UNIVERSITY OF CALIFORNIA SAN DIEGO

Utility of Biomarkers and Genetic Risk Assessments for Predicting Clinical and Neuropathological Outcomes in Older Adults

A dissertation submitted in partial satisfaction of the requirements for the degree

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in

Neurosciences

by

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The dissertation of Barbara Elizabeth Spencer is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

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Chair

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DEDICATION

For Mom, Dad, and Katie

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Chapter 1, in full, is a reprint of the material as it appears in: Spencer BE, Jennings RG, Brewer JB, Alzheimer's Disease Neuroimaging Initiative (2019) Combined biomarker prognosis of mild cognitive impairment: An 11-year follow up study in the Alzheimer's Disease Neuroimaging Initiative. *J Alzheimers Dis* **68**, 1549–1559. The dissertation author was the primary investigator and author of this paper.

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ABSTRACT OF THE DISSERTATION

Utility of Biomarkers and Genetic Risk Assessments for

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by

Barbara Elizabeth Spencer

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Historically, a clinical diagnosis of Alzheimer's disease could only be confirmed upon the discovery of β-amyloid plaques and tau tangles post-mortem. Today, biomarkers (imaging and biofluid measurements of β-amyloid, tau, and neurodegeneration) are being used to define the disease in vivo. Chapter 1 of this dissertation examines the long-term performance of baseline cognitive, neuroimaging, and cerebrospinal fluid biomarker-assisted prognoses in patients with mild cognitive impairment. Concordant atrophy, memory impairment, and abnormal β-amyloid and tau was associated with the highest risk for conversion to dementia, while individuals with concordant negative risk remained stable for up to 11 years. These results

suggest that baseline biomarker-assisted predictions of decline to dementia are stable over the long term, and that combinations of complementary biomarkers can improve the accuracy of these predictions. Though individuals with mild cognitive impairment are still in the prodromal stages of clinical Alzheimer's disease, converging evidence suggests the pathologic changes underlying Alzheimer's disease begin up to 15 years before cognitive impairment. Chapter 2 of this dissertation tests whether the combination of the polygenic and age-specific risk for Alzheimer's disease can predict elevated β-amyloid in clinically unimpaired individuals in order to enroll these individuals into preclinical Alzheimer's disease trials. Alzheimer's disease incidence rates and a polygenic hazard score were used to create a gene- and age-defined ADAge. The ADAge-enrichment screening method identified clinically unimpaired individuals with elevated β-amyloid and lowered screening costs in real-world preclinical Alzheimer's disease trial data. These results demonstrate the utility of ADAge enrichment as a more efficient and cost-effective means to enroll clinically normal individuals with elevated β-amyloid in clinical trials. Clinically, distinctions between Alzheimer's disease and related dementias are suboptimal and complicated by shared genetic risk factors and frequent co-pathology. Chapter 3 of this dissertation analyzes the utility of polygenic risk assessments for Alzheimer's disease, dementia with Lewy bodies, and Parkinson's disease to differentiate between individuals with distinct underlying pathologies. Polygenic scores were specifically associated with either dementia with Lewy bodies or Alzheimer's disease pathology, indicating that an assessment of genetic risk may be useful to clinically distinguish between Alzheimer's disease and dementia with Lewy bodies.

INTRODUCTION

Alois Alzheimer first described the presence of abnormal clumps of protein outside of neurons (β-amyloid [Aβ] plaques) and twisted strands of protein inside of neurons (neurofibrillary tangles of hyperphosphorylated tau) that became the hallmark pathological changes of Alzheimer's disease (AD) [1]. Historically, a clinical diagnosis of AD could only be confirmed upon the discovery of Aβ plaques and tau tangles post-mortem. In addition to quantifying the extent of neurodegeneration, which, while not specific to AD, is closely related to cognitive decline [2,3], recent advances have led to the development of tools that allow researchers to assess the amount of these abnormal proteins in living people. In light of these advances, a recent set of guidelines [4] suggests that researchers should use biomarkers (imaging and biofluid measurements of Aβ, tau, and neurodegeneration) to define the disease in vivo. This dissertation will explore whether 1) baseline biomarker-assisted predictions of progression from mild cognitive impairment to clinical AD are stable over the long term, 2) genetic- and age-associated risk for AD can help identify asymptomatic individuals with abnormal AD biomarkers, and 3) genetic risk assessments can improve pathological specificity in AD and related dementias.

BIOMARKER-ASSISTED PREDICTION OF PROGRESSION TO CLINICAL AD

As biomarkers for Aβ, tau, and neurodegeneration are incorporated into research and clinical frameworks, they may be used to predict decline in older individuals. The presence of an abnormal biomarker for either Aβ or tau is associated with clinical decline, and this association becomes stronger when both are abnormal [5–7]. Evaluating neurodegeneration in addition to

biomarkers for Aβ and tau improves the prediction of near-term progression to dementia [2,8,9]. However, extended follow-up studies are needed to determine the stability of these biomarker-based predictions of progression. The question of stability is vital to future clinical applications, such as how frequently biomarkers would need to be reassessed to maintain predictive power. Given the field's rapid evolution and only recent development of such biomarkers, long-term follow-up studies are rare.

Chapter 1 of this dissertation examines the long-term performance of baseline cognitive, neuroimaging, and cerebrospinal fluid biomarker-assisted prognoses in patients with mild cognitive impairment. The influence of each risk factor or combination of factors on progression to dementia was evaluated in biomarker-defined groups of participants characterized at baseline and followed for up to 11 years. Importantly, attention was given to the stability of a negative biomarker result.

GENE- AND AGE-INFORMED SCREENING OF BIOMARKER STATUS IN PRECLINICAL AD

Despite the fact that individuals with mild cognitive impairment are still in the prodromal stages of clinical AD, converging evidence suggests the pathologic changes underlying AD begin up to 15 years before cognitive impairment [10]. The concept of preclinical AD was long ago supported by the observation that some non-demented individuals had pathology at autopsy that was indistinguishable from that seen early in the course of AD [11], and is now reflected in the most recently proposed neuropathological and biomarker-based criteria for AD, which do not require clinical symptoms [4,12].

In clinically unimpaired individuals, this preclinical stage is defined by elevated Aβ, which is considered to be the earliest detectable indication of AD neuropathologic change [13]. Leveraging the ability to assess pathology in vivo with biomarkers, some recent clinical trials have begun enrolling clinically unimpaired individuals with elevated Aβ to test whether decreasing Aβ would slow AD-related decline. This approach postulates that clinical trials may have more success with early intervention, rather than attempt to reverse the significant accumulated damage present in individuals with mild cognitive impairment. However, an estimated 40–65% of healthy adults over 80 have elevated Aβ [14,15], and most individuals with preclinical AD will never develop dementia in their lifetime [16]. Then, it is difficult to both define and detect clinically meaningful change in a cohort of individuals who may never experience cognitive changes associated with these brain abnormalities.

Still, ongoing preclinical AD trials are hoping to detect change in cognitive decline as a consequence of decreasing Aβ. Identifying such clinically unimpaired individuals with elevated Aβ, at least until a plasma biomarker is widely available, requires screening with Aβ positron emission tomography (PET) scans or lumbar puncture. This screening process is both inefficient, enrolling only ~30% of those PET scanned, and expensive. Novel enrichment methods are needed to improve efficiency of enrollment of these individuals by identifying those clinically unimpaired individuals who are at greatest risk for AD. Though not diagnostic itself, an assessment of genetic risk for AD may inform the likelihood of underlying AD pathology.

The largest single genetic risk factor for late onset AD is the apolipoprotein E (*APOE*) ε4 allele, though genome-wide association studies have identified additional, more common variants that confer risk for AD [17]. Polygenic scores capture an individual's risk for AD as an aggregate of their risk across many variants. Recently, a polygenic hazard score based on 31

variants and *APOE* was developed and validated that better predicts the age of AD onset than does *APOE* alone [18]. Notably, the hazard score employs a survival analysis method that accounts for the age of onset of AD. This is distinct from the typical case-control framework in which a clinically unimpaired individual is treated as a control despite the fact that they may develop AD in the future. For a given individual, aging is the single most important risk factor for AD, regardless of genetic background. Therefore, instantaneous risk for developing AD is better understood as a function of both genetic and baseline age-specific risk for AD.

Chapter 2 of this dissertation tests whether the combination of the polygenic and age-specific risk for AD can predict elevated Aβ in clinically unimpaired individuals. AD incidence rates and the polygenic hazard score were used to create a gene- and age-defined ADAge. The efficiency of ADAge-enriched Aβ screening for preclinical AD trials was examined and the impact of ADAge enrichment on screening costs was evaluated in real-world clinical trial screening data.

GENETIC RISK ASSESSMENT FOR IMPROVED DIAGNOSTIC SPECIFICITY IN AD AND RELATED DEMENTIAS

AD is the most common type of dementia, but Lewy bodies (aggregated α-synuclein) have been found in up to 25% of older individuals in community-based studies [19–22]. Along with vascular damage and tau, the presence of neocortical Lewy bodies was one of the three independent pathological correlates of dementia in a population-based sample [19]. Lewy body disease is an umbrella term that includes Parkinson's disease (PD), dementia with Lewy bodies (DLB), and PD with dementia, which are all characterized by the presence of Lewy bodies. DLB became the term [23] for what early case reports described as a new, diffuse neorcortical type of

Lewy body disease [24–28]. Early work noted the frequent presence of Aβ plaques in such cases in addition to the Lewy bodies [29–31], and often designated the clinical and pathological entity now known as DLB as a variant on either the AD [31] or PD [30] spectrum.

Clinically, the distinction between AD and DLB is suboptimal despite great effort to correlate variations in clinical presentation with pathological diagnoses [31–36]. Core features of DLB include fluctuations in cognition, visual hallucinations, REM sleep behavior disorder, and parkinsonism [32]. Attempts to tease apart the presentations of AD and DLB have found executive and visuospatial impairments and hallucinations most useful [33,37–40]. However, cases of mixed pathologies are common [41,42], with clinically well-characterized AD cases often presenting with unsuspected Lewy pathology [31,43], and the majority of pathologically defined DLB cases presenting with AD co-pathology [21]. Clinical distinctions are complicated by such mixed underlying pathologies [44], which can influence clinical presentation [32]. AD co-pathology in DLB is associated with a more AD-like clinical presentation and an accelerated, more severe disease course [32,36,45–47].

An assessment of genetic risk may improve clinical-neuropathological correlations, but the large-scale genetic studies that identify such risk typically rely on clinical diagnoses, which may limit their specificity. In addition to the clinical and pathological overlap, DLB also shares genetic risk factors with both AD and PD. As in AD, the *APOE* ε4 allele increases risk for dementia across the Lewy body disease spectrum [48–53]. The discovery of a mutation in the α-synuclein gene in familial PD [54] and identification of α-synuclein as the main protein component of Lewy bodies [55] revealed its importance across the Lewy body disease spectrum, though there are apparent differences in its associations to DLB and PD [56,57]. Additional common genetic risk factors between DLB and PD have since been reported [57,58]. Chapter 3

of this dissertation analyzes the utility of polygenic risk assessments for AD, DLB, and PD to differentiate between individuals with distinct underlying pathologies.

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CHAPTER 1. COMBINED BIOMARKER PROGNOSIS OF MILD COGNITIVE IMPAIRMENT: AN 11-YEAR FOLLOW-UP STUDY IN THE ALZHEIMER'S DISEASE NEUROIMAGING INITIATIVE

INTRODUCTION

Biomarkers inform predictive prognosis in mild cognitive impairment (MCI) and are under consideration for wider use in clinical [1] and research [2, 3] settings. Incorporation of available biomarkers into research and clinical frameworks requires long-term follow-up studies, which are rare, given the field's rapid evolution and only recent development of such biomarkers.

Combining across biomarkers may allow biological staging in Alzheimer's disease (AD). Longitudinal studies of unimpaired individuals demonstrated that amyloid positivity may appear years before clinically relevant decline [4] and does not predict decline as well alone as it does in combination with abnormal tau [5, 6], suggesting a benefit to evaluating multiple biomarkers. In MCI, the combination of medial temporal lobe atrophy, memory impairment, and abnormal amyloid and tau predicts near-term conversion to dementia [7]. Within amyloid positive individuals, hippocampal atrophy, alone [8] and in the presence of abnormal tau [9], predicts progression to dementia.

It remains unknown how stable these biomarker-based predictions of progression from MCI to dementia are over the long term. The question of stability is vital to future clinical applications, such as how frequently biomarkers would need to be reassessed to maintain predictive power. The current study follows to dementia conversion or dropout an established cohort of MCI patients from the Alzheimer's Disease Neuroimaging Initiative (ADNI) [10] that

was characterized at baseline using cognitive, volumetric magnetic resonance imaging (vMRI), and cerebrospinal fluid (CSF) biomarker cutoffs. In this long-term follow-up investigation, we examined the performance of each baseline assessment of risk for decline, with attention to time-to-progression to dementia and stability of a negative biomarker result.

METHODS

ADNI

All data used in preparation of this article were obtained from the ADNI database (http://adni.loni.usc.edu). In 2003, the ADNI began a large-scale, multi-site observational study of cognitively normal older adults and participants with MCI and AD that included the collection of clinical, neuroimaging, and other biomarker data [10]. Participants in ADNI are longitudinally followed and clinically evaluated at regular intervals, providing a well-characterized MCI cohort. Now that few ADNI phase 1 MCI subjects remain, this unique cohort can be evaluated across their entirety of observations to examine the stability of these biomarker-assisted predictions.

Subjects were limited to the original Heister et al. cohort, for which criteria have been described [7]. Briefly, these subjects were diagnosed as MCI at baseline, based on an MMSE score between 24 and 30, a CDR rating of 0.5, both a subjective memory complaint and an objective memory impairment, intact activities of daily living, and absence of dementia. Only the subset of subjects who completed a baseline vMRI, lumbar puncture, and Rey Auditory Verbal Learning Test (AVLT) were included. Clinical follow-up assessments occurred at 6-month intervals up to 2 years after baseline, and at 1-year intervals from year 2 onward, for a maximum of 15 visits over 11.5 years. Diagnosis of dementia at follow-up was determined by the study clinician.

Participants

For the present analysis, subjects from the original cohort of 192 were excluded if they did not complete at least one follow-up visit, leaving 185 individuals. For subjects who had a dementia diagnosis at their final follow-up visit, the date of conversion was calculated as the point halfway between the last visit at which the subject maintained an MCI diagnosis and the first visit at which the subject was diagnosed with dementia.

Standard protocol approvals, registrations, and patient consents

The research protocol was approved by each local institutional review board and written informed consent was obtained from each participant or participant's guardian.

MRI acquisition and analysis

For details on the original ADNI MRI acquisition protocol and processing pipeline, see Jack et al. [11]. For each subject, the initial baseline MP-RAGE sequence was used for analysis. The baseline MRI visit occurred between August 26, 2005 and September 24, 2007. Raw DICOM files for each subject were downloaded from ADNI. NeuroQuant® software (version 1.4) was used for automated segmentation [12]. This process corrects for spatial distortion and intensity variation due to gradient nonlinearities and B1 field inhomogeneity and derives volumes of subcortical structures based on a probabilistic atlas. This processing pipeline received FDA 510K clearance for clinical use in measuring volumes of brain structures in MRI images [13] and thus matches that available in clinical practice.

Hippocampal occupancy (HOC), an estimate of medial temporal lobe atrophy, was calculated as the ratio of hippocampal volume to the sum of hippocampal and surrounding

inferior lateral ventricle volume. Lower scores indicate an expansion of the inferior lateral ventricle as a function of tissue loss. Right and left occupancy scores were averaged for each individual. HOC was compared to hippocampal volume corrected for intracranial volume (HC % ICV), a commonly used measure of neurodegeneration. All imaging measures were normalized for age and sex.

