performance 5 years prior on both the MMSE and DRS global cognitive measures (**Figure 2.1**). A similar pattern was observed on the DRS Attention, Initiation, Conceptualization, and Construction subscales. DRS Memory subscale performance, in contrast, showed similar slopes of decline for HS and AD, but with a persistent longitudinal profile of less severe memory impairment in HS.

Longitudinal performance on domain-specific neuropsychological tests showed that HS produced a slower rate of decline than AD on measures of language (BNT, FAS fluency) and memory for word lists (i.e., CERAD Word List, CVLT). In each case, the HS and AD groups did not differ 5 years prior to the final visit, but HS were less impaired than AD at the last visit. HS and AD declined at similar rates on two additional memory tests (i.e., Visual Reproduction and Logical Memory) and tests of category fluency, attention (Digit Span) and visuospatial ability (Block Design), even though HS were less impaired than AD on these measures overall (i.e., less impaired both 5 years prior to the last visit and at the last visit). There was no difference in overall impairment or rate of decline on tests of executive function (i.e., Digit Symbol Substitution, Trail Making Test parts A and B); the HS and AD groups did not differ 5 years prior to the last visit or at the last visit.

Performance on individual neuropsychological tests 5 years prior to the final visit provides a look at the differential effects of early HS and AD pathology on various cognitive processes at a time when global mental status is equivalent and near normal levels in the two groups. Despite comparable impairment on the CVLT and CERAD Word List memory tests at that point, AD was more impaired than HS on the immediate recall conditions of the Logical Memory and Visual Reproduction tests (see **Figure 2.2**). This discrepancy suggests that these later two tasks engage cognitive processes beyond episodic memory that may be affected more

prominently in early AD than in early HS. One possibility, for example, is that the Logical Memory test engages both memory and semantic processing (since the story provides a semantic structure), and language deficits are greater in early AD than in early HS. This is consistent with our finding that AD was more impaired than HS on category fluency but not letter fluency tests, a pattern that is thought to reflect a semantic language deficit[34]. Similarly, the Visual Reproduction test may engage both memory and visuospatial abilities (to process the geometric forms), and visuospatial deficits are greater, as shown by our results with the Block Design test, in early AD than in early HS.

A number of our findings are consistent with previous cross-sectional studies that have compared patterns of cognitive deficits in HS and AD. As reported by Corey-Bloom *et al.*[7], we found that performance on tests of attention and visuospatial abilities were less impaired in HS than in AD throughout the course of disease. Consistent with Nelson *et al.*[4], we found that 5 years prior to the last visit HS were less impaired than AD on the category fluency test, while the two groups were equally impaired on the CERAD Word List memory test, although we used the immediate recall condition while Nelson *et al*. used delayed recall. This pattern was not maintained over time, however, as HS showed slower decline than AD on the CERAD memory test and declined at the same rate as AD on the category fluency test, thus causing the ratio of CERAD memory (either immediate or delayed) to category fluency to not differ in HS and AD in the later stages of disease. In contrast to Corey-Bloom *et* al.[7] and other investigators[3], we did not detect differences on measures of executive function at any time point.

Our findings indicate that HS is a progressive pathological process that results in gradual cognitive decline, albeit at a rate slower than in AD. The involvement of all cognitive domains in HS suggests that the effects of HS pathology are not localized to the hippocampus, as the name might imply, but likely involve diffuse neocortical regions either directly or through disruption of networks that support the affected cognitive functions. Worse deficits in AD than HS on tasks that require visuospatial and semantic processing may reflect greater disruption of fronto-

temporal semantic[35,36] and occipito-parietal visual networks[37] that are known to be altered by AD. In contrast, the striking similarity in performance of HS and AD on five measures from three different tests of executive function indicates that HS results in similar disruptions to frontal executive networks as AD. Indeed, these executive measures are the only measures to not demonstrate less impairment in HS at the final visit, suggesting that relative to the levels of impairment in other cognitive functions, executive deficits may actually be more prominent in HS than AD.

Despite some of the group differences we observed at various time points in the course of disease, the overall similarity of cognitive impairments between HS and AD makes it difficult to confidently distinguish these pathologies on clinical grounds alone. As Nelson *et al.*[4] suggest, the overlap in the distribution of scores on those cognitive measures that did show group differences means that no measure tested had the discriminatory power necessary for individual classification.

Some investigators suggest that the presence of dementia with a clinical and cognitive profile consistent with AD and neuroimaging evidence of neurodegeneration in an elderly person (e.g., over age 80), but in the absence of positive PET-imaging or CSF biomarkers of amyloid or tau pathology, suggests the presence of HS[38]. However, without a positive biomarker for HS, such a diagnosis must remain speculative and may rise only to the level of “probable” or “possible” HS. A pattern of circumscribed medial temporal lobe hypometabolism on FDG PET imaging has been proposed as a potential marker of HS[38] but has yet to be validated. The presence of the TDP-43 protein has been proposed as a possible pathological marker of the disease, but there is considerable overlap in TDP-43 deposition across AD and other neurodegenerative diseases (e.g., Amyotrophic Lateral Sclerosis, Fronto-Temporal Lobar Degeneration), and there is currently no PET imaging ligand or CSF marker to measure TDP-43 deposition in the brain. Our results, in conjunction with those of Nelson *et al.*[4], show that the pattern of cognitive deficits at a single time point may provide some supportive positive

evidence for the diagnosis of HS, but this does not rise to the level where it would be useful for identifying an individual patient, and any diagnostic utility of the pattern may depend upon the stage of disease. Without a positive biomarker of HS pathology, it will remain very difficult to differentiate the disease from AD.

We observed very few differences between AD and HS+AD on cognitive measures in terms of overall severity or rate of decline. These results suggest that the contributions of the two pathologies to the development of dementia are not simply additive. Since the AD and AD+HS groups had a similar degree of AD pathology (i.e., all were Braak stage V-VI and had moderate to severe neuritic plaque density), the addition of HS pathology might be expected to increase the severity of cognitive impairment or the rate of cognitive decline, but this was not the case. The interpretation of these results is limited, however, as the temporal order of onset of the pathologies is not known, and HS+AD were more likely than AD participants to be taking acetylcholinesterase inhibitors at the first modeled visit. The only measure that demonstrated a difference between AD and HS+AD was the Boston Naming Test, traditionally considered a measure of cortical (rather than hippocampal) function. This is further evidence for the wide-ranging effects of HS on cognitive functions beyond those thought to be regulated by the hippocampus.

Strengths of this work include the relatively large numbers of participants compared to most previous studies, the longitudinal nature of the analysis, and the consistency of neuropsychological, clinical, and pathological evaluation. We report performance on a broad range of well-established cognitive instruments, allowing comparison to existing literature. Additionally, when we restricted the analysis to AD participants that were age-matched to HS, the results were essentially unchanged.

There are also some limitations. First, previous work suggests that HS occurs unilaterally in some cases, yet the ADRC neuropathology protocol assesses only the left hemisphere. Second, given the legacy nature of this study, TDP-43 immunostaining was not

routinely performed at the time of autopsy for the vast majority of cases. Though TDP-43 protein inclusions in the hippocampus have been reported in 70 - 90% of HS cases[3,4], this has proved to be a non-specific pathological marker for HS that also occurs in 20 - 60% of cases with pure AD[39,40], and is not part of the diagnostic criteria for either disease. Furthermore, a recent study examining the effects of neural pathologies on cognition found that TDP-43 and HS pathologies independently affect distinct cognitive domains, suggesting that they may not be manifestations of the same pathologic process[41]. Nonetheless, the association of TDP-43 with cognitive decline is intriguing (both in the context of HS and dementia in general), and we will examine the complex relationship between TDP-43 and cognition in future studies.

2.5 Conclusion

HS and AD both result in progressive impairments in all cognitive domains, although HS generally appears to decline at a slower rate. Compared to HS, AD disproportionally affects semantic and visuospatial abilities at an early stage of overall cognitive impairment. In contrast, by the final evaluation, HS demonstrated less impairment in all domains except executive function. Yet despite these group-level differences, the differing trajectories of decline and the extensive overlap in cross-sectional scores between groups meant that no cognitive measure had the discriminatory power to differentiate HS and AD clinically.

2.6 Disclosures

Mr. Smirnov reports no disclosures. Dr. Galasko serves as editor for Alzheimer's Research and Therapy, and as a paid consultant on Data Safety Monitoring Boards for Pfizer, Inc., Elan, Inc., and Balance Pharmaceuticals, Inc. Dr. Hansen reports no disclosures. Dr. Edland serves as a paid consultant on Data Safety Monitoring Boards for Lilly USA, LLC and Suven Life Sciences Ltd. Dr. Brewer has served on advisory boards for Elan, Bristol-Myers Squibb, Avanir, Novartis, Genentech, and Eli Lilly and holds stock options in CorTechs Labs, Inc and Human Longevity, Inc. Dr. Salmon serves as a consultant for Takeda Pharmaceuticals, Inc.

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Table 2.1 Participant Demographics and Pathology

	AD n = 195	***AD + HS n = 18***	***HS n = 21***	***P Value***
Age at Last Visit, y, mean ± SD	78.1 ± 7.1	84.9 ± 5.2	85.2 ± 7.0	<.001 [a,b]
Age at Death, y, mean ± SD	79.7 ± 7.0	86.6 ± 5.1	86.7 ± 7.1	<.001 [a,b]
Last Visit to Death Interval, y, mean ± SD	1.6 ± 1.0	1.6 ± 1.0	1.5 ± 0.9	.89
Age at Symptom Onset, y, mean ± SD	70.3 ± 8.1	76.5 ± 5.9	78.3 ± 7.1	<.001 [a,b]
Disease Duration, y, mean ± SD	9.3 ± 3.6	10.3 ± 4.9	8.2 ± 3.6	.25
Education, y, mean ± SD	14.7 ± 3.0	15.3 ± 2.4	15.3 ± 2.8	.55
Gender, n (%):				.60
Male	115 (59%)	9 (50%)	14 (67%)	
Female	80 (41%)	9 (50%)	7 (33%)	
# Apo E4 Alleles*, n (%):				<.05 [a]
0 alleles	65 (34%)	4 (22%)	12 (57%)	
1 alleles	93 (48%)	13 (72%)	9 (43%)	
2 alleles	34 (18%)	1 (6%)	0 (0%)	
Clinical History, n (%):				
Hypertension	100 (51%)	9 (50%)	12 (57%)	.87
Diabetes	20 (10%)	0 (0%)	4 (19%)	.13
Stroke	30 (15%)	3 (17%)	3 (14%)	.99
Braak Stage, n (%):				<.001 [a,b,c]
I	-	-	6 (29%)	
II	-	-	2 (10%)	
III	-	-	4 (20%)	
IV	-	-	6 (29%)	
V	65 (33%)	13 (72%)	1 (5%)[#]	
VI	130 (67%)	5 (28%)	2 (10%)[#]	
CERAD Neuritic Plaque Score, n (%):				<.001 [a,c]
None	-	-	6 (29%)	
Mild	-	-	3 (14%)	
Moderate	43 (22%)	7 (39%)	8 (38%)	
Severe	152 (78 %)	11 (61%)	4 (19%)	
Vascular Pathology*, n (%):				
Arterial or Lacunar Infarct	30 (16%)	0 (0%)	2 (10%)	.19
Microinfarct	15 (8%)	3 (17%)	6 (29%)	.008 [a]
Hemorrhage/Microbleed	11 (6%)	1 (6%)	0 (0%)	.70
Cerebral Amyloid Angiopathy (Moderate/Severe)	121 (63%)	12 (67%)	8 (38%)	.08
Arteriolosclerosis (Moderate/Severe)	27 (18%)	5 (42%)	6 (35%)	.06
At Least 1 of Above	142 (73%)	13 (72%)	15 (71%)	.99

Abbreviations: AD = Alzheimer's disease; HS = Hippocampal Sclerosis; MCI = Mild Cognitive Impairment; DLB = Dementia with Lewy Bodies; CDR = Clinical Dementia Rating; POD = Pfeffer Outpatient Disability scale

Table 2.1 Participant Demographics and Pathology, Continued

*Missing data: APOE genotype (n = 4, 1.7%), Arterial or Lacunar Infarct (n = 2, <1%), Microinfarcts (n = 1, <1%), Hemorrhage or Microbleed (n = 1, <1%), Amyloid Angiopathy (n = 2, <1%), Arteriolosclerosis (n = 56, 24%)

Despite Braak Stages of V or VI, these 3 participants did not meet the diagnostic criteria for AD due to having “none” or “mild” CERAD neuritic plaque scores.
P Values from Fisher Exact or ANOVA tests as appropriate
[a]Significant post-hoc pairwise comparison between HS and AD
[b]Significant post-hoc pairwise comparison between HS+AD and AD
[c]Significant post-hoc pairwise comparison between HS+AD and HS

Table 2.2 Global Cognitive Performance at Last Visit by Pathologic Diagnosis

	AD n = 195	***AD + HS n = 18***	***HS n = 21***	***P Value***
Clinical Diagnosis at Last Visit, n (%):				.49
Prob/Poss AD	173 (89%)	17 (94%)	18 (86%)	
MCI	2 (1%)	0 (0%)	1 (5%)	
DLB	17 (9%)	1 (6%)	1 (5%)	
Other	3 (1%)	0 (0%)	1 (5%)	
CDR at Last Visit*, n (%):				<.001 [a,c]
0	0 (0%)	0 (0%)	0 (0%)	
0.5	5 (4%)	0 (0%)	3 (21%)	
1.0	15 (12%)	3 (23%)	7 (50%)	
2.0	61 (49%)	4 (31%)	4 (29%)	
3.0	44 (35%)	6 (46%)	0 (0%)	
POD at Last Visit*, mean ± SD:	16.7 ± 3.9	18.0 ± 3.3	11.9 ± 6.3	<.001 [a,c]
MMSE, mean ± SD (/30)	10.5 ± 8.0	10.7 ± 7.7	18.4 ± 6.1	< .001 [a,c]
DRS Total, mean ± SD (/144)	63.4 ± 37.9	59.7 ± 41.3	101.0 ± 26.1	< .001 [a,c]
DRS Attention, mean ± SD (/37)	23.2 ± 11.3	23.2 ± 12.2	32.5 ± 5.9	.002 [a,c]
DRS Initiation, mean ± SD (/37)	11.1 ± 9.8	11.0 ± 11.2	21.9 ± 9.6	< .001 [a,c]
DRS Construction, mean ± SD (/6)	2.2 ± 2.1	1.8 ± 1.6	4.1 ± 1.6	< .001 [a,c]
DRS Conceptualization, mean ± SD (/39)	19.7 ± 13.0	17.9 ± 13.6	30.5 ± 9.0	.001 [a,c]
DRS Memory (/25)	7.1 ± 4.8	5.7 ± 5.5	12.0 ± 4.9	< .001 [a,c]

Abbreviations: AD = Alzheimer's disease; HS = Hippocampal Sclerosis; MMSE = Mini-Mental State Exam, DRS = Mattis Dementia Rating Scale.

* Missing Data: CDR (n = 82, 35%), POD (n = 47, 20%)

P Values from Fisher Exact or ANOVA tests as appropriate
[a]Significant post-hoc pairwise comparison between HS and AD
[b]Significant post-hoc pairwise comparison between HS+AD and AD
[c]Significant post-hoc pairwise comparison between HS+AD and HS

Table 2.3 Global Cognitive Performance at First Modeled Visit by Pathologic Diagnosis

	AD n = 195	***AD + HS n = 18***	***HS n = 21***	***P Value***
Interval from First Modeled Visit to Last Visit, y, mean ± SD	4.0 ± 1.3	4.3 ± 1.2	4.1 ± 1.7	.15
Clinical Diagnosis at First Modeled Visit, n (%):				.44
Cognitively Normal	10 (5%)	1 (6%)	1 (5%)	
Prob/Poss AD	161 (83%)	15 (83%)	15 (71%)	
MCI	10 (5%)	1 (6%)	3 (14%)	
DLB	7 (4%)	1 (6%)	0 (0%)	
Other	7 (4%)	0 (0%)	2 (10%)	
CDR at Last Visit*, n (%):				.04 [c]
0	4 (4%)	0 (0%)	1 (6%)	
0.5	24 (23%)	0 (0%)	7 (41%)	
1.0	63 (59%)	11 (73%)	9 (53%)	
2.0	15 (14%)	4 (27%)	0 (0%)	
3.0	0 (0%)	0 (0%)	0 (0%)	
Medications used*, n (%):				
AChE Inhibitors	59 (30%)	12 (67%)	9 (43%)	.007 [b]
NMDA Antagonists	9 (5%)	3 (17%)	0 (0%)	.06
Antipsychotics	10 (5%)	1 (6%)	0 (0%)	.68
Antidepressants	47 (24%)	4 (22%)	5 (24%)	.99
POD at First Modeled Visit, mean ± SD	10.2 ± 5.2	12.2 ± 5.7	7.4 ± 5.3	.02 [c]
MMSE*, mean ± SD (/30)	21.7 ± 5.4	22.1 ± 3.8	24.4 ± 2.5	.07
DRS Total*, mean ± SD (/144)	112.2 ± 20.1	113.9 ± 12.4	121.7 ± 10.4	.09
DRS Attention, mean ± SD (/37)	33.8 ± 3.9	35.3 ± 1.4	35.5 ± 1.5	.06
DRS Initiation, mean ± SD (/37)	26.1 ± 7.8	26.4 ± 6.2	29.4 ± 5.2	.17
DRS Construction, mean ± SD (/6)	4.6 ± 1.5	4.5 ± 1.2	4.8 ± 1.3	.80
DRS Conceptualization, mean ± SD (/39)	33.0 ± 5.9	33.5 ± 3.4	34.2 ± 2.6	.63
DRS Memory (/25)	14.6 ± 5.4	14.2 ± 5.3	17.8 ± 4.7	.03 [a]

Abbreviations: AD = Alzheimer's disease; HS = Hippocampal Sclerosis; MMSE = Mini-Mental State Exam, DRS = Mattis Dementia Rating Scale.

* Missing Data: MMSE (n = 3, 1 %), DRS (n = 8, 3%), Medications (n = 1, <1%), CDR (n = 95, 41%), POD (n = 14, 6%)

P Values from Fisher Exact or ANOVA tests as appropriate
[a]Significant post-hoc pairwise comparison between HS and AD
[b]Significant post-hoc pairwise comparison between HS+AD and AD
[c]Significant post-hoc pairwise comparison between HS+AD and HS

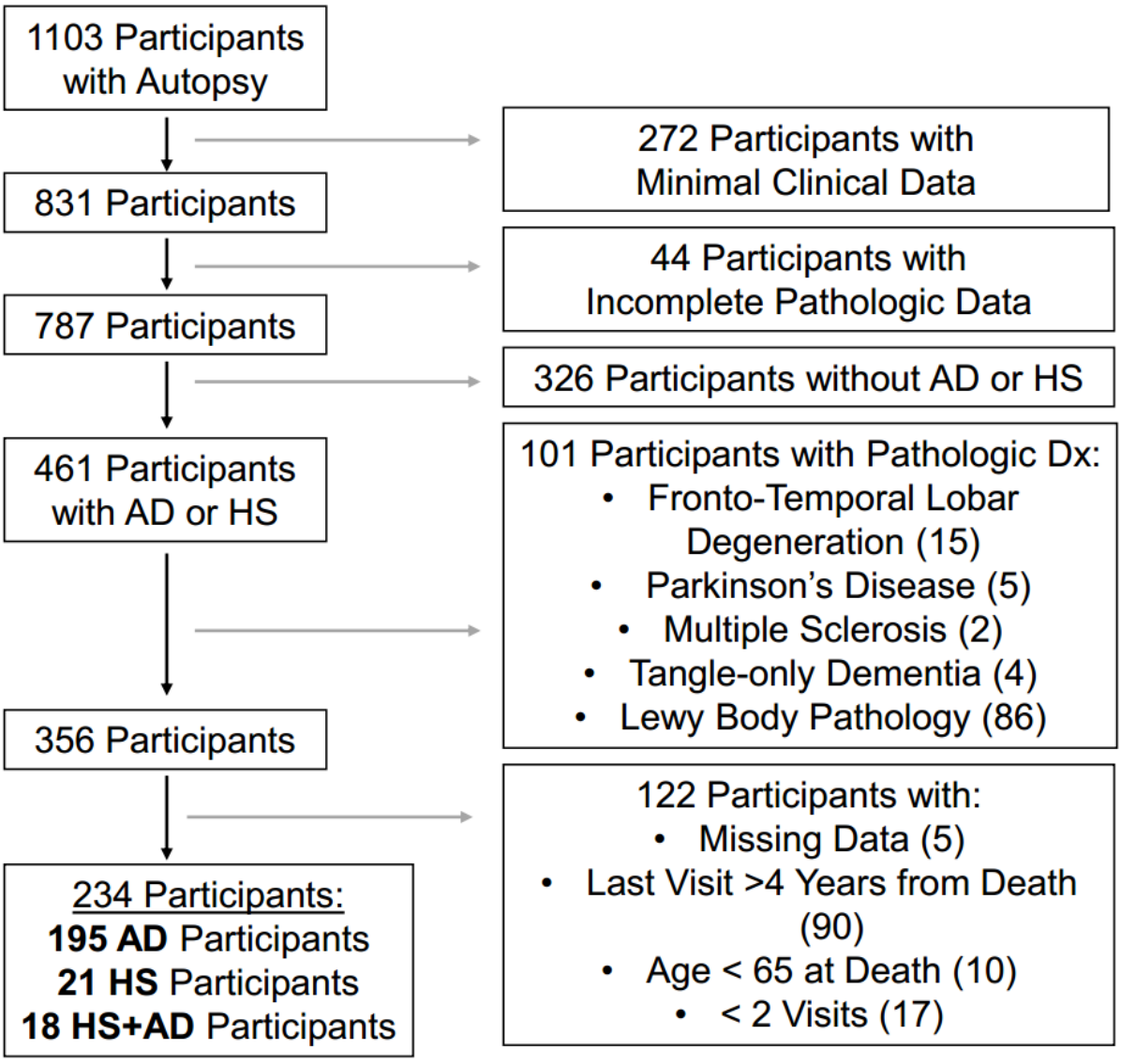
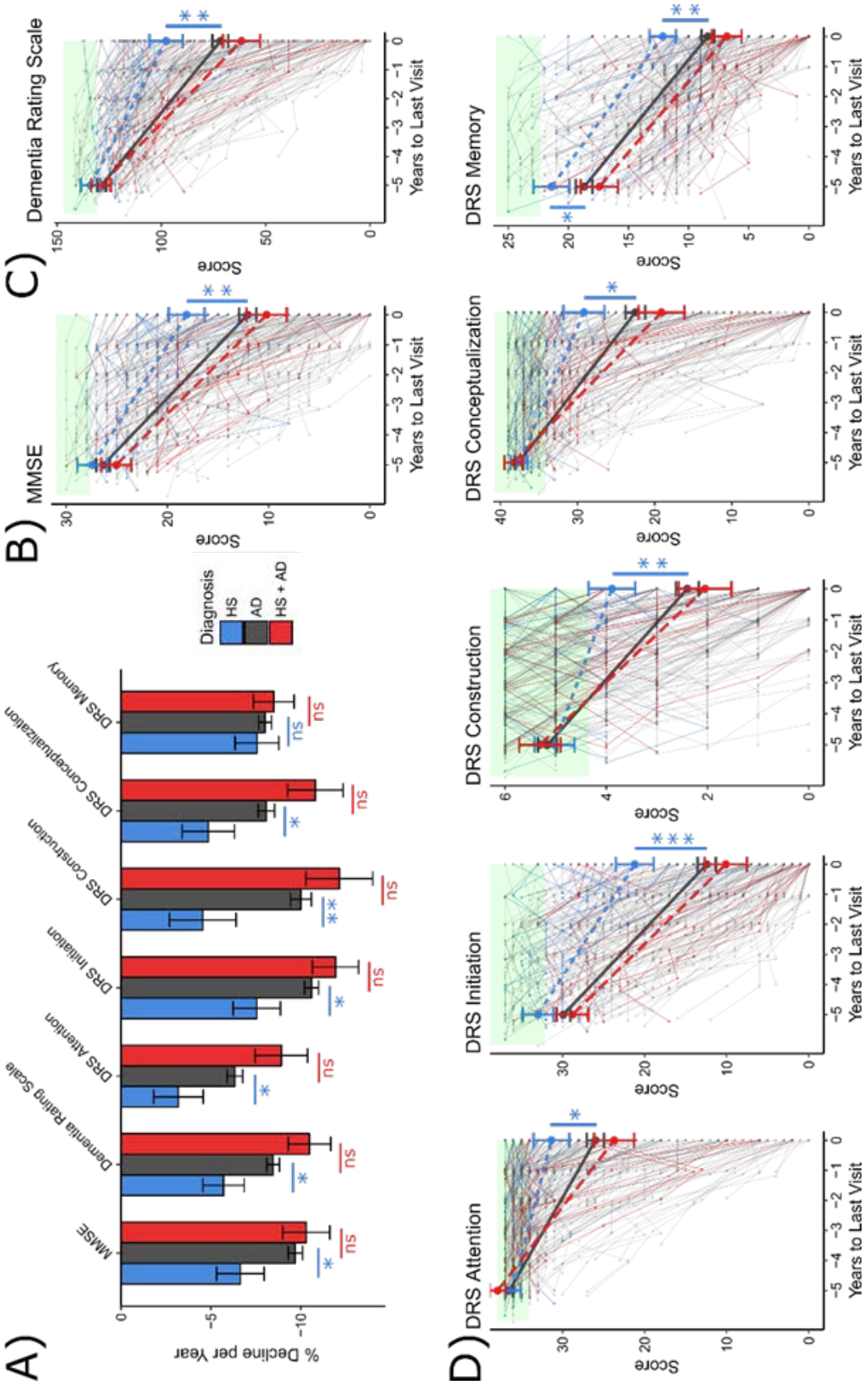


Figure 2.1 Participant Selection and Exclusion

Flowchart of the identification of participants for this analysis from the brain bank at the UCSD Shiley-Marcos Alzheimer's Disease Research Center. Exclusion criteria along with the number of subjects excluded, are presented at each stage of selection.

Figure 2.2 Trajectories of Global Cognitive Decline by Pathologic Diagnosis

Linear mixed modeling of the trajectories of cognitive decline due to Hippocampal Sclerosis (blue), Alzheimer's disease (grey), or both pathologies (red). Average rates of cognitive decline per year (expressed as percentage of maximal score), stratified by eventual pathology, are presented for each cognitive measure **(A)**. Spaghetti plots of data used for modeling, overlaid with model predictions in bold are presented for the MMSE **(B)**, total DRS **(C)**, and each of the DRS subscales **(D)**. Green shading represents the reference range for normal performance, defined as being within 1.5 standard deviations of mean performance of ADRC robust normal controls. Comparisons of modeled performance (in reference to AD) were made at last visit and 5 years prior. *p < .05; **p < .01; ***p < .001 as calculated using the Satterwaithe approximation for degrees of freedom.


A)
% Decline per Year
0
−5
−10
MMSE
Dementia Rating Scale
DRS Attention
DRS Initiation
DRS Construction
DRS Conceptualization
DRS Memory
ns
*
Diagnosis
HS
AD
HS + AD
B)
MMSE
C)
Dementia Rating Scale
D)
DRS Attention
DRS Initiation
DRS Construction
DRS Conceptualization
DRS Memory
Score
Years to Last Visit
−5 −4 −3 −2 −1 0

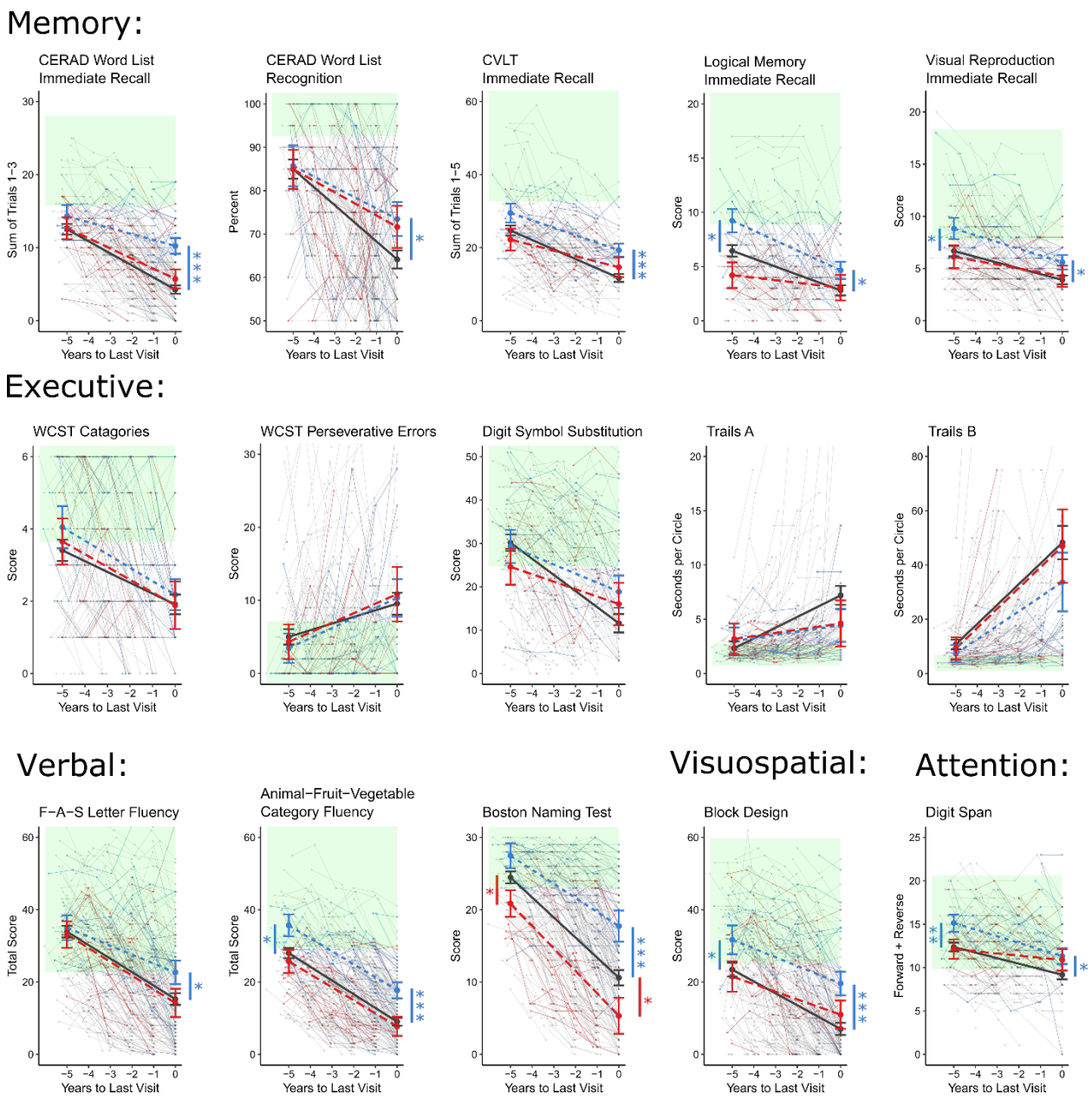


Figure 2.3 Trajectories of Cognitive Decline on Domain-Specific Measures by Pathologic Diagnosis

Linear mixed modeling of decline in domain-specific measures due to Hippocampal Sclerosis (blue), Alzheimer's disease (grey), or both pathologies (red). Green shading represents the reference range for normal performance, defined as being within 1.5 standard deviations of mean performance of ADRC robust normal controls. Comparisons of modeled performance (in reference to AD) were made at last visit and 5 years prior. *p < .05; **p < .01; ***p < .001 as calculated using the Satterwaithe approximation for degrees of freedom.

Chapter 3

Cognitive Decline Profiles Differ in Parkinson's Disease Dementia and Dementia with Lewy Bodies

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Abstract

Objective: To examine whether domain specific patterns of cognitive impairment and trajectories of decline differed in patients with clinically-diagnosed PDD (N = 29) and autopsy-confirmed DLB (N = 58) or AD (N = 174). Further, to determine the impact of pooling PDD and DLB patients in clinical trials targeting cognition.

Methods: Patients were matched on demographics and level of global cognitive impairment. Patterns of cross-sectional performance and longitudinal decline were examined in 4 cognitive domains: Visuospatial, Memory, Executive, and Language. Power analyses were performed to determine the numbers of participants needed to adequately power a hypothetical clinical trial to slow cognitive decline in pure PDD, pure DLB, or a mixed PDD/DLB group.

Results: Both DLB and PDD were more impaired and declined more rapidly than AD in the Visuospatial domain. PDD patients exhibited the most impairment and fastest decline in Executive, though DLB patients also declined faster than AD. Memory was more impaired in AD than DLB, and in both compared to PDD, however all 3 groups declined at comparable rates. In contrast, PDD declined at a slower rate on Language measures than DLB or AD. Power analyses suggest that Visuospatial and Executive outcome measures would be most sensitive in PDD, but Memory and Language in DLB.

Conclusions: DLB and PDD differ from each other, and from AD, in a cognitive domain-specific manner. As such, different outcome measures may be most sensitive to detecting changes in DLB versus PDD, suggesting that the two should be analyzed separately in clinical trials.

3.1 Introduction

Parkinson's Disease Dementia (PDD) and Dementia with Lewy Bodies (DLB) are disorders characterized by cognitive impairment and motor symptoms associated with α-synuclein pathology in brainstem nuclei, neocortex, and paralimbic regions[1–4]. While subtle neuropathologic differences may exist, no hallmark features easily distinguish the two, so the disorders are often grouped as Lewy Body Disease (LBD)[5,6]. The clinical distinction is primarily order of symptom onset: in PDD dementia begins at least one year after onset of Parkinson's disease, while in DLB dementia precedes or co-occurs with parkinsonism. When both motor and cognitive symptoms are present, the disorders appear remarkably similar, and both may exhibit psychiatric symptoms, autonomic symptoms, REM-sleep behavior disorder, and cognitive fluctuations[7–12]. There are, however, potential differences in patterns of cognitive deficits in PDD and DLB[9,13–18], though the extent of these differences and how they evolve over time remains largely unknown. This presents a pressing problem as anti-synuclein therapeutic trials applicable to both disorders develop[19]. If the conditions differ substantially in profiles of cognitive impairment and decline, pooling patients with PDD and DLB in an LBD trial may substantially reduce power to detect a targeted cognitive response by increasing within-group variability. With these issues in mind, the present study compares cognitive profiles and trajectories of decline in PDD and DLB, and assesses the impact of pooling these groups in a hypothetical LBD clinical trial. Comparisons are also made to Alzheimer's disease (AD), as concomitant AD is more common in DLB than PDD and may impact cognitive decline[15,20,21].

3.2 Methods

3.2.1 Standard Protocol Approvals, Registrations, and Patient Consents

The research protocol was reviewed and approved by the human subject's review board at the University of California, San Diego (UCSD). Informed consent was obtained at the point

of entry into the ADRC longitudinal study from all patients or their caregivers consistent with California State law. Informed consent for autopsy was obtained at the time of death from the next of kin.

3.2.2 Participants

Participants for this study were selected from the longitudinal study and brain bank of the UCSD Shiley-Marcos Alzheimer's Disease Research Center (ADRC), recruited from 1985 to 2014. A “baseline” visit was identified for all potential cases as the first ADRC evaluation that warranted a diagnosis of dementia. Cases with significant concomitant pathological diagnoses (e.g., Fronto-Temporal Lobar Degeneration, Hippocampal Sclerosis, etc.) were excluded from the selection process.

We identified 29 patients who met clinical diagnostic criteria for PDD[2]: all initially presented with PD defined by the presence of 2 of 3 cardinal features[22] and developed dementia more than one year later. The diagnosis of PD is likely to be highly accurate in these patients given the excellent sensitivity and specificity provided by the clinical criteria[23,24]. Other neurologic conditions that could produce parkinsonism were ruled out by neurologic examination. The average interval between the onset of parkinsonism and development of dementia was 8.9 years (sd=8.0). Twelve of the 29 PDD cases were autopsied and all had neuropathologic changes consistent with idiopathic PD.

Autopsy-confirmed cases with DLB were matched 2:1 to PDD cases on demographics (age, education) and global cognitive performance at baseline using nearest neighbor propensity score matching[25]. This resulted in 58 DLB cases. All initially presented with dementia only, and those that developed parkinsonism (i.e., at least 2 cardinal signs of PD) did so 4.7 years (sd=3.6) after dementia onset. Autopsy confirmed patients with AD were similarly matched 2:1 to the combined PDD/DLB groups, resulting in 174 AD cases.

3.2.3 Neuropathology

The brain was divided sagittally, and left hemibrain was fixed in 10% buffered formalin, while right hemibrain was sectioned coronally and then frozen at −70°C. Right hemibrain tissue blocks from midfrontal, inferior parietal and superior temporal cortices, primary visual cortex in the occipital cortex, hippocampus, amygdala, basal ganglia, substantia nigra, and cerebellum were removed and placed in 2% paraformaldehyde for subsequent thick sectioning by vibratome. Tissue blocks adjacent to these were stored at −70°C for subsequent immunoblot analysis for synaptic proteins and Aβ species (soluble and oligomers). Vibratome sections (40 μm thick) were stored in cryoprotective medium at −20°C for subsequent immunochemical studies. The formalin-fixed left hemibrain was serially sectioned in 1 cm slices, and tissue blocks from the regions described above were processed for histopathological examination by H&E, Thioflavin-S (Thio-S), and immunohistochemistry with antibodies to detect tau and β-amyloid deposits.

Brains were staged for degree of neurofibrillary tangle pathology by one pathologist (L.A.H.) using a modification of the Braak staging scheme[26]. Estimates of neuritic plaque density were calculated using methods recommended by CERAD[27]. Alzheimer's disease was operationalized using the NIA-Reagan consensus criteria for the postmortem diagnosis of AD, wherein Braak stage V-VI with moderately to severely dense neuritic plaques corresponds to "high likelihood" that dementia is due to AD[28]. None of the AD cases had Lewy bodies or abnormal a-synuclein immunostaining in the neocortex or pigmented brainstem nuclei.

The DLB cases fell into either the limbic (transitional) or neocortical subtypes proposed in the 1996 consensus guidelines for the pathological diagnosis of DLB[4], based on hematoxylin-eosin staining and immunostaining with antibodies against α-synuclein[1,3,4]. Cases were not classified as DLB if Lewy bodies were found only in the amygdala. Some of the DLB cases had sufficient concomitant AD pathology to warrant a secondary diagnosis of AD (historically called

"Lewy body variant of AD"[20]). In a secondary analysis, cases were divided by the likelihood that a given combination of DLB subtype and Braak stage would result in a typical clinical DLB syndrome, determined according to the latest DLB criteria[1]. Twelve PDD patients from this cohort were autopsied and in all cases Lewy bodies were found in the locus ceruleus, substantia nigra, and/or nucleus basalis of Meynert, as well as in the neocortex.

3.2.4 Clinical Evaluation

Participants had annual standardized clinical, neurological, and neuropsychological evaluations as previously described[29,30]. The clinical evaluation included review of history with the patient and/or informant, mental status testing, assessment of psychiatric symptoms (e.g., depression, psychosis including hallucinations), and assessment of functional impairment using the Pfeffer Outpatient Disability (POD) scale[31] or the Functional Assessment Questionnaire (FAQ) (converted to corresponding POD scores). Clinical Dementia Rating (CDR) total score and scores for each of six subdomains were computed (i.e., CDR sum of boxes). Hoehn and Yahr staging scores were determined for those with PDD or DLB[32].

The physical portion of the structured ADRC neurological examination was used to assess degree of motor impairment. Many patients in the cohort were examined prior to the implementation of the Uniform Parkinson's Disease Rating Scale (UPDRS), but the vast majority of the ADRC structured neurological examination overlapped features of the UPDRS. A 20-point motor impairment scale[33] was derived from the ADRC examination based on the presence (1 point) or absence (0 points) of parkinsonian features (see **Table 3.2**).

Global cognitive function was assessed with the Dementia Rating Scale (DRS)[34]. Further neuropsychological assessment included standardized measures of Memory (Wechsler Memory Scale (WMS) Visual Reproduction Test immediate and delayed recall; WMS-Revised Logical Memory Test; Verbal List Learning Test), Language (30-item Boston Naming Test; Letter

Fluency Test (F-A-S); Category Fluency Test ("animals", "fruits", "vegetables"); Wechsler Adult Intelligence Scale-Revised (WAIS-R) Vocabulary Test), Executive functions (modified Wisconsin Card Sorting Test; Trail Making Test Parts A and B; WAIS-R Digit Symbol Substitution Test), and Visuospatial abilities (Wechsler Intelligence Scale for Children-Revised (WISC-R) Block Design Test; Visual Reproduction Test copy; Clock Drawing Test; Cube Drawing Test). The Verbal List Learning Test was derived as the z-score for the immediate recall condition (summed across trials) of the Buschke Selective Reminding Task (12% of cases), the CERAD Word List Learning Test (2% of cases), or the California Verbal Learning Test (CVLT) (86% of cases). Performance on all measures was transformed to z-scores using reference values from a pool of 228 "robust" normal controls who were diagnosed as normal on their first ADRC evaluation and remained normal for the duration of their participation in the ADRC longitudinal study.

Consensus clinical diagnoses based on published criteria were made by two or more board-certified neurologists with expertise in dementia and movement disorders. Diagnosing neurologists were informed whether the neuropsychological assessment identified deficits in two or more domains of cognition, but not of individual test or cognitive domain scores. Probable DLB was diagnosed clinically based on the presence of dementia and at least two of three additional core features of mild parkinsonism, well-formed visual hallucinations, and fluctuations in consciousness or attention[3,4]. REM sleep behavior disorder was also considered, but was not systematically assessed prior to its inclusion in the latest DLB guidelines[1]. Cognitive decline had to precede or occur in conjunction with mild parkinsonism. The clinical diagnosis of PDD was based on the presence of at least two of the cardinal motor signs of PD, as well as objective cognitive deficits on neuropsychological tests and functional decline due to cognitive problems[2]. Motor signs had to precede cognitive decline by more than one year. Probable AD was diagnosed according to NINCDS-ADRDA[35] or NIA-AA criteria[36].

3.2.5 Principal Component Analysis and Generation of Participant-Level Domain Scores

The Very Simple Structure criteria[37] suggested 4 as the optimal number of interpretable factors to extract from the baseline scores for the selected cognitive measure of the entire sample (n=261). Principal component analysis (PCA) with varimax rotation resulted in 4 orthogonal rotated components, which were conceptually labeled “Visuospatial”, “Memory”, “Executive”, and “Language” based on the highest loadings for each measure (**Table 3.3**).

The PCA loadings matrix was used to generate individual domain scores for each participant at their baseline visit. A small number of missing values (less than 5% per test, except for Logical Memory, Cube, and Vocabulary which were missing up to 13% of values) were imputed using fully conditional specification[38] as implemented by the *mice* R statistical package[39]. Five parallel imputations were performed and carried forward through the modeling analysis, before being pooled for the final result. Each imputation was guided by diagnostic grouping, participant demographics, global cognitive test scores, and scores on other cognitive measures in the test’s domain.

While imputation of a small portion of baseline data adds little bias to the analysis[38], we were concerned about longitudinal imputation of missing values since the amount of missing data increased when multiple evaluations were considered. Thus, we did not generate longitudinal PCA-derived domain scores. We used an alternative approach wherein each test at each visit was assigned to only one cognitive domain, guided by the highest PCA loadings. Z-scores for all tests in that domain were averaged to create a domain composite score. If less than half of measures in any given domain were available for a patient, the visit for that patient was dropped from the analysis. The rate of dropped visits did not differ by diagnostic group.

3.2.6 Statistical Analysis

Demographics and clinical characteristics were compared across groups using a 3-group ANOVA for continuous variables with post-hoc Tukey's HSD tests for significant results, or a 3-group Fisher Exact Test for categorical variables with post-hoc pairwise Fisher Exact comparisons for significant results. Cross-sectional comparisons of cognitive domain scores across groups were performed using linear least squares regression adjusting for age, sex, and education.

Analyses of trajectories of cognitive decline across groups used data from baseline and 2 annual follow-up evaluations. Longitudinal linear mixed-effects models were used to assess how performance in each cognitive domain composite declined with time. Performance was modeled with fixed effects of diagnostic group, sex, years of education, age at baseline, baseline score on the measure of interest, and each term's interaction with time. Participant-specific intercepts and slopes were included as random effects. All analyses were performed in R version 3.6.0 using the *lme4* package with restricted maximal likelihood (REML) estimation. Degrees of freedom for fixed effects were estimated by the Satterwaithe approximation as implemented in the package *lmerTest*.

Power analyses for Mixed Models with Repeated Measures (MMRM) were performed as implemented in the *longpower* package[40]. We determined the sample sizes needed in a hypothetical 2-year trial to detect a 50% reduction in decline on each cognitive domain composite (power 0.8, significance 0.05) if the sample consisted of a pooled group of DLB and PDD patients (in a 1:1 ratio) or separate groups of DLB or PDD patients. Each analysis assumed 15% attrition per year.

3.2.7 Data Availability Statement

Anonymized data and related documents such as study protocols and statistical analysis plans will be shared with any qualified investigator upon request.

3.3 Results

3.3.1 Participant Demographics

The PDD, DLB and AD groups did not differ significantly in age, education, or global cognition at baseline (**Table 3.1**). The PDD group had a higher median Hoehn and Yahr PD staging score than the DLB group. On our 20-point motor impairment scale AD patients averaged 1.5 (sd=2.7), DLB patients 4.5 (sd=5.1), and PDD patients 14.7 points (sd=3.0) (tabulations in **Table 3.2**). The PDD group had a higher percentage of males than the other groups, consistent with the known greater prevalence of PD in males than females[41]. The AD group had a higher percentage of individuals with one or more APOE ε4 allele than the PDD group, but did not differ from the DLB group. All PDD patients were on dopaminergic medication at baseline, with 86% taking L-DOPA, compared to only 7% of DLB patients and no AD patients. The percentage of DLB patients taking acetylcholine esterase inhibitors at baseline was higher than in AD, and the percentage taking NMDA antagonists was higher than in the PDD group (in which no one was prescribed this medication). Antidepressant and antipsychotic use did not differ across groups.

Since many participants enrolled prior to the development of the DLB diagnostic criteria[4], only 26% of the pathologically-confirmed DLB cases were clinically diagnosed with Probable or Possible DLB at baseline. However, retrospective chart review of all DLB cases revealed that 31% met diagnostic criteria for Probable DLB and 12% for Possible DLB (i.e. presence of 1 core feature[4]) at baseline, and 35% meet these criteria at a subsequent visit, bringing the total of those ever meeting clinical criteria for DLB to 78%.

3.3.2 Cross-sectional Cognitive Profiles

Separate regression analyses for each domain at baseline revealed significant group differences in the Visuospatial, Memory, and Executive domains, but not in the Language

domain (**Figure 3.1**). PDD (β±se=-0.81±0.27, p=0.003) and DLB (β±se=-1.11±0.27, p=0.003) were more impaired than AD in the Visuospatial domain, but did not differ from each other. In contrast, AD performed worse than DLB (β±se=0.26±0.27, p=0.016), and much worse than PDD (β±se=1.26±0.15, p =$2.6x10^{-15}$), in the Memory domain. Furthermore, DLB performed worse than PDD (β±se=-0.98±0.16, p=$5.9x10^{-9}$) in the Memory domain. PDD were more impaired than DLB (β±se=0.59±0.27, p=0.029) or AD (β±se=-0.84±0.24, p=$4.7x10^{-4}$) in the Executive domain, while AD and DLB did not differ from each other (p=0.14).

3.3.3 Longitudinal Cognitive Decline

Linear mixed effects modeling, adjusted for demographics and baseline performance, identified differences in the *slope* of 2-year decline in Visuospatial, Executive, and Language domain composites, but not the Memory domain (**Figure 3.2**). DLB (β±se=-0.52±0.14, p=0.001) and PDD (β±se=-0.85±0.20, p= $1.6x10^{-4}$) declined more rapidly than AD in the Visuospatial domain, but did not differ from each other. In contrast, PDD declined more rapidly than DLB (β±se=0.41±0.18, p=0.024) or AD (β±se=-0.66±0.16, p=$1.3x10^{-4}$) in the Executive domain, and DLB also declined more rapidly than AD (β±se=-0.25±0.11, p=0.027). DLB declined more rapidly than PDD (β±se=-0.62±0.28, p=0.04) in the Language domain. Despite large cross-sectional differences in the Memory domain composite, rates of decline in Memory did not differ across groups.

3.3.4 Power Calculations for a 2-year Treatment Trial with Cognitive Outcomes in LBD

Power calculations for MMRM analyses suggest that different numbers of LBD participants would be needed to detect a 50% reduction in decline in cognition over two years (power=.80; p=.05) depending on the cognitive domain assessed and the make-up of the LBD sample (**Table 3.4**). A 50% reduction in decline on the Visuospatial composite score could be reliably detected with 38 PDD participants per group, whereas 125 DLB participants or 93

participants with a 1:1 mixture of PDD and DLB would be needed per group. The Executive domain composite score would require only 36 PDD patients per group, but 202 DLB patients or 158 PDD and DLB patients with a 1:1 mixture. In contrast, despite a lack of difference in average rate of decline between groups, an outcome based on the Memory composite score would require 127 PDD patients per group to detect a 50% reduction in decline, but only 51 DLB patients or 74 PDD and DLB patients with a 1:1 mixture. This is due to the much larger variance in the PDD group trajectories compared to DLB. Similarly, the Language composite score would require 827 PDD patients per group, compared to only 33 DLB or 58 PDD and DLB patients with a 1:1 mixture.

3.3.5 Secondary Analyses

Secondary analyses were carried out to assess how stricter definitions of groups affected the results. First, if the analysis was restricted to pathologically confirmed PDD cases the pattern of cross-sectional results remained unchanged, however differences in decline between DLB and PDD no longer reached significance. Similarly, if the analysis was restricted to the 78% of DLB cases that met clinical DLB criteria in life, the only changes were the loss of significance in the cross-sectional difference in memory between DLB and AD (now p=0.09), and loss of the cross-sectional and longitudinal differences in executive function between DLB and PDD (now p=0.08 and 0.10).

Finally, we divided the DLB group based on likelihood of each combination of DLB stage and Braak stage being associated with a typical DLB clinical syndrome[1]. This resulted in significant differences, such that Visuospatial ability was more impaired in “high likelihood” DLB (p=0.03), while Memory was more impaired in “low/intermediate likelihood” DLB (p = 0.02). However, both DLB groups remained more impaired than the PDD group in Memory (both $p < 1x10^{-10}$). Meanwhile, only “high likelihood” DLB and PDD differed from AD on Visuospatial, Memory, and Executive abilities (all $p < 0.05$).

Longitudinally, the two DLB groups did not differ from each other in any rates of decline. However, only PDD and “high likelihood” DLB differed from AD in Visuospatial (both p=0.0001), while “low/intermediate likelihood” DLB declined more rapidly than AD in Memory (p=0.03). All three other groups declined less rapidly on Executive function than PDD (all p <0.05) and did not differ from each other. Finally, PDD declined marginally less rapidly than each of the other three groups on Language (all p=0.05-0.06).

3.4 Discussion

While DLB and PDD may be nearly indistinguishable pathologically, our work adds to the small literature suggesting that the two may differ cognitively in important ways, adding to the current debate on whether the conditions should be pooled or treated separately[5-13]. Our findings showed that DLB and PDD patients with comparable levels of global cognition differed in their domain-specific profiles of impairment and trajectories of decline. Specifically, we replicated previous work suggesting greater impairment and/or decline in Executive function in both DLB and PDD relative to AD[15]. However, we also observed a previously unreported greater impairment and more rapid decline (adjusted for baseline score) of Executive function in PDD than DLB (PDD < DLB < AD). PDD and DLB were relatively more impaired and declined more rapidly than AD in Visuospatial ability, but did not differ from each other (DLB ≈ PDD < AD). These findings are in line with previous work suggesting that visuospatial impairments are similar in PDD and DLB, but are less pronounced in AD[42]; however, we did not observe the greater visuospatial impairments in DLB compared to PDD reported in other studies[43,44].

Although there were large cross-sectional differences in Memory with AD and DLB worse than PDD (AD < DLB << PDD) consistent with previous reports[13,43,44], the three groups declined at nearly identical rates on average. This may be a function of the relatively mild level of dementia of the participants – a point when AD and DLB have already experienced most of their early memory decline, but PDD has not reached the memory deficits associated with later

stages of this disorder[45]. Conversely, while the three groups performed similarly in Language ability cross-sectionally at this mild level of global impairment, the PDD patients declined less rapidly than either the DLB or AD patients, in line with previous reports of greater language/verbal memory impairments in DLB and AD versus PDD in the later stages of disease[46].

The observed double-dissociations in both our cross-sectional and longitudinal results (i.e., worse Executive in PDD than DLB; worse Language and Memory in DLB than PDD) are likely a reflection of the subtle differences in pathology between DLB and PDD. Interestingly, both cortical and subcortical pathology appear at comparable rates in the disorders, and as a result the disorders do not differ in the proportion that may be characterized with a "cortical" or "subcortical" cognitive presentation[47]. Nevertheless, the generally more severe brainstem pathology in PDD may account for the disproportionate impairments in Executive ability. In contrast, the greater concomitant AD pathology of the DLB cases may shift the cognitive impairment profile to be more similar to AD with greater Memory and Language impairments.

It should be noted, however, that differences in degree of AD pathology do not account for all of the observed cognitive differences between DLB and PDD. Despite a substantial reduction in statistical power, in secondary analyses separating the DLB group by level of concomitant AD pathology into those with "high likelihood" or "low/intermediate" likelihood of expressing a typical DLB syndrome, both remained more impaired cross-sectionally in Memory and declined slower in Executive and (marginally) Language than the PDD group. Further, the two DLB groups did not differ from each other in any rates of decline, diverging only in cross-sectional Visuospatial and Memory impairments at baseline.

The observed differences in cognitive patterns and rates of decline are essential to consider in the design of clinical trials for LBD that may be targeting the common underlying α-synuclein pathology of PDD and DLB[48]. If the baseline impairments and longitudinal trajectories

of cognitive decline differ by domain, any clinical response to a compound may be apparent in one group but not the other, depending on the domain-weighting of the cognitive outcome measure – resulting in a loss of statistical power to detect a change. To assess the impact of pooling DLB and PDD in clinical trials, we performed power calculations for the sample sizes that would be needed to detect a 50% reduction in decline over 2 years in only DLB patients, only PDD patients, and a 1:1 mixture of the two. We find that due to the higher variability (SD) in the rates of decline in DLB over PDD in the Executive and Visuospatial domains, far fewer participants were needed to reach the desired power in a pure PDD sample than in either a pure DLB or a combined cohort. In contrast, the tiny effect size in the Language domain would require massive numbers of PDD patients, despite the lower variability in PDD, while only a small handful of DLB patients would be required to reach the same power. A somewhat similar picture emerged with Memory, where both groups had nearly identical rates of decline, but the greater variability in the PDD patients resulted in the need for more than twice as many patients to reach the same power as for DLB patients.