CSF acquisition and analysis

Collection and processing of baseline CSF samples followed the standardized ADNI protocol, which has been described [14]. Amyloid-$\beta_{1–42}$ (Aβ), total tau (t-tau), and phosphorylated tau (p-tau) were measured using a multiplex immunoassay.

Rey auditory-verbal learning test

Administration of the AVLT has been described [15]. Initially, the subject immediately recalls as many words as she can remember from a 15-word list over a series of 5 trials in which the same list is presented. Here, the sum of the scores from these 5 immediate learning trials of the AVLT was used to assess the degree of memory impairment.

Risk stratification

Subjects were stratified into risk and non-risk groups using the same cutoff thresholds established in Heister et al. [7]. Briefly, vMRI thresholds were generated by identifying optimal separation of healthy and AD cohorts using an independent sample of subjects from ADNI [7]. Published threshold values for CSF measures [14] and for the sum of scores from the immediate learning trials of the AVLT [16] were used, each determined using receiver operating

characteristic analyses to maximize the diagnostic accuracy between mild AD cases (mean MMSE 23.5 and 23.4, respectively) and normal controls.

Statistical analyses

Differences in subject demographics and clinical characteristics by conversion status were tested with Pearson's chi-squared test with Yates' continuity correction or Welch's two sample t-test. Kaplan-Meier survival curves were used to determine dementia-free survival time. Cox proportional hazard analyses, controlling for age, were used to examine the influence of each risk factor or combination of factors on dementia-free survival. Hazard ratios (HR) were always calculated in comparison to the negative risk group. In order to further investigate the effect of neurodegeneration on survival, some analyses were repeated in a subset of subjects classified as prodromal AD [2], defined as positive for CSF Aβ and p-tau. For subjects who did not complete an 11-year visit and did not convert to dementia over the course of follow-up, time to censoring was examined by risk group with Welch's two sample t-test, corrected for multiple comparisons, to test for informative dropout. All analyses were done using R (version 3.3.3, https://www.r-project.org/). Significance was set to $p<0.05$.

Data availability

A request for access to data can be submitted and approved by the ADNI Data and Publications Committee (ida.loni.usc.edu/collaboration/access/appLicense.jsp).

RESULTS

Subject demographics and clinical characteristics, split by conversion status, are

summarized in Table 1.1. In this study 185 MCI subjects were followed longitudinally for a mean (SD) 4.3 (2.8) years. 59% of participants converted to dementia within the follow-up period, with a median dementia-free survival time of 2.8 years (95% CI, 2.5–3.7). Subjects who did not convert to dementia were lost to follow-up at an average of 3.8 years. Sex was not a significant predictor of conversion when included as a covariate in the analysis for any single biomarker or biomarker combination. While the Cox proportional hazard analyses controlled for age, it was only a significant predictor of conversion in the HOC positive, CSF negative combination (HR 1.1, 95% CI, 1.0–1.2) and the CSF negative, AVLT positive combination (HR 1.1, 95% CI, 1.0–1.2). There was no significant difference in apolipoprotein E *(APOE)* ε4 allele carriers by conversion status. However, there was a significant relationship between *APOE* ε4 allele dose and age at conversion (Figure 1.1). Of the subjects who did not complete their 11-year visit but retained an MCI diagnosis at their last completed clinical evaluation, the time to censoring was associated with risk factor group (FDR-corrected $p < 0.05$), with subjects in the positive risk group for HOC, HC % ICV, t-tau, p- tau/Aβ ratio, p-tau, t-tau/Aβ ratio, or Aβ remaining in the study an average 1.6 years shorter than their negative risk counterparts.

Each individual risk factor predicted conversion to dementia, with HRs ranging from 1.9 to 3.7 (Table 1.2). Figure 1.2 shows Kaplan-Meier survival curves for subjects stratified into positive and negative risk groups based on individual vMRI and CSF measures and AVLT scores. HOC (HR 3.7) and the t-tau/Aβ ratio (HR 3.6) outperformed HC % ICV (HR 2.4) and any other CSF measure (HR 1.9–3.5), and were therefore the vMRI and CSF measure, respectively, used in joint risk analyses.

The joint presence of any two risk factors increased risk for conversion (HR 7.1–11.0), with the presence of both medial temporal lobe atrophy and memory impairment on the AVLT

showing the greatest risk for decline. Figure 1.3 shows Kaplan-Meier survival curves for subjects stratified into positive and negative risk groups based on combinations of joint HOC, t-tau/Aβ ratio, and AVLT risk.

Concordant HOC, AVLT, and t-tau/Aβ ratio risk was associated with the highest risk for conversion to dementia (HR 15.1) (Figure 1.3). Subjects with concordant positive risk on all three factors (n = 54) converted to dementia at a median 1.3 (95% CI, 0.9–1.8) years (Figure 1.4). The 13% of concordant positive subjects who did not convert were lost to follow-up at a mean (SD) 1.7 (1.3) years, and their subsequent cognitive status is therefore unknown.

As was previously reported [7], the presence of medial temporal lobe atrophy was associated with the shortest median dementia-free survival time, both alone (1.3 [95% CI, 1.3–1.8] years) and in combination with AVLT impairment (1.3 [95% CI, 1.2–1.8] years), abnormal CSF (1.3 [95% CI, 1.2–1.8] years), or both (1.3 [95% CI, 0.9–1.8] years). When limited to those with prodromal AD, atrophy remained predictive of conversion (HR 2.5 [95% CI, 1.6–3.8]), shifting median dementia-free survival time over 2 years (1.3 [95% CI, 1.2–1.8] years HOC+, 3.5 [95% CI, 2.5–7.3] years HOC–).

Conversely, the presence of two negative biomarkers at baseline was associated with long-term stability. Individuals testing negative for AVLT and either CSF t-tau/Aβ ratio or medial temporal lobe atrophy risk retained their MCI diagnosis for a median 8.5 (95% LCI 7.5) or 9.8 (95% LCI 7.3) years, respectively. Due to the high survival of subjects with joint negative HOC and t-tau/Aβ ratio risk and concordant negative HOC, AVLT, and t-tau/Aβ subjects, the median time to conversion is undefined greater than the last study timepoint (11.5 years). 78% of subjects with concordant negative HOC, AVLT, and t-tau/Aβ ratio risk (n = 18) remained stable over the follow-up period. The mean (SD) time to conversion in the four concordant negative

subjects who received diagnoses of dementia was 6.2 (2.2) years. Two of these subjects remained amyloid negative on florbetapir (AV-45) scans acquired an average 4.5 years after baseline, suggesting a dementing amyloid negative illness.

DISCUSSION

In this longitudinal investigation, we demonstrate the stability of baseline cognitive, biofluid, and neuroimaging biomarker-assisted predictive prognosis in MCI. While each individual risk factor predicted conversion to dementia, individuals with three risk factors were at higher risk of conversion than individuals with only one or two risk factors. Subjects with concordant positive risk on all three factors (n = 54) converted to dementia at a median 1.3 (95% CI, 0.9–1.8) years, while subjects with concordant negative risk had such high survival that they retained their MCI diagnoses for a median time that is undefined greater than the last study time-point (11.5 years). These findings align with the mounting body of evidence suggesting the predictive prognostic benefit of assessing multiple complementary biomarkers, which may provide nonoverlapping information about disease progression.

Subjects with medial temporal lobe atrophy showed the greatest hazard of converting to dementia and, as previously demonstrated, those with medial temporal lobe atrophy remained dementia free for the shortest amount of time [7]. Neurodegeneration—as measured by atrophy in MRI—better correlates with clinical impairment and progression from MCI to dementia than does amyloid plaque burden [7, 17]. Neither neurodegeneration nor progression to dementia is AD specific. Nevertheless, atrophy on MRI provides a useful indication that the underlying disease process is neurodegenerative and is the most effective predictor of near-term clinical progression. Even within subjects with prodromal AD, time to conversion shifted over 2 years

with the amount of baseline atrophy.

Within the amyloid positive MCI subjects in this cohort (n = 139), 31% did not convert to dementia over the follow-up period, a mean (SD) 3.2 (2.0) years after baseline. Converging evidence suggests the pathologic changes underlying AD occur continuously over a long period. Reports indicate 10–65% [14, 18–20] of healthy older adults are amyloid positive, a figure that increases with age from 10–18% of those in their 60s to 40–65% of those in their 80s. If the goal is to identify individuals at greatest risk for cognitive decline, amyloid status alone fails to provide the clinician guidance regarding near-term outcomes, and, in fact, in this slowly developing disease, may lead to misattribution of the current complaint to AD with possible distraction from concurrent treatable etiologies.

International Working Group 2 (IWG-2) research diagnostic criteria consider the combination of elevated tau and low Aβ in CSF to be *in vivo* evidence of AD pathology [21], noting the improved predictive and discriminative accuracy of such a combination over CSF Aβ alone. In normal controls followed longitudinally, only those with both low amyloid and elevated tau had significantly greater cognitive decline than those without either [5]. This aligns with converging evidence that amyloid is necessary but not sufficient for AD dementia, and that both Aβ-associated neurodegeneration [22] and clinical decline [23] occur only in the presence of tau. Here, we find the ratio of t-tau to Aβ predicts risk for decline better than any other CSF measure.

In patients with progressive or persistent unexplained MCI, amyloid positron emission tomography (PET) positivity is said to increase the certainty that this impairment represents early AD [24]. However, the patient's MCI may not be solely caused by or even related to this amyloid positivity, and, hence, even in the setting of a positive amyloid test and cognitive impairment, it remains imperative to screen for presence of disease mimics and retain vigilance

for concurrent depression, sleep apnea, polypharmacy (e.g., opiate or anticholinergic medications), and other frequent causes of cognitive impairment in older adults. Conversely, amyloid negative status was the strongest single predictor of stability in this cohort, with the caveat that, in the broader context of clinical practice, a significant proportion of individuals destined for dementia are amyloid negative [25–27].

Despite interest in identifying underlying pathology, the question of highest clinical relevance in current practice is whether or not a patient will progress and decline. In the current study, eight of the twelve amyloid negative subjects with medial temporal lobe atrophy at baseline converted to dementia at a mean (SD) 1.6 (1.3) years. To ensure the amyloid negative status of these subjects was not merely a consequence of our CSF cutoff selection, we used Cohen's kappa to quantify the agreement between positive and negative classifications for CSF and PET measures, taking agreement by chance into account. For subjects who eventually underwent an AV-45 scan (n = 52, mean (SD) 4.6 (0.7) years after baseline), there was excellent agreement between PET categorization (SUVRs calculated using a whole cerebellum reference region, cutoff 1.11) [28] and baseline Aβ CSF risk group (Cohen's kappa = 0.92). Consistent with the results from Heister et al. [7], subjects with this biomarker profile, which has come to be labeled suspected non-Alzheimer's pathophysiology (SNAP) [29], showed risk for decline that warrants close monitoring. In clinical trials, focus may be placed on the earliest changes, such as amyloid deposition, rather than those most proximal to cognitive decline. While the approach may change when disease modifying therapies are available, identification of subgroups likely to progress in the near term provides valuable information in clinical decision-making. Biomarker-informed prognostic information, distinct from amyloid status, may be particularly valuable to individuals in prodromal stages of dementing amyloid negative illnesses, who might otherwise

have little information about their likelihood of progression, and it may also provide clinicians with improved risk-benefit analysis for patients when treatments become available. In moving toward incorporating precision medicine techniques, additional information such as genetic background might be incorporated to better understand how outcomes differ in these biomarker-defined groups.

Structural imaging is already used in clinical assessment to rule out potentially treatable etiologies and, with small modifications to protocols, images can be collected that allow for fully-automated segmentation [12, 17]. Implementing vMRI in clinical practice allows for the assessment of AD-predominant structural changes, such as medial temporal lobe atrophy, and should improve predictive prognosis for patients with MCI. Algorithms can be trained to identify AD patterns of brain atrophy in non-demented subjects that correlate with poorer cognitive performance and aid in predictive prognosis [30–32]. Such patterns, perhaps difficult for most community radiologists to consistently identify, could be built into algorithms to allow standard imaging devices to assist in their detection. Their refinement can leverage the wealth of clinical informatics now available, allowing the algorithm to gain enduring experience that extends well beyond any individual clinician.

One important limitation to this study is that the highly selected amnestic MCI cohort in ADNI does not represent the variety of underlying pathologies seen in clinical practice. Yet, biomarker-assisted predictive prognosis might be expected to yield better performance in cohorts matching clinical practice, where greater heterogeneity of MCI is seen. Further, 97% of the converters in this cohort received a clinical diagnosis of probable AD, but without neuropathological confirmation it is unknown what mixture of underlying pathologies is truly present in these subjects as heterogeneity is common, even within amnestic MCI cohorts [25]. In

our sample, time to censoring is biased by a few, disproportionately biomarker negative subjects who continue ADNI visits for a decade or longer without converting to dementia. Nevertheless, time to censoring is not significantly different between risk groups before year 10. As subjects in biomarker positive risk groups are more likely to convert to dementia and were observed for a shorter period of time, our results represent a conservative estimate in these latest timepoints. There is also a need for diversity in cohorts to generalize these findings beyond the well-educated subjects of European ancestry who make up both this cohort and the ones upon which the cutoffs used here were initially established. Further, while there is a need to establish cut-points in certain cases, such as to determine eligibility in clinical trials, it is also clear that AD is a continuum and potentially relevant information is lost when biomarkers are reduced to a binary classification of *positive* or *negative*.

The current study demonstrates that predictive prognosis in MCI is more accurate when supplemented by an assessment of baseline cognitive, biofluid, and neuroimaging biomarkers and supports incorporating multiple complementary biomarkers in future clinical and research frameworks. Our data demonstrate the long-term stability of such baseline biomarker-assisted predictive prognosis in MCI.

Table 1.1 Subject demographics and clinical characteristics split by conversion status. Subject demographics and clinical characteristics split by conversion status. Reported as mean (SD) unless otherwise noted. P-value based on Pearson's chi-squared test with Yates' continuity correction or Welch's two sample t-test.

	Stable (n=75)	**Converted (n=110)**	**p-value**
Women, No. (%)	24(32)	39(35)	0.74
Age, y	74.6(7.1)	74.6(7.5)	0.99
Education, y	15.5(3.0)	15.8(3.0)	0.58
APOE ε4 allele carrier, No. (%)	36(48)	66(60)	0.14
MMSE	27.2(1.7)	26.7(1.8)	0.07
CDR-SB	1.3(0.8)	1.7(0.9)	5.26×10^{-4}
ADAS-Cog 11	10.3(4.4)	12.6(4.4)	6.18×10^{-4}
AVLT	33.5(9.5)	28.2(7.1)	7.17×10^{-5}
HOC	0.70(0.10)	0.65(0.11)	6.94×10^{-4}
HC % ICV	0.49(0.07)	0.46(0.06)	5.12×10^{-3}
CSF Aβ, pg/mL	186.0(61.1)	147.1(43.2)	5.20×10^{-6}
CSF p-tau, pg/mL	29.5(15.8)	39.4(17.0)	7.51×10^{-5}
CSF t-tau, pg/mL	91.6(56.9)	109.1(51.8)	0.04

Table 1.2 Results of Cox proportional hazards regressions controlling for age. Each hazard ratio is reported relative to the negative risk group. Asterisks indicate p-values that survive Bonferroni correction.

Individual Risk Factors	**No. positive (%)**	**HR (95% CI)**	**p-value**
HOC	83 (45)	3.7 (2.5–5.5)	1.82×10^{-10} *
t-tau/Aβ ratio	130 (70)	3.6 (2.2–6.1)	9.83×10^{-7} *
Aβ	139 (75)	3.5 (2.0–6.1)	1.63×10^{-5} *
p-tau/Aβ ratio	145 (78)	3.3 (1.9–5.9)	4.03×10^{-5} *
AVLT	129 (70)	3.1 (1.9–5.1)	3.17×10^{-6} *
p-tau	132 (71)	2.9 (1.7–4.7)	3.08×10^{-5} *
HC % ICV	109 (59)	2.4 (1.6–3.6)	2.19×10^{-5} *
t-tau	81 (44)	1.9 (1.3–2.8)	1.15×10^{-3} *
Risk Factor Combinations	**No. (%)**	**HR (95% CI)**	**p-value**
HOC, AVLT, & t-tau/Aβ ratio Positive	54 (29)	15.1 (5.1–44.7)	8.98×10^{-7} *
HOC, AVLT, & t-tau/Aβ ratio Negative	18 (10)		
HOC & AVLT			
HOC & AVLT Positive	64 (35)	11.0 (5.3–22.8)	1×10^{-10} *
HOC Positive, AVLT Negative	19 (10)	6.2 (2.2–17.2)	4.83×10^{-4} *
HOC Negative, AVLT Positive	65 (35)	3.4 (1.7–7.0)	6.9×10^{-4} *
HOC & AVLT Negative	37 (20)		
HOC & t-tau/Aβ ratio			
HOC & CSF Positive	65 (35)	10.0 (4.7–21.0)	1.47×10^{-9} *
HOC Positive, CSF Negative	18 (10)	7.6 (2.4–23.6)	4.78×10^{-4} *
HOC Negative, CSF Positive	65 (35)	4.3 (2.0–9.2)	1.3×10^{-4} *
HOC & CSF Negative	37 (20)		
t-tau/Aβ ratio & AVLT			
CSF & AVLT Positive	100 (54)	7.1 (3.2–15.7)	1.46×10^{-6} *
CSF Positive, AVLT Negative	30 (16)	2.6 (1.0–6.7)	0.04
CSF Negative, AVLT Positive	29 (16)	1.9 (0.7–4.8)	0.21
CSF & AVLT Negative	26 (14)		

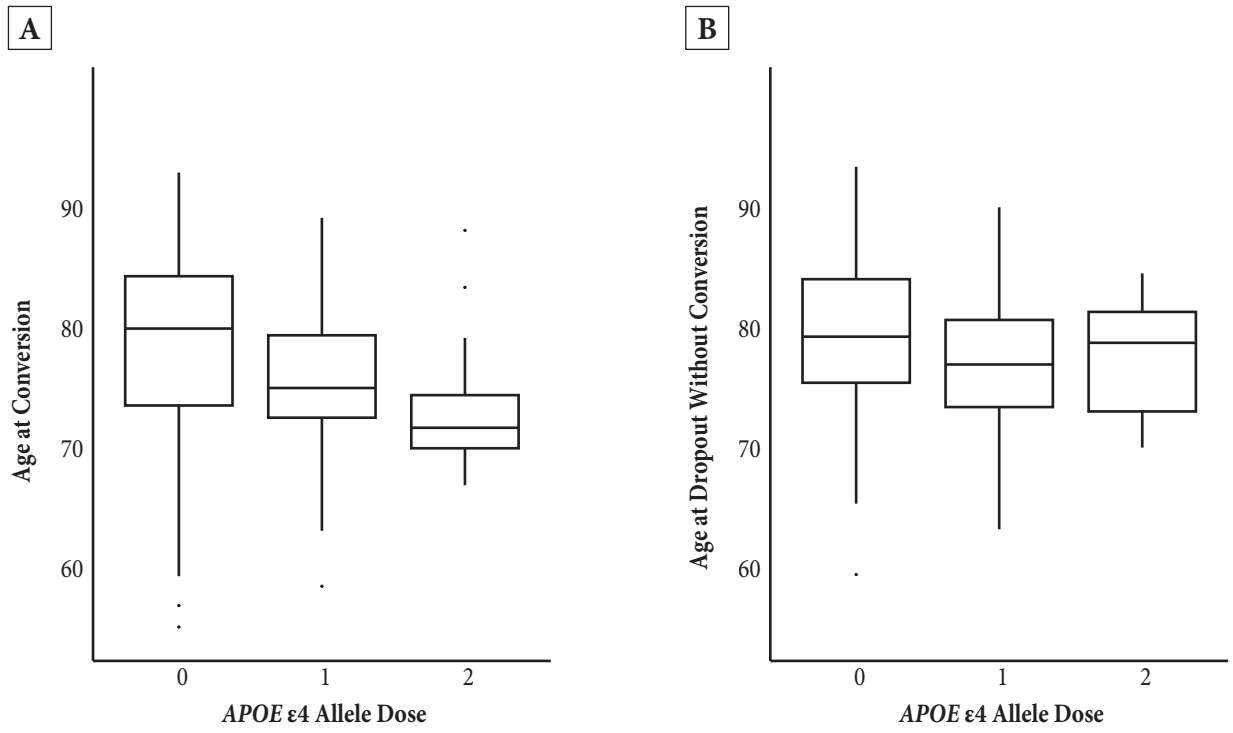

Figure 1.1 *APOE* is related to age at conversion. A) There was a significant relationship between age at conversion and *APOE* ε4 allele dose (p=.02). **B)** However, *APOE* ε4 allele dose had no effect on the time to dropout in cases of dropout without conversion (p=.29). The y-axis shows the age at event. The x-axis shows *APOE* ε4 allele dose.

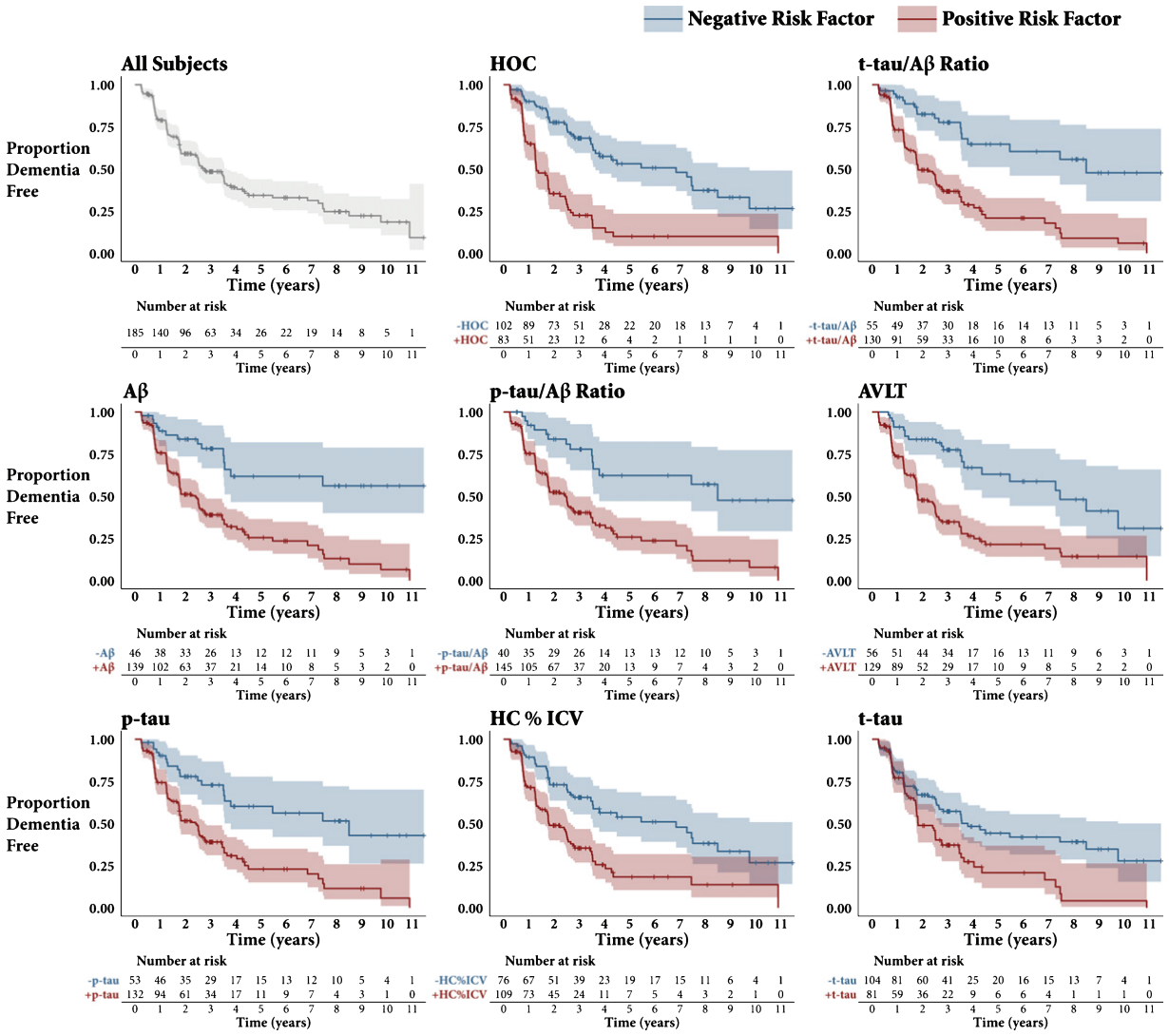

Figure 1.2 Kaplan-Meier survival curves for each individual risk factor. Kaplan-Meier survival curves, which estimate the probability that a subject will remain dementia free at a given time, are displayed for the entire cohort and stratified by positive (red) or negative (blue) risk for each individual risk factor. The y-axis shows the proportion of stable subjects. The x-axis shows time in years. Vertical drops indicate conversion. Tick marks indicate censoring. Shading represents 95% confidence intervals. Only subjects who have not yet converted or dropped out are considered at risk at a given time point.

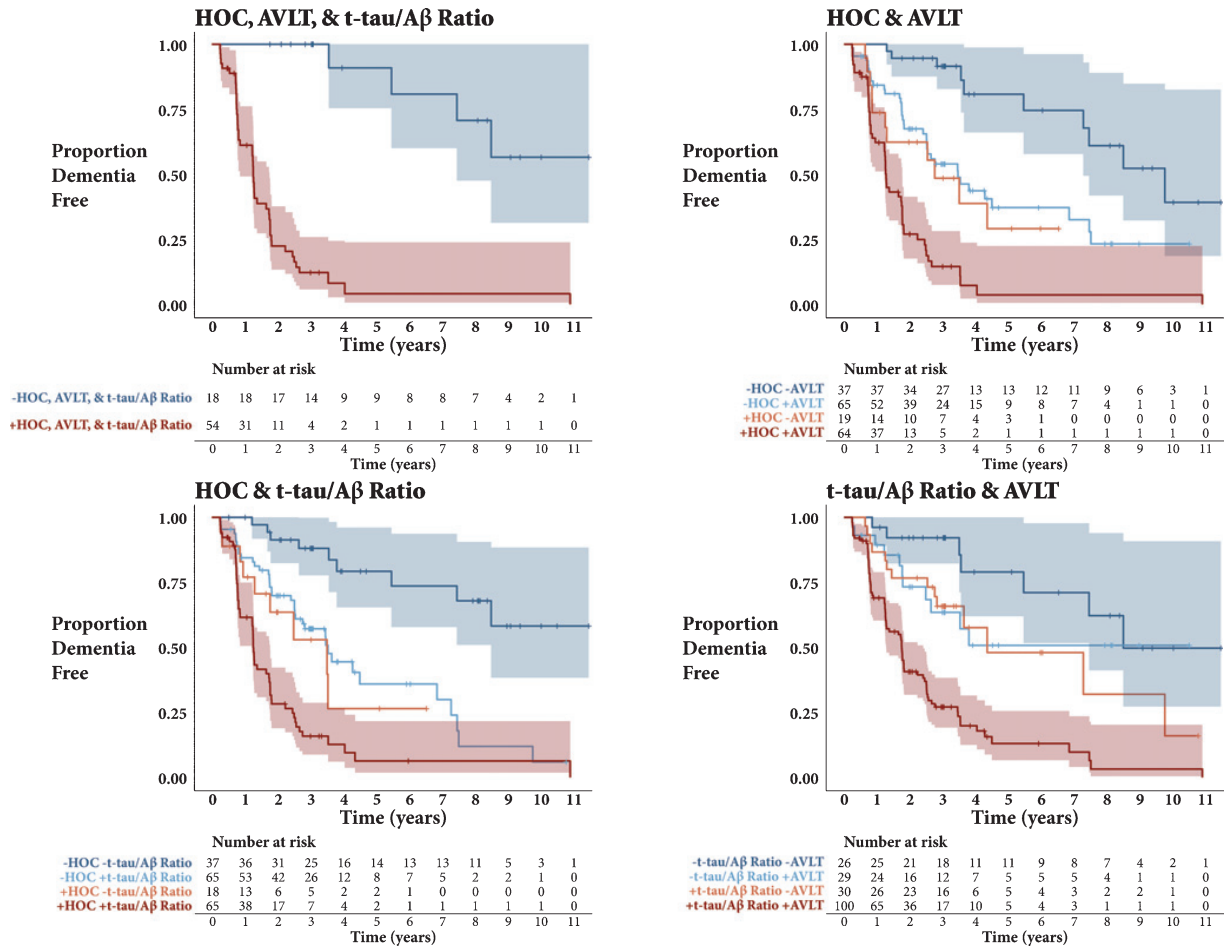

Figure 1.3 Kaplan-Meier survival curves stratified by risk factor combinations. Kaplan-Meier survival curves estimate the probability that a subject will remain dementia free at a given time. The y-axis shows the proportion of stable subjects. The x-axis shows time in years. Vertical drops indicate conversion. Tick marks indicate censoring. Shading represents 95% confidence intervals. Only subjects who have not yet converted or dropped out are considered at risk at a given time point.

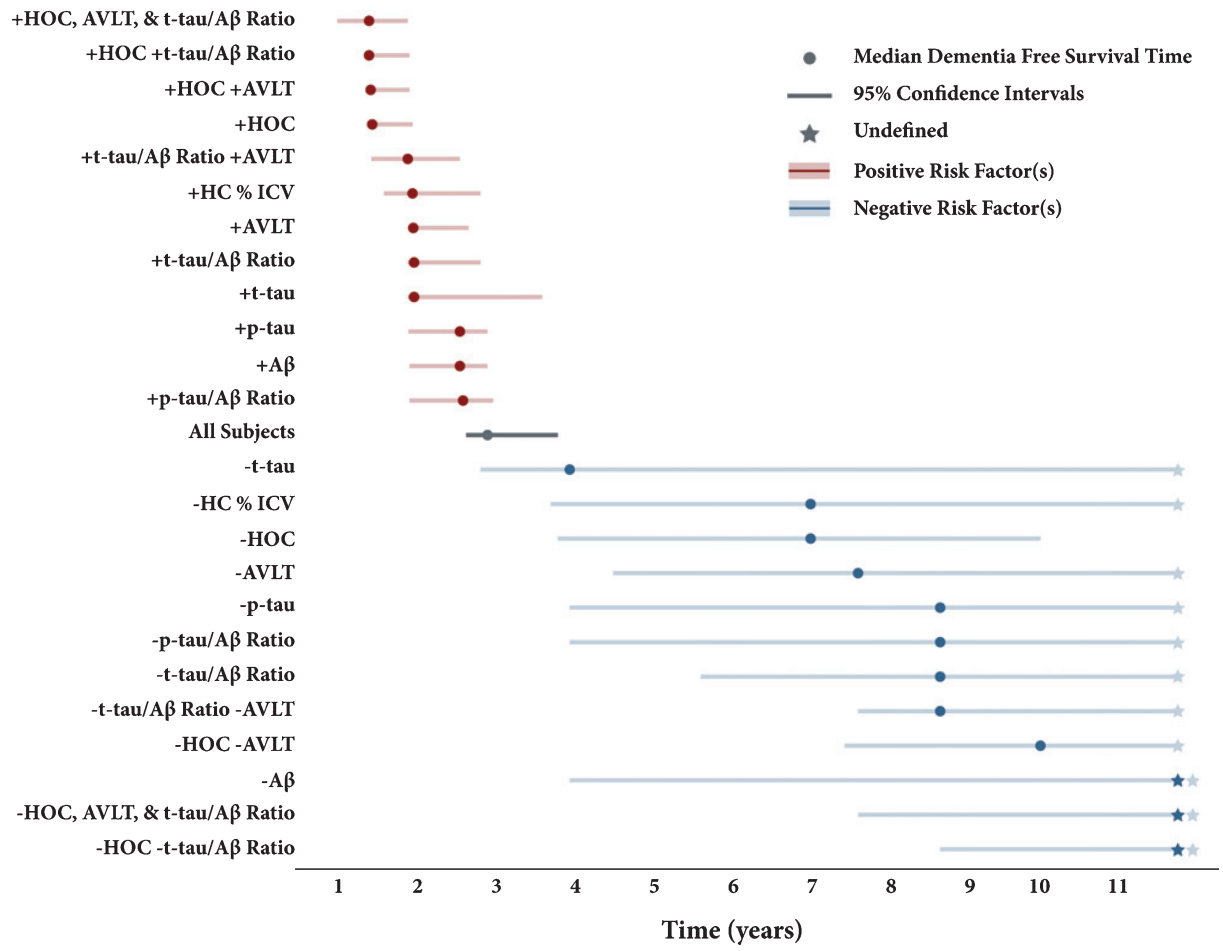

Figure 1.4 Median dementia-free survival time stratified by individual risk factors and risk factor combinations. All combinations represent concordant positive or negative risk. The x-axis shows time in years. Error bars represent 95% confidence intervals. Positive risk factors are displayed with dashed error bars; negative risk factors are displayed with dotted error bars. Due to high survival, three groups (–Aβ; –HOC, AVLT, and t-tau/Aβ ratio; – HOC – t-tau/Aβ ratio) never reached a median dementia-free survival time, and in several groups the upper limit of the 95% confidence interval was undefined past the last timepoint of 11.5 years. Stars represent such undefined estimates past the study completion.