Our power analysis indicated that the most efficient approach to detecting changes in cognition is to focus on Visuospatial and Executive measures in PDD, but Memory and Language in DLB. For example, based on the high PCA loadings in both Visuospatial and Executive domains (**Table 3.3**), the Wisconsin Card Sorting Test, Digit Symbol Substitution, and the Trail Making Test would make good choices to track changes in PDD. In contrast, measures with high loadings on Memory (e.g. Logical Memory) or Language (e.g. category fluency, confrontation naming) would provide the most power to track changes in DLB. Thus, despite the similarity in underlying pathology and drug-targets, separating DLB and PDD groups and examining appropriate outcome measures should substantially improve the power of a trial to detect an effect on cognition.

Because a portion (22%) of our pathologically-confirmed DLB group was never clinically diagnosed with DLB in life, it is unlikely they would be selected for a clinical trial of LBD. Therefore, we repeated our primary analysis restricting to the 78% of DLB cases that met DLB criteria during life. The pattern of results and effect sizes was largely unchanged, albeit with reduced statistical power. We note that with promising work on effective α-synuclein biomarkers[49,50], it will soon be possible to restrict the DLB group in a clinical trial to those accurately diagnosed during life, and our full primary analysis is representative of the cognitive profiles and rates of decline that can be expected.

A major strength of this study is the use of autopsy-confirmed cases of DLB and AD given the marginal performance of the DLB clinical diagnostic criteria. The majority of previous work relied on these clinical diagnoses resulting in nearly guaranteed inclusion of pure AD cases in their DLB groups, and inclusion of DLB cases with very subtle neurological features of DLB in their AD groups. An additional strength of this study is the examination of multiple cognitive domains using multiple measures, which have not previously been studied longitudinally. Finally, longitudinal analyses allowed us to perform power calculations with direct implications for clinical trial design as well as patient care.

Several caveats should be considered. First, while the groups were carefully matched on demographics and global cognition, there were differences in degree of motor impairment and the use of parkinsonian and cognitive medications. It should be noted, however, that baseline performance and rate of decline for each cognitive domain did not correlate with degree of motor impairment in the combined DLB/PDD group. Further, many of the DLB participants enrolled prior to the most recent DLB criteria, and some even before the first DLB criteria were published. A chart review was preformed to retrospectively apply criteria to all DLB cases, but some information (especially RBD) may not have been systematically collected. Finally, regarding power calculations, we note that neuropathological diagnosis will not be available to

clinical trials, and specificity of diagnoses in trials will generally be lower than those used here. Hence, power of clinical trials in practice will be lower than suggested by our power calculations. We also note the domain-specific composite scores reported here have not been validated as primary endpoints for clinical trials of neurodegenerative diseases. The purpose of the power calculation exercise is simply to characterize the potential improvement in trial efficiency to be gained by choosing the domains of cognitive function most sensitive to the respective neurodegenerative processes. While specific sample sizes reported here are not reliable, the relative efficiency of domain-specific endpoint scales is well represented by this analysis.

In summary, this work characterized both the cross-sectional and longitudinal differences in the cognitive profiles of patients with PDD, DLB, and AD, and examined the resulting effects on a hypothetical clinical trial. Our findings suggest that there may be substantial benefits to a trial by separately examining DLB and PDD patients with outcome measures targeting the cognitive processes most affected in each.

3.5 Acknowledgements

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Table 3.1 Baseline Demographics

	AD (N=174)	DLB (N=58)	PDD (N=29)	P value
Age at Baseline	73.9 ± 6.9	74.1 ± 6.3	72.9 ± 6.1	0.70
Male	90 (52%)	33 (57%)	24 (83%)	0.006[b,c]
Race:				
White	172 (99%)	58 (100%)	29 (100%)	
Black	1 (<1%)	0 (0%)	0 (0%)	0.66
Pacific Islander	1 (<1%)	0 (0%)	0 (0%)	
Hispanic/Latino	9 (5%)	2 (3%)	1 (3%)	0.90
Education (years)	14.6 ± 3.3	15.2 ± 3.0	15.2 ± 3.3	0.44
Parkinsonism-Cognition Interval (years)[e]		3.6 ± 3.0	-8.9 ± 8.0	
DRS (/144)	120.7 ± 8.5	121.9 ± 9.2	122.8 ± 9.1	0.40
CDR-sb (/18)[d]	4.8 ± 1.8	4.9 ± 2.3	5.2 ± 2.5	0.75
CDR Global Score[d]:				
0.5	33 (29%)	17 (40%)	7 (47%)	
1.0	76 (67%)	20 (48%)	7 (47%)	0.10
2.0	5 (4%)	5 (12%)	1 (7%)	
POD (/20)[d]	9.2 ± 4.3	8.6 ± 5.4	7.6 ± 5.3	0.24
APOE ε4 Allele Frequency:				
0 ε4 alleles	58 (33%)	26 (45%)	13 (45%)	
1 ε4 alleles	80 (46%)	26 (45%)	8 (28%)	0.003[b]
2 ε4 alleles	36 (21%)	6 (10%)	1 (3%)	
Unknown			7 (24%)	
NIA-Reagan Criteria for Pathologic Diagnosis of AD:				
Low Likelihood	0 (0%)	13 (22%)	7 (58%)	
Intermediate Likelihood	0 (0%)	12 (21%)	3 (25%)	
High Likelihood	174 (100%)	33 (57%)	2 (17%)	
Hoehn and Yahr (/5)	0	0 [0-3][g]	3 [2-5][g]	<0.001[f]
Clinical Dx at Baseline Visit:				
AD	174 (100%)	41 (71%)	0 (0%)	
DLB	0 (0%)	15 (26%)[h]	0 (0%)	
PDD	0 (0%)	0 (0%)	29 (100%)	
Other	0 (0%)	1 (2%)	0 (0%)	
Medications at Baseline:				
L-DOPA	0 (0%)	4 (7%)	25 (86%)	
DA Agonists	0 (0%)	0 (0%)	12 (41%)	
MAO-B Inhibitors	0 (0%)	0 (0%)	8 (28%)	
COMT Inhibitors	0 (0%)	0 (0%)	3 (10%)	
Amantadine	0 (0%)	0 (0%)	2 (7%)	
AChE Inhibitors	65 (37%)	32 (55%)	6 (21%)	0.004[a]
NMDA Antagonists	13 (7%)	9 (16%)	0 (0%)	0.03[c]
Antidepressants	45 (26%)	21 (36%)	10 (34%)	0.23
Antipsychotics	8 (5%)	4 (7%)	3 (10%)	0.36

Table 3.1 Baseline Demographics, Continued

Abbreviations: DRS = Dementia Rating Scale, CDR-sb = Clinical Dementia Rating – sum of boxes, POD = Pfeffer Outpatient Disability scale,
Values are mean ± sd or N (%) as appropriate, unless otherwise specified.
Post-hoc significant: [a]*AD-DLB,* [b]*AD-PDD,* [c]*DLB-PDD*
[d]Missing *Data:* CDR (34% AD; 28% DLB; 48% PDD); POD (2% AD; 10% PDD)
[e]Positive numbers indicate that Onset of Cognitive Decline was prior to Parkinsonism, while negative numbers indicate the inverse.
[f]Comparison only between DLB and PDD
[g]Values are: median [range]
[h]Note that some cases were diagnosed before the development of DLB criteria. See text for discussion.

Table 3.2 Motor Symptoms by Diagnostic Group

	AD (N=174)	DLB (N=58)	PDD (N=29)
R Finger Taps	18 (10%)	16 (28%)	24 (83%)
L Finger Taps	19 (11%)	17 (29%)	23 (79%)
R Rapid Alt Movement	16 (9%)	10 (17%)	22 (76%)
L Rapid Alt Movement	18 (10%)	13 (22%)	21 (72%)
Neck Rigidity	6 (3%)	10 (17%)	23 (79%)
R Arm Rigidity	11 (6%)	14 (24%)	24 (83%)
L Arm Rigidity	11 (6%)	16 (28%)	24 (83%)
R Leg Rigidity	6 (3%)	8 (14%)	20 (69%)
L Leg Rigidity	6 (3%)	9 (16%)	19 (66%)
Parkinsonian Tremor	2 (1%)	5 (9%)	19 (66%)
R Action Tremor	13 (7%)	10 (17%)	13 (45%)
L Action Tremor	13 (7%)	12 (21%)	13 (45%)
Parkinsonian Speech	2 (1%)	12 (21%)	19 (66%)
Masked Facies	18 (10%)	21 (36%)	28 (97%)
Bradykinesia	15 (9%)	19 (33%)	27 (93%)
Parkinsonian Gait	12 (7%)	19 (33%)	29 (100%)
Other Gait Disorder	31 (18%)	18 (31%)	22 (76%)
Stooped Posture	19 (11%)	23 (40%)	28 (97%)
Postural Instability	26 (15%)	13 (22%)	17 (59%)
Abnormal Rise from Chair	10 (6%)	9 (16%)	24 (83%)

[a] While these were assessed bilaterally, numbers are presented for those with at least unilateral involvement

Table 3.3 Principal Component Analysis Factor Loadings

	RC1 (Visuospatial)	RC2 (Memory)	RC3 (Executive)	RC4 (Language)
Visual Reproduction - Copy	**0.804**	-0.010	0.006	0.061
Visual Reproduction – Immediate Recall	**0.639**	0.478	-0.114	0.159
Cube	**0.560**	0.111	-0.036	0.277
Block Design	**0.780**	-0.077	0.218	0.216
Clock	**0.706**	0.038	0.008	0.016
Trails A	**0.746**	0.006	0.328	-0.068
Wisconsin Card Sorting Test	0.353	-0.012	**0.530**	0.215
Trails B	0.514	-0.020	**0.516**	0.213
Letter Fluency	0.230	-0.048	**0.579**	0.250
Digit Symbol Substitution	0.503	-0.033	**0.646**	0.086
Logical Memory – Immediate Recall	-0.029	**0.800**	0.258	0.144
Logical Memory – Delayed Recall	-0.098	**0.876**	-0.018	0.053
Verbal List Learning Test	-0.038	**0.551**	0.538	0.096
Visual Reproduction – Delayed Recall	0.213	**0.761**	-0.113	0.024
Vocabulary	0.090	0.043	0.193	**0.858**
Boston Naming Test	0.127	0.246	0.021	**0.743**
Category Fluency	0.170	0.211	0.484	**0.539**

Table 3.4 MMRM power calculations estimating number of participants needed to detect a 50% reduction in decline in a hypothetical trial.

Calculations were performed for a power of 80% and a significance level of 0.05. 15% dropout per year was assumed. Delta is 50% of the average decline for the group. SD is the standard deviation of the decline.

		Visuospatial	Memory	Executive	Language
Combined	SD	1.45	0.39	0.92	1.30
	Delta	0.69	0.21	0.33	0.78
	N	**93**	**74**	**158**	**58**
PDD only	SD	1.13	0.58	0.43	0.60
	Delta	0.85	0.23	0.34	0.10
	N	**38**	**127**	**36**	**827**
DLB only	SD	1.56	0.31	1.04	1.20
	Delta	0.64	0.20	0.33	0.96
	N	**125**	**51**	**202**	**33**

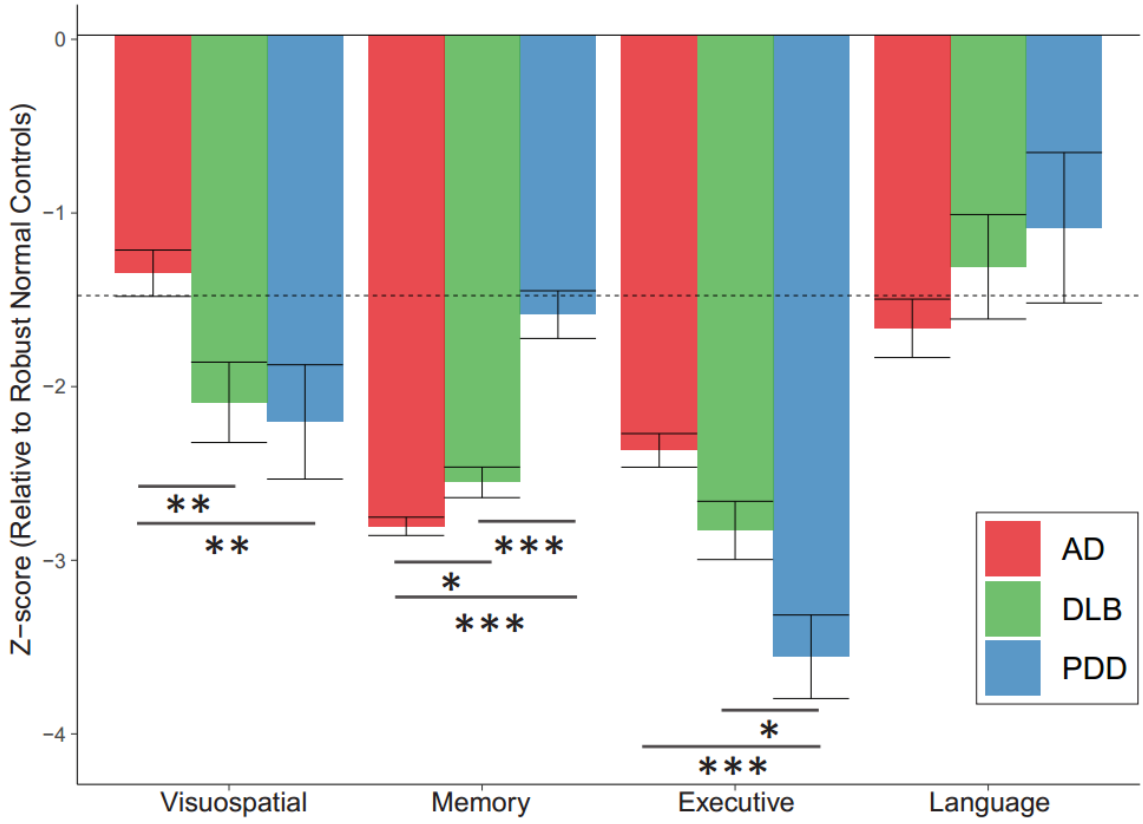

Figure 3.1 Cross-Sectional Cognitive Profiles

Cross-sectional cognitive domain scores of the Dementia with Lewy Bodies (DLB), Parkinson's Disease Dementia (PDD), and Alzheimer's Disease (AD) groups at baseline, matched on demographics and global cognitive impairment. Statistical comparisons are made based on linear least squares regression adjusted for age, sex, and education.

*** p < 0.05; ** p < 0.01; *** p < 0.001**

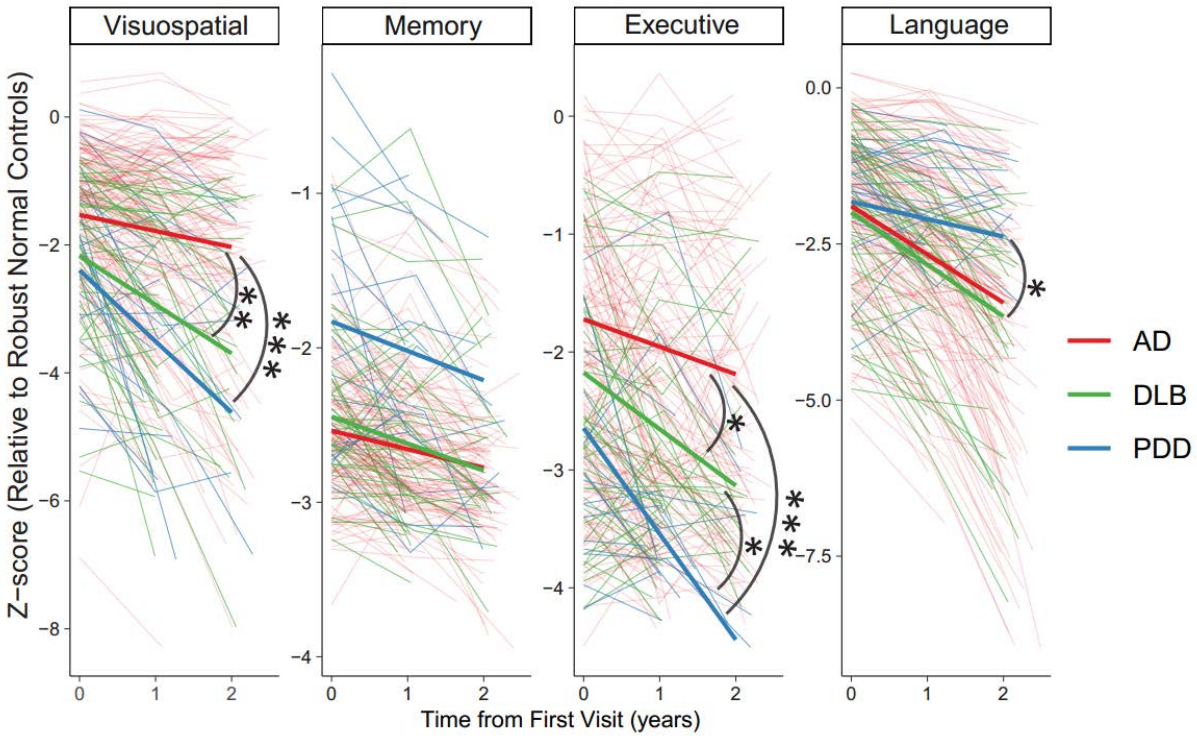

Figure 3.2 Longitudinal Decline

Longitudinal cognitive decline on each domain composite score in Dementia with Lewy Bodies (DLB), Parkinson's Disease Dementia (PDD), and Alzheimer's Disease (AD) over 2 years. Statistical comparisons are made between the *slopes* of decline, rather than absolute values, based on mixed effects models adjusted for age, sex, education, and baseline performance.

*** p < 0.05; ** p < 0.01; *** p < 0.001**

Chapter 4

Age-of-Onset and APOE Related Heterogeneity in Pathologically Confirmed Sporadic Alzheimer's Disease

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Abstract

Objective: To characterize age-related clinical heterogeneity in Alzheimer's disease (AD) and determine if it is modified by APOE genotype or concomitant non-AD pathology, we analyzed data from 1750 patients with sporadic, pathologically-confirmed severe AD.

Methods: In this retrospective cohort study, regression and mixed effects models assessed effects of estimated age of onset, APOE genotype, and their interaction on standardized clinical, cognitive and pathologic outcome measures from the National Alzheimer's Coordinating Center (NACC) database.

Results: A bimodal distribution of age of onset frequency in APOE ε4- cases showed best separation at age 63. Using this age cut-off, cases were grouped as early onset (EO) AD ε4- (n=169), EOAD ε4+ (n=273), late onset (LO) AD ε4- (n=511), and LOAD ε4+ (n=797). EOAD were more likely than LOAD patients to present with non-cognitive behavioral or motor symptoms or non-memory cognitive complaints, and had more executive dysfunction, but less language impairment on objective cognitive testing. Age of onset and ε4- genotype were independently associated with lower baseline MMSE and greater functional impairment, and EOAD had faster cognitive and functional decline than LOAD regardless of APOE genotype. EOAD were more likely than LOAD patients to receive a non-AD clinical diagnosis even though they were more likely to have "pure" AD without concomitant vascular or other non-AD neurodegenerative pathology.

Conclusions: Early onset sporadic AD is associated with a greater likelihood of an atypical, non-memory dominant clinical presentation, especially in the absence of the APOE ε4 allele, which may lead to misattribution to non-AD underlying pathology.

4.1 Introduction

Despite a shared basic pathology across the age range[1], Alzheimer's disease (AD) is known for age-related heterogeneity in its clinical and neuropathologic presentation[2,3], posing significant diagnostic challenges for clinicians. Studies suggest that individuals with early onset (EO) AD (typically defined as age < 65) have faster cognitive decline than those with late onset AD (LOAD)[4–7], less prominent memory impairment[8–11], and more often present as focal cortical syndromes such as primary progressive aphasia (PPA)[12], posterior cortical atrophy (PCA)[13–16], or frontal variant AD[17–19]. These "atypical" presentations of EOAD are especially common in the absence of the APOE ε4 AD risk allele[20,21], and are susceptible to being misattributed to non-AD causes. Because many of these studies did not include autopsy verification, it is impossible to know if age-related clinical and cognitive heterogeneity occurred in the context of typical AD pathology, or if it depended upon differences in the extent and severity of AD pathology, non-AD pathology superimposed on AD, or non-AD pathology alone. Therefore, we examined the relationship between age of onset and the clinical presentation of dementia in individuals with autopsy-confirmed severe AD in the large, multi-center National Alzheimer's Coordinating Center (NACC) database. The contribution of concomitant non-AD neuropathology to potential age-related differences was also examined. We hypothesized that those with EOAD, especially without the APOE ε4 risk allele, are more likely than those with LOAD to have atypical clinical and cognitive presentations, resulting in greater misattribution of the underlying pathology to non-AD causes.

4.2 Methods

4.2.1 Case Selection

Data were drawn from the September 2019 data freeze of the NACC database[22,23], which collects standardized data from Alzheimer's Disease Centers (ADCs) across the United States. Due to major changes in data collected under version 3 of the NACC Uniform Data Set (UDS), this study was restricted to those with clinical data collected under UDS versions 1 and 2

spanning the years 2005 to 2015. Within these parameters, we identified 2830 cases with a pathologic diagnosis of severe AD, defined as Braak Stage V-VI with moderate/severe density of neuritic plaques (i.e. “High Likelihood” by NIA-Reagan Criteria[24]). As our focus was sporadic AD, cases were excluded if they had a known dominantly-inherited mutation for AD (e.g. APP; N=42), a family history of such a mutation (N=8), Down’s syndrome (N=4), or a reported age of onset younger than 50 (N=115) which may indicate an unknown/*de novo* autosomal-dominant mutation[25]. Cases were also excluded if essential data were missing, such as reported age of onset (N=209) or APOE genotype (N=315), or if the last valid Mini-Mental State Exam (MMSE) score was more than 5 years before death (N=494). Ultimately, 1750 cases with pathologically confirmed severe AD at death were included in this study.

4.2.2 Neuropathological Analysis

Data reported to NACC by each ADC neuropathologist included a diagnosis and specific pathological findings defined in a NACC Coding Guidebook (https://www.alz.washington.edu). In addition to Braak staging and staging of neuritic plaque density (CERAD score), we utilized information on the presence/absence of arteriosclerosis, micro-infarcts, macro-infarcts/lacunae, and hemorrhages/microbleeds. Atherosclerosis of the Circle of Willis and amyloid angiopathy were each rated on a 4-point scale (none, mild, moderate, severe), and are dichotomized as moderate/severe vs. none/mild in our analyses. Lewy Body Disease (LBD) pathology included brainstem-predominant, limbic (i.e., transitional), and neocortical (i.e., diffuse) subtypes. Data on the Amygdala predominant and Olfactory bulb subtypes was available in a subset of 809 (46%) participants. Hippocampal Sclerosis was not identified separately in the earlier NACC UDS 1 and 2, but instead included under a diagnosis of Medial Temporal Lobe (MTL) Sclerosis. Due to extensive changes in reporting practices in NACC over the years[26], all Frontotemporal Lobar Degeneration (FTLD) pathologies (e.g. Pick’s disease, Progressive Supranuclear Palsy, Corticobasal Degeneration, Tangle-only Disease, Argyrophilic Grain Dementia, FTLD-TDP43) are grouped together in analyses. Data on TDP-43 immunostaining in the Amygdala,

Hippocampus, and Neocortex was available in a subset of 368 (21%) participants for staging of Limbic-predominant Age-related TDP-43 Encephalopathy (LATE)[27].

4.2.3 Clinical Assessment and Diagnosis

At baseline and subsequent visits that occurred at approximately one-year intervals, participants received a standardized dementia evaluation[28] that included medical history (active or remote), family history, physical and neurologic examination, review of medications, neuropsychological testing, and functional assessment with the Clinical Dementia Rating (CDR® Dementia Staging Instrument) and the Functional Assessment Questionnaire (FAQ). Psychiatric symptoms were assessed with the Geriatric Depression Scale (GDS) and the Neuropsychiatric Inventory (NPI). Cognition was assessed with a neuropsychological test battery[29] that consisted of the MMSE and measures of verbal learning and memory (Logical Memory Test [story A only] I and II), attention and executive function (Digit Span Forward and Digit Span Backward; Trail-Making A and B; Digit Symbol Substitution), and language/semantic memory (30-item Boston Naming Test, Animal and Vegetable Fluency). The predominant symptom first recognized in “Cognition”, “Behavior,” or “Motor” domains (when present) was recorded. Estimated age of onset of cognitive decline was determined by the clinician through an interview with a knowledgeable informant/study partner.

At each visit a clinical diagnosis was assigned based on a review of all available clinical data. The clinical diagnostic procedure included determining if Mild Cognitive Impairment (MCI), Dementia, or Cognitive Impairment-not MCI was present. Next, presence was noted of conditions such as probable or possible AD, probable or possible vascular dementia, FTD (including bvFTD, PPA, PSP, CBD), Lewy Body Disease (DLB or PD), or various other conditions that result in cognitive impairment. Of those conditions present, one was chosen as the “primary” contributor to the cognitive impairment, while others could be listed as “contributing” or “present but not contributing” to cognitive impairment.

Baseline visit was defined as the first visit at which the participant received a non-normal diagnosis. Baseline was the first ADC visit for a vast majority of the cases; however, some cases (n=140; 8%) entered the study as cognitively normal and were only classified as impaired at a later ADC visit. Last visit was defined as the last visit prior to death during which UDS version 1 or 2 data were collected. The baseline visit was also the last visit for 18.2 % of the cases.

4.2.4 Statistical Analysis

Density distributions of the estimated age of onset by the number of APOE ε4 alleles were plotted for the 1750 cases. The bimodal distribution in the ε4-negative (ε4-) population was fit well by a 2-component gaussian mixture model ($p<0.001$) using an expectation maximization (EM) algorithm with the *mixtools* package for R[30]. The point of intersection between the two distributions was determined to be approximately 63 years of age, which was used to dichotomize cases as EOAD (age $\leq$ 63) or LOAD (age > 63). Although the cut-point was derived from the ε4- population only, it was also applied to the ε4+ cases to allow us to more easily probe separate effects of APOE and age of onset and their interaction. APOE genotype was collapsed into ε4- (ε3ε3; ε2ε3; ε2ε2) and ε4+ (ε2ε4; ε3ε4; ε4ε4) categories in analyses. In order to illustrate the results of the various analyses, cases were divided into four groups and group means and standard deviations are presented in tables and figures: EOAD ε4- (n=169), EOAD ε4+ (n=273), LOAD ε4- (n=511), and LOAD ε4+ (n=797).

Effects of Age-of-Onset, APOE Genotype, and the Age-of-Onset X APOE Genotype interaction were examined by linear regression for continuous variables and logistic regression for dichotomous clinical and neuropathological variables. If the interaction term was not significant it was dropped from the model and only main effects were reported to allow for simple interpretation. Sex distributions differed by age of onset (see **Table 4.1**), so sex was included as a covariate in all subsequent analyses. We report the Beta coefficients and 99%

confidence intervals (CI) for linear regression and the Odds Ratio (OR) and 99% CI for logistic regression.