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CHAPTER 2. GENE- AND AGE-INFORMED SCREENING FOR PRECLINICAL ALZHEIMER'S DISEASE TRIALS

INTRODUCTION

In clinically normal (CN) individuals, elevated brain β-amyloid (Aβ) is considered to be the earliest detectable indication of Alzheimer's disease (AD) neuropathologic change. Recently, preclinical AD trials began targeting Aβ in CN individuals to test whether decreasing Aβ would slow AD-related decline. As such, these trials require biomarker confirmation of elevated Aβ for enrollment. However, these CN individuals are only identified through the inefficient process of Aβ positron emission tomography (PET) screening, which enrolls only ~30% of those screened. Novel enrichment methods leveraging genetic and age-specific risk for AD may improve speed and efficiency of preclinical AD trial enrollment while reducing screening costs by identifying those CN individuals who are at greatest risk for AD.

Genetic variants such as the apolipoprotein E (*APOE*) ε4 allele are known to modulate AD risk. Risk for AD increases and age of AD onset decreases with an increasing number of *APOE* ε4 alleles. Recently, a polygenic hazard score (PHS) based on 31 variants and *APOE* was developed and validated that better predicts the age of AD onset than does *APOE* alone [1]. Previous work has demonstrated the utility of the PHS for predicting risk of clinical progression and cognitive decline in CN individuals [2,3]. However, for a given individual, aging is the single most important risk factor for AD, regardless of genetic background. Therefore, instantaneous risk for developing AD is better understood as a function of both genetic and baseline age-specific risk for AD.

We hypothesized that by combining PHS- and age-specific risk for AD we could predict elevated Aβ in CN individuals. We developed a gene- and age-defined ADAge enrichment method and tested the efficiency of such gene- and age-informed Aβ screening for preclinical AD trials in an independent cohort, while evaluating its impact on hypothetical trial population demographics. We then applied the ADAge enrichment method to real-world clinical trial screening data and compared the screening and associated costs necessary to enroll CN participants with elevated Aβ into the trial with or without ADAge enrichment.

METHODS

Participants

The development cohort comprised 939 participants (306 CN, 469 with mild cognitive impairment, and 164 with AD) from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). ADNI participants who completed a Florbetapir PET scan and had PHS calculated were included.

For the validation cohort, an independent sample of 80 participants was selected from the Shiley-Marcos Alzheimer's Disease Research Center (ADRC) of the University of California, San Diego. Inclusion was limited to CN participants who had undergone a lumbar puncture and genotyping.

The real-world clinical trial cohort comprised 3322 screened participants from the Anti-Amyloid Treatment in Asymptomatic Alzheimer's Disease (A4) trial who had undergone genotyping and a Florbetapir PET scan.

The research protocol was approved by each local institutional review board and written informed consent was obtained from each participant or participant's guardian.

Alzheimer's Disease Neuroimaging Initiative (ADNI)

In 2003, the ADNI began a large-scale, multi-site observational study of cognitively normal older adults and participants with mild cognitive impairment (MCI) and Alzheimer's disease (AD) that included the collection of clinical, neuroimaging, and other biomarker data. For up-to-date information, see www.adni-info.org.

Genetic Data

All participants in the ADNI development, ADRC validation, and A4 clinical trial cohorts were genotyped using a commercially available Illumina BeadChip array. In the ADRC validation cohort, genetic data was accessed through the National Alzheimer's Coordinating Center database, preprocessed with PLINK to exclude samples with a missingness rate greater than 10% and to perform strand flips as necessary, and imputed using the Michigan Imputation Server [4], which removes duplicate sites, non-single-nucleotide polymorphism (SNP) sites, monomorphic sites, and SNPs with a call rate < 90%, and achieves imputation with minimac3. We used the Haplotype Reference Consortium (HRC, version r1.1 2016) as the reference panel and Eagle (v2.3) phasing. Post-imputation the data was filtered to exclude genotype calls with an estimated posterior genotype probability < 0.9.

In the A4 clinical trial cohort, 3329 samples were available following pre-imputation quality control (QC). For a complete description of the A4 GWAS data QC and imputation pipeline see ida.loni.usc.edu. Genotype data was imputed using the Michigan Imputation Server, using the HRC (r1.1 2016) reference panel. Post-imputation the data was filtered to exclude genotype calls with an estimated posterior genotype probability < 0.9. 7 samples were excluded

following post-imputation QC that precluded the calculation of the polygenic hazard score, leaving 3322 samples to analyze.

PHS

PHS was downloaded from ADNI for each participant in the ADNI development cohort and calculated as described for the ADRC validation and A4 clinical trial cohorts [1].

Briefly, AD-associated SNPs were identified in the International Genomics of Alzheimer's Project (IGAP) cohort at $p < 10^{-5}$. These SNPs were then integrated into a stepwise Cox proportional hazards model using a subset of the Alzheimer's Disease Genetics Consortium (ADGC) phase 1 genetic data, which excluded individuals from the National Alzheimer's Coordinating Center and ADNI samples. This stepwise procedure identified 31 SNPs that most improved the model prediction (Table 2.1).

A PHS was calculated for each participant as the vector product of that individual's genotype for the 31 SNPs and the corresponding parameter estimates from the ADGC phase 1 Cox proportional hazard model, choosing the effect allele to be consistent with the direction of the beta in the IGAP summary statistics, in addition to the *APOE* effects.

Because the development and validation of the PHS has been largely limited to white, non-Hispanic cohorts, for the purposes of this analysis only white, non-Hispanic participants were included in the ADNI development and ADRC validation cohorts. This resulted in the exclusion of 29 otherwise eligible ADNI participants and 10 ADRC participants. However, all participants were included in the real-world A4 clinical trial cohort, which comprised 340 participants that were Hispanic, not white, or both.

Aβ Status Classification

We classified participants as having normal Aβ (Aβ-) or as Aβ positive (Aβ+) based on Florbetapir PET or cerebrospinal fluid (CSF) quantification (Table 2.2). For the ADNI development cohort, Florbetapir PET summary data were downloaded from ADNI (see Landau et al. [5] for acquisition and processing details). Images were acquired between May 25, 2010 and July 19, 2016. Aβ positivity was determined using a cutoff of 1.11 standardized uptake value ratio (SUVR, whole cerebellum reference region) for the summary cortical grey matter region of interest. For the ADRC validation cohort, CSF was collected between June 30, 2011 and November 17, 2017. CSF sample collection and Aβ quantification by liquid chromatography-tandem mass spectrometry has been described [6]. Aβ positivity was determined using an Aβ1-42 to Aβ40 ratio cutoff of 0.16, a threshold value determined to optimally distinguish CN participants from those with AD [6]. Though not completely overlapping, most individuals have concordant amyloid biomarker results when assessed with both PET and CSF [7], with even better agreement when using the Aβ42/40 ratio than Aβ42 alone [8]. For the A4 clinical trial cohort, screening Florbetapir PET summary data were downloaded from A4. Aβ positivity was determined using a cutoff of 1.11 SUVR for the composite summary region of interest.

ADAge

We used the US population baseline AD incidence rate [9] in combination with PHS to calculate an individualized genetic assessment of age-specific AD risk in the form of a predicted annualized incidence rate. For a given rate, the ADAge is defined as the age at which there is an equivalent risk in the baseline population.

At chronological age *t,* where IR is the US population baseline AD incidence rate [9],

$$IR = 0.084e^{0.142(t\text{-}60)}$$

This population baseline incidence rate can be combined with the PHS to generate an individualized predicted annualized incidence rate (PAIR).

$$PAIR = e^{PHS} \times IR$$

Like the IR, the PAIR gives an estimate of an individual's age-associated AD risk, but, in this case, also incorporates genetic information. For a given individual's PAIR, the ADAge is defined as the age at which there is equivalent risk in the baseline population.

$$ADAge = \frac{\ln\left(\frac{PAIR}{0.084}\right)}{0.142} + 60$$

For example, if a participant whose chronological age is 67 has a PAIR that is equivalent to the population baseline IR for an 81-year-old, this participant's ADAge would be 81 (Figure 2.1).

Statistical Analyses

We used Meng's test for comparing two or more correlated correlations to assess whether the ADAge was more correlated with Aβ than chronological age was in the ADNI development cohort. This test was repeated within CN and *APOE* ε4 carrier subsets of the ADNI development cohort to further evaluate this difference. We then chose an ADAge cutpoint by maximizing the Youden index for predicting Aβ positivity in CN participants in the ADNI development cohort.

We applied the ADNI development cohort derived ADAge cutpoint to 1000 bootstrap samples of the ADRC validation cohort. Using these bootstrap samples, 95% confidence intervals (CI) for the differences in means between samples enrolled by each strategy (ADAge enrichment vs no enrichment) were calculated to determine the efficiency of ADAge-informed Aβ screening for preclinical AD trials as well as to assess demographic differences between such

samples. These comparisons were repeated in the theoretical, Aβ+ end trial populations that would be enrolled by each strategy within the ADRC validation cohort to determine the impact of ADAge enrichment on trial population demographics.

A4 Clinical Trial Screening Scenario

We compared the screening necessary to enroll Aβ+ CN participants in the A4 clinical trial cohort using each enrollment strategy (ADAge enrichment vs no enrichment). For both strategies, the number needed to PET scan to verify Aβ status is a function of Aβ positivity within the A4 clinical trial cohort. The proportion of individuals who were Aβ+ under each enrollment strategy was compared, and this comparison was repeated in *APOE* ε3 homozygotes to evaluate the impact of enrichment beyond *APOE*. For the ADAge enrichment strategy, the number needed to genotype (to recruit those with an ADAge greater than the cutpoint) is a function of the proportion of the sample with an ADAge greater than the cutpoint within the A4 clinical trial cohort. The screening cost assumes $4285 per PET scan [10] and $150 per genotype.

Data availability

A request for access to ADNI data can be submitted and approved by the ADNI Data and Publications Committee (ida.loni.usc.edu/collaboration/access/appLicense.jsp). A request for resources, materials, or participant referrals from the Shiley-Marcos ADRC can be made by emailing Christina Gigliotti, Ph.D at cgigliotti@ucsd.edu. A request for access to pre-randomization study data collected by the A4/Longitudinal Evaluation of Amyloid Risk and Neurodegeneration Study for the purpose of scientific investigation, teaching or the planning of

clinical research studies can be submitted (https://ida.loni.usc.edu/collaboration/access/appLicense.jsp).

RESULTS

Cohort demographics are displayed in Table 2.2. Spearman's correlations were calculated for the relationships between Florbetapir PET SUVR and either chronological age or ADAge across ADNI development cohort subsets. Compared to chronological age, ADAge was more correlated with Florbetapir SUVR in the entire cohort (difference in correlation [95% CI] 0.28 [0.25 – 0.33], $p < .001$) as well as within CN (0.14 [0.08 – 0.21], $p < .001$) and *APOE* ε4 carrier (0.06 [0.02 – 0.11], $p = .006$) subsets of the cohort. Figure 2.2 shows scatterplots for the relationships between Florbetapir SUVR and either chronological age or ADAge.

Figure 2.3 shows the relationships between Aβ positivity and chronological age or ADAge (Figure 2.4 shows this relationship in each diagnostic group). The optimal ADAge cutpoint for predicting Aβ positivity in CN participants in the ADNI development cohort was determined to be 76.4 (Figure 2.5).

We generated 1000 bootstrap samples of the ADRC validation cohort (Figure 2.6). To test whether ADAge enrichment increased the proportion of CN individuals with elevated Aβ in the sample, we applied the ADAge cutpoint to each of these bootstrap samples and compared these enriched samples to the original, unenriched samples. The ADAge-enriched sample had a higher proportion of Aβ+ individuals (mean [95% CI] 0.46 [0.27 – 0.66] vs 0.28 [0.18 – 0.37], difference 0.19 [0.07 – 0.33]).

Next, we compared the theoretical, Aβ+ trial populations enrolled by each strategy (ADAge enrichment vs no enrichment) within the ADRC validation cohort to determine the

impact of ADAge enrichment on trial cohort demographics. The ADAge-enriched sample was older than the unenriched sample (76.70 [72.71 – 80.68] vs 73.69 [70.78 – 76.61], difference 3.00 [0.94 – 5.22]). However, the samples were similar in the proportion of *APOE* ε4 carriers (0.75 [0.48 – 1.02] vs 0.64 [0.43 – 0.84], difference 0.11 [-0.04 – 0.28]), the dementia rating scale score (141.33 [140.37 – 142.29] vs 140.95 [140.02 – 141.89], difference 0.38 [-0.28 – 1.14]), and the proportion of female participants (0.75 [0.50 – 1.00] vs 0.59 [0.38 – 0.80], difference 0.16 [0.00 – 0.33]).

Finally, using the A4 clinical trial cohort, we examined the screening necessary to enroll Aβ+ CN participants in the A4 clinical trial with and without ADAge enrichment (Figure 2.7 and Table 2.3), assuming a cost of $4285 per PET scan [10] and $150 per genotype. Similar to what we observed in the ADRC validation cohort, ADAge enrichment increased the proportion of Aβ+ individuals in the A4 clinical trial cohort from 0.34 (95% CI 0.32 – 0.35) to 0.52 (0.49 – 0.56) (Figure 2.8). When limited to *APOE* ε3 homozygotes, ADAge enrichment again increased the proportion of Aβ+ individuals in the cohort from 0.22 (0.20 – 0.24) to 0.37 (0.31 – 0.43). By leveraging low-cost genetic screening as inclusion criteria for subsequent high-cost PET, the ADAge-enriched sample reduced the number of PET scans needed by 1196.68 (36.02%). Despite needing to genetically screen a large number of participants, ADAge enrichment lowered the total screening cost by $3.91 million (27.50%). This reduction in total screening cost assumes no genetic screening was completed in the A4 clinical trial. In fact, the 3322 participants were genotyped through the trial, meaning the ADAge enrichment approach would have lead to a total screening savings of $4.41 million (31.00%).

DISCUSSION

Given the high-cost, effort-intensive process of broad screening for Aβ positivity and the availability of low-cost genetic screening, ADAge warrants further evaluation as an enrichment method to address the current inefficiency in preclinical AD trial enrollment. We demonstrated that ADAge was more correlated with Aβ than chronological age and could be used to enrich the proportion of Aβ+ individuals in a sample, leading to an estimated $4.41 million (31.00%) savings.