Cognitive domain scores were created to examine cognitive profiles at baseline. Patients' raw scores on each cognitive test were converted to z-scores relative to robust normal control participants (individuals who remained cognitively normal throughout all of their evaluations in NACC). Matching performed with the *MatchIt* R package[31] using exact sex matching and nearest-neighbor age matching in a ratio of 5 robust controls to 1 case was done separately for EOAD and LOAD populations. A small percentage of cognitive data (<5% per test) was imputed using the *missMDA* R package[32]. Principal Component Analysis with Varimax rotation identified 4 components that were conceptually labeled as "Executive", "Memory", "Language", and "Attention" based on the primary loadings. The four component scores (i.e., cognitive domain scores) were generated for each participant (centered and scaled relative to matched robust normal controls) and separately examined in regression models with linear adjustments for sex and education.

To examine progression, longitudinal change on the MMSE and CDR-Sum of Boxes (SOB) for up to 5.5 years from baseline was examined using linear mixed effects models with terms for Age-of-Onset, APOE Genotype, Age-of-Onset X APOE Genotype, and their interactions with Time. Models were adjusted for sex, education, baseline score, and their interactions with Time. MMSE scores of 0 after the first 0, and CDR-SOB scores of 18 after the first 18, were dropped before model fitting to minimize floor effects.

4.2.5 Standard protocol approvals, registrations, and patient consents

The research protocol was approved at each ADC's Institutional Review Board, and written informed consent was obtained from participants at each ADC.

4.2.6 Data availability policy

NACC has developed and maintains a large relational database of standardized clinical and neuropathologic research data collected from the National Institute on Aging-funded ADCs

across the United States. NACC data are freely available to all researchers at https://www.alz.washington.edu/.

4.3 Results

4.3.1 Participant Demographic Characteristics

The participants with severe AD pathology selected for this study (n=1750) had a mean ± standard deviation age of 75.6±9.4 years at their baseline ADC clinical assessment, 15.4±3.1 years of education, and 43% were female. The average baseline MMSE score was 19.5±7.9. The APOE genotype distribution was 61% ε4+ and 39% ε4-. The average estimated age of onset was 70.5±9.7 years, age of death was 80.3±9.5 years, and overall duration of illness was 9.7±3.9 years. The average age at the last ADC evaluation was 78.5±9.6 years, the last MMSE score was 13.9±8.2, and the interval between the last evaluation and death was 1.8±1.3 years.

4.3.2 Distribution of Age of Onset by APOE Genotype

Density distributions of the estimated ages of onset by the number of APOE ε4 alleles (**Figure 4.1A**) showed the expected dose-dependent shift of the major peaks to an earlier age of onset as the number of ε4 alleles increased. A 2-component gaussian mixture model fit only to the ε4- individuals ($p<0.001$) identified 2 underlying distributions (see **Figure 4.1B**), one peaking at age 76.7±7.5 years and accounting for 78% of the cases, and another peaking at age 57.2±3.8 years and accounting for 22% of the cases. The point of intersection between these two distributions (the age of onset which is equally likely to belong to both) was age 63.0 years.

4.3.3 Participant Demographic Characteristics as a Function of Age-of-Onset and APOE Genotype

EOAD cases had longer duration of illness than LOAD cases by 0.80 years [99% CI: 0.25 – 1.34], and a longer interval from last evaluation to death by 0.22 years [99% CI: 0.03 – 0.41] (**Table 4.1**). Presence of an ε4 allele was associated with a longer duration of illness by 0.89 years [99% CI: 0.40 – 1.37]. EOAD cases were less likely to be female [OR=0.57; 99% CI:

0.42 – 0.77] than LOAD cases. When ε4+, EOAD cases were more likely than LOAD cases to have 2 rather than 1 ε4 allele [OR=1.55; 1.05 – 2.25]. APOE ε4+ cases were more likely than ε4- cases to have had a first degree relative with dementia [OR 2.02; 99% CI: 1.53 – 2.67]. There were no significant interactions of APOE genotype with age of onset.

4.3.4 Clinical Assessment and Diagnosis

4.3.4.1 Presenting Symptoms. Most cases reported that cognitive symptoms occurred first; however, EOAD cases were more likely than LOAD cases to report non-cognitive (i.e., behavioral or motor) symptoms first [OR=2.70; 99% CI: 1.17 – 6.13], and this was particularly true for ε4- EOAD cases as shown by a significant age of onset X APOE genotype interaction effect (p<0.01) (**Figure 4.2A**). When cognitive symptoms occurred first, the first symptom was usually memory impairment (**Figure 4.2B**), but EOAD cases were more likely than LOAD cases to report non-memory cognitive symptoms first [OR=5.56; 3.32 – 9.39]. This was again particularly true for ε4- EOAD cases as shown by a significant age of onset X APOE genotype interaction effect (p<0.01).

4.3.4.2 Clinical Rating Scales. EOAD cases had lower MMSE scores by 2.50 points [99%CI: 1.38 – 3.62], higher (i.e., worse) FAQ scores by 3.31 points [99% CI: 1.54 – 5.07], higher (i.e., worse) CDR-SOB scores by 0.94 points [99% CI: 0.22 – 1.66], and higher (i.e., worse) NPI scores by 1.27 points [99% CI: 0.68 – 1.95] than LOAD cases (**Table 4.2**). Presence of an ε4 allele was associated with lower MMSE scores by 1.29 points [99% CI: 0.30 – 2.28], higher FAQ scores by 2.24 points [99% CI: 0.66 – 3.82], higher CDR-SOB scores by 0.91 points [99% CI: 0.21 – 1.55], and higher NPI scores by 0.68 points [99% CI: 0.07 – 1.28]. GDS scores did not differ by age of onset or APOE genotype. There was no age of onset X APOE genotype interaction effect for any of these measures. This pattern of results did not change at the final evaluation, except that NPI scores no longer differed by APOE genotype (see **Table 4.3**).

EOAD cases progressed more rapidly than LOAD cases by approximately 1.27 points per year [99% CI: 0.65 – 1.76] on the MMSE and by 0.32 points per year [99% CI: 0.07 – 0.56]

(higher scores are worse) on the CDR-SOB, even after accounting for differences in baseline performance (**Figure 4.3B and 4.3C**). APOE genotype or its interaction with age of onset had no significant effect on MMSE and CDR-SOB rate of decline.

4.3.4.3 Medical History and Medication Use. Baseline reports of neurologic conditions that could affect cognition were infrequent, but history of stroke [OR=0.20; 99% CI: 0.06 – 0.49] or transient ischemic attack (TIA) [OR=0.34; 99% CI: 0.13 – 0.74] was less often reported for EOAD than LOAD cases, while history of traumatic brain injury (TBI) was more often reported for EOAD than LOAD cases [OR=2.04; 99% CI: 1.34 – 3.07] (**Table 4.2**). Baseline reports of psychiatric history of depression in the past 2 years were more frequent in ε4- than ε4+ cases in EOAD, but more frequent in ε4+ than ε4- cases in LOAD [$p < 0.01$ for the age of onset X APOE genotype interaction]. Depression older than 2 years was more common in EOAD than LOAD [OR=1.71; 99% CI: 1.19 – 2.43], while alcohol abuse and other psychiatric disorders did not differ by group.

Use of antidepressants was more common at baseline in EOAD than LOAD cases [OR=2.11; 99% CI: 1.58 – 2.83] (**Table 4.2**). Neither anxiolytics/sedatives/hypnotics as a group nor antipsychotics differed by age of onset or APOE genotype at baseline. Use of FDA-approved medications for the treatment of AD was more common in EOAD than LOAD cases [OR=1.92; 99% CI: 1.39 – 2.69], and in ε4+ than ε4- cases [OR=1.72; 99% CI: 1.32 – 2.25], with no evidence for an interaction.

4.3.4.4 Cognitive Testing. APOE ε4+ cases had significantly worse baseline Memory domain scores than ε4- cases [β=-0.28; 99% CI: -0.40 – -0.15] (**Figure 4.3A**). Executive domain scores were profoundly more impaired in EOAD cases than LOAD cases, and this was particularly true for those with an ε4- genotype [$p<0.01$ for the age of onset X APOE genotype interaction]. Language domain scores were worse in LOAD cases than EOAD cases [β=0.30; 99% CI: 0.07 – 0.53]. Attention domain scores were unimpaired overall, but slightly worse in EOAD cases than LOAD cases [β=-0.44; 99% CI: -0.72 – -0.16].

4.3.4.5 Clinical Diagnosis. The proportion of cases diagnosed with dementia at baseline ranged from 73% to 91% across groups (**Table 4.2**). EOAD cases were more likely than LOAD cases to receive a clinical diagnosis of dementia rather than MCI [OR=3.02, 99% CI: 1.94 – 4.90]. This same pattern of results was observed at the final evaluation before death, although the proportion of cases diagnosed with dementia increased in all groups and ranged from 92% to 100% (**Table 4.3**).

4.3.4.6 Presumed etiology. The presumed etiology of the cognitive impairment was assigned based on the results of the baseline evaluation. The proportion of cases with an etiology thought to be "primary" or "contributing" to the cognitive impairment are shown as a function of age of onset and APOE genotype in **Figure 4.2C**. Approximately 80-90% of all cases were presumed to have AD pathology as "primary" or "contributing" to the cognitive impairment. In approximately 10-20% of cases, cognitive impairment was incorrectly attributed to an etiology other than AD. EOAD cases were more likely than LOAD cases [OR 0.64; 99% CI: 0.43 – 0.97] to have their cognitive impairment incorrectly attributed to non-AD pathology. FTLD pathology was the second most common presumed etiology after AD and was more likely to be assigned as "primary" or "contributing" to the cognitive impairment in EOAD cases than LOAD cases [OR 4.25; 99% CI: 2.74 – 6.65]. Vascular pathology, in contrast, was less likely to be assigned to EOAD cases than LOAD cases [OR 0.18; 99% CI: 0.06 – 0.46], although it was rarely assigned as a cause of cognitive impairment.

There was little change in the presumed etiology of cognitive impairment between the baseline and final evaluations (see **Figure 4.2D**). EOAD cases continued to be less likely than LOAD cases to have their cognitive impairment attributed to AD, although this was now only true for ε4- EOAD cases [$p<0.01$ for the APOE genotype X age of onset interaction]. FTLD remained the second most common presumed etiology after AD, and was more likely to be assigned as "primary" or "contributing" to cognitive impairment in EOAD cases than LOAD cases, particularly in those without an APOE ε4 allele [$p<0.01$ for the APOE genotype X age of

onset interaction]. Vascular pathology, though rarely assigned, remained more likely to be assigned to LOAD cases than EOAD cases [OR 0.25; 99% CI: 0.10 – 0.53].

4.3.5 Neuropathological Analysis and Concomitant Pathology

A greater percentage of ε4+ than ε4- cases had concomitant moderate-to-severe amyloid angiopathy [OR=2.22; 99% CI: 1.71 – 2.89] (**Figure 4.4A**). A lower percentage of EOAD cases than LOAD cases had concomitant moderate-to-severe atherosclerosis [OR=0.39; 99% CI: 0.28 – 0.53], arteriolosclerosis [OR=0.65; 99% CI: 0.47 – 0.88], infarcts/lacunae [OR=0.34 99% CI:; 0.20 – 0.55], and microinfarcts [OR=0.49; 99% CI: 0.32 – 0.73].

The proportion of individuals with pure severe AD with no identified concomitant pathologies was higher among EOAD cases than LOAD cases [OR=1.41; 99% CI: 1.06 – 1.87] (**Figure 4.4B**). There was no significant effect of age of onset, APOE genotype, or their interaction on the prevalence of concomitant LBD, FTLD, or other pathology. Medial Temporal Lobe Sclerosis (including Hippocampal Sclerosis) was less common in EOAD cases than in LOAD cases [OR = 0.46; 99% CI: 0.27 – 0.74], but there was no significant effect of APOE genotype or age of onset X APOE genotype interaction.

In exploratory sub-analyses of individuals with full staging for LBD (N=809; 46%; Figure 4C) and LATE (N=368; 21; Figure 4D), presence of any Lewy bodies was more likely in ε4+ than ε4- [OR 1.48; 99% CI: 1.02 – 2.17], though none of the individual stages differed by APOE genotype. Presence of any TDP-43 pathology was less likely in EOAD than LOAD [OR = 0.37; 99% CI: 0.18 – 0.72], driven by lower likelihood specifically of Hippocampal TDP-43 [OR = 0.24; 99% CI: 0.08 – 0.57]. The remaining effects and interactions were not significant.

4.4 Discussion

We identified several examples of age-related heterogeneity in patients with sporadic, pathologically-confirmed severe AD. Consistent with previous studies of clinically diagnosed patients[8], we found that EOAD patients were more likely than LOAD patients to report non-cognitive changes (e.g., behavioral dysfunction) or non-memory cognitive decline (i.e., language

or executive function impairment) as their initial symptom. They were also more likely to have self-reported history of TBI, but less likely to have a history of stroke or TIA. EOAD patients were more impaired than LOAD patients on mental status and functional activity measures at their initial ADC evaluation. Objective cognitive testing showed that EOAD patients had far worse executive function impairment, but less language impairment, than LOAD patients. As in previous studies with clinically diagnosed AD[4–7], EOAD patients declined more rapidly than LOAD patients on cognitive and functional measures. Ultimately, EOAD patients were more likely than LOAD patients to receive a non-AD clinical diagnosis (e.g., FTD, DLB) at their initial ADC evaluation; however, this was only true for APOE ε4- EOAD by the last ADC evaluation.

Our results confirm in a relatively large cohort of patients with autopsy-proven severe AD greater likelihood of an initial atypical, non-memory focal cortical presentation in EOAD than in LOAD. Despite this, EOAD patients were more likely than LOAD patients to have "pure" AD pathology without concomitant non-AD pathology. LOAD were more likely than EOAD to have cerebrovascular pathology, MTL/Hippocampal Sclerosis, and in a sub-analysis, hippocampal TDP-43, consistent with a more common history of vascular disease in LOAD than EOAD, and the known age-related increase in vascular[33,34], Hippocampal Sclerosis[35], and LATE[27] pathology. Thus, even though EOAD were *more* likely than LOAD patients to have an atypical clinical presentation, concomitant non-AD pathologies were *less* common in EOAD than LOAD. This finding suggests that co-pathologies are not responsible for the atypical clinical presentation or faster cognitive decline in EOAD.

We also identified heterogeneity related to APOE genotype. Regardless of age of onset, ε4+ patients performed worse than ε4- patients on mental status and memory tests, functional activity scales, and psychiatric measures. APOE genotype was not associated with clinical diagnosis at the initial ADC evaluation, but ε4+ patients were significantly more likely than ε4- patients to be correctly assigned an AD diagnosis at their final evaluation. This increased

accuracy may be because ε4+ patients exhibited a more AD-like profile with more prominent memory impairment. It is possible, however, that patients known to be ε4+ were more likely to be called AD since some ADCs may use genetic information when assigning diagnoses. There was no difference in the likelihood of concomitant pathologies in ε4+ and ε4- patients, except for a higher likelihood of amyloid angiopathy in ε4+ patients as previously reported[36]. It is unclear if amyloid angiopathy significantly contributes to cognitive decline[37,38]. Additionally, in a sub-analysis, LBD pathology (not specific to any particular LBD stage) was more likely in ε4+ than ε4- patients, consistent with findings that the APOE ε4 allele is associated with LBD pathology independent of AD[39].

Frequency distributions of age of onset were different for APOE ε4+ and ε4- patients. Estimated age of onset appeared to be normally distributed for those with one or two ε4 alleles, with an expected shift toward younger onset in those with two ε4 alleles[40,41]. In contrast, the frequency distribution of age of onset for those who were ε4- appeared to have two separate peaks at 57 and 76 years of age. This finding is consistent with the possibility that EOAD ε4- patients represent a subgroup that has increased likelihood of atypical clinical features[2,3]. This possibility is supported by several age of onset by APOE genotype interaction effects we observed. For example, the EOAD ε4- group was more likely than other groups to have non-cognitive or non-memory cognitive presenting symptoms, greater executive function deficits on objective testing, and were most likely to be clinically assigned an etiology other than AD (particularly FTD). By the last evaluation before death, only 76% of EOAD ε4- patients, but over 90% of EOAD ε4+, LOAD ε4+ and ε4- patients, were assigned an etiology of AD.

There are several possible explanations for why EOAD patients, and especially those who are APOE ε4-, have an increased likelihood of an atypical clinical profile even in the absence of concomitant pathology. First, there may be genetic contributions to the clinical manifestation of EOAD beyond APOE in the form of polygenic risk or unknown mutations. A

genetic explanation is unlikely, however, since the EOAD ε4- group had the lowest percentage (at 51%) of individuals reporting a first-degree family member with dementia.

Second, the EOAD ε4- group was more likely (at 50% prevalence) than other groups to report a history of depression within the past 2 years. Although active depression was not evident on the GDS, past depression may have influenced the nature of the initial presentation of symptoms, leading to increased reports of initial behavioral or non-memory cognitive changes and more prominent executive function deficits in EOAD ε4- patients. It is not clear whether depression in the past 2 years is a component of the dementia syndrome in EOAD or a reaction to awareness of cognitive impairment at a young age. However, if it is a reaction, it might be expected to equally impact ε4+ and ε4- EOAD patients.

Third, there could be age-related differences in concomitant pathologies not fully examined or reported in the ADC neuropathological evaluations. Indeed, our exploratory sub-analysis showed associations of TDP-43 pathology with older age of onset, consistent with the criteria for LATE[27] and with a recent neuropathologic cluster-analysis of TDP-43 cases in the NACC dataset showing that of two clusters with severe concomitant AD, the cluster with more extensive TDP-43 had older ages of onset and death[42].

Finally, distribution of AD pathology may differ by age of onset and APOE genotype. Previous research shows that there can be an atypical distribution of tangle pathology with neocortical predominance and “hippocampal sparing” even in those with severe AD pathology (i.e., Braak stage V-VI)[20,43]. While these studies did not specifically address age of onset, they found that this atypical distribution was most likely to occur in younger patients (< age 65 on average) who were ε4-. Similarly, structural imaging and Tau PET both support the idea of greater frontal and parietal atrophy and pathologic burden in EOAD[44–46]. Unfortunately, the distribution of AD pathology (beyond Braak staging) was not examined in the UDS neuropathological evaluation so we cannot determine if EOAD patients (particularly ε4-) were

more likely than LOAD patients to have “hippocampal sparing” or some other atypical topography of AD pathology.

Several limitations of the present study should be noted. The multi-center nature of the study may enhance generalizability, but it introduces variability since the 33 contributing ADCs may differ slightly in inclusion/exclusion criteria, assessment measures used to reach a diagnosis, and neuropathologic methodology. ADC cohorts are not representative of the general population, with over-representation of rare dementias (e.g. FTD) and possible under-representation of vascular dementia. The original NACC neuropathologic evaluation did not include updated methods (e.g., α-synuclein antibodies) and diagnostic classifications (e.g., LATE), so only exploratory analysis could be carried out in some instances. Finally, our cohort was intentionally limited to those with severe AD pathology which may have precluded our ability to observe subtle contributions of concomitant pathology to atypical disease presentations. However, applying strict pathological criteria for AD allowed us to avoid the pitfalls of including non-AD mimics (e.g. Hippocampal Sclerosis[47,48]), while also providing a more accurate picture than clinical studies that may underestimate heterogeneity because patients with atypical presentations of AD have been misdiagnosed and excluded.

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Table 4.1 Demographics

	LO ε4-	LO ε4+	EO ε4-	EO ε4+	Early Onset Effect [99% CI]	APOE ε4+ Effect [99% CI]	EO x ε4+ Interaction [99% CI]
n	511	797	169	273			
Age at Onset	77.3 ± 7.1	73.3 ± 6.0	56.9 ± 3.5	58.0 ± 4.0			
Age at Baseline	81.7 ± 6.4	78.6 ± 6.1	62.3 ± 4.8	64.0 ± 5.4			
Age at Last Visit	84.7 ± 6.5	81.5 ± 6.2	65.1 ± 5.5	66.7 ± 5.6			
Age at Death	86.3 ± 6.5	83.2 ± 6.1	66.9 ± 5.4	68.6 ± 5.7			
Duration (y)	8.9 ± 3.6	9.9 ± 3.8	10.0 ± 3.8	10.5 ± 4.2	**0.80 [0.25 – 1.34]**	**0.89 [0.40 – 1.37]**	n.s.
Last Visit - Death Interval (y)	1.6 ± 1.3	1.8 ± 1.3	1.9 ± 1.4	2.0 ± 1.3	**0.22 [0.03 – 0.41]**	0.14 [-0.03 – 0.31]	n.s.
Education	15.1 ± 3.4	15.4 ± 3.0	15.6 ± 2.9	15.8 ± 2.8	0.43 [-0.01 – 0.87]	0.27 [-0.18 – 0.72]	n.s.
Female	241 (47%)	361 (45%)	61 (36%)	84 (31%)	**0.57 [0.42 – 0.77]**	0.89 [0.69 – 1.15]	n.s.
0 APOE ε4 alleles	511 (100%)	0 (0%)	169 (100%)	0 (0%)			
1 APOE ε4 allele	0 (0%)	627 (79%)	0 (0%)	190 (70%)			**1.55 [1.05 – 2.25]&**
2 APOE ε4 alleles	0 (0%)	170 (21%)	0 (0%)	83 (30%)			
1st Degree Family History: Dementia	263 (58%)	530 (71%)	79 (51%)	187 (74%)	0.95 [0.70 – 1.31]	**2.02 [1.53 – 2.67]**	n.s.

Comparison of 2 vs 1 ε4 alleles between the EO and LO ε4+ groups only.
Missing Data: Education (n=14; <1%), Family History of Dementia (n=141; 8%)
Effects are β [99% CI] for linear regression or Odds Ratios [99% CI] for logistic regression. Effects are bolded when the 99% CI for β does not include 0, and when the 99% CI for the OR does not include 1 (i.e. $p<0.01$).

Table 4.2 Clinical Assessment and History at Baseline

	LO ε4-	LO ε4+	EO ε4-	EO ε4+	Early Onset Effect [99% CI]	APOE ε4+ Effect [99% CI]	EO x ε4+ Interaction [99% CI]
n	511	797	169	273			
MMSE	21.1 ± 7.2	19.5 ± 8.0	18.0 ± 7.8	17.6 ± 8.3	**-2.50 [-3.62 – -1.38]**	**-1.29 [-2.28 – -0.30]**	n.s.
FAQ	15.6 ± 10.8	18.1 ± 10.4	19.5 ± 8.9	21.0 ± 8.0	**3.31 [1.54 – 5.07]**	**2.24 [0.66 – 3.82]**	n.s.
CDR Sum of Boxes	5.8 ± 5.0	6.7 ± 5.2	6.6 ± 4.6	7.5 ± 5.1	**0.94 [0.22 – 1.66]**	**0.91 [0.21 – 1.55]**	n.s.
GDS	2.4 ± 2.4	2.3 ± 2.4	2.6 ± 2.2	2.5 ± 2.4	0.21 [-0.16 – 0.58]	-0.09 [-0.40 – 0.24]	n.s.
NPI	4.1 ± 4.4	4.8 ± 4.5	5.5 ± 5.1	6.0 ± 5.3	**1.27 [0.68 – 1.95]**	**0.68 [0.07 – 1.28]**	n.s.
Cognitive Status: MCI	140 (27%)	160 (20%)	15 (9%)	25 (9%)	**3.02 [1.94 – 4.90]**	1.42 [0.98 – 1.97]	n.s.
Cognitive Status: Dementia	371 (73%)	637 (80%)	154 (91%)	248 (91%)			
Medical History: Stroke	46 (9%)	47 (6%)	5 (3%)	2 (1%)	**0.2 [0.06 – 0.49]**	0.59 [0.34 – 1.01]	n.s.
Medical History: TIA	34 (7%)	53 (7%)	2 (1%)	9 (3%)	**0.34 [0.13 – 0.74]**	1.11 [0.64 – 1.96]	n.s.
Medical History: Seizures	10 (2%)	19 (2%)	9 (5%)	11 (4%)	2.1 [0.95 – 4.5]	0.99 [0.47 – 2.2]	n.s.
Medical History: TBI	45 (9%)	71 (9%)	33 (20%)	45 (17%)	**2.04 [1.34 – 3.07]**	0.91 [0.61 – 1.37]	n.s.
Medical History: Alcohol Abuse	22 (4%)	45 (6%)	18 (11%)	23 (8%)	1.69 [0.97 – 2.87]	1.05 [0.62 – 1.82]	n.s.
Medical History: Depression (past 2 years)	160 (32%)	298 (38%)	97 (58%)	127 (47%)	**3.03 [1.90 – 4.89]**	1.31 [0.96 – 1.79]	**0.49 [0.27 – 0.89]**
Medical History: Depression (older)	70 (14%)	139 (18%)	38 (23%)	65 (24%)	**1.71 [1.19 – 2.43]**	1.27 [0.91 – 1.79]	n.s.
Medical History: Other Psych. Disorder	20 (4%)	28 (4%)	9 (5%)	16 (6%)	1.62 [0.82 – 3.09]	0.86 [0.48 – 1.56]	n.s.
AD Medication Use	278 (55%)	530 (67%)	116 (69%)	223 (82%)	**1.92 [1.39 – 2.69]**	**1.72 [1.32 – 2.25]**	n.s.
Antidepressant Use	157 (31%)	273 (35%)	89 (53%)	135 (49%)	**2.11 [1.58 – 2.83]**	1.08 [0.83 – 1.41]	n.s.
Anxiolytic/Sedative/Hypnotic Use	50 (10%)	66 (8%)	14 (8%)	35 (13%)	1.32 [0.82 – 2.09]	1.01 [0.65 – 1.56]	n.s.
Antipsychotic Use	36 (7%)	87 (11%)	17 (10%)	31 (11%)	1.19 [0.73 – 1.87]	1.47 [0.95 – 2.32]	n.s.

Effects are β [99% CI] for linear regression or Odds Ratios [99% CI] for logistic regression. Effects are bolded when the 99% CI for β does not include 0, and when the 99% CI for the OR does not include 1 (i.e. $p<0.01$).

Missing Data if > 1%: MMSE (n = 19; 1%), FAQ (n = 585; 33%), GDS (n=245; 14%), NPI (n=64; 4%), TBI (n=31, 2%)

Table 4.3 Clinical Assessment at Last Visit

	LO ε4-	LO ε4+	EO ε4-	EO ε4+	Early Onset Effect *[99% CI]*	APOE ε4+ Effect *[99% CI]*	EO x ε4+ Interaction *[99% CI]*
n	511	797	169	273			
MMSE	16 ± 7.9	14 ± 8.3	10.9 ± 7.8	10.7 ± 7.5	**-4.05 [-5.40 – -2.69]**	**-1.59 [-2.77 – -0.41]**	n.s.
FAQ	25.1 ± 7.4	26.2 ± 6.4	26.4 ± 6.2	27.1 ± 4.7	**1.07 [0.05 – 2.09]**	**1.05 [0.12 – 1.98]**	n.s.
CDRSUM	10.9 ± 5.5	11.8 ± 5.1	12.4 ± 5.4	12.8 ± 4.7	**1.27 [0.53 – 2.02]**	**0.75 [0.09 – 1.41]**	n.s.
GDS	2.8 ± 2.7	2.4 ± 2.5	2.6 ± 2.1	2.6 ± 2.7	0.10 [-0.43 – 0.63]	-0.29 [-0.72 – 0.16]	n.s.
NPI	5.8 ± 5.1	6.4 ± 5.3	7.7 ± 5.6	8.2 ± 6.4	**1.79 [1.00 – 2.59]**	0.52 [-0.19 – 1.23]	n.s.
Cognitive Status: MCI	38 (7%)	36 (5%)	3 (2%)	1 (0%)	**6.78 [2.20 – 35.7]**	1.88 [0.91 – 3.44]	n.s.
Cognitive Status: Dementia	471 (92%)	761 (95%)	166 (98%)	272 (100%)			

Effects are β [99% CI] for linear regression or Odds Ratios [99% CI] for logistic regression. Effects are bolded when the 99% CI for β does not include 0, and when the 99% CI for the OR does not include 1 (i.e. $p<0.01$).