Strategies that combine age and genetics may be further optimized to enhance efficiency, such as determining age cutoffs for administering genetic screening. An estimated 40–65% [11,12] of healthy adults over 80 are Aβ+. Hence, the likelihood of enrolling Aβ+ participants in this age group is high and less dependent on genetic risk, diminishing the value of genetic prescreening in this older population. However, with advanced age also comes a higher likelihood of concomitant pathologies such as vascular disease and the recently described Limbic-predominant age-related TDP-43 encephalopathy [13].

Previous work modeled the prediction of Aβ positivity by PHS in CN individuals using logistic regression [3]. Here, we translated this prediction to a practical strategy in which we demonstrated that we can enrich a cohort for Aβ positivity based on inclusion decisions made at an individual level. Further, ADAge enrichment does not rely on PHS alone. Rather, older individuals with low to average genetic risk are included alongside younger individuals with high risk under the ADAge enrichment method.

We found a similar proportion of *APOE* ε4 carriers in our theoretical trial populations with and without ADAge enrichment. As ~60% [14,15] of individuals with late-onset AD are *APOE* ε4 carriers, an approach that sought to enrich for Aβ positivity by enrolling only *APOE* ε4

carriers would disproportionately represent this group in a trial. Given the heterogeneous nature of AD, it is important to consider additional variants that modulate AD risk. The PHS includes genes associated with multiple biological processes implicated in AD, such as inflammation, synaptic function, and epigenetic regulation [16]. Further, previous work has demonstrated the value of the PHS beyond *APOE* [1–3], and ADAge enrichment increased the proportion of Aβ+ individuals even within *APOE* ε3 homozygotes in the A4 clinical trial cohort.

Although we validated our findings in an independent research sample and real-world clinical trial data, these cohorts were relatively homogeneous. Due to observed differences in AD genetic risk across racial and ethnic groups [17–19], findings are largely limited to white, non-Hispanic individuals. Future work is needed to develop AD polygenic scores in diverse populations. Further validation in more diverse samples would provide more evidence that an ADAge enrichment strategy could be successfully implemented into screening for clinical trials, especially those whose cohort demographics better reflect the underlying population diversity. Large-scale population-based samples are needed to clarify the true prevalence of Aβ positivity across the lifespan and examine differences between genders and between racial and ethnic groups. However, the proportion of Aβ+ CN individuals in our ADRC validation cohort (.28) closely matches what has been observed in the screening process of the A4 trial (.29) in similarly aged participants, with a similar proportion of *APOE* ε4 carriers [20].

In conclusion, ADAge enrichment provides for a more efficient and cost-effective method to enroll Aβ+ CN participants in clinical trials. Enrolled cohorts are expected to be 3 years older on average than their unenriched counterparts, but similar in sex, cognition, and *APOE* ε4 status.

Table 2.1 PHS Parameters. The table displays the 31 SNPs, their chromosomes, positions, and closest genes, and the corresponding parameter estimates from the ADGC phase 1 Cox proportional hazard model, in addition to the *APOE* effects. This table is adapted from Table 2 of Desikan et al. [1].

	Chr	**Position**	**Gene**	**β**
ε2 allele	19		*APOE*	-0.47
ε4 allele	19		*APOE*	1.03
rs4266886	1	207685786	*CR1*	-0.09
rs61822977	1	207796065	*CR1*	-0.08
rs6733839	2	127892810	*BIN1*	-0.15
rs10202748	2	234003117	*INPP5D*	-0.06
rs115124923	6	32510482	*HLA-DRB5*	0.17
rs115675626	6	32669833	*HLA-DQB1*	-0.11
rs1109581	6	47678182	*GPR115*	-0.07
rs17265593	7	37619922	*BC043356*	-0.23
rs2597283	7	37690507	*BC043356*	0.28
rs1476679	7	100004446	*ZCWPW1*	0.11
rs78571833	7	143122924	*AL833583*	0.14
rs12679874	8	27230819	*PTK2B*	-0.09
rs2741342	8	27330096	*CHRNA2*	0.09
rs7831810	8	27430506	*CLU*	0.09
rs1532277	8	27466181	*CLU*	0.21
rs9331888	8	27468862	*CLU*	0.16
rs7920721	10	11720308	*CR595071*	-0.07
rs3740688	11	47380340	*SPI1*	0.07
rs7116190	11	59964992	*MS4A6A*	0.08
rs526904	11	85811364	*PICALM*	-0.2
rs543293	11	85820077	*PICALM*	0.3
rs11218343	11	121435587	*SORL1*	0.18
rs6572869	14	53353454	*FERMT2*	-0.11
rs12590273	14	92934120	*SLC24A4*	0.1
rs7145100	14	107160690	*abParts*	0.08
rs74615166	15	64725490	*TRIP4*	-0.23
rs2526378	17	56404349	*BZRAP1*	0.09
rs117481827	19	1021627	*C19orf6*	-0.09
rs7408475	19	1050130	*ABCA7*	0.18
rs3752246	19	1056492	*ABCA7*	-0.25
rs7274581	20	55018260	*CASS4*	0.1

Table 2.2 Demographics and clinical characteristics of the ADNI development, ADRC validation, and A4 clinical trial cohorts split by Aβ status. Reported as mean (SD) unless otherwise noted. P-value based on Pearson's chi-squared test or Welch's two sample t-test.

	ADNI Development Cohort			
	Entire Cohort n=939	Aβ+ n=507	Aβ- n=432	*P* value Aβ+ vs Aβ-
Clinical Diagnosis				< .001
CN, No. (%)	306 (32.59)	102 (20.12)	204 (47.22)	
MCI, No. (%)	469 (49.95)	264 (52.07)	205 (47.45)	
AD, No. (%)	164 (17.47)	141 (27.81)	23 (5.32)	
Age, years	74.05 (7.47)	74.79 (7.19)	73.18 (7.71)	.001
ADAge	76.29 (8.49)	79.21 (7.91)	72.86 (7.84)	< .001
Female, No. (%)	423 (45.05)	236 (46.55)	187 (43.29)	.32
APOE ε4 carrier, No. (%)	414 (44.09)	329 (64.89)	85 (19.68)	< .001
PHS	0.32 (0.76)	0.63 (0.76)	-0.05 (0.58)	< .001
Florbetapir SUVR*	1.21 (0.23)	1.39 (0.17)	1.00 (0.06)	< .001
	ADRC Validation Cohort			
	Entire Cohort n=80	Aβ+ n=22	Aβ- n=58	*P* value Aβ+ vs Aβ-
Age, years	72.92 (5.95)	73.69 (6.88)	72.63 (5.59)	.52
ADAge	73.01 (8.34)	77.28 (8.77)	71.40 (7.65)	.009
Female, No. (%)	53 (66)	13 (59)	40 (69)	.40
APOE ε4 carrier, No. (%)	29 (36)	14 (64)	15 (26)	.002
PHS	0.01 (0.88)	0.51 (0.92)	-0.18 (0.80)	.004
Aβ 42/40 Ratio†	0.20 (0.06)	0.13 (0.02)	0.22 (0.05)	.001
	A4 Clinical Trial Cohort			
	Entire Cohort n=3322	Aβ+ n=1115	Aβ- n=2207	*P* value Aβ+ vs Aβ-
Age, years	71.34 (4.74)	71.98 (4.88)	71.02 (4.64)	< .001
ADAge	71.86 (7.20)	74.77 (7.13)	70.40 (6.78)	< .001
Female, No. (%)	1992 (59.96)	674 (60.45)	1318 (59.72)	.69
APOE ε4 carrier, No. (%)	1166 (35.10)	651 (58.39)	515 (23.33)	< .001
PHS	0.07 (0.84)	0.40 (0.88)	-0.09 (0.77)	< .001
Florbetapir SUVR*	1.09 (0.19)	1.31 (0.17)	0.98 (0.06)	< .001

*Aβ positivity was determined using a cutoff of 1.11 SUVR.
†Aβ positivity was determined using an Aβ1-42 to Aβ40 ratio cutoff of 0.16.

Table 2.3 Cost of ADAge-enriched Aβ screening in the A4 clinical trial. The table shows the cost of enrolling 1115 Aβ+ CN participants in the A4 clinical trial with and without ADAge enrichment, split by chronological age bins. The number needed to PET scan to verify Aβ status is shown for each enrollment strategy (unenriched vs ADAge-enriched). The number needed to genotype to recruit those with an ADAge greater than the cutpoint is shown for the ADAge-enriched strategy. The number needed to PET scan is a function of Aβ positivity within the A4 clinical trial cohort. The number needed to genotype is a function of the proportion of the sample with an ADAge greater than the 76.4 cutpoint within the A4 clinical trial cohort. The cost assumes $4285 per PET scan and $150 per genotype. Reported as mean (95% CI).

Entire A4 Clinical Trial Cohort

	Unenriched	**ADAge-Enriched**
Proportion ADAge > 76.4	–	0.26 (0.25–0.28)
No. Needed to Genetic Screen	–	8087.40 (7404.07–8909.68)
Genetic Screen Cost (million USD)	–	1.21 (1.11–1.34)
Proportion Aβ+	0.34 (0.32–0.35)	0.52 (0.49–0.56)
No. Needed to PET Scan	3322 (3161.33–3499.87)	2125.32 (1995.51–2273.19)
PET Scan Cost (million USD)	14.23 (13.55–15.00)	9.11 (8.55–9.74)
Total Cost (million USD)	14.23 (13.55–15.00)	10.32 (9.66–11.08)

A4 Clinical Trial Cohort: Chronological Age ≥70

	Unenriched	**ADAge-Enriched**
Proportion ADAge > 76.4	–	0.40 (0.38–0.43)
No. Needed to Genetic Screen	–	5493.57 (5004.76–6088.19)
Genetic Screen Cost (million USD)	–	0.82 (0.75–0.91)
Proportion Aβ+	0.37 (0.35–0.39)	0.51 (0.47–0.54)
No. Needed to PET Scan	3004.16 (2824.49– 3208.24)	2204.94 (2051.09–2383.76)
PET Scan Cost (million USD)	12.87 (12.10–13.75)	9.45 (8.79–10.21)
Total Cost (million USD)	12.87 (12.10–13.75)	10.27 (9.54–11.13)

A4 Clinical Trial Cohort: Chronological Age ≥75

	Unenriched	**ADAge-Enriched**
Proportion ADAge > 76.4	–	0.62 (0.58–0.65)
No. Needed to Genetic Screen	–	3872.29 (3458.78–4398.11)
Genetic Screen Cost (million USD)	–	0.58 (0.52–0.66)
Proportion Aβ+	0.40 (0.37–0.44)	0.47 (0.42–0.51)
No. Needed to PET Scan	2758.16 (2525.59–3037.90)	2389.29 (2166.94–2662.47)
PET Scan Cost (million USD)	11.82 (10.82–13.02)	10.24 (9.29–11.41)
Total Cost (million USD)	11.82 (10.82–13.02)	10.82 (9.80–12.07)

Table 2.3 Cost of ADAge-enriched Aβ screening in the A4 clinical trial, Continued.

A4 Clinical Trial Cohort: Chronological Age ≥80

	Unenriched	ADAge-Enriched
Proportion ADAge > 76.4	–	0.82 (0.76–0.87)
No. Needed to Genetic Screen	–	3253.36 (2736.44–4011.04)
Genetic Screen Cost (million USD)	–	0.49 (0.41–0.60)
Proportion Aβ+	0.40 (0.33–0.47)	0.42 (0.35–0.49)
No. Needed to PET Scan	2794.06 (2393.59–3355.45)	2657.67 (2257.70–3229.87)
PET Scan Cost (million USD)	11.97 (10.26–14.38)	11.39 (9.67–13.84)
Total Cost (million USD)	11.97 (10.26–14.38)	11.88 (10.08–14.44)

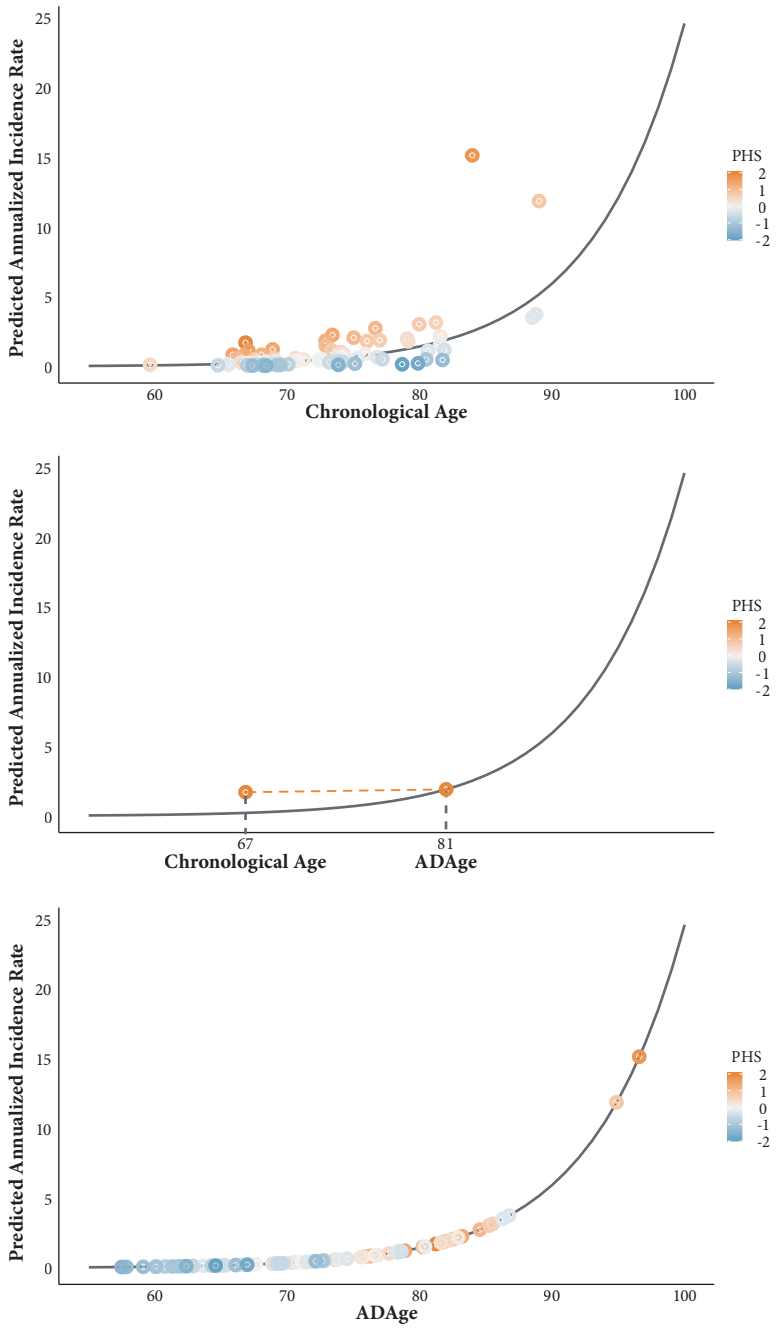


Figure 2.1 Generating ADAge. When combined with the population baseline AD incidence rate, the PHS can be used to calculate an individualized genetic assessment of age-associated AD risk in the form of a predicted annualized incidence rate. For a given rate, the age at which there is equivalent risk in the baseline population is the ADAge. Each dot is an individual from the ADRC validation cohort. Colors represent the PHS. The gray line represents the population baseline estimate from previously reported age-specific incidence rates of AD in the United States population.