Missing Data: MMSE (n = 436; 25%), FAQ (n = 390; 22%), GDS (n=805; 46%), NPI (n=74; 4%),

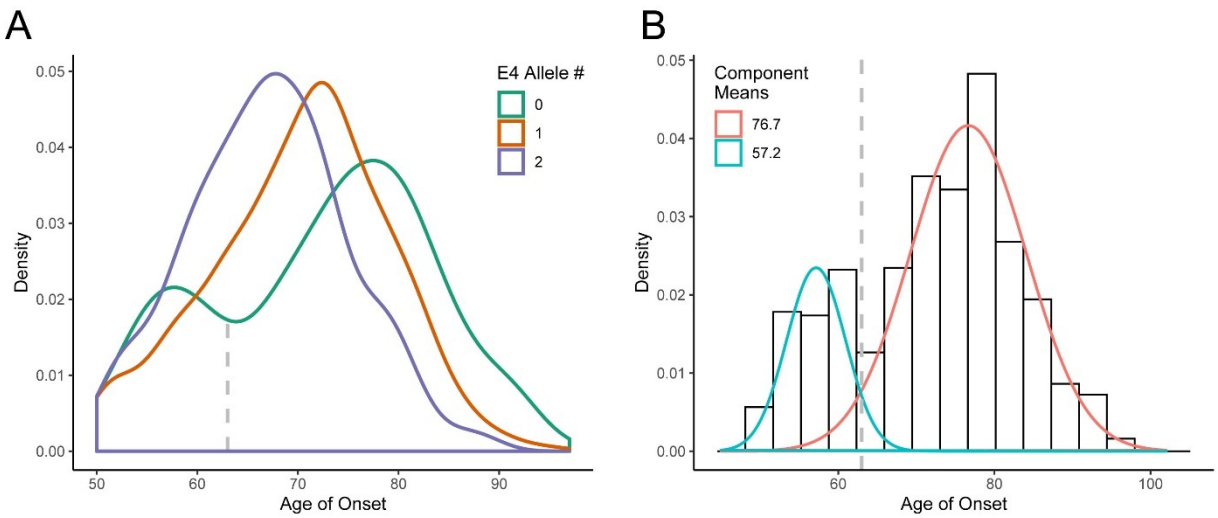

Figure 4.1 Distribution of Ages of Onset by APOE genotype.

(A) A plot of the density distributions split by the number of APOE ε4 alleles (smoothed histograms that integrate to 1 for each group) shows a marked bimodal distribution for the ε4-negative population. **(B)** A 2-component gaussian mixture model only of this ε4-negative population fit the data well and identified 2 underlying normal distributions: the larger distribution (red) accounted for 78% of the cases with a peak at age 76.7 and a standard deviation of 7.5, while the smaller distribution (blue) accounted for 22% of the cases with a peak at age 57.2 and a standard deviation of 3.8. The point of intersection between these two distributions was age 63.0 (vertical grey dotted lines in both panels) – this point was chosen as the distinction between Early Onset and Late Onset in this study regardless of APOE genotype.

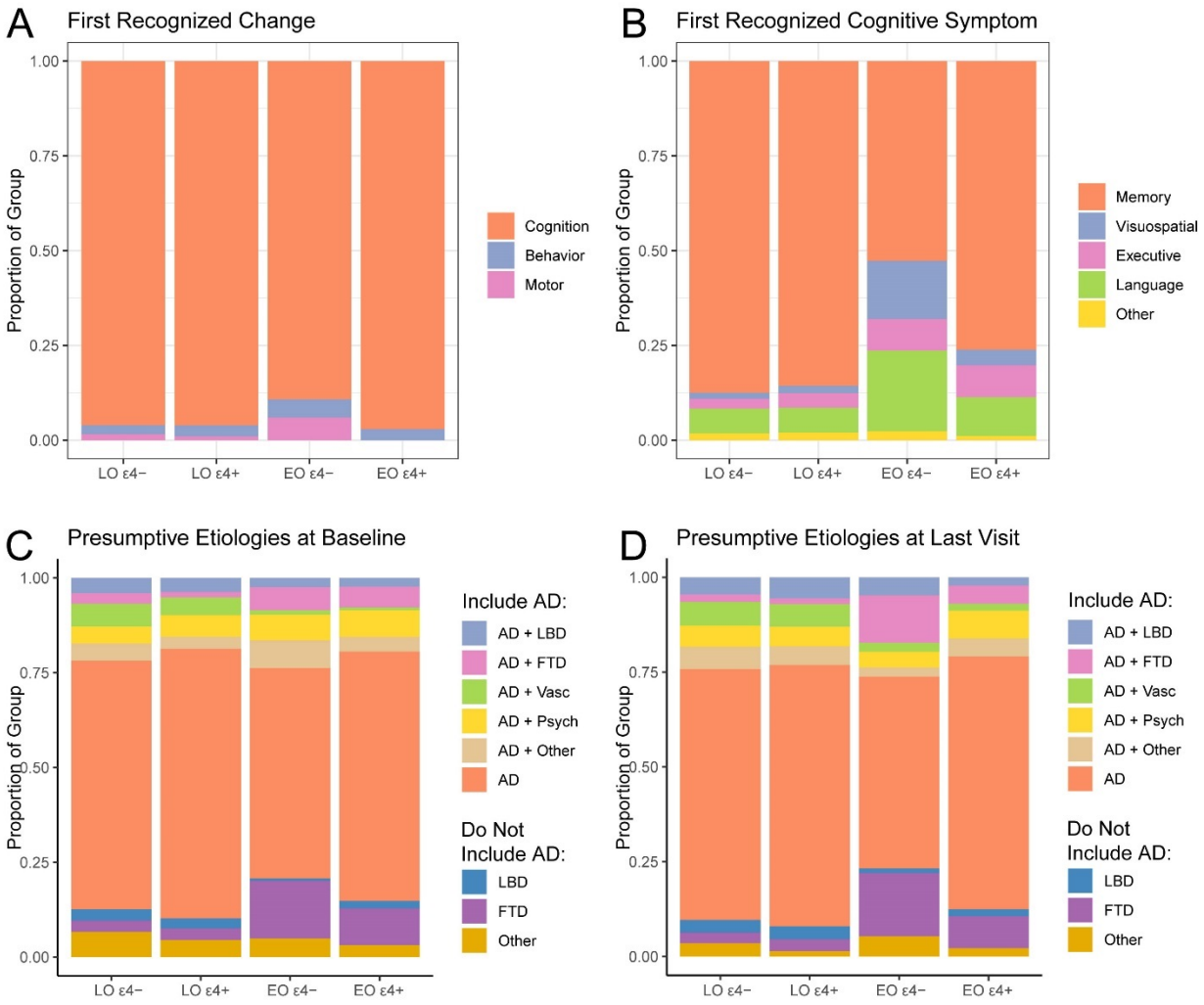

Figure 4.2 Clinical Presentation and Diagnosis

(A) The proportion of cases reporting cognition, behavior, or motor impairments as the first recognized change, plotted as a function of age of onset and APOE genotype. The colors of the bars represent the proportion of which mutually-exclusive outcome was reported in each group. Missing data for n=22 (1%). **(B)** The distribution of which specific cognitive symptom was first recognized in the participant, plotted as a function of age of onset and APOE genotype. The "Other" group includes rare reports of the first symptom being Orientation (n=1; <1%), Attention/concentration (n=23; 1%), and other write-ins (n=8; <1%). Missing data for n=8 (<1%). **(C, D)** The clinically-assigned presumptive etiologies reported as either "primary" or "contributing" to the cognitive impairment at Baseline and Last Visit (closest to death). The etiology combinations that correctly included AD are represented with pastel colors at the top, while those that missed AD in error are in dark tones at the bottom of the figure. The "AD + Other" and non-AD "Other" groups includes other combinations in the presence of absence of AD, respectively, that account for <5% of the total sample. Missing data for n=109 (6%) at baseline, and n=10 (<1%) Final Visit.

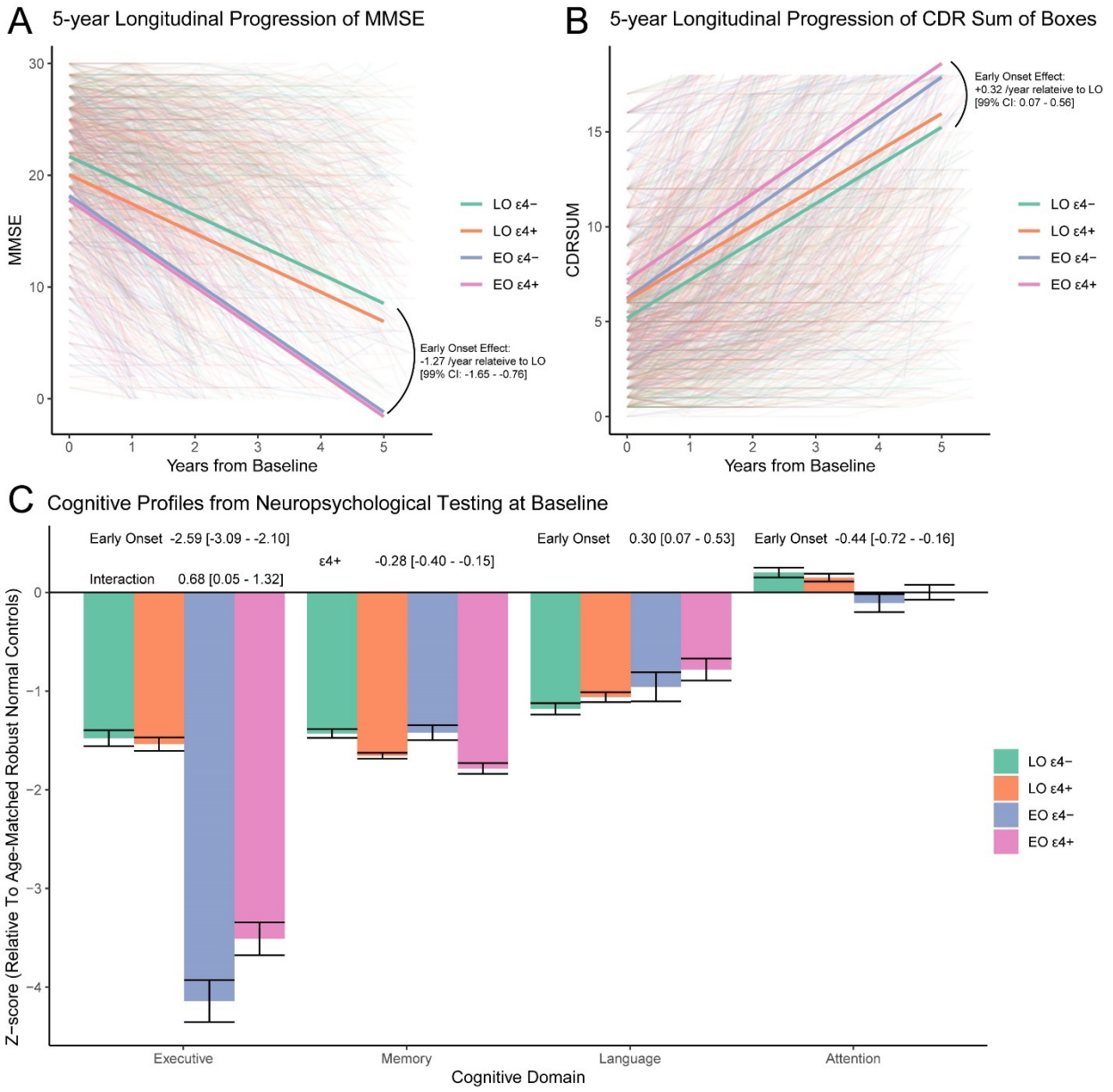

Figure 4.3 Objective Cognitive Assessment and Progression

(A,B) 5-year longitudinal progression on the MMSE and CDR sum-of-boxes scales from baseline, modeled with linear mixed effects models. The effect of age of onset on the longitudinal slope of decline is reported, while the effect of APOE and its interaction with age of onset were not significant. **(C)** The baseline cognitive performance by age of onset and APOE genotype in 4 domains derived via PCA from the full NACC neuropsychological battery. Performance is expressed as Z-scores relative to separate age and gender matched robust normal control participants (those that remained normal for the all NACC visits) for early and late onset groups. Effects for being early rather than late onset, for the presence of an ε4 allele, and for their interaction are presented above the bars, when significant.

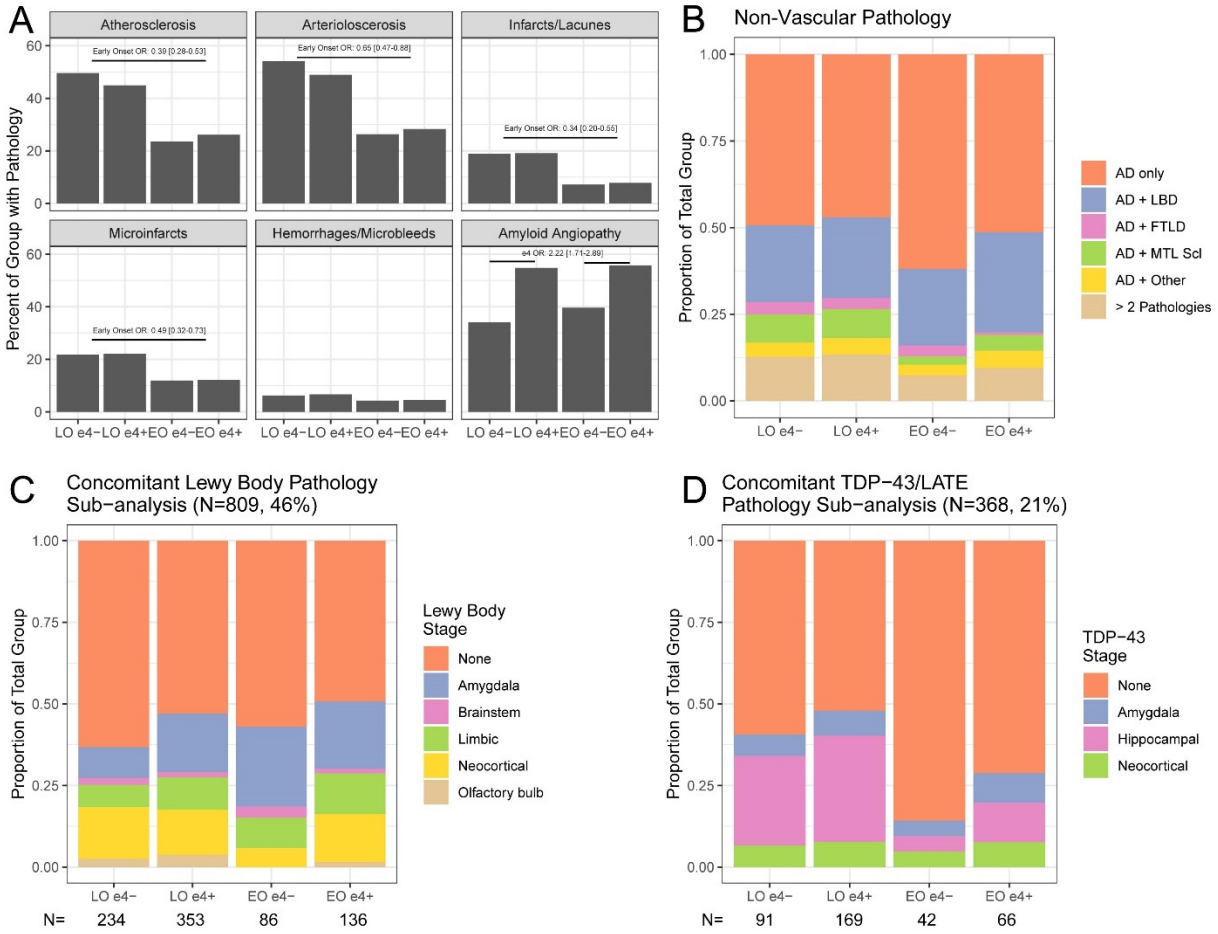

Figure 4.4 Concomitant Pathology

(A) Presence of each of the 6 vascular pathologies assessed is separately shown as a proportion of each APOE genotype and age of onset group. While the interaction of age of onset with APOE genotype was not significant for any measures, effects of age of onset or presence of ε4 alleles separately are reported above the bars. **(B)** The distribution of non-vascular pathologies assessed in NACC by Age of Onset and APOE genotype. **(C)** Sub-analysis of LBD stage in cases with UDS 3 neuropathologic evaluation including all 5 categories (N=809; 46%). **(D)** Sub-analysis of TDP-43/LATE stage in cases that had complete reported TDP-43 data in the Amygdala, Hippocampus, and Neocortex (N=368, 21%).

Chapter 5

Distribution of Neurofibrillary Tangle Pathology Mediates Age of Onset Related Clinical Heterogeneity in Sporadic Alzheimer's Disease

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Abstract

In sporadic Alzheimer's Disease (AD), patients with an earlier age of onset are more likely to present with atypical clinical and cognitive features and exhibit more rapid progression than those with a later onset. The pathologic basis of these age-related clinical differences is unknown; however, there is some evidence that the distribution of tau neurofibrillary tangles (NFTs) in AD also changes with age of onset. We examined the relationship between distribution of NFTs, presence of non-AD co-pathology, and clinical features of a cohort of patients with sporadic autopsy-confirmed severe (NIA-Reagan "high") AD and age of onset of 51-60 (n=40), 61-70 (n=41), and >70 (n=40). At baseline, global cognitive measures did not differ by age of onset, but those with an earlier age of onset were more likely to present with a non-memory complaint, exhibited more psychiatric symptoms, and showed greater functional impairment in activities of daily living. At autopsy, α-synuclein co-pathology did not differ by age of onset, but both TDP-43 and microvascular pathology were less common in those with earlier onset, consistent with prior studies. Importantly, the burden of NFT pathology in the middle frontal gyrus relative to the hippocampus (Mid. Frontal / Hippocampal tangle ratio) was greater in those with earlier onset. This was driven by an inverse relationship between middle frontal NFT density and age of onset, with patients with earlier onset having more middle frontal NFTs. In contrast, NFT density in the hippocampus was greater in those with an APOE ε4 allele and in those with concomitant TDP-43 pathology, but was not related to age of onset. On detailed neuropsychological evaluation, patients with earlier onset showed greater impairment in Executive and Visuospatial abilities, and exhibited more rapid longitudinal decline. Using mediation analyses, we show that the Mid. Frontal / Hippocampal tangle ratio, but not the concomitant pathologies, appears to mediate the effects of age of onset on Executive function and decline. Overall, this suggests that an altered NFT distribution, with increased cortical NFTs in earlier onset patients, may contribute to changes in the clinical presentation of AD with age.

5.1 Introduction

Clinical studies suggest there is age-related heterogeneity in the clinical and cognitive presentation of Alzheimer's disease (AD)[1–3]. Patients with earlier age of symptom onset are more likely to have atypical presentations with prominent non-memory cognitive deficits[1,4,5], greater psychiatric involvement, and more rapid cognitive decline[6,7]. However, the pathologic basis for these clinical differences is not well explained. We recently examined the relationship of clinical and pathologic features to age of onset in 1,750 patients with sporadic, pathologically-confirmed severe AD using data from the National Alzheimer's Coordinating Center (NACC). Patients with an early onset of symptoms (age $\leq$ 63), especially without an APOE ε4 risk allele, were more likely than those with late onset to report non-cognitive (i.e. behavioral) or non-memory cognitive decline as their initial symptom, to exhibit much greater executive function impairment on objective neuropsychological testing, and to decline more rapidly on cognitive and functional measures. These atypical features resulted in greater misattribution of the underlying etiology to non-AD causes, despite the fact that at autopsy these early-onset patients had *less* concomitant non-AD vascular and non-vascular (e.g., α-synuclein, TDP-43) neurodegenerative pathology.

Given that atypical clinical and cognitive presentations in early-onset AD are not a function of concomitant non-AD pathology, we hypothesized that this paradoxical finding might be explained by age-related differences in the distribution of AD pathology. This hypothesis was prompted by Murray and colleagues'[8] report of substantially different average ages of symptom onset in distinct neuropathologic subtypes of AD defined by comparing relative NFT density in hippocampal versus neocortical brain regions. Patients with disproportionally high neocortical tangle densities were classified as "Hippocampal Sparing", those with disproportionally high hippocampal tangle densities as "Limbic Predominant", and all others were considered "Typical AD". Hippocampal Sparing patients had an estimated age of onset of 63, compared to 69 for

Typical patients and 76 for Limbic Predominant patients. These findings support the possibility that age-related diversity in the distribution of neocortical tangles mediates age-related heterogeneity in clinical presentations of AD during life[9].

To further examine how distribution of NFT burden relates to age of onset, we examined 121 patients with sporadic, autopsy-confirmed severe AD who varied in reported age of onset of symptoms, and inspected the distribution of NFT pathology between the hippocampus and middle frontal gyrus. We also measured concomitant α-synuclein (i.e., Lewy body), TDP-43, and microvascular pathologies across the age of onset spectrum, and determined if they interacted with the distribution of tangle pathology. We then performed mediation analyses to test the hypotheses that the distribution of neurofibrillary tangle pathology, or one of the concomitant pathologies, would explain the age of onset effects on the cognitive features displayed.

5.2 Methods

5.2.1 Standard Protocol Approvals, Registrations, and Patient Consents

The research protocol was reviewed and approved by the human subjects review board at the University of California, San Diego (UCSD). Informed consent was obtained at the point of entry into the ADRC longitudinal study from all patients or their caregivers consistent with California State law. Informed consent for autopsy was obtained from the participant during life, or their legally authorized representative in accordance with California state law.

5.2.2 Participants

Participants with sporadic pathologically-confirmed severe AD were selected from the autopsy series of the UCSD Shiley-Marcos Alzheimer's Disease Research Center (ADRC). The ADRC has followed subjects since its inception in 1985 and has maintained an autopsy rate of approximately 90% among people clinically characterized during life. Severe AD was defined using NIA-Reagan Criteria as “high likelihood” of AD being responsible for the clinical dementia

syndrome (i.e., Braak stage V-VI with moderate/severe neuritic plaques)[10]. Participants were selected without regard to concomitant pathologies or clinical diagnoses. Included participants had to have clinical and cognitive data available from a baseline evaluation (defined as the first visit at which they received a non-“normal” clinical diagnosis) and at least 1 annual follow-up evaluation. The interval from estimated onset of symptoms to the baseline evaluation, and the interval from last evaluation to death, had to be within 3 SD of the sample mean (14 and 4 years, respectively). As our focus was on sporadic AD, participants were excluded if they had a known dominantly-inherited mutation for AD (e.g. PSEN1), a family history of such a mutation, or a reported age of onset younger than 50. Participants were also excluded for essential missing data such as age of onset, APOE genotype, or pathologic findings necessary to identify and classify AD neuropathology.

After applying these inclusion and exclusion criteria, we identified 46 participants with sporadic, pathologically-confirmed severe AD and estimated onset of symptoms between 51 and 60 years of age (early onset). This represented all early onset individuals in the larger autopsy series meeting our criteria. In order to generate a demographically matched sampling through the rest of the age range, we then matched an equal number of participants meeting our inclusion and exclusion criteria with ages of onset of 61 to 70 years (n=46 from a possible 81) and 70 years and above (n=46 from a possible 186) to the early onset participants, matching on sex, years of education, and the year the autopsy was completed (to ensure comparability of clinical and pathologic procedures over the years). Matching was performed using a partial means matching approach using the *MatchIt* package for R, blind to all other clinical, cognitive and neuropathological information. During the process of performing retrospective immunostaining for TDP-43 and α-synuclein pathology, it was discovered that some of the cases had missing tissue or the tissue was not of adequate quality for immunohistochemistry and 6 cases with onset before 60, 5 cases with onset of 61-70, and 6

cases with onset after 70 had to be excluded from the study. This resulted in a final tally of 40 cases with onset before 60, 41 cases with onset between 61 and 70, and 40 cases with onset after age 70. The groups remained well-matched on the initial matching variables.

5.2.3 Neuropathological Evaluation

Autopsy was performed using a previously described protocol[11]. Each brain was divided sagittally and the left hemibrain was fixed in 10% buffered formalin, while the right hemibrain was sectioned coronally and frozen at -70°C. Standardized neuropathologic assessment, including gross and microscopic evaluation, was then performed by one of two neuropathologists (either A.H. or L.A.H.). The formalin-fixed left hemibrain was serially sliced into 1 cm slices, and tissue sections were obtained for paraffin embedding. Cortical sections included at least the following regions: middle frontal gyrus, rostral superior temporal gyrus, and inferior parietal lobule. Additional sections routinely obtained included hippocampus (CA1-CA4 and dentate gyrus), entorhinal cortex, basal ganglia, midbrain with substantia nigra, pons with locus coeruleus, and cerebellar cortex with dentate nucleus. All tissue sections were stained with hematoxylin and eosin (H&E) for histopathological examination. Additional histochemical and immunohistochemical staining included thioflavin-S, Aβ (Ab 69D, rabbit polyclonal from Edward Koo, 1:400), PHF1 tau (1:200), phospho-synuclein 81A (1: 5,000) and TDP-43 (1:4,000).

5.2.3.1 AD pathology. Neuritic and diffuse plaques, and neurofibrillary tangles (NFT), were identified either by thioflavin-S stains under polarized light or by Aβ and PHF-1 immunohistochemistry. For thioflavin-S evaluation, lesions were evaluated visually in 10 µm-thick sections stained with thioflavin-S and viewed with ultraviolet illumination and a 440 µm bandpass wavelength excitation filter. A Braak stage for NFT pathology was determined for each case using previously detailed methods[11]. Estimates of neuritic plaque density were calculated using methods recommended by CERAD[12]. Pathological diagnosis of AD was made

using the NIA-Reagan consensus criteria for the postmortem diagnosis of AD[10], wherein Braak stage V-VI with moderate to severe neuritic plaque density corresponds to “high likelihood” that dementia is due to AD.

Hippocampal and neocortical NFT density was approximated based on counts in the CA1 sector of the hippocampus (“Hippocampal tangle count”) and in the middle frontal gyrus of the neocortex (“Mid. Frontal tangle count”). Entire sections were surveyed to find areas with the heaviest pathologic burden, and these were utilized for lesion counting. Three high magnification fields were counted and averaged to provide a single NFT count per 0.1 mm^2 microscopic field for each brain region. To account for differences in the sensitivity of the two staining procedures (PHF-1 and Thioflavin-S, **Supplemental Figure 5.1**), regional NFT counts were z-transformed separately for each method, centering the mean to 0 and the standard deviation to 1. Participant groups evaluated by the two staining methods were well matched in age of onset, age of death, disease duration, sex, education, and APOE genotype distribution. The ratio of raw counts (“Mid. Frontal / Hippocampal tangle ratio”), with a range of 0 to 2.2, did not differ by staining method across age of onset (**Supplemental Figure 5.1**) and was directly pooled and used as a single continuous measure to approximate the relative distribution of pathology while accounting for overall pathologic burden.

Based on regional NFT counts and the Mid. Frontal / Hippocampal tangle ratio, patients were classified into “Hippocampal Sparing”, “Limbic Predominant”, or “Typical” neuropathologic subtypes using an approximation of the Murray et al.[8] criteria. Hippocampal Sparing was defined as: 1) Mid. Frontal tangle density above the median of the sample, 2) Hippocampal tangle density below the median of the sample, and 3) the Mid. Frontal / Hippocampal tangle ratio above the 75th percentile of the sample. Limbic Predominant was defined as: 1) Mid. Frontal tangle density below the median of the sample, 2) Hippocampal tangle density above

the median of the sample, and 3) the Mid. Frontal / Hippocampal tangle ratio below the 25th percentile of the sample. All other cases were considered Typical.

5.2.3.2 Lewy body pathology. Lewy body pathology was identified by H & E staining and immunostaining with antibodies against α-synuclein, and was staged according to consensus guidelines for the pathologic diagnosis of Lewy Body Disease (LBD)[13] into “brainstem”, “limbic” (transitional), or “neocortical” subtypes. Individuals with Lewy bodies only in the amygdala were not considered LBD given their unclear clinical impact.

5.2.3.3 TDP-43 pathology. TDP-43 pathology was identified by TDP-43 immunohistochemical staining and staged according to the latest consensus guidelines for the diagnosis of Limbic-predominant Age-related TDP-43 Encephalopathy (LATE)[14] into “amygdala”, “hippocampal”, or “neocortical” stages. Although hippocampal sclerosis (HS) often occurs in the presence of TDP-43 pathology, HS was diagnosed independently of TDP-43 pathology when neuronal loss in the CA1 and subiculum was out of proportion with the degree of AD pathology.

5.2.3.4 Vascular pathology. Vascular pathology was assessed by examining the brain for large arterial and lacunar infarcts, microinfarcts, and hemorrhages. Arteriolosclerosis, atherosclerosis of the circle of Willis, and amyloid angiopathy were each rated as “none”, “mild”, “moderate”, or “severe” using a semi-quantitative 4-point scale.

5.2.4 Clinical and Neuropsychological Evaluation

Participants had annual standardized clinical, neurological, and neuropsychological evaluations as previously described[15,16]. The clinical evaluation included review of history with the patient and/or informant, mental status testing, assessment of psychiatric symptoms using the Neuropsychiatric Inventory (NPI), and assessment of functional impairment using the Pfeffer Outpatient Disability (POD) scale[17] or the Functional Assessment Questionnaire (FAQ)

(converted to corresponding POD scores). Clinical Dementia Rating (CDR) total score, and its six subdomain scores, were computed (i.e., CDR sum of boxes).

The neuropsychological assessment included the Dementia Rating Scale (DRS; a measure of global cognitive function)[18] and standardized measures of *Memory* (Wechsler Memory Scale (WMS) Visual Reproduction Test immediate recall; WMS-Revised Logical Memory Test immediate recall; California Verbal Learning Test (CVLT) immediate recall and recognition; CERAD Word List immediate recall and recognition), *Language* (30-item Boston Naming Test; Letter Fluency Test (F-A-S); Category Fluency Test ("animals", "fruits", "vegetables"); Wechsler Adult Intelligence Scale-Revised (WAIS-R) Vocabulary Test), *Executive functions* (modified Wisconsin Card Sorting Test; Trail Making Test Parts A and B; WAIS-R Digit Symbol Substitution Test), and *Visuospatial abilities* (Wechsler Intelligence Scale for Children-Revised (WISC-R) Block Design Test; Visual Reproduction Test copy; Clock Drawing Test; Cube Drawing Test).

Using methods previously described[19], principal component analysis (PCA) with varimax rotation was performed with all test data to generate baseline cognitive domain scores. A small number of missing values were imputed using fully conditional specification[20], guided by diagnostic grouping, demographics, and other cognitive scores, as implemented by the *mice* R statistical package[21]. The PCA resulted in 4 orthogonal rotated components (based on the scree plot) which were conceptually labeled "Visuospatial", "Memory", "Executive", and "Language" based on the highest loadings for each measure (**Supplemental Table 5.1**). The baseline PCA-derived domain scores were transformed to z-scores using reference values from a pool of 497 "robust" normal controls who were diagnosed as normal on their first ADRC evaluation and remained normal for the duration of their participation in the ADRC longitudinal study.

Consensus clinical diagnoses were made according to published criteria by two or more board-certified neurologists with expertise in dementia and movement disorders. The

diagnosing neurologists were told whether the neuropsychological assessment identified deficits in two or more domains of cognition, but were not given individual test or cognitive domain scores. Probable or Possible AD or Mild Cognitive Impairment (MCI) was diagnosed according to NINCDS-ADRDA[22] or NIA-AA criteria[23]. Probable DLB was diagnosed clinically based on the presence of dementia and at least two of three additional core features of mild parkinsonism, well-formed visual hallucinations, and fluctuations in consciousness or attention[13,24,25]. Primary Progressive Aphasia (PPA) was diagnosed clinically based on prominent difficulty with language, which is the principal cause of impaired daily living activities, with aphasia being the most prominent deficit at symptom onset[26,27]. At baseline, no participants were clinically diagnosed at Posterior Cortical Atrophy, behavioral variant Frontotemporal Dementia, or any other forms of Frontotemporal Lobar Degeneration.

5.2.5 Statistical Analysis

Effects of age-of-onset on demographic and clinical features were examined by linear regression for continuous variables (e.g., age, education, mental status exam scores), and by logistic regression for categorical variables (e.g., APOE genotype, baseline clinical diagnosis). In order to illustrate the results of these analyses, means and standard deviations are presented by age-of-onset groups in **Table 5.1**.

Continuous neuropathologic outcomes were analyzed using linear regression models with terms for age-of-onset, sex, and APOE genotype (ε4+ or ε4-). Categorical neuropathologic outcomes were analyzed using logistic regression with terms for age-of-onset, sex, and APOE genotype (ε4+ or ε4-). Beta coefficients with standard errors for linear regression models or odds ratios for logistic regression models are reported in **Supplemental Table 5.2**. Coefficients for age-of-onset are standardized for a 10 year change in age.

Effects of concomitant non-AD pathologies on regional NFT counts and the Mid. Frontal / Hippocampal tangle ratio, beyond the effects of age-of-onset alone, were examined with separate linear regression models for each type of concomitant pathology (α-synuclein, TDP-43 or vascular). In these models, regional NFT counts or Frontal / Hippocampal tangle ratio were predicted by age-of-onset, sex, APOE genotype (ε4+ or ε4-), and the degree/stage of concomitant pathology.

Effects of age-of-onset on baseline cognitive domain scores were examined using linear regression models adjusting for sex, education, and APOE genotype (ε4+ or ε4-). When a significant age-of-onset effect on a particular cognitive domain was observed, mediation analyses were performed to determine if this effect was mediated by the distribution of tangle pathology (measured by the Frontal / Hippocampal tangle ratio) or by the presence (any level vs none) of a concomitant pathology. As a first step, a full linear model was fit for the particular cognitive domain score, with terms for age-of-onset, sex, education, APOE genotype, and one of the pathologic measures (either Mid. Frontal / Hippocampal tangle ratio or presence of a concomitant pathology). A mediation model[28] was then fit with the pathologic measure predicted by age-of-onset, sex, education, and APOE genotype: linear regression was used for the Mid. Frontal / Hippocampal tangle ratio and logistic regression was used for the presence of a concomitant pathology. The *mediation* R package[29] was used to evaluate the direct effect of age-of-onset on the cognitive domain score, and the portion of the effect mediated by the pathologic measure. 95% confidence intervals were determined using 10,000 non-parametric bootstrap simulations.

Because missing data precluded generation of longitudinal cognitive domain scores, we examined performance on a series of individual cognitive tests for which more than 50% of participants had data for 3 follow-up visits: DRS, MMSE, CERAD Word List, Block Design, Verbal Fluency, and Boston Naming Test. For these analyses, the subject-specific slope (i.e.,

rate of decline) across 2 to 3 annual evaluations was calculated for each test. As with the baseline cognitive domains scores, the effects of age-of-onset on slopes of decline were examined using linear regression models adjusting for sex, education, and APOE genotype (ε4+ or ε4-). When a significant age-of-onset effect on rate of decline (i.e., slope) was observed for a particular cognitive test, mediation analyses were performed (as described above) to determine if the effect was mediated by the distribution of tangle pathology (measured by the Frontal / Hippocampal tangle ratio) or by the presence (any level vs none) of a concomitant pathology.

5.3 Results

5.3.1 Participant Demographics at Baseline

The overall sample had a mean ± standard deviation age of 70.3±7.7 years at their baseline ADRC clinical evaluation, 14.5±2.7 years of education, and 36% were female. In accordance with our matching procedure, sex distribution and years of education did not differ by age of onset (as a continuous variable models, though data is presented by age of onset tercile in **Table 5.1**). The average baseline MMSE score was 22.1±4.9, DRS score was 112.7±17.1, and CDR-sum score was 5.7±2.5. None of these global cognitive measures varied by age of onset. The average basic ADL score was 6.9±1.5 and instrumental ADL score (measured by the POD) was 9.4±5.0. Both ADL measures were more impaired at baseline in those with earlier onset (β=-0.35 per 10 years [95% CI: -0.68 – -0.02], p=0.04 and β=-1.25 [-2.34 – -0.16], p=0.02, respectively). The APOE genotype distribution was 63% ε4+ and 37% ε4- and did not differ by age of onset.

The overall average reported age of onset was 66.0±8.1 years, age of death was 76.7±8.3 years, interval from age of onset to the baseline visit was 4.2±2.7 years, interval from baseline visit to death was 6.5±3.1 years, and total duration of illness was 10.7±3.7 years. The intervals from age of onset to the baseline visit (β=-1.06 [95% CI: -1.63 – -0.49] per 10 years, p=0.001), and from age of onset to death (i.e., duration of illness) (β=-0.78 [-1.60 – -0.04],

p=0.03), were longer in those with earlier onset. The other characteristics did not differ by age of onset.

The first cognitive symptom reported at onset was usually Memory (84%), but this was less likely to be true with earlier onset (Odds Ratio [OR]=2.22 per 10 years [95%CI: 1.16 – 4.60]; p=0.02). Psychiatric symptoms reflected by Neuropsychiatric Inventory (NPI) scores were more common in those with earlier onset (β=-0.66 [-1.15 – -0.17], p=0.01); however, use of antidepressants, antipsychotics, FDA-approved medications for the treatment of AD, or other medications with CNS activity did not differ by age of onset.

The most frequent baseline clinical diagnosis was probable AD (73%) followed by possible AD (12%), and the probability of receiving these diagnoses did not vary with age. The likelihood of being clinically diagnosed with LBD was relatively rare (6%), but increased with earlier onset (OR = 0.24 [95%CI: 0.06 – 0.71] per 10 years, p=0.02). In contrast, the likelihood of being diagnosed with Mild Cognitive Impairment (MCI) was greater with later age of onset (OR = 2.49 [1.09 – 6.29], p=0.04). The likelihood of being clinically diagnosed with some other non-AD cause of cognitive impairment (n=1 PPA; n=1 Depression), was very rare (2%) and did not differ with age of onset.

5.3.2 Concomitant Non-AD Neuropathology

To begin to define pathologic features that could drive age of onset-related clinical variability, we first examined the extent to which concomitant non-AD neuropathology changed with age in our cohort. The proportion of participants with various concomitant pathologies, including α-synuclein, TDP-43, and vascular pathology, is shown as a function of age of onset and APOE genotype in **Figure 5.1.** Full logistic models that examined the effect of age of onset, APOE genotype, and sex on the presence and severity/stage of each type of concomitant pathology are in **Supplemental Table 5.2**. Concomitant α-synuclein pathology was present in

21% of the overall sample, but the likelihood of its presence or severity/stage did not differ by age of onset, APOE genotype, or sex (**Figure 5.1A & 5.1B**).

Concomitant TDP-43 pathology was present in 41% of the overall sample. In keeping with other studies[30–33], TDP-43 pathology was more likely to be present in those with later onset (Odds Ratio [OR] = 2.00 [95% CI: 1.23 - 3.35], p=0.007), and this was driven by Neocortical TDP-43 (OR = 1.94 [1.1 - 3.57], p=0.03) (**Figure 5.1C**). There was also a greater likelihood of concomitant TDP-43 pathology (OR = 2.46 [1.10 - 5.78], p=0.03) in those with an APOE ε4 allele, driven by TDP-43 in the amygdala (OR = 4.41 [1.37 - 19.82], p=0.02) (**Figure 5.1D**). There was no effect of sex on the likelihood of concomitant TDP-43 pathology. In our cohort of pathologically severe AD patients, concomitant hippocampal sclerosis (diagnosed independently of TDP-43 pathology) was present in 7% of the overall sample, and its likelihood did not differ by age of onset, APOE genotype, or sex.

Concomitant vascular pathology such as significant infarcts, microinfarcts, and hemorrhages were rare in this sample and did not differ by age of onset, APOE genotype, or sex. In terms of microvascular disease, arteriolosclerosis was present in 36% of the overall sample and was more likely to be present in those with later onset (OR = 2.02 [1.24 – 3.40], p<0.001), driven by moderate severity arteriolosclerosis (OR = 2.50 [1.37 – 4.91], p = 0.004) (**Figure 5.1E & 5.1F**). Atherosclerosis of the circle of Willis was present in 73% of the overall sample and was also more likely to be present in those with later onset (OR = 3.02 [1.70 – 5.74], p=0.006), driven by moderate severity atherosclerosis (OR = 2.17 [1.3 - 3.8], p=0.005). Amyloid angiopathy was present in 90% of the overall sample, but its likelihood did not differ by age of onset. There was no effect of APOE genotype or sex on the likelihood of concomitant arteriolosclerosis, atherosclerosis, amyloid angiopathy, or any of the other vascular pathologies.

5.3.3 Distribution of NFT Neuropathology

We performed regional counts to quantify the NFT density in the middle frontal gyrus ("Mid Frontal tangle count") and hippocampus ("Hippocampal tangle count") and calculate the relative distribution of pathology in the two regions ("Mid. Frontal / Hippocampal tangle ratio"). Consistent with previous reports[8,34], we observed a wide range of NFT densities, including cases with markedly disproportionate amounts of NFT pathology in these two regions. Sample micrographs of cases with low Mid. Frontal / Hippocampal tangle ratio (**Figure 5.2A**) and high Mid. Frontal / Hippocampal tangle ratio (**Figure 5.2B**) are shown in **Figure 5.2**.

The relationships between regional NFT densities or relative distribution of NFT pathology with age of onset or APOE genotype was examined (**Figure 5.3** and separately by staining method in **Supplemental Figure 5.1**). Full linear or logistic models with effect of age of onset, APOE genotype, and sex are in **Supplemental Table 5.3**. Importantly, the Mid. Frontal / Hippocampal tangle ratio decreased with increasing age of onset, indicating a greater relative neocortical burden with earlier age of onset (β = -0.18 [-0.26 – -0.10] per 10 years, p<0.001) (**Figure 5.3A, left panel**). This effect appears driven by a significant decrease in the density of NFT pathology in the Mid. Frontal cortex with increasing age of onset (β = -0.51 [-0.72 – -0.31], p<0.001, **Figure 5.3A, middle panel**), with density of NFT pathology in the hippocampus not significantly associated with age of onset (**Figure 5.3A, right panel**). APOE4 genotype did not affect Mid. Frontal / Hippocampal tangle ratio (**Figure 5.3B, left panel**) or density of NFT in Mid. Frontal cortex (**Figure 5.3B, middle panel)**. However, density of NFT pathology in Hippocampus was greater in those with an APOE ε4 allele than in those without an ε4 allele (β = 0.49 [0.13 – 0.86], p=0.009) (**Figure 5.3B, right panel**). Finally, there was no association of sex with Mid Frontal/ Hippocampal tangle ratio or with density of NFT pathology in either Mid. Frontal cortex or Hippocampus. In post-hoc analyses that added a model term for NFT staining method (PHF-1 vs Thioflavin-S), the term was not significant and the pattern of results was unchanged.

Based on regional NFT counts and Mid. Frontal / Hippocampal tangle ratio, patients were classified into “Hippocampal Sparing”, “Limbic Predominant”, or “Typical” neuropathologic subtypes using an approximation of the Murray et al.[8] criteria (**Figure 5.3C**). The two cases highlighted in **Figure 5.3C** correspond to micrographs from **Figure 5.2**, with **Figure 5.2a** representative of the Hippocampal Sparing subtype and **Figure 5.2b** representative of the Limbic Predominant subtype. The “Limbic Predominant” subtype was associated with later age of onset (OR = 6.11 [2.61 – 18.14] per 10 years; $p < 0.001$) and the “Hippocampal Sparing” subtype was associated with earlier age of onset (OR = 0.46 [0.24 – 0.85], $p = 0.018$) (**Figure 5.3C**). Neither sex nor APOE genotype affected membership in these subtypes. Overall, these data corroborate Murray and colleagues’[8] report of substantially different average ages of onset of symptoms in hippocampal sparing versus limbic predominant AD.

5.3.4 Relationship between Concomitant Non-AD Neuropathology and the Distribution of NFT Neuropathology

Relationships between the regional density or relative distribution of NFT pathology and presence or absence of concomitant Lewy body, TDP-43, or vascular (arteriolosclerosis) pathology were examined (**Figure 5.4**)**.** After adjusting for the effect of age of onset, sex, and APOE genotype, neither Lewy body pathology (**Figure 5.4A**) nor vascular pathology (**Figure 5.4C**) was associated with density of NFT pathology in the hippocampus or Mid. Frontal cortex, or with the Mid. Frontal / Hippocampal tangle ratio. Concomitant TDP-43 pathology was associated with greater density of NFT pathology in the hippocampus ($\beta = 0.60$ [0.24 – 0.97], $p=0.001$), but not in the Mid. Frontal cortex (**Figure 5.4B**). Accordingly, the presence of TDP-43 pathology was associated with a lower Mid. Frontal / Hippocampal tangle ratio ($\beta = -0.17$ [-0.31 – -0.04], $p=0.01$) indicating a greater relative hippocampal NFT burden in those with concomitant TDP-43 pathology.

5.3.5 Effect of Age of Onset on Baseline Cognitive Performance and its Mediation by Neuropathology

Relationships between age of onset and baseline cognitive performance indexed by the Memory, Executive, Visuospatial and Language domain scores are shown in **Figure 5.5A**. Linear models that examined these relationships while controlling for sex and APOE genotype are in **Supplemental Table 5.3**. Baseline performance in the Executive ($\beta = 0.48$ [0.09 – 0.90], $p= 0.013$) and Visuospatial ($\beta = 0.97$ [0.46 – 1.46], $p<0.001$) domains was worse in those with earlier onset (**Figure 5.5A**), while age of onset was not related to performance in the Memory or Language domains.

Mediation analyses were carried out to mathematically quantify the extent to which the presence of concomitant non-AD pathology (Lewy pathology, TDP-43, or arteriolosclerosis), or the distribution of NFT pathology (i.e., the Mid. Frontal / Hippocampal tangle ratio), transmitted the effects of age of onset onto baseline cognitive performance in the Executive and Visuospatial domains. None of the concomitant Non-AD pathologies mediated the effect of age of onset on baseline Executive or Visuospatial performance (**Supplemental Table 5.4**). There was a significant mediation effect of the Mid. Frontal / Hippocampal tangle ratio between the effect of age of onset and baseline performance in the Executive domain ($p<0.001$) (**Figure 5.5B, Supplemental Table 5.4**). This mediation analysis indicates that, in our cohort, age of onset produced *indirect* effects on Executive cognitive abilities (i.e., worse Executive performance with earlier age of onset). This indirect effect occurred primarily via age of onset's direct effects on distribution of NFT pathology (i.e., higher Mid. Frontal / Hippocampal tangle ratio with earlier age of onset) which, in turn, affected Executive domain performance. In contrast, there was no mediation effect of the distribution of NFT pathology between the effect of age of onset and baseline performance in the Visuospatial domain ($p=0.62$), only a strong direct relationship between age of onset and Visuospatial cognitive abilities ($p=0.008$) (**Figure**

5.5C). In post-hoc analyses that added a model covariate for NFT staining method (PHF-1 vs Thioflavin-S), the term was not significant and the pattern of results was unchanged.

5.3.6 Effect of Age of Onset on Longitudinal Cognitive Decline and its Mediation by Neuropathology

Relationships between age of onset and rate (i.e., slope) of cognitive decline on specific neuropsychological tests were examined if longitudinal data over 2 to 3 annual evaluations were available for at least 50% of the participants on each measure. Linear models that examined these relationships while controlling for sex and APOE genotype are in **Supplemental Table 5.5**. There was faster decline with earlier age of onset on the MMSE, the DRS and each of its subscales (except memory), and the category fluency test (all p-values < .03) (**Figure 5.6**). Mediation analyses were used to determine if the presence of concomitant Non-AD pathology or the distribution of NFT pathology (i.e., the Mid. Frontal / Hippocampal tangle ratio) mediated the effects of age of onset on rate of cognitive decline. None of the concomitant Non-AD pathologies mediated the effect of age of onset on rate of decline on any of the cognitive measures. In contrast, the relationship between age of onset and rate of decline on the MMSE, category fluency, total DRS score, and DRS conceptualization, attention and initiation subscales (but not the DRS construction subscale) was mediated by the distribution of NFT pathology (**Supplemental Table 5.5**).

5.4 Discussion

We identified age-related heterogeneity in the neuropathological and clinical features in a cohort of 121 patients with sporadic, pathologically-confirmed severe AD. Individuals with earlier onset of AD were less likely than those with later onset to have concomitant non-AD neurodegenerative (i.e., TDP-43) or vascular (i.e., atherosclerosis of the circle of Willis, microvascular arteriolosclerosis) pathology, and more likely to have a distribution of NFT

pathology characterized by greater neocortical burden (i.e., a higher Mid. Frontal / Hippocampal tangle ratio). At their baseline clinical assessment, those with earlier age of onset were more likely than those with later onset to report non-memory cognitive impairment as their initial presenting symptom, exhibit greater functional impairment in activities of daily living, report more psychiatric symptoms, and have greater cognitive deficits and faster decline in executive functions and visuospatial abilities. Mediation analyses showed that the effect of age of onset on executive functions was indirect and mediated primarily through its effect on the distribution of NFT pathology (i.e., higher Mid. Frontal / Hippocampal tangle ratio with earlier age of onset) and not through its effect on presence or absence of concomitant non-AD pathology.

These results replicate and extend our recent findings from the large multi-center NACC database (ref) which showed a similar paradoxical pattern of more atypical clinical features in patients with early age of onset ($\leq$ 63 years of age) AD than in those with late age of onset (>63 years of age) despite less concomitant non-AD neurodegenerative or vascular pathology. We now extend those findings by showing that NFT density in midfrontal neocortex, and the ratio of midfrontal neocortical NFT to hippocampal NFT, are strongly inversely related to estimated age of onset. This finding is consistent with imaging studies which show greater Tau-PET tracer uptake in neocortical regions with earlier ages of symptom onset[35,36] or with atypical clinical presentations[37]. We also show that hippocampal tangle density is not related to age of onset, instead showing greater density in those with an APOE ε4 risk allele, in agreement with studies showing FDG hypometabolism[38,39] or increased Tau-PET tracer uptake[40] in limbic regions in those who are APOE ε4+.

Mediation analyses were performed to formally test if the effect of age of onset on the cognitive presentation of AD could be partially explained by age-related differences in the distribution of NFT pathology or concomitant non-AD pathologies. We found that Mid. Frontal / Hippocampal tangle ratio, but not concomitant Lewy body pathology, TDP-43 pathology, or

microvascular pathology, was a significant mediator of the observed effect of age of onset on cognitive performance in the Executive domain. This finding suggests that age of onset produced indirect effects on Executive cognitive abilities primarily through its direct effects on distribution of NFT pathology which, in turn, affected Executive domain performance. In contrast, there was no mediation effect of the distribution of NFT pathology between the effect of age of onset and baseline performance in the Visuospatial domain. This may be related to our choice of midfrontal neocortex in calculating the distribution of NFT pathology (i.e., the Mid. Frontal/Hippocampal tangle ratio) since deficits in Visuospatial abilities may better map onto pathology in the posterior temporo-occipital cortex which shows the greatest hypometabolism and atrophy in AD-type PCA on imaging[41–43].

The present results are consistent with previous findings of Murray and colleagues[8], who identified three pathologic subtypes of AD defined by the relative distribution of Hippocampal and Neocortical NFTs. These investigators showed that a Hippocampal Sparing subtype of AD was associated with earlier ages of onset and less co-pathology, and was more likely to have an atypical clinical presentation, more rapid decline, and receive a non-AD clinical diagnosis. The inverse clinical presentation (e.g., later age of onset, more concomitant non-AD pathology, typical cognitive presentation) was observed for patients classified as Limbic predominant in both studies. Our work here supports the notion that age of onset is a major determinant of NFT distribution, and in turn the pathologic subtype of AD[9], and extend prior efforts by demonstrating a relationship between pathology and specific domains of cognitive performance and decline.

While we did observe increasing presence and severity of concomitant TDP-43 and vascular pathologies with age of onset, they did not significantly mediate the age-related variability in cognitive performance or decline. This suggests that their additional effects are relatively minor and are likely swamped out by the presence of severe (Braak stage 5-6) AD pathology. Consistent with previous studies, the presence of concomitant Lewy pathology was

approximately 20-25% and did not vary by age of onset. Unexpectedly, hippocampal sclerosis did not show the well-established relationship with age, but this may be due to the difficulty of diagnosing neuronal loss and gliosis “out of proportion to AD pathology” in the context of severe AD pathology – which is likely accentuated in the later onset patients with higher hippocampal tangle burden. Also unexpectedly, we did not observe an effect of APOE genotype on the presence of amyloid angiopathy, which may be due to its high overall prevalence (90%) in our sample.

We examined possible synergistic effects between concomitant non-AD pathologies and NFT tangle distribution. We showed that those with TDP-43 (LATE) neuropathology had significantly greater Hippocampal NFT density, and in turn a lower Mid. Frontal / Hippocampal tangle ratio, even after co-varying for the effect of age of onset, sex, and APOE genotype. This suggests that the two pathologies co-occur at a level more likely than chance. This finding is consistent with reports of co-occurrence of these two pathological features in amygdala and hippocampus[44–46], as well as with hypothesized common upstream mechanisms[47]. We observed significantly greater hippocampal tangle burden and greater TDP-43 immunoreactivity in those with an APOE ε4 allele, which could be one such possible mechanism. It is possible, for example, that the APOE ε4 allele confers selective vulnerability of the hippocampus to both AD and TDP-43 pathology.

The decrease in the Mid. Frontal / Hippocampal NFT ratio with increasing age of onset that we observed suggests that either the neocortex is more vulnerable to NFT pathology in younger individuals who develop severe AD, or the hippocampus is more vulnerable to NFT pathology in older individuals who develop severe AD. The latter possibility is the more likely of the two given numerous studies that show an increase in the selective vulnerability of the hippocampus to ischemia, hypoglycemia, hyperexcitability, metabolic stresses, and neurodegenerative diseases with increasing age[48–51]. Furthermore, age-related microglial

senescence[52,53] and increased pro-inflammatory signaling in the hippocampus may be mechanisms that lead to impaired homeostasis[54] and the development of AD neuropathology[55]. Less hippocampal vulnerability in the younger onset patients we studied may seem counterintuitive since we did not observe a relationship between hippocampal NFT density and age of onset. However, this may reflect the fact that we selected our patients to have similar baseline scores on mental status test (e.g., MMSE) across ages of onset. These mental status tests predominantly assess memory and may largely reflect hippocampal dysfunction. Thus, if the hippocampus is less vulnerable in younger patients, they would have to be further along in the disease course to have the same level of hippocampal NFT burden, and same mental status test scores, as the older patients. This would make them more likely to have greater neocortical NFT burden which would increase the Mid. Frontal / Hippocampal ratio. Consistent with this possibility, patients with earlier onset had significantly longer intervals from estimated onset to baseline which suggests that it took a longer period of time for them to develop the same hippocampal NFT burden as those with later age of onset.