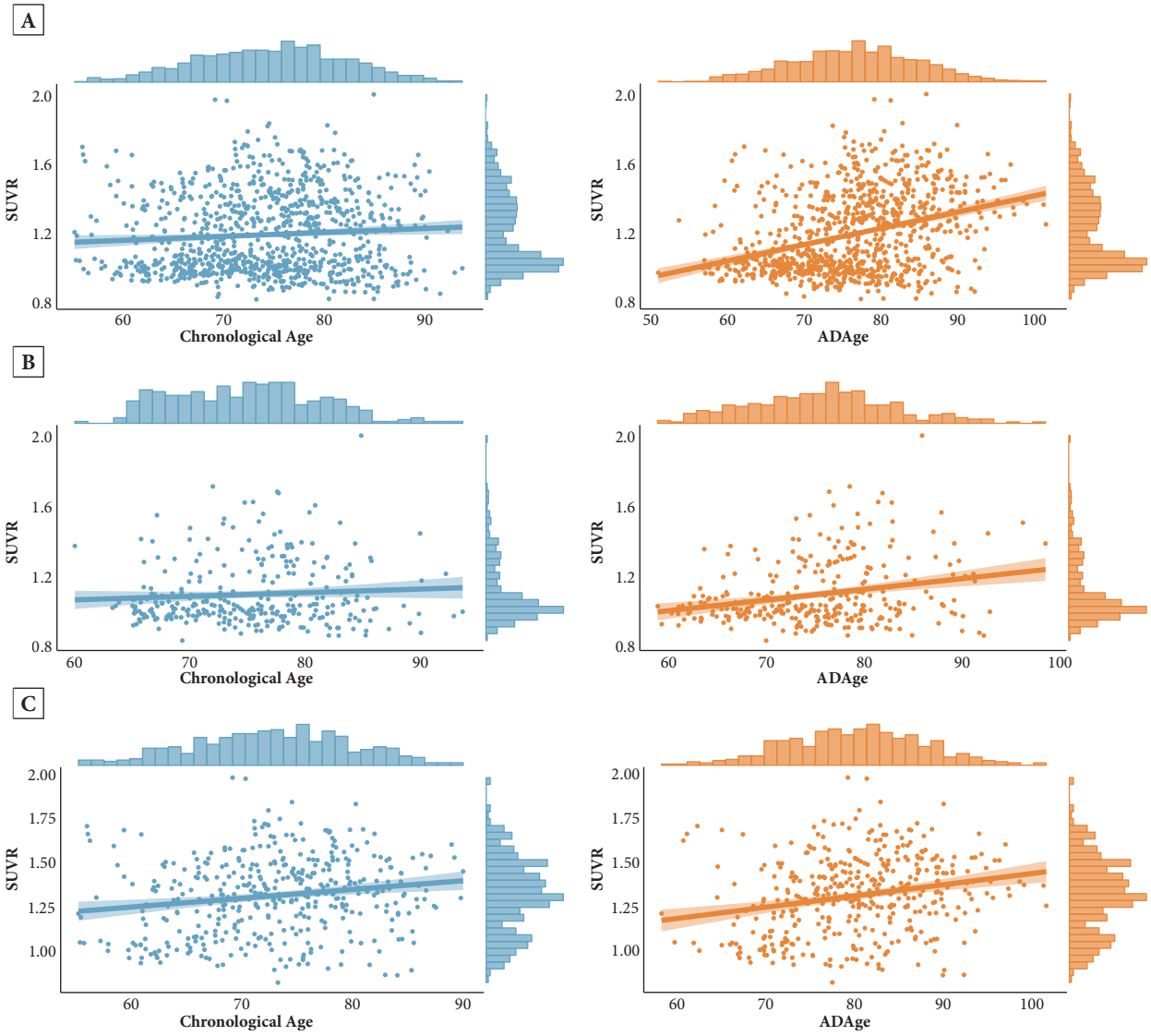


Figure 2.2 Compared to chronological age, ADAge is more correlated with Florbetapir SUVR. Scatterplots for the relationships between SUVR and either chronological age or ADAge within **A)** the entire ADNI development cohort as well as within **B)** CN or **C)** *APOE* ε4 carrier subsets of the cohort are shown. The distributions of SUVR and either chronological age or ADAge are displayed as histograms along the vertical and horizontal axes, respectively. Spearman's correlations were calculated for the relationships between SUVR and either chronological age or ADAge across cohort subsets. Meng's test for comparing correlated correlations found that, compared to chronological age, ADAge was more correlated with Florbetapir SUVR in the entire ADNI development cohort (difference in correlation [95% CI] .28 [.25 – .33], $p < .001$) as well as within CN (.14 [.08 – .21], $p < .001$) and *APOE* ε4 carrier (.06 [.02 – .11], $p = .006$) subsets of the cohort.

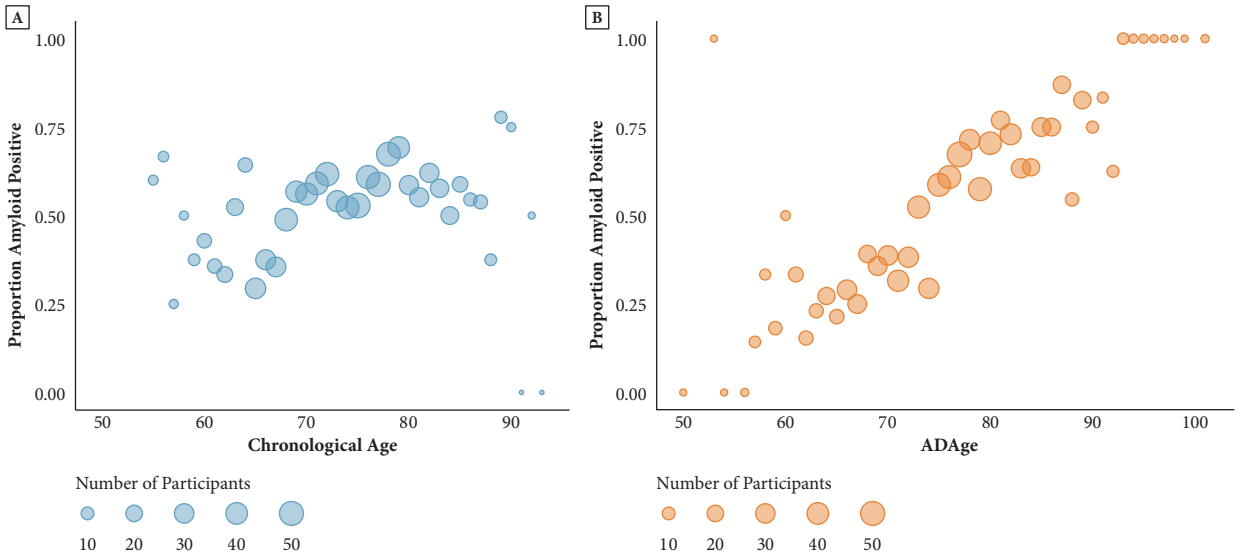


Figure 2.3 Visualization of the relationships between Aβ positivity and A) chronological age (blue) or B) ADAge (orange) in the ADNI development cohort. Dot sizes are proportional to the number of participants in each age bin.

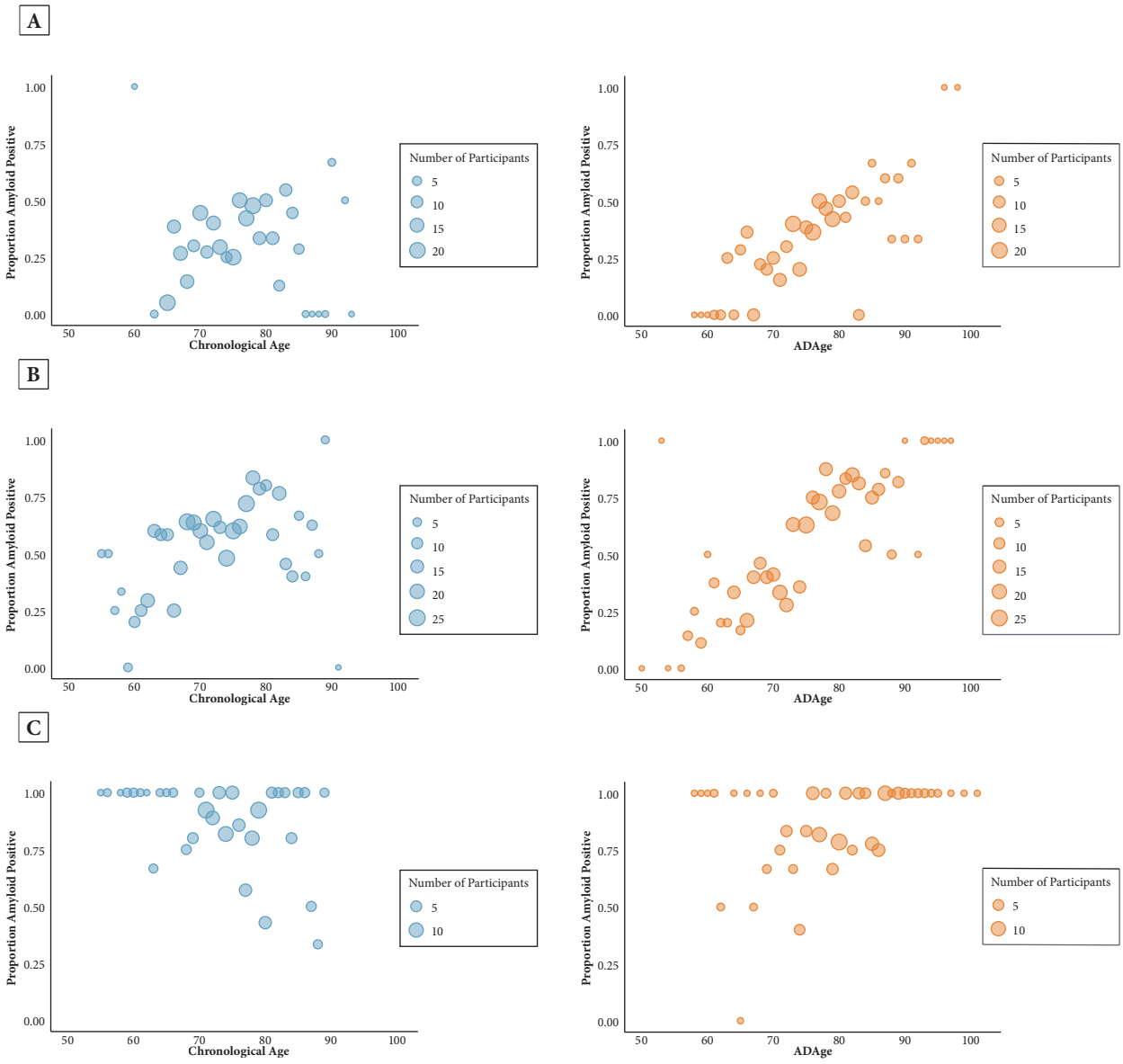


Figure 2.4 Visualization of the relationships between Aβ positivity and chronological age (blue) or ADAge (orange) in subsets of the ADNI development cohort clinically diagnosed as A) CN, B) MCI, or C) AD. Dot sizes are proportional to the number of participants in each age bin.

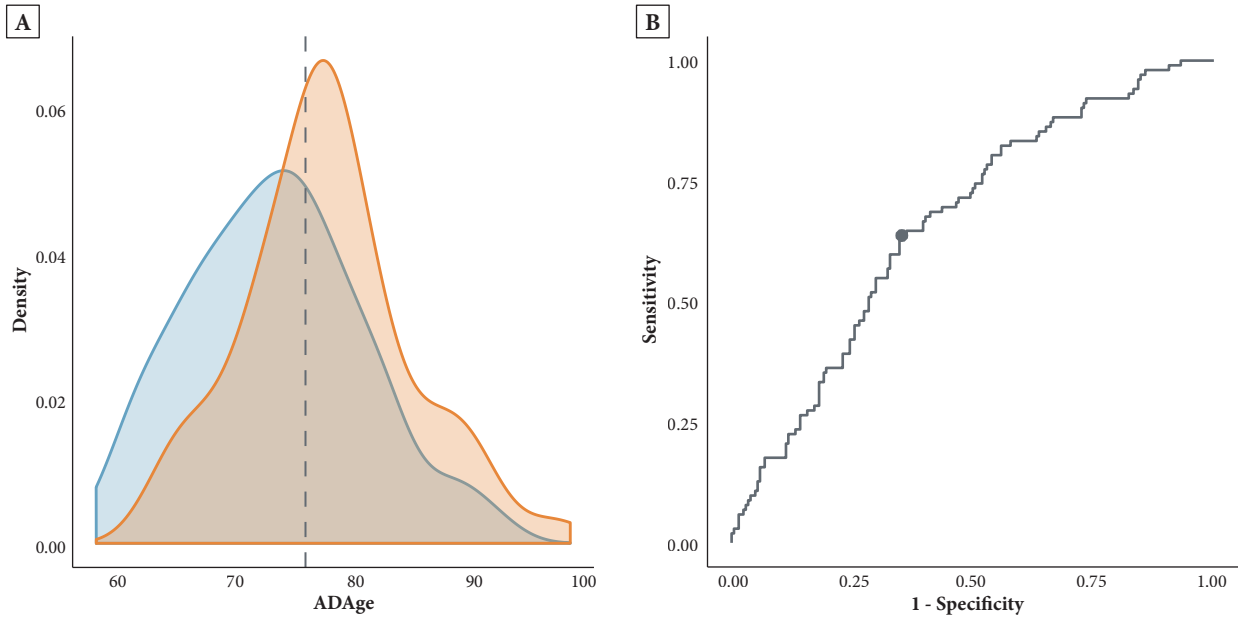
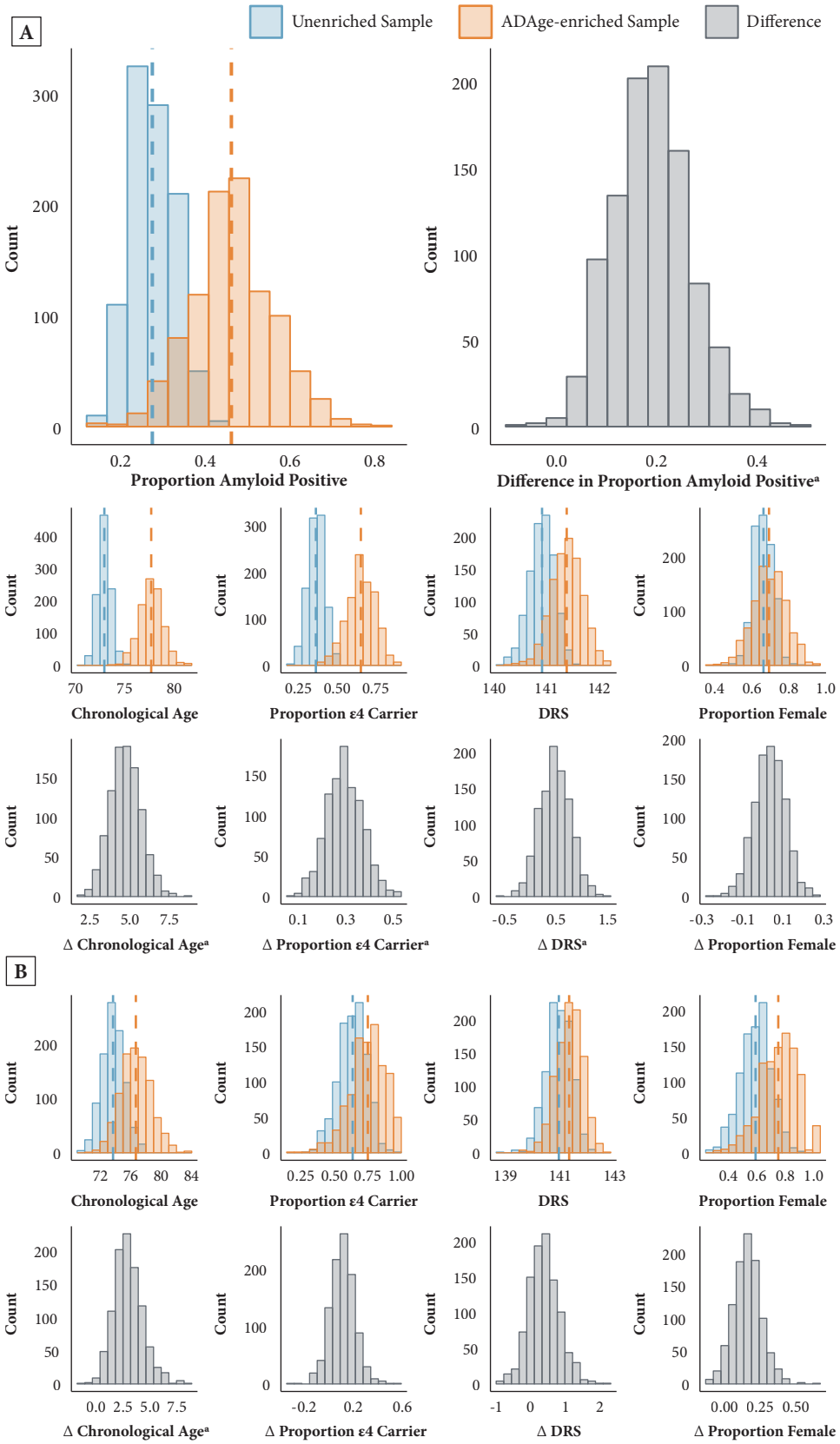


Figure 2.5 The optimal ADAge cutpoint was determined to be 76.4. A) The distribution of ADAges in Aβ- (blue) and Aβ+ (orange) CN participants in the ADNI development cohort is shown. The dashed line represents the ADAge cutpoint. **B)** The ROC curve illustrates the sensitivity (0.64) and specificity (0.65) of the ADAge for predicting Aβ positivity (AUC 0.66).

Figure 2.6 Demographic characteristics of the unenriched (blue) and ADAge-enriched (orange) ADRC validation samples. Histograms display the means of 1000 bootstrap samples of the ADRC validation cohort and the difference in those means (gray). **A)** At baseline, the ADAge-enriched sample had a higher proportion of Aβ+ individuals (mean [95% CI] 0.46 [0.27 – 0.66] vs 0.28 [0.18 – 0.37], difference 0.19 [0.07 – 0.33]), was older (77.67 [75.34 – 80.01] vs 72.92 [71.61 – 74.23], difference 4.75 [3.16 – 6.36]), had a greater proportion of *APOE* ε4 carriers (0.65 [0.47 – 0.84] vs 0.36 [0.25 – 0.47], difference 0.29 [0.17 – 0.42]), and had a higher dementia rating scale score (141.38 [140.79 – 141.98] vs 140.91 [140.43 – 141.39], difference 0.47 [0.01 – 0.97]). The proportion of female participants was similar between ADAge-enriched and unenriched samples (0.69 [0.51 – 0.87] vs 0.66 [0.56 – 0.77], difference 0.03 [-0.09 – 0.15]). **B)** The theoretical, Aβ+ end trial populations that would be enrolled by each strategy (ADAge enrichment vs no enrichment) were compared. The ADAge-enriched sample remained older than the unenriched sample (76.70 [72.71 – 80.68] vs 73.69 [70.78 – 76.61], difference 3.00 [0.94 – 5.22]). However, the samples were similar in the proportion of *APOE* ε4 carriers (0.75 [0.48 – 1.02] vs 0.64 [0.43 – 0.84], difference 0.11 [-0.04 – 0.28]), the dementia rating scale score (141.33 [140.37 – 142.29] vs 140.95 [140.02 – 141.89], difference 0.38 [-0.28 –1.14]), and the proportion of female participants (0.75 [0.50 –1.00] vs 0.59 [0.38 – 0.80], difference 0.16 [0.00 – 0.33]).

[a] The difference in means is statistically significant


A
Unenriched Sample
ADAge-enriched Sample
Difference
Count
Proportion Amyloid Positive
Difference in Proportion Amyloid Positive[a]
Chronological Age
Proportion ε4 Carrier
DRS
Proportion Female
Δ Chronological Age[a]
Δ Proportion ε4 Carrier[a]
Δ DRS[a]
Δ Proportion Female
B
Chronological Age
Proportion ε4 Carrier
DRS
Proportion Female
Δ Chronological Age[a]
Δ Proportion ε4 Carrier
Δ DRS
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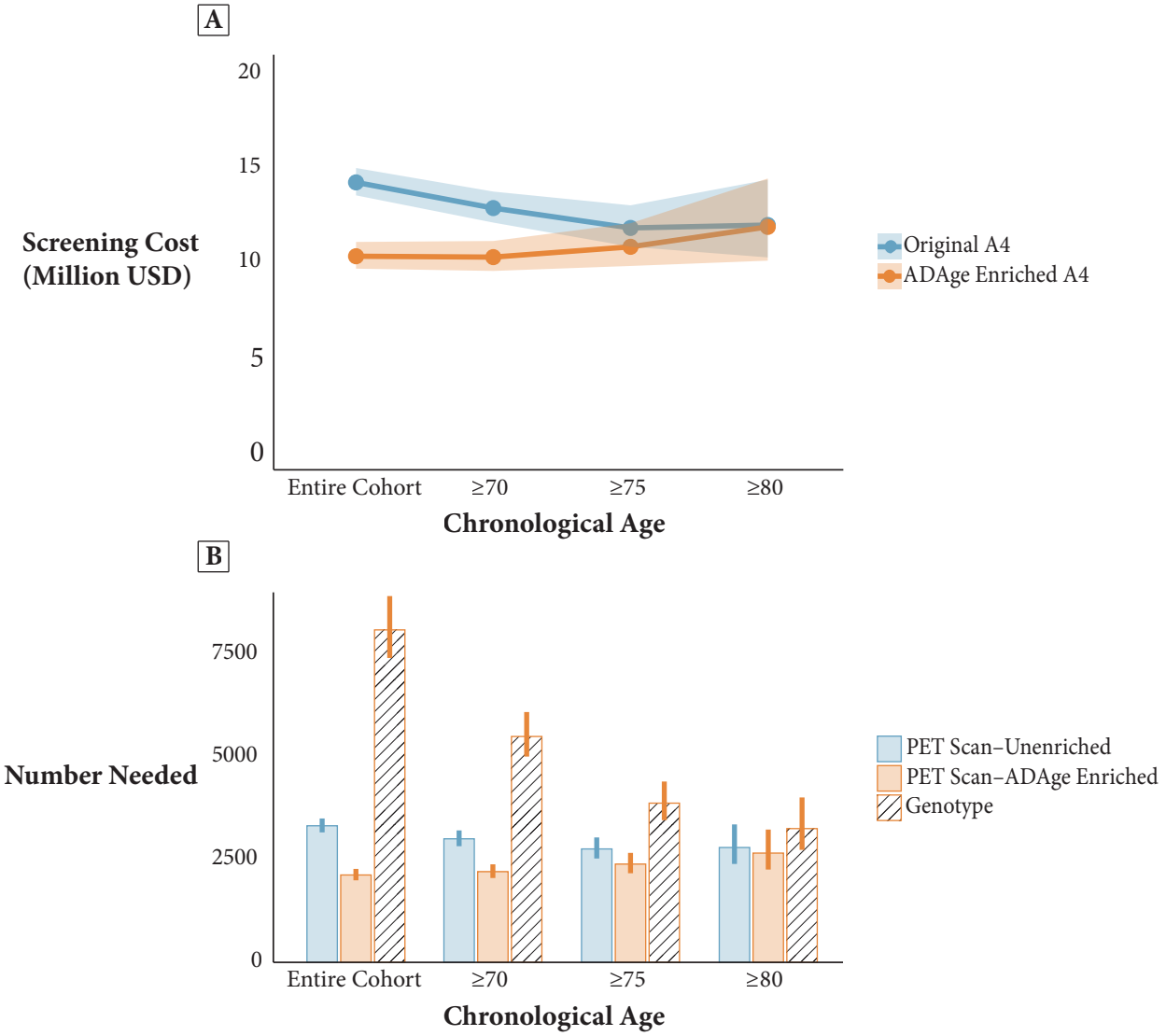

Figure 2.7 ADAge-enriched Aβ screening more efficiently enrolls clinically normal individuals in the A4 preclinical AD trial. The figure outlines the screening necessary to enroll 1115 Aβ+ CN participants in the A4 clinical trial for each enrollment strategy, split into bins by chronological age. **A)** The screening cost for each strategy (unenriched [blue] vs ADAge-enriched [orange]), assumes $4285 per PET scan and $150 per genotype. Shading represents 95% confidence intervals. **B)** The number needed to PET scan to verify Aβ status is shown for each enrollment strategy (unenriched [blue] vs ADAge-enriched [orange]) by chronological age bin. Error bars represent 95% confidence intervals. The number needed to genotype to recruit those with an ADAge greater than the cutpoint is shown for the ADAge-enriched strategy (diagonal pattern) by each age bin. The number needed to PET scan is a function of the Aβ positivity within the A4 clinical trial cohort. The number needed to genotype is a function of the proportion of the sample with an ADAge greater than the 76.4 cutpoint within the A4 clinical trial cohort.

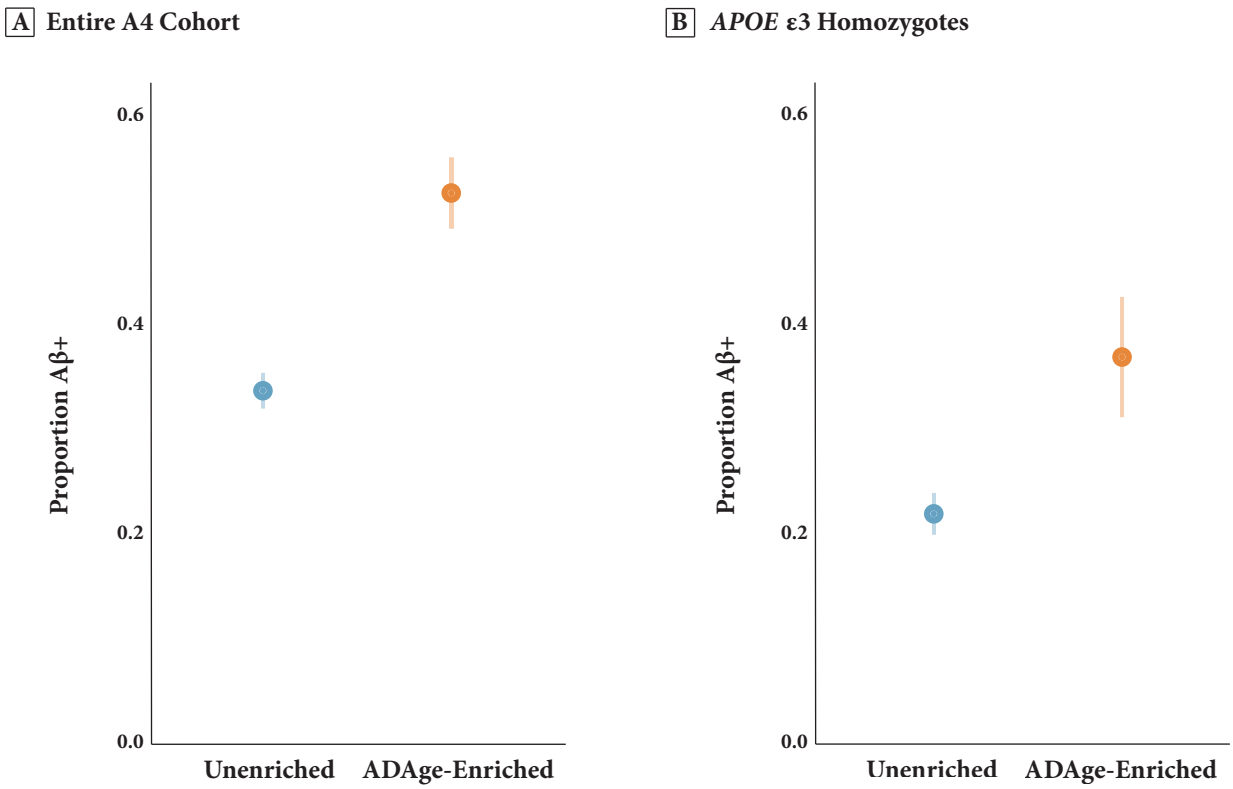

Figure 2.8 ADAge enrichment increased the proportion of CN individuals with elevated Aβ in the A4 clinical trial cohort. The ADAge-enriched sample had a higher proportion of Aβ+ individuals **A)** overall (0.52 [95% CI 0.49 – 0.56] vs 0.34 [0.32 – 0.35]) and **B)** in *APOE* ε3 homozygotes (0.37 [0.31 – 0.43] vs 0.22 [0.20 – 0.24])

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CHAPTER 3. ASSESSMENT OF GENETIC RISK FOR IMPROVED CLINICAL-NEUROPATHOLOGICAL CORRELATIONS

INTRODUCTION

In heterogeneous disease cohorts, accurate distinctions between Alzheimer's disease (AD) and related dementias may improve precision in care delivery and thus lead to better outcomes. In the diagnosis of Dementia with Lewy bodies (DLB), distinction from AD is suboptimal and complicated by the frequent co-occurrence of AD neuropathologic changes (NC) with Lewy bodies (LBs). Patients clinically diagnosed with AD often present with concurrent LB pathology at autopsy, though many studies have attempted to tease apart the differences in clinical presentations to better reflect underlying pathology [1–6].

Incorporating information about genetic risk into a difficult differential diagnosis may improve clinical-neuropathological correlations. A recently developed AD polygenic hazard score (PHS) is associated with the hallmark ADNCs, neuritic plaques and neurofibrillary tangles. However, the large-scale genetic studies that identify such risk typically rely on clinical diagnoses, which are imperfect proxies for the often mixed underlying pathologies [7,8]. While the AD PHS has reported associations with LBs [9], it is unclear whether this reflects a shared genetic risk between pathologies, a byproduct of the common presence of LB co-pathology with AD, since the level of ADNC was not controlled for in the analysis, or a lack of specificity in the AD PHS due to the presence of LBs or other mixed pathologies in those clinically diagnosed with AD.

DLB has both clinical features and genetic risk factors that overlap with both AD and Parkinson's disease (PD). The apolipoprotein E (*APOE*) ε4 allele is the strongest genetic risk

factor for late onset AD and is also overrepresented in pure DLB and PD with dementia [10]. The SNCA, GBA, and BCL7C/STX1B genes are implicated in risk for both DLB and PD [11,12], though the associations at the SNCA locus differ between the two [13,12]. Given the relatively low accuracy of a DLB diagnosis, genetic studies typically examine relatively small, neuropathologically confirmed DLB cohorts [12,14], with few genome-wide significant variants identified. Then, genetic risk for PD, discovered in well-powered studies, may be better suited to predict the underlying LB pathology in DLB cases. In a pathologically defined cohort we will test the hypothesis that the AD PHS, a DLB polygenic risk score (PRS), and a PD PRS can differentiate individuals with DLB from those who have AD.

METHODS

Participants

A sample of 437 participants was selected from the Shiley-Marcos Alzheimer's Disease Research Center (ADRC) of the University of California, San Diego (UCSD). An independent sample of 3982 participants evaluated at other ADRCs was selected from the National Alzheimer's Coordinating Center (NACC). Inclusion was limited to participants who had undergone genotyping and a neuropathological assessment at autopsy. All data were collected through the NACC uniform data set, minimum data set, or neuropathology data set, except where otherwise specified. For submission of data to the NACC, each local institutional review board approved the research protocol and written informed consent was obtained from each participant or participant's guardian.

Pathological Diagnosis

The 4419 participants were categorized based on diagnostic criteria for AD, DLB, frontotemporal lobar degeneration (FTD), medial temporal lobe sclerosis (MTLS), and other major pathological diagnoses as follow.