This study has a number of strengths. First, strict pathologic definitions of AD avoid the potential pitfalls of including non-AD patients in the analysis or, perhaps more critically, excluding individuals with atypical clinical presentations as non-AD. Second, while Tau PET imaging is becoming increasingly common and has the ability to assess the distribution of NFT pathology, our study included extensive assessment of microvascular, TDP-43, and Lewy pathology which cannot currently be identified by biomarkers or imaging. It is important to show that atypical features of early onset AD cannot be explained away by presence of other non-AD neuropathology. Third, the detailed cognitive phenotyping available enabled us to assess the impact of age of onset on profiles of cognition, how they change over time, and the specific contribution of regional NFT distribution and co-pathologies.

Several limitations should be considered. As a retrospective analysis, over 30 years of pathologic data was assembled to establish a substantial cohort of patients with early age of onset of sporadic, autopsy-confirmed severe AD. Such a long time-frame entailed changes in both clinical and neuropathologic practices and criteria, as well as in the clinicians and pathologists who applied them. To minimize any bias this may have caused, patients were matched on the year in which the neuropathologic analysis occurred. Another limitation is that the mediation analyses we performed violated the temporal order implied by the method – the cognitive outcomes were collected several years before the pathologic mediators. While the pathologic burden may grow from the time of the cognitive testing to death, the pattern and distribution of pathology at death is likely to be representative of the pattern and distribution of pathology at the time of clinical diagnosis of dementia. Future work on detailed evaluation of regional tau PET data across age of onset could allow these findings to be replicated more proximal to the clinical evaluation during life.

In summary, we have shown that there is substantial clinical and pathologic heterogeneity related to the age of onset in patients with severe AD at autopsy. Patients with earlier onset were more likely to have atypical (non-memory) clinical presentations, as well as more functional and psychiatric impairment, but paradoxically were less likely to have concomitant non-AD neuropathology (i.e. TDP-43 and arteriolosclerosis). However, these same patients with earlier onset had a relatively greater Neocortical to Hippocampal NFT burden, which was shown to significantly mediate their cognitive performance on measures of executive function and rates of longitudinal decline. This suggests that, at least in the context of severe AD, the distribution of AD neuropathology rather than concomitant pathology is the major determinant of age-related heterogeneity in clinical phenotype and the greater prevalence of atypical presentations in early onset sporadic AD. Further work is needed to see if these findings extend to patients with less severe AD pathology, or if age-related concomitant

neuropathologies have a greater influence on their clinical and cognitive profiles across age of onset.

5.5 Acknowledgements

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Table 5.1 Participant Demographics

Age of Onset	**< 60**	**61 - 70**	**> 70**	**Age of Onset p-value** [a]
n	40	41	40	
Age at onset	56.9 ± 2.9	66.0 ± 2.6	75.2 ± 3.9	
Age at baseline	61.8 ± 4.1	70.8 ± 3.8	78.1 ± 3.6	
Age at death	68.0 ± 5.1	77.5 ± 4.7	84.7 ± 4.6	
Onset-Baseline Int.	5.0 ± 2.6	4.8 ± 2.7	3.0 ± 2.3	**0.001**
Onset-Death Int.	11.1 ± 4.0	11.5 ± 3.8	9.5 ± 3.1	**.03**
Baseline-Death Int.	6.2 ± 3.4	6.7 ± 3.0	6.5 ± 2.9	.11
Education	14.2 ± 2.5	14.5 ± 2.7	14.8 ± 3.0	.82
Female	15 (38%)	14 (34%)	14 (35%)	.95
APOE Genotype:				
0 ε4 alleles	18 (45%)	13 (32%)	14 (35%)	.91
1 ε4 allele	20 (50%)	17 (41%)	22 (55%)	.76
2 ε4 alleles	2 (5%)	11 (27%)	4 (10%)	.55
First Recognized Cognitive Symptom:				
Memory	29 (72%)	37 (90%)	36 (90%)	**.02**
Language	5 (12%)	1 (2%)	2 (5%)	.18
Visuospatial	3 (8%)	1 (2%)	0 (0%)	.06
Other	3 (8%)	2 (5%)	2 (5%)	.49
NPI	3.33 ± 2.22	3.44 ± 2.13	2.10 ± 2.31	**.01**
Global Cognition:				
MMSE [b]	20.95 ± 5.07	22.88 ± 4.75	22.5 ± 4.84	.10
DRS Total	108.62 ± 15.71	115.05 ± 17.84	114.33 ± 17.46	.13
CDR-sob [b]	6.36 ± 2.63	5.73 ± 2.05	5.15 ± 2.76	.11
Functional Ability:				
Basic ADLs [b]	7.27 ± 1.77	6.72 ± 1.41	6.69 ± 1.2	**.04**
POD (iADLs)	10.28 ± 5.1	10 ± 3.82	7.92 ± 5.62	**.02**
Medications:				
AD medications	22 (55%)	15 (37%)	18 (45%)	.42
Antidepressants	14 (35%)	16 (39%)	8 (20%)	.56
Antipsychotics	2 (5%)	3 (7%)	2 (5%)	.76
Clinical Diagnosis at Baseline:				
MCI	1 (2%)	5 (12%)	4 (10%)	**.04**
Probable AD	29 (72%)	27 (66%)	32 (80%)	.92
Possible AD	4 (10%	8 (20%)	2 (5%)	.47
LBD	6 (15%)	0 (0%)	1 (2%)	**.02**
Other [c]	0 (0%)	1 (2%)	1 (2%)	.20

Table 5.1 Participant Demographics, Continued

Abbreviations: APOE = Apolipoprotein E, NPI = Neuropsychiatric Inventory, MMSE = Mini Mental State Exam, DRS = Dementia Rating Scale, CDR-sob = Clinical Dementia Rating – sum of boxes, ADL = Activities of Daily Living, POD = Pfeffer Outpatient Disability scale, iADLs = Instrumental Activities of Daily Living, MCI = Mild Cognitive Impairment, LBD = Lewy Body Disease

[a] p-values are reported for the effect of Age of Onset as a continuous variable as a predictor of each outcome in linear or logistic regression (as appropriate)

[b] Missing data: MMSE (n=1, <1%), CDR sum of boxes (n=32, 26%), basic ADLs (n=6, 5%)

[c] Other diagnoses included Primary Progressive Aphasia (n=1 with an age of onset of 68) and Pseudodementia/Depression (n=1 with an age of onset of 86)

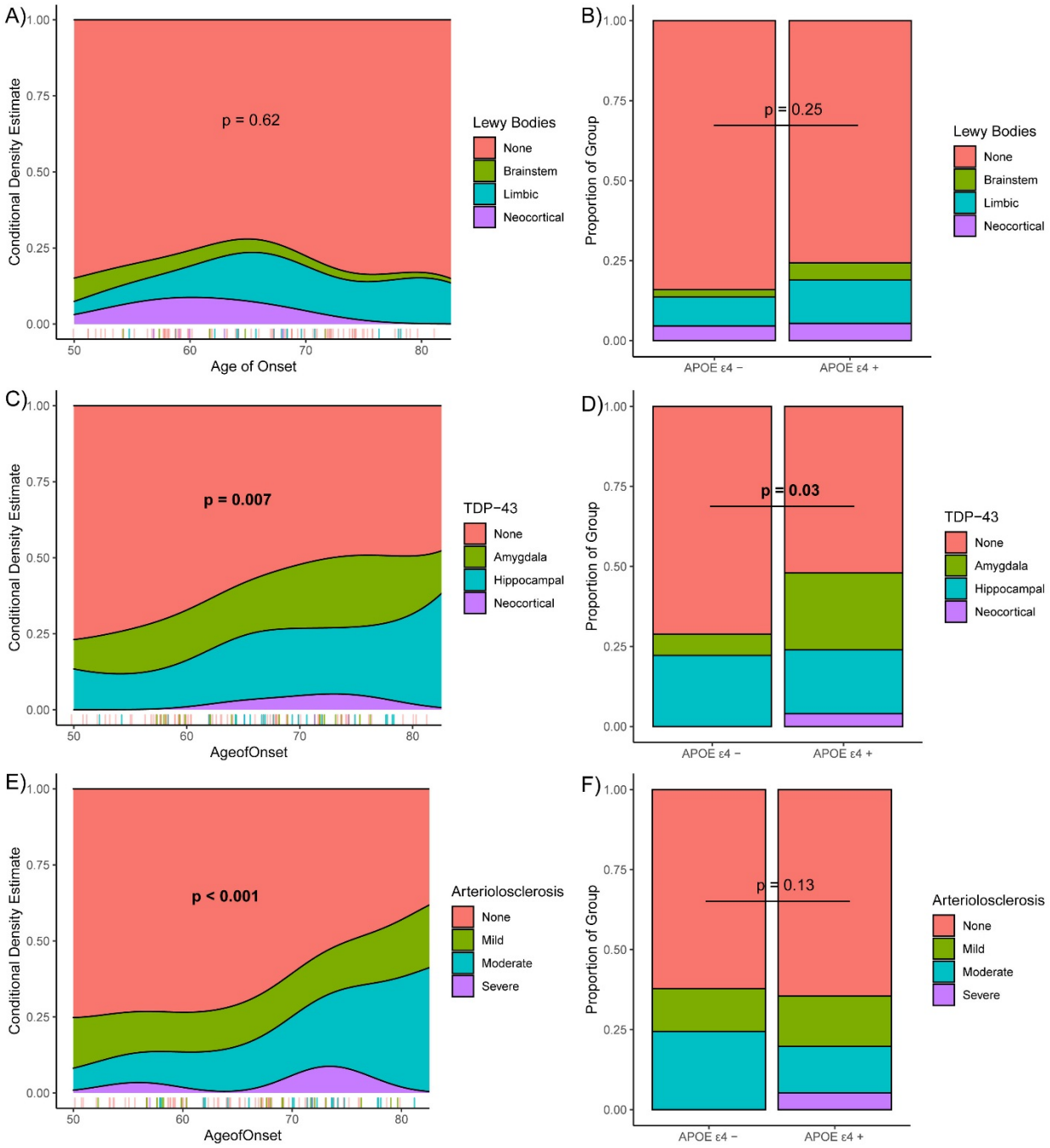


Figure 5.1 Concomitant Pathologies by Age of Onset and APOE Genotype

Age of Onset and APOE effects on prevalence of Lewy Bodies (A, B), TDP-43 (C, D), and Arteriolosclerosis (E, F). p-values on figure represent differences by Age of Onset or the presence of an APOE ε4 allele in the presence of any level of the pathology (vs none). Full model results can be found in **Supplemental Table 5.2**.

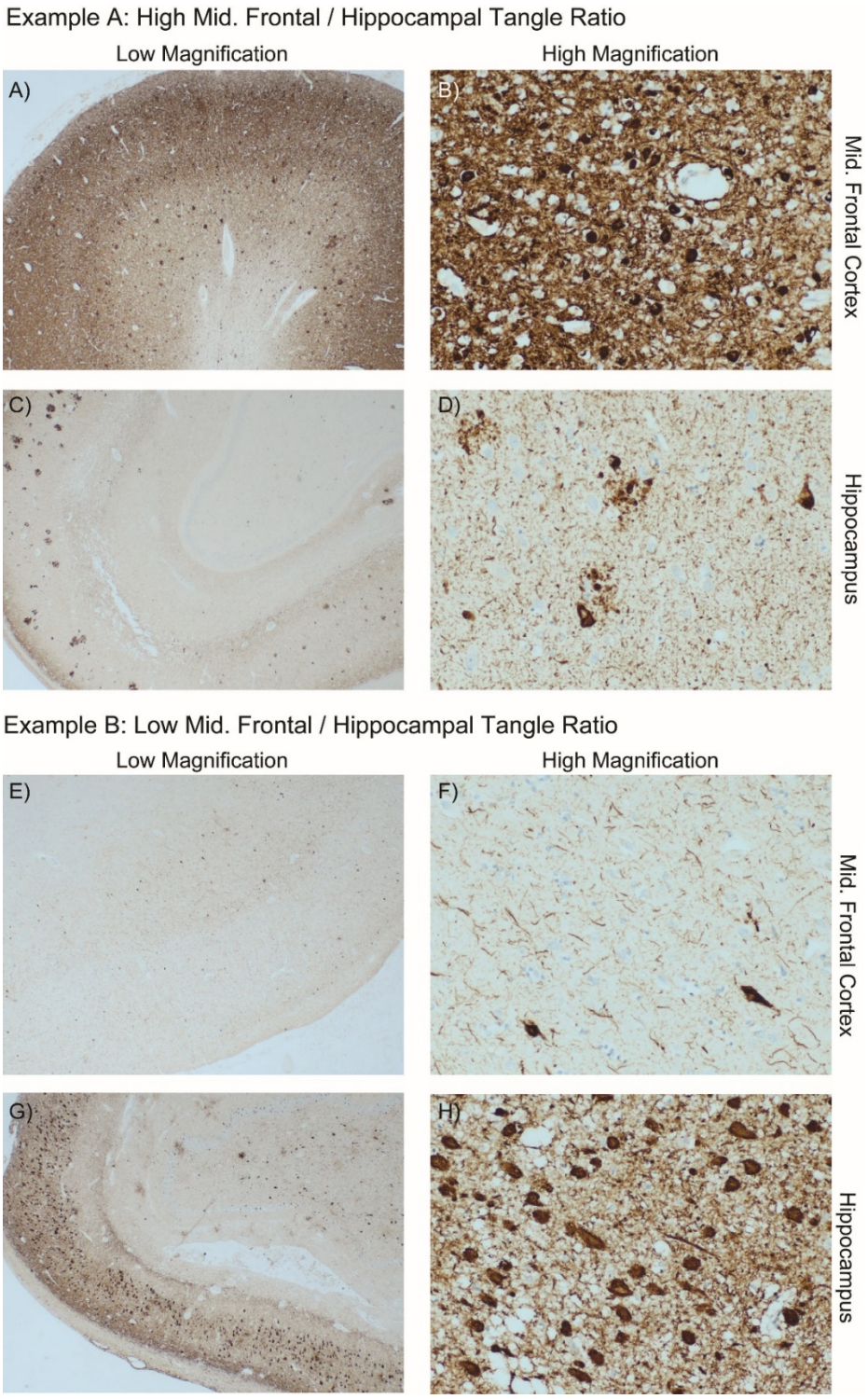
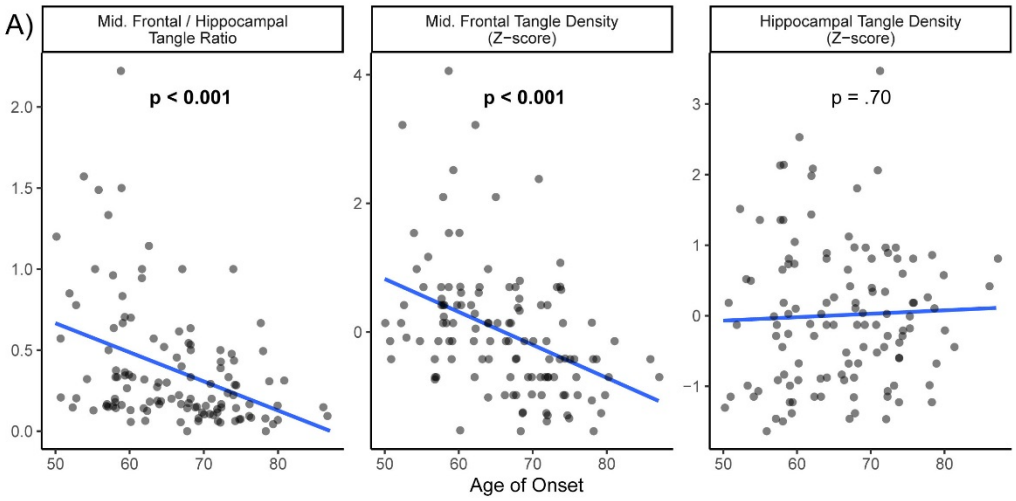


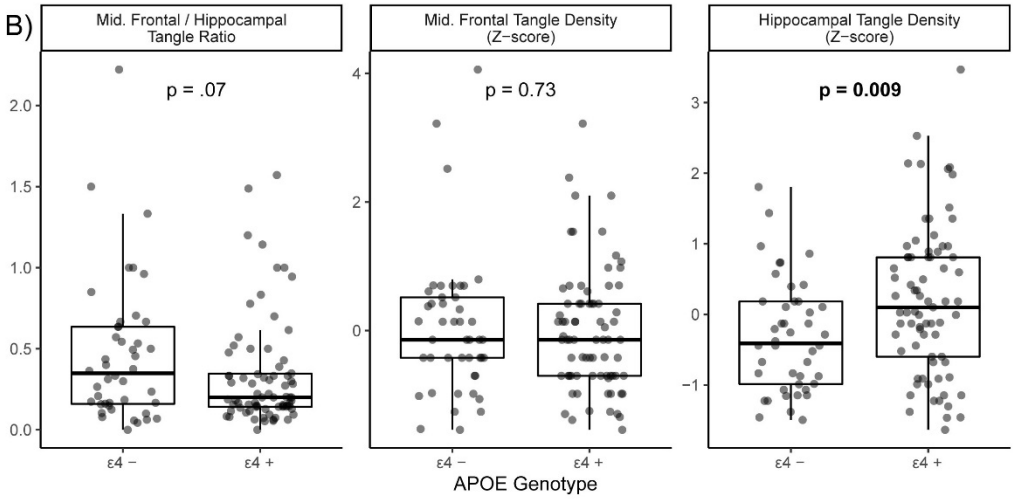
Figure 5.2 Sample Micrographs of NFT Pathology Distribution with PHF-1 Tau Immunostaining in Two Participants

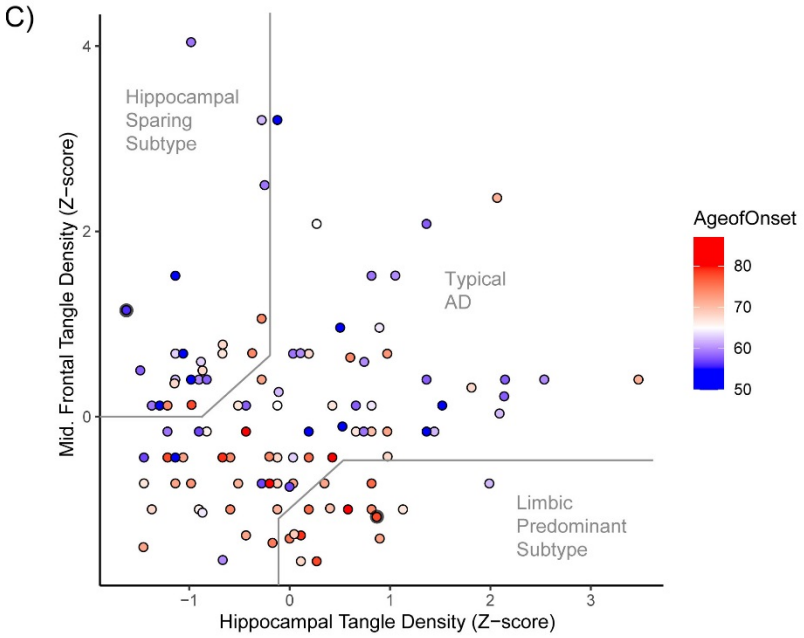
Example A (panels A-D) corresponds with a relatively high Mid. Frontal / Hippocampal tangle ratio, while Example B (panels E-H) corresponds with a relatively low Mid. Frontal / Hippocampal tangle ratio.

Figure 5.3 AD Neuropathology by Age of Onset and APOE Genotype

(A) Association of Hippocampal tangle density, Mid. Frontal density, or their ratio with Age of Onset. (B) Associations of the same variables with presence of an APOE e4 allele. (C) Scatterplot of Mid Frontal tangles against Hippocampal tangles. Colder (blue) colors correspond with earlier onset, while warmer (red) colors correspond with later ages of onset. Delineations of our approximations of the Murray et al. “Hippocampal Sparing”, “Limbic Predominant”, or “Typical” AD neuropathologic subtypes are shown on the plot. Two points outlined with thick black circles correspond to the Examples A and B from **Figure 5.2**. Full model results can be found in **Supplemental Table 5.3**.


A)
Mid. Frontal / Hippocampal Tangle Ratio
p < 0.001
Mid. Frontal Tangle Density (Z−score)
p < 0.001
Hippocampal Tangle Density (Z−score)
p = .70
Age of Onset


B)
Mid. Frontal / Hippocampal Tangle Ratio
p = .07
Mid. Frontal Tangle Density (Z−score)
p = 0.73
Hippocampal Tangle Density (Z−score)
p = 0.009
ε4 −
ε4 +
APOE Genotype


C)
Hippocampal Sparing Subtype
Typical AD
Limbic Predominant Subtype
Mid. Frontal Tangle Density (Z−score)
Hippocampal Tangle Density (Z−score)
AgeofOnset
80
70
60
50

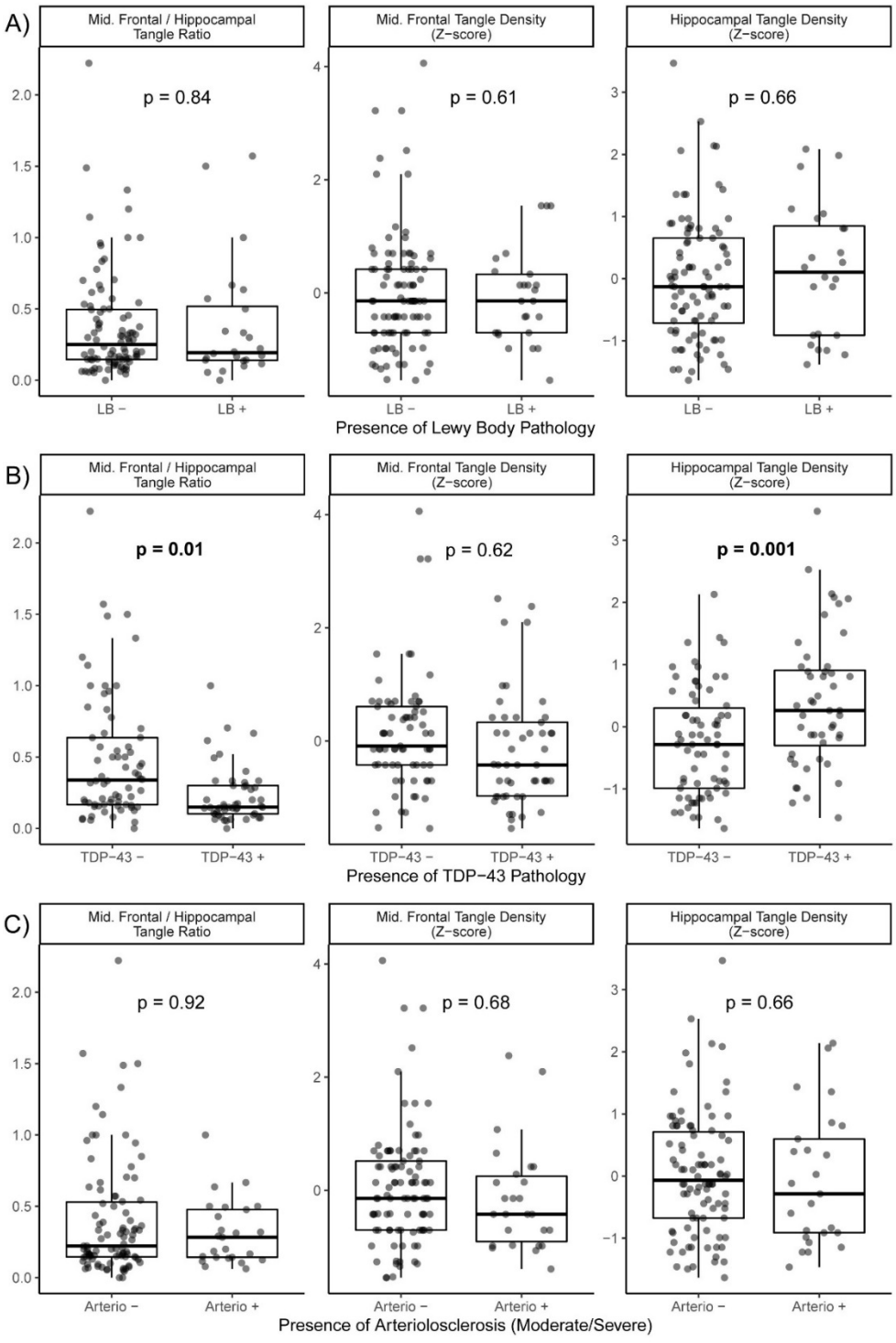


Figure 5.4 Interaction of concomitant pathologies with distribution of NFT pathology

The Mid. Frontal tangle density, Hippocampal tangle density, and Mid. Frontal / Hippocampal tangle ratio are shown by the presence of concomitant Lewy body pathology (A), TDP-43 pathology (B), or arteriolosclerosis (C). P-values on the figure correspond to the effect of each concomitant pathology (any level vs none), after adjusting for age of onset, sex, and APOE genotype.

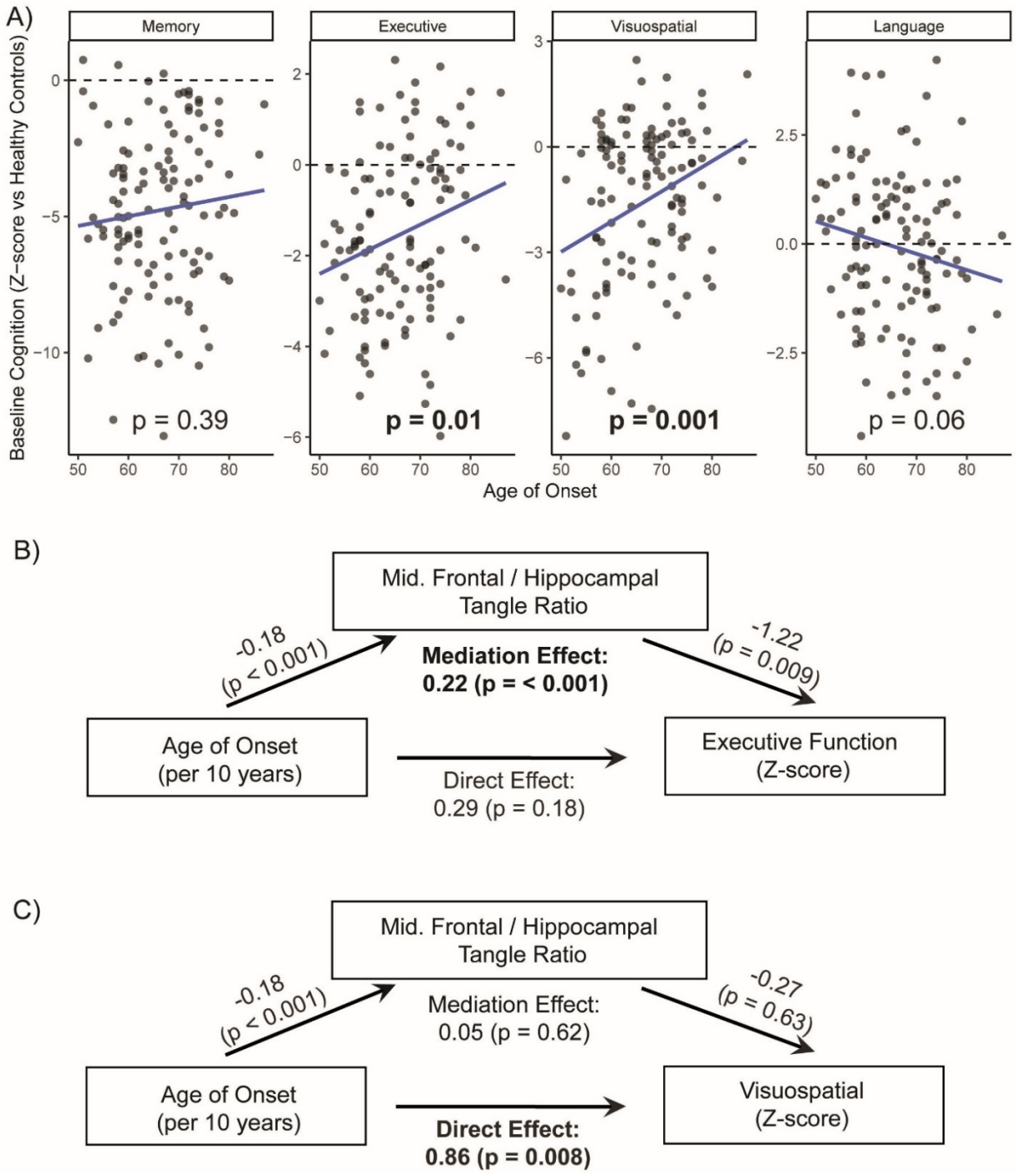
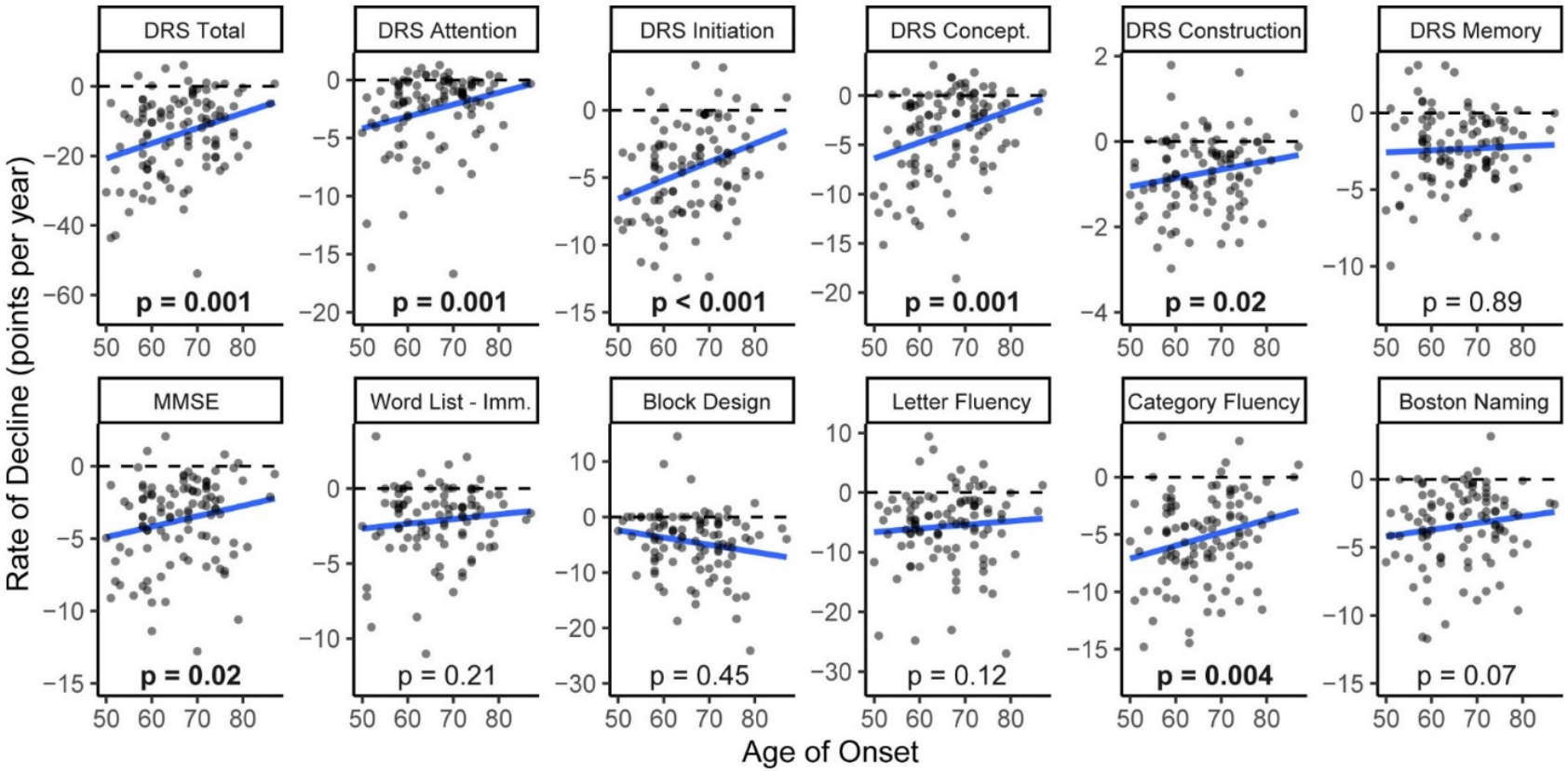


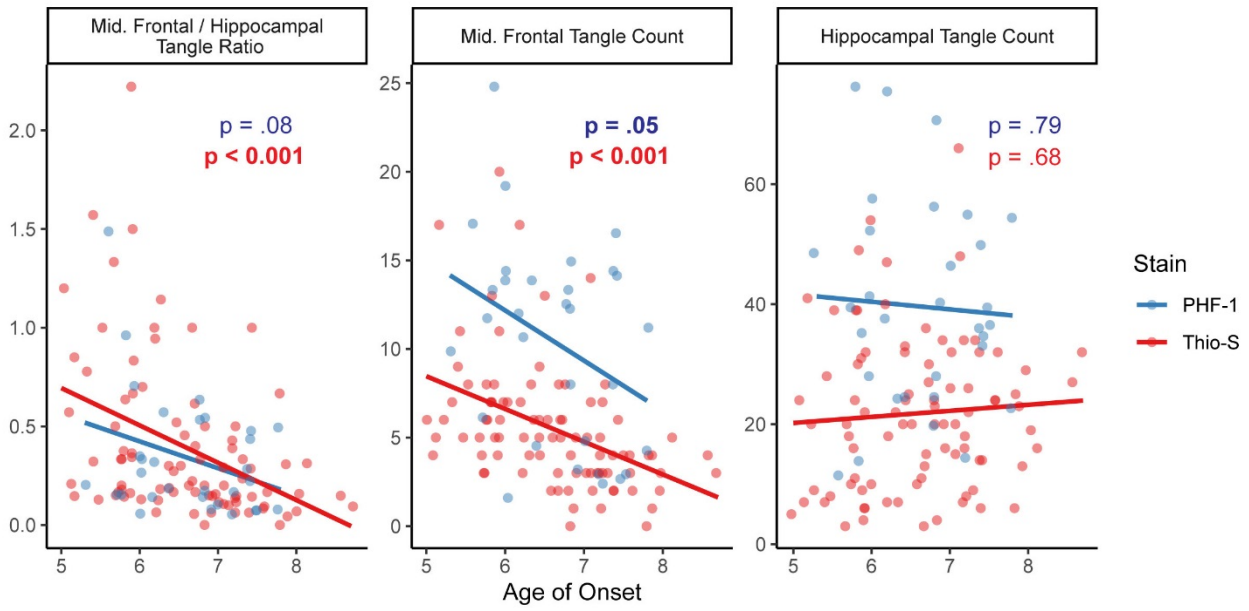
Figure 5.5 Age of Onset Effects on Cognition

Baseline cognitive performance in each cognitive domain in plotted against Age of Onset. P-values represent associations from models adjusted for sex, education, and APOE genotype. (B) Significant mediation effect of the Mid. Frontal / Hippocampal tangle ratio on Executive Function score. (C) Non-significant mediation effect of the Mid. Frontal / Hippocampal tangle ratio on Visuospatial scores. Full model and mediation results can be found in **Supplemental Table 5.4**.

Figure 5.6 Age of Onset Effects on Longitudinal Cognitive Decline

Annualized rates of decline calculated from 2-3 longitudinal evaluations on each measure are plotted against age of onset. P-values on the plot represent associations from models adjusted for sex, education, and APOE genotype. Full model and mediation results can be found in **Supplemental Table 5.5**.


DRS Total
DRS Attention
DRS Initiation
DRS Concept.
DRS Construction
DRS Memory
MMSE
Word List - Imm.
Block Design
Letter Fluency
Category Fluency
Boston Naming
p = 0.001
p = 0.001
p < 0.001
p = 0.001
p = 0.02
p = 0.89
p = 0.02
p = 0.21
p = 0.45
p = 0.12
p = 0.004
p = 0.07
Rate of Decline (points per year)
Age of Onset



Supplemental Figure 5.1 Tangle Counts by Staining Method

The Mid. Frontal / Hippocampal Tangle Ratio did not differ in its relationship with age of onset when using either PHF-1 immunostaining or Thioflavin-S, and was pooled directly. In contrast, while the Hippocampal and Mid. Frontal tangle counts individually also showed generally consistent relationships with age of onset, they differed in absolute value by staining method and they were Z-transformed before being pooled for primary analyses in **Figure 5.3** and **Supplemental Table 5.3**. The demographics of those analyzed by each method were well matched, and did not differ in age of onset, age at death, duration of illness, sex distribution, or APOE genotype.

Supplemental Table 5.1 Principle Components Analysis (PCA) of all Cognitive Measures.

Component loadings for the PCA with Varimax rotation of the available neuropsychological tests. Significant loadings (>0.4) are bolded.

	Visuospatial	Language	Executive	Memory
Component Eigenvalue	(10.0)	(2.3)	(1.4)	(1.3)
Visual Reproduction – Imm. Recall	**.62**	.24	.19	.28
Logical Memory – Imm. Recall	.05	**.55**	.23	**.50**
CERAD Word List – Imm. Recall	.24	**.63**	0.20	**.54**
CERAD Word List – Recognition	.10	.15	.12	**.89**
CVLT – Immediate Recall Trials 1-5	.12	**.57**	.18	**.62**
CVLT – Recognition	.21	.06	.14	**.83**
WCST – Categories	.12	.32	**.83**	.17
WCST – Total Correct	.10	.28	**.81**	.08
Digit Symbol Substitution	**.57**	.07	**.61**	.19
TMT – Trail A	**-.64**	.28	**-.47**	-.08
TMT – Trail B	**-.44**	-.18	**-.67**	-.21
Block Design	**.65**	.26	**.52**	.17
Visual Reproduction – Copy	**.85**	.11	.03	-.03
Cube	**.75**	.25	.08	.24
Clock – Copy	**.70**	.30	.09	.08
Clock – Command	**.54**	.36	.34	.13
Letter Fluency (F-A-S)	13	**.46**	**.49**	.29
Category Fluency (Animal-Fruit-Veg)	.26	**.63**	.39	.25
Boston Naming Test	.26	**.75**	.00	-.04
Vocabulary	.12	**.67**	.36	.20

Supplemental Table 5.2 Concomitant Pathology Outcomes Models

The effects of Age of Onset (per 10 years), Sex, and presence of an APOE ε4 allele on NFT density and classification by Murray et al.[8] subtypes, from linear or logistic regression models with those three terms.

	Age of Onset Odds Ratio (95% CI)	**Age of Onset p-value**	**Sex Odds Ratio (95% CI)**	**Sex p-value**	**APOE ε4 Odds Ratio (95% CI)**	**APOE ε4 p-value**
Lewy Body Pathology:						
Any Stage	0.87 (0.49 - 1.52)	0.619	0.64 (0.23 - 1.64)	0.366	1.78 (0.7 - 5.03)	0.245
Brainstem	0.49 (0.13 - 1.58)	0.251	0.4 (0.02 - 2.91)	0.429	2.85 (0.39 - 57.62)	0.362
Limbic	1.36 (0.67 - 2.8)	0.390	1.03 (0.29 - 3.24)	0.963	1.56 (0.48 - 6.02)	0.481
Neocortical	0.53 (0.16 - 1.54)	0.263	0.34 (0.02 - 2.24)	0.335	1.38 (0.25 - 10.51)	0.720
TDP-43 Pathology:						
Any Stage	**2.00 (1.23 - 3.35)**	**0.007**	0.84 (0.37 - 1.86)	0.668	**2.46 (1.1 - 5.78)**	**0.032**
Amygdala	1.28 (0.68 - 2.44)	0.439	1.35 (0.49 - 3.6)	0.555	**4.41 (1.37 - 19.82)**	**0.024**
Hippocampal	**1.94 (1.1 - 3.57)**	**0.025**	0.61 (0.21 - 1.6)	0.335	0.91 (0.36 - 2.36)	0.845
Neocortical	2.19 (0.45 - 13.37)	0.350	0.89 (0.04 - 10.29)	0.929	- [a]	- [a]
Hippocampal Sclerosis	1.48 (0.58 - 4.08)	0.422	1.44 (0.32 - 6.08)	0.617	- [a]	- [a]
Infarcts	1.01 (0.38 - 2.63)	0.987	1.77 (0.39 - 7.98)	0.441	4.3 (0.73 - 82.04)	0.180
Microinfarcts	2.1 (0.76 - 6.52)	0.167	1.40 (0.26 - 6.92)	0.674	4.08 (0.64 - 80.21)	0.207
Arteriolosclerosis						
Any Stage	**2.02 (1.24 - 3.4)**	**0.006**	1.49 (0.67 - 3.33)	0.327	0.87 (0.39 - 1.95)	0.734
Mild	1.08 (0.57 - 2.03)	0.808	2.01 (0.72 - 5.62)	0.177	1.16 (0.41 - 3.6)	0.782
Moderate	**2.50 (1.37 - 4.91)**	**0.005**	1.06 (0.37 - 2.89)	0.916	0.51 (0.19 - 1.37)	0.179
Severe	1.58 (0.42 - 6.87)	0.510	0.57 (0.03 - 4.81)	0.632	- *	- *
Atherosclerosis						
Any Stage	**3.02 (1.7 - 5.74)**	**<0.001**	0.87 (0.35 - 2.2)	0.768	1.98 (0.82 - 4.85)	0.130
Mild	1.03 (0.64 - 1.67)	0.894	0.54 (0.23 - 1.24)	0.160	0.9 (0.41 - 2.03)	0.799
Moderate	**2.17 (1.3 - 3.8)**	**0.004**	1.77 (0.76 - 4.1)	0.183	1.27 (0.54 - 3.08)	0.593
Severe	1.43 (0.69 - 3.07)	0.339	0.96 (0.27 - 3.06)	0.944	4.1 (1.04 - 27.34)	0.075

Supplemental Table 5.2 Concomitant Pathology Outcomes Models, Continued

Amyloid Angiopathy						
Any Stage	0.53 (0.24 - 1.12)	0.105	0.34 (0.09 - 1.17)	0.090	1.25 (0.33 - 4.36)	0.732
Mild	0.79 (0.46 - 1.31)	0.364	0.69 (0.27 - 1.63)	0.405	0.79 (0.34 - 1.86)	0.583
Moderate	0.68 (0.42 - 1.07)	0.101	1.1 (0.51 - 2.38)	0.799	0.89 (0.42 - 1.92)	0.770
Severe	1.58 (0.93 - 2.76)	0.097	0.74 (0.29 - 1.79)	0.510	1.83 (0.74 - 4.86)	0.204

[a] only individuals with APOE ε4 alleles had neocortical TDP43, Hippocampal Sclerosis, or Severe Arteriolosclerosis

Supplemental Table 5.3 Tau Pathology Outcomes Models

The effects of Age of Onset (per 10 years), Sex, and presence of an APOE ε4 allele on NFT density and classification by Murray et al.[8] subtypes, from linear or logistic regression models with those three terms.

	Age of Onset β (95% CI)	**Age of Onset p-value**	**Sex β (95% CI)**	**Sex p-value**	**APOE ε4 β (95% CI)**	**APOE ε4 p-value**
Regional NFT Density:						
Hippocampal Tangle Density (Z-score)	0.04 (-0.18 - 0.26)	0.704	0.18 (-0.19 - 0.55)	0.345	**0.49 (0.13 - 0.86)**	**0.009**
Mid. Frontal Tangle Density (Z-score)	**-0.51 (-0.72 - -0.31)**	**<0.001**	0.13 (-0.22 - 0.48)	0.460	-0.06 (-0.4 - 0.28)	0.728
Mid. Frontal / Hippocampal Tangle Ratio	**-0.18 (-0.26 - -0.10)**	**<0.001**	0.00 (-0.13 - 0.14)	0.951	-0.12 (-0.26 - 0.01)	0.069
	Age of Onset Odds Ratio (95% CI)	**Age of Onset p-value**	**Sex Odds Ratio (95% CI)**	**Sex p-value**	**APOE ε4 Odds Ratio (95% CI)**	**APOE ε4 p-value**
Murray *et al.* Subtypes:						
Hippocampal Sparing	**0.46 (0.24 - 0.85)**	**0.018**	1.13 (0.4 - 3.04)	0.818	0.52 (0.2 - 1.39)	0.189
Typical	0.74 (0.46 - 1.19)	0.215	0.74 (0.33 - 1.64)	0.451	1.23 (0.55 - 2.7)	0.611
Limbic Predominant	**6.11 (2.61 - 18.14)**	**<0.001**	1.7 (0.54 - 5.41)	0.360	2.43 (0.7 - 10.21)	0.186

Supplemental Table 5.4 Cognitive Models

The effect of Age of Onset on baseline cognitive performance in each domain is first examined in models adjusting for sex, education, and APOE genotype. Those with significant effects were tested for mediation by each of the pathologic variables separately

	Total Effect:		Mediation Effects:							
	Age of Onset Effect (95% CI)	**Age of Onset p-value**	**NFT Ratio Effect (95% CI)**	**Tangle Ratio p-value**	**Lewy Body Effect (95% CI)**	**Lewy Body p-value**	**TDP-43 Effect (95% CI)**	**TDP-43 p-value**	**Vascular Effect (95% CI)**	**Vascular p-value**
Language	-0.35 (-0.74 – -0.02)	0.059								
Visuospatial	**0.97 (0.46 – 1.46)**	**0.001**	0.05 (-0.16 – 0.27)	0.63	0.02 (-0.09 – 0.07)	0.94	0.10 (-0.04 – 0.29)	0.17	0.04 (-0.16 – 0.20)	0.66
Executive	**0.48 (0.09 – 0.90)**	**0.013**	**0.21 (0.08 – 0.38)**	**0.003**	0.01 (-0.06 – 0.07)	0.97	0.04 (-0.06 – 0.19)	0.48	-0.07 (-0.23 – 0.05)	0.37
Memory	0.38 (-0.30 – 0.90)	0.294								

Supplemental Table 5.5 Longitudinal Cognitive Decline and Mediation

The effect of Age of Onset on 2-3 year slopes of decline is first examined in models adjusting for sex, education, and APOE genotype. Those with significant effects were tested for mediation by each of the pathologic variables separately.

	Total Effect:		Mediation Effects:							
	Age of Onset Effect (95% CI)	**Age of Onset p-value**	**NFT Ratio Effect (95% CI)**	**Tangle Ratio p-value**	**Lewy Body Effect (95% CI)**	**Lewy Body p-value**	**TDP-43 Effect (95% CI)**	**TDP-43 p-value**	**Vascular Effect (95% CI)**	**Vascular p-value**
Δ DRS Total Score	**0.36 (0.15 - 0.57)**	**0.001**	**0.13 (0.05 - 0.23)**	**<0.001**	0.00 (-0.02 - 0.02)	0.96	0.04 (-0.01 - 0.12)	0.12	0.03 (-0.03 - 0.12)	0.30
Δ DRS Attention	**0.37 (0.15 - 0.60)**	**0.001**	**0.14 (0.02 - 0.28)**	**0.01**	0.01 (-0.02 - 0.05)	0.97	0.01 (-0.04 - 0.09)	0.69	0.02 (-0.04 - 0.10)	0.38
Δ DRS Initialization	**0.43 (0.21 - 0.65)**	**<0.001**	**0.11 (0.02 - 0.21)**	**0.03**	0.00 (-0.02 - 0.03)	0.95	0.04 (-0.06 - 0.11)	0.33	0.00 (-0.08 - 0.08)	0.98
Δ DRS Conceptualization	**0.27 (0.04 - 0.50)**	**0.001**	**0.14 (0.05 - 0.25)**	**0.002**	0.00 (-0.02 - 0.03)	0.96	0.03 (-0.02 - 0.11)	0.30	0.04 (-0.02 - 0.13)	0.26
Δ DRS Construction	**0.37 (0.16 - 0.58)**	**0.02**	0.03 (-0.10 - 0.17)	0.66	0.00 (-0.02 - 0.02)	0.94	0.07 (-0.02 - 0.16)	0.14	0.06 (-0.04 - 0.17)	0.35
Δ DRS Memory	0.02 (-0.20 - 0.23)	0.89								
Δ MMSE	**0.28 (0.04 - 0.51)**	**0.02**	**0.15 (0.06 - 0.26)**	**<0.001**	0.00 (-0.02 - 0.03)	0.86	-0.02 (-0.02 - 0.06)	0.22	0.04 (-0.01 - 0.12)	0.14
Δ CERAD Word List – Immediate Recall	0.17 (-0.10 - 0.43)	0.21								
Δ Block Design	-0.1 (-0.37 - 0.17)	0.45								
Δ F-A-S Letter Fluency	0.19 (-0.05 - 0.43)	0.12								
Δ Animal-Fruit-Veg Category Fluency	**0.34 (0.11 - 0.56)**	**0.004**	**0.12 (0.02 - 0.27)**	**0.02**	0.00 (-0.01 - 0.03)	0.97	-0.02 (-0.06 - 0.02)	0.71	0.03 (-0.04 - 0.11)	0.42
Δ Boston Naming Test	0.22 (-0.02 - 0.45)	0.07								

Chapter 6

General Conclusion

The clinicopathologic studies presented here provide essential clinical and cognitive characterization and comparisons of the dementia syndromes associated with neuropathologically-verified AD, Hippocampal Sclerosis, Lewy body disease and their interactions. These studies have also shown how differences in both the type and distribution of neuropathology can be associated with overlapping and distinct impairments in cognition and clinical features. Gathering this information is a necessary step in refining the tools of clinical diagnosis and in developing more accurate clinical criteria.

Findings from Chapter 2 indicate that Hippocampal Sclerosis results in global dementia with gradual decline in all higher order cognitive domains (i.e., memory, language executive functions, visuospatial abilities). The cognitive deficits and clinical features of HS are virtually indistinguishable from those of AD. However, the rate of decline in HS is slower than in AD. The global nature of cognitive impairment in HS suggests that the effects of HS pathology are not localized to the hippocampus, as the name might imply, but likely involve diffuse neocortical regions either directly or through disruption of networks that support the affected cognitive functions. One explanation may be the strong association between HS and TDP-43 proteinopathy that often extends beyond the hippocampus into association cortices[1,2]. TDP-43 is a relatively new pathologic entity and is currently staged using LATE criteria[3] independently of HS pathology. Studies suggest that TDP-43 and HS pathology may have dissociable effects on cognition[4]. Future work is necessary to identify the underlying cause of hippocampal sclerosis in the absence of TDP-43, and to identify additional clinical features that could be used to identify it and differentiate it from AD during life, both in the presence or absence of TDP-43.

Findings from Chapter 3 reveal both cross-sectional and longitudinal double-dissociations in patterns of cognitive deficits and declines between DLB and PDD that most likely reflect subtle differences in pathology. The generally more severe brainstem pathology in PDD than DLB may account for the disproportionate impairments in Executive ability in PDD,

whereas greater cortical Lewy body pathology and concomitant AD pathology in DLB may shift the cognitive impairment profile to be more similar to AD with greater Memory and Language impairments than in PDD. These differences are important to consider when designing clinical trials targeting Lewy body pathology. We show a substantial increase in statistical power to detect a change in rate of decline due to a treatment when DLB and PDD are considered separately and when the appropriate cognitive domain for each is targeted by the clinical trial outcome measure. Given the high rate of co-occurrence of AD pathology in DLB (and to a lesser extent in PDD), future work should expand on previous efforts[5,6] to identify the separate contributions of α-synuclein and NFT pathologies to clinical profiles of DLB and PDD, and compare the synuclein-driven clinical and cognitive features of the two disorders.

Finally, Chapters 3 and 4 demonstrate considerable variability in clinical and cognitive presentation across age of onset within those with severe AD pathology, and show that this variability is (at least partly) mediated by the distribution of NFT pathology. These findings help explain the paradox that those patients with younger onset of symptoms tend to have higher likelihood of atypical clinical presentations of AD, despite the fact that they tend to have less concomitant non-AD neuropathology. Instead, they have disproportionately greater neocortical NFT pathology relative to the degree of hippocampal NFT pathology they bear. Greater influence of NFT neocortical pathology in younger age of onset AD could lead to a higher rate of misattribution of the etiology of impairment since it may affect the same neocortical circuits impaired in FLTD, leading to non-memory presentations, rapid decline, and greater functional and psychiatric impairments that are commonly associated with FLTD[7,8]. These findings emphasize the importance of fully exploring the clinical, cognitive and pathological variability within AD, including age-related variability, particularly since the extraordinarily high prevalence of AD means that even atypical presentations may occur with high absolute frequency.

Overall, the results of these studies highlight the challenges that exist in making a clinical diagnosis of a specific etiology of dementia. At the older end of the age spectrum, Hippocampal Sclerosis presents as an AD mimic, so clinical and cognitive features do not provide much information to help clinically differentiate it from AD. At the younger end of the spectrum, early onset AD may cause a strong dysexecutive cognitive profile with psychiatric involvement that might normally point to FLTD[7]. In both of these cases, a biomarker for AD obtained in mild stages of dementia would help rule in or rule out AD, which might greatly improve the ability to identify unique early clinical and cognitive features that might help diagnose the etiology of dementia during life. Future work should focus on identifying the most unique features of each disease when AD is excluded from the picture.

Given the high prevalence of multiple co-occurring pathologies (which increases with advancing age), it is also essential to consider the impact of non-AD pathologies on the clinical and cognitive presentation of dementia when they occur along with severe AD. This is especially important in the context on many ongoing AD clinical trials where a response to an effective anti-AD therapy may be attenuated in the context of concomitant non-AD pathology that is unaffected by the drug. The present series of studies suggest there is minimal impact of concomitant pathologies on detectable differences in the profiles of impairment in the presence of severe AD. In Chapter 2, the only difference between AD and AD plus HS groups across all neuropsychological measures was slightly more impaired performance on the Boston Naming Test by the AD plus HS group. In Chapter 3, DLB patients with more concomitant AD pathology had more AD-like clinical profiles than the PDD patients. In Chapter 4, we paradoxically observed the highest rates of atypical clinical features of AD in those with earlier ages of onset, which was the group with the lowest rates of concomitant non-AD pathologies. We confirmed this finding in Chapter 5 by showing that age-related variability in the cognitive profiles of AD patients was mediated by the distribution of AD pathology, and unaffected by the presence of

concomitant alpha-synuclein, TDP-43, or vascular pathology. Taken together, these results suggest that while many of these non-AD pathologies can cause impairment in cognition on their own, their effects are often overshadowed by the effects of AD pathology when it is concurrently present. This will continue to pose challenges for accurate diagnosis until more specific diagnostic characteristics or biomarkers for non-AD pathologies are developed.

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