AD: If Thal phase was assessed, an "ABC" score indicating intermediate or high ADNC [15] constituted an AD diagnosis. Otherwise, pathological diagnosis of AD followed NIA-Reagan criteria (i.e., at least Consortium to Establish a Registry for Alzheimer's Disease (CERAD) moderate and Braak stage III/IV) [16].

DLB: Pathological diagnosis of DLB followed criteria outlined in the fourth consensus report of the DLB Consortium (i.e., requires limbic (transitional) or diffuse neocortical Lewy-related pathology) [6].

MTLS: MTLS (including hippocampal sclerosis) was determined based on pathologist report to the NACC as present or absent.

FTD and other tauopathies: Evidence of FTD with tau pathology (including Pick's disease, corticobasal degeneration, and progressive supranuclear palsy), FTD and parkinsonism with tau-positive or argyrophilic inclusions, other tauopathies (including tangle-only dementia and argyrophilic grain dementia), FTD with ubiquitin-positive (tau-negative) inclusions, FTD with TDP-43 pathology, and FTD with no distinctive histopathology present or not otherwise specified constituted an FTD pathological diagnosis.

Other Pathological Diagnoses: Cases with "other pathological diagnoses" were excluded. Specifically, in versions 1-9 of the NACC neuropathology data, this constituted evidence of prion-related disorders and other major pathologic disorders (e.g. infectious, immunologic, metabolic, neosplastic, toxic, or degenerative). In version 10, this constituted ALS/motor neuron

disease, Pigment-spheroid degeneration/NBIA, multiple system atrophy, prion disease, trinucleotide disease (Huntington disease, SCA, or other), malformation of cortical development, metabolic/storage disorder, leukodystrophy, multiple sclerosis or other demyelinating disease, contusion/traumatic brain injury of any type (acute or chronic), neoplasm (primary or metastatic), infectious process of any type (encephalitis, abscess, etc), herniation (any site), or other pathologic diagnosis, Down syndrome, AD-related genes (dominantly inherited), FTLD related genes (dominantly inherited), or other known genetic mutation. Neuron loss in the substantia nigra was additionally considered except in the case of a DLB pathological diagnosis.

To disentangle the effects of genetic risk on pathology in light of frequently occurring co-pathology, we restricted analysis to individuals who met the above criteria for only AD (n=1854), DLB (n=57), or FTD (n=65) without meeting criteria for any other pathological diagnosis, those who met the criteria for both AD and DLB but no other pathological diagnosis (AD+DLB, n=455), and those who met the criteria for both AD and MTLS but no other pathological diagnosis (AD+MTLS, n=182). Also, individuals who did not meet criteria for any of the above pathological diagnoses were included (control, n=245). This lead to the exclusion of 1561 participants based on pathological criteria, either with mixed pathology inconsistent with the above groups, or with pathology documented in a way that precluded categorization (e.g. LBs present in an unspecified region).

Clinical Diagnosis

Clinical diagnosis was assessed at the final visit before death. Individuals in the control group (who did not meet criteria for any of the pathological diagnoses) were further limited to clinically normal participants without PD, resulting in the exclusion of 68 additional participants.

For all other pathologically defined groups, no clinical criteria were imposed except to exclude individuals with PD dementia, following the one-year rule, excluding an additional 14 participants (1 DLB and 13 AD+DLB), and those with Parkinsonism who did not meet pathological criteria for DLB, excluding an additional 63 participants (55 AD, 7 AD+MTLS, and 1 FTD).

Genetic Data

Genetic data for UCSD ADRC participants was accessed through the NACC database or obtained locally. Alzheimer's disease Genetics Consortium (ADGC) data for participants evaluated at other ADRCs was accessed through the National Institute on Aging Genetics of Alzheimer's disease Data Storage Site (NIAGADS). All participants were genotyped using a commercially available Illumina BeadChip array. Genetic data was imputed using the Michigan Imputation Server [17].

AD Polygenic Hazard Score Calculation

The PHS was calculated as described for all participants [18]. Briefly, AD-associated single-nucleotide polymorphisms (SNPs) were identified in the International Genomics of Alzheimer's Project (IGAP) cohort at $p < 10^{-5}$. These SNPs were then integrated into a stepwise Cox proportional hazards model using a subset of the ADGC phase 1 genetic data, excluding individuals from the NACC. This stepwise procedure identified 31 SNPs that most improved the model prediction. The PHS used in the current study was calculated for each participant as the vector product of that individual's genotype for the 31 SNPs and the corresponding parameter estimates from the ADGC phase 1 Cox proportional hazard model, in addition to the *APOE* effects.

PD Polygenic Risk Score Calculation

The PD PRS was calculated for each participant as the vector product of that individual's genotype for the 90 independent genome-wide significant variants identified by the most recent meta-analysis of PD genome-wide association study (GWAS) data and the corresponding parameter estimates using data from all available studies [19].

DLB Polygenic Risk Score Calculation

The DLB PRS was calculated as the vector product of that individual's genotype for the 5 independent genome-wide significant variants identified by the first DLB GWAS and the corresponding parameter estimates from the discovery stage [12]. To ensure there was no participant overlap between samples, the DLB PRS was only analyzed in the subset of 2282 participants in the present study who were assuredly not included in the DLB GWAS.

Unified Parkinson Disease Rating Scale (UPDRS)

The UPDRS part III was used to quantify the extent of motor manifestations of PD. We specifically examined the items concerning resting tremor (of the face, hands, or feet), rigidity (of the neck, upper extremities, or lower extremities), posture stability, and bradykinesia.

Statistical Analysis

Binary logistic regression was used to examine the relationship between the PD PRS and a clinical diagnosis of PD, controlling for age at death and sex. Ordinal logistic regression models were used to examine the relationship between the PD PRS and the UPDRS item scores, controlling for age at death and sex, and Bonferroni corrected for multiple comparisons. Brant's

test was used to test the proportional odds assumption. Proportional odds models were used except in the case where the proportional odds assumption was violated, in which cases partial proportional odds models were used.

Clinical and demographic differences between pathologically defined groups were examined using either Welch's two sample t-test or Pearson's chi-squared test as appropriate, corrected for multiple comparisons using the Benjamini-Hochberg procedure. *APOE* ε4 allele frequency was examined in across pathologically defined groups using Pearson's chi-squared test. Pairwise comparisons between groups were corrected for multiple comparisons using the Benjamini-Hochberg procedure.

Multinomial logistic regression models were used to examine the relationship between the AD PHS, PD PRS, or DLB PRS and the pathological diagnosis group, controlling for age at death and sex, and Bonferroni corrected for three comparisons. Given that the *APOE* ε4 allele is a known risk factor for both AD and DLB, multinomial logistic regression models were used to examine the relationship between either the AD PHS or DLB PRS without its *APOE* component weights and the pathological diagnosis group, controlling for age at death and sex.

Ordinal logistic regression models were used to examine the relationship between either the AD PHS, PD PRS, or DLB PRS and AD pathological outcome variables (i.e, Braak stage for neurofibrillary tangles or CERAD score for neuritic plaques), controlling for age at death and sex. Multicollinearity was evaluated. Brant's test was used to test the proportional odds assumption. Proportional odds models were used except in the case where the proportional odds assumption was violated, in which cases partial proportional odds models were used. Binary logistic regression models were used to examine the relationship between the AD PHS, PD PRS, or DLB PRS and the presence of at least limbic (transitional) Lewy-related pathology,

controlling for age at death and sex. For each pathological outcome variable, results were Bonferroni corrected for three comparisons. To examine the effect of the AD PHS and DLB PRS on pathology beyond *APOE*, analyses were repeated using versions of the AD PHS or DLB PRS without its *APOE* component weights.

Data Availability

The datasets supporting the conclusions of this article are available in the NACC (alz.washington.edu) or NIAGADS (niagads.org) repositories. A request for resources, materials, or participant referrals from the Shiley-Marcos ADRC can be made by emailing Christina Gigliotti, Ph.D at cgigliotti@ucsd.edu.

RESULTS

In all 4419 participants, regardless of pathological diagnosis, the PD PRS predicted a clinical diagnosis of PD (odds ratio (OR) = 1.52, 95% confidence interval (CI) = 1.16–1.98, p =0.002). 3661 participants were evaluated on the UPDRS part III. The PD PRS was not associated with motor symptom severity in any of the 12 items examined.

2713 participants were categorized into one of six pathologically defined groups: FTD (n=64), DLB (n=56), AD+DLB (n=442), AD (n=1799), AD+MTLS (n=175) or control (n=177). Clinical and demographic characteristics of these pathologically defined groups are reported in Table 3.1. Notable in Table 3.1 is the following: the age at death in each pathologically defined group, with the exception of AD+MTLS, was younger than in the control group, more men were in the DLB and AD+DLB groups than in the other groups, all AD groups (AD, AD+DLB, and AD+MTLS) had worse cognitive impairment than the FTD or DLB groups, and, within AD

groups, those with mixed pathology (AD+DLB or AD+MTLS) had worse cognitive impairment than those with only AD.

APOE ε4 allele frequency was different between pathologically defined groups (overall $\chi^2 = 174.5$, $p < .001$, Table 3.2). Pairwise chi-squared tests revealed that *APOE* ε4 allele frequency was greater in all AD groups (AD, AD+DLB, and AD+MTLS) and the DLB group than in the control group (FDR adjusted $p < .05$). Additionally, *APOE* ε4 allele frequency was greater in all AD groups than in either the FTD or DLB groups (FDR adjusted $p < .001$).

Higher AD PHS was associated with increased relative risk ratios for the AD, AD+DLB, and AD+MTLS pathological diagnosis groups, compared to the control group ($p < .001$, Table 3.2 and Figure 3.1). Higher AD PHS was also associated with increased relative risk ratios for the DLB and FTD groups compared to the control group ($p < .01$), though with lower relative risk ratios than any of the AD groups, as confirmed by non-overlapping confidence intervals. Higher PD PRS was not associated with significant increased relative risk ratios for any pathological group compared to the control group. Higher DLB PRS was associated with increased relative risk ratios for the AD, AD+DLB, AD+MTLS, and DLB groups compared to the control group ($p < .001$).

Without the *APOE* ε4 or ε2 dosage weights, we observed similar results for the AD PHS, which was associated with increased relative risk ratios for the AD (1.86 95% CI [1.47–2.37]), AD+DLB (1.83 [1.39–2.40]), AD+MTLS (1.91 [1.37–2.65]), DLB (2.03 [1.26–3.26]), and FTD (1.78 [1.14–2.78]) groups compared to the control group. Without the *APOE* component weight, the specificity of the DLB PRS emerged, as it was only associated with increased relative risk ratios for the DLB (3.58 [1.20–10.66]) and AD+DLB (3.15 [1.47–6.77]) groups compared to the control group.

The odds of having tau pathology at or above a given Braak stage increased with the AD PHS ($p < .001$, Table 3.3 and Figure 3.2). The PD PRS was not associated with Braak stage. Beginning with Braak stage II, the odds of having tau pathology at or above a given Braak stage increased with DLB PRS ($p < .001$). Without the *APOE* ε4 or ε2 dosage weights, we observed similar results for the association between Braak stage and the AD PHS (OR 1.20 95% CI [1.08–1.34]). Yet, without the *APOE* component weight, the DLB PRS was not associated with Braak stage (0.89 [0.73–1.08]).

The odds of having neuritic plaques at or above a given density increased with both the AD PHS and DLB PRS ($p < .001$, Table 3.3 and Figure 3.2). The PD PRS was not associated with neuritic plaque density. Without the *APOE* ε4 or ε2 dosage weights, we observed similar results for the association between the AD PHS and neuritic plaque density (OR 1.25 95% CI [1.10–1.43]). Conversely, without the *APOE* component weight, the odds of having neuritic plaques at or above a given density decreased with increasing DLB PRS (OR 0.76 [0.60–0.96])

None of the polygenic scores were associated with the presence of at least limbic (transitional) LB pathology (Table 3.3 and Figure 3.2). Similarly, without the *APOE* ε4 or ε2 dosage weights, the AD PHS was not associated with the presence of at least limbic (transitional) LB pathology (OR 1.04 95%CI [0.89–1.22]). However, without the *APOE* component weight, the DLB PRS was associated with the odds of having at least limbic (transitional) LB pathology (3.41 [1.93–6.03]).

DISCUSSION

We tested whether genetic risk for AD, DLB, or PD may be useful to clinically distinguish between AD and DLB. The AD and DLB polygenic scores were associated with their

respective pathological diagnostic categories, though not exclusively. We replicated the finding of an overrepresentation of the *APOE* ε4 allele in the pure DLB group compared to controls [10], but found that the ε4 allele frequency was higher in all AD groups (AD, AD+DLB, or AD+MTLS) than in the DLB group. Given this increased frequency and the strong, dose-dependent risk of the ε4 allele in AD, the inclusion of an *APOE* weight in the DLB PRS diminished its ability to specifically predict LB pathology. When the *APOE* weight was removed, the DLB PRS was associated with only DLB and AD+DLB pathological diagnosis groups, and was associated with increased LB but not AD pathology.

However, the dose-dependent weighting of the *APOE* ε4 allele is an important feature of the AD PHS, and enabled the distinction between AD groups and the non-AD groups. When removing the *APOE* ε2 and ε4 dosage weights, the AD PHS maintained its association with Braak stage and neuritic plaque density, but it also maintained associations with all pathological groups relative to the control group. This suggests that the AD PHS may capture risk for processes such as inflammation, synaptic function, and epigenetic regulation disrupted in both AD and non-AD dementias.

The PD PRS was associated with a clinical PD diagnosis, but not any pathological diagnosis or variable examined in this study. These results align with recent work finding genetic risk for PD explained only a small amount (.37%) of variance in DLB [20]. In this heterogeneous cohort, the PD PRS was not associated with motor symptom severity on the UPDRS part III items, but the majority (67%) of those participants with abnormal UPDRS scores did not have any LB pathology. It is known that motor symptoms in AD can exist outside of associations with LBs [21,22], in which case it is unlikely the PD PRS would be a useful predictor.

Despite striking group-level results, one limitation to the clinical utility of this work is the amount of individual variation in polygenic scores within pathological groups. It remains unclear whether assessing polygenic risk for AD and DLB in combination with clinical features and biomarkers improves the accuracy of clinical diagnoses at the individual level. Further, the polygenic scores used in these analyses were predominantly derived and tested on individuals of European ancestry. Known differences in genetic risk across racial and ethnic groups [23–25] suggest these findings may not generalize. Future work is required to develop polygenic scores in diverse populations.

Despite few identified genome-wide significant variants, the DLB PRS without the *APOE* component weight was specifically associated with LB pathology and a pathological diagnosis of DLB, either alone or in combination with AD. The AD PHS was associated with ADNC but not LB pathology, and most strongly predicted a pathological diagnosis of AD, either alone or in combination with DLB or MTLS. Together, these results and the lack of associations with the PD PRS align with evidence that genetic risk for DLB is not simply situated in the middle of an AD-PD continuum, but has a distinct signature that can be exploited along with risk for AD to improve clinical diagnoses.

Table 3.1 Demographics and clinical characteristics split by pathological diagnosis group. Reported as mean (SD) unless otherwise noted. Primary clinical diagnosis of AD included probable or possible AD, LBD included DLB, Lewy body variant of AD, and LBD, and FTD included FTD, Pick's disease, CBD, PSP, and PPA. FDR adjusted $p < .05$ for differences from *Control, †FTD, ‡DLB, §AD+DLB, ¶AD, or #AD+MTLS based on pairwise Pearson's chi-squared tests or Welch's t-tests. Small numbers in certain subgroups prevented the pairwise comparison of primary clinical diagnoses across pathologically defined groups.

	Pathological Diagnosis Group					
	Control	FTD	DLB	AD+DLB	AD	AD+MTLS
Participants, N	177	64	56	442	1799	175
Women, N (%)	94 (53)	25 (39)	12 (21)*§¶#	181 (41)*¶#	959 (53)	94 (54)
Age at Death, y	83.7 (8.7)	78.5 (11.5)*#	80.1 (8.6)*#	79.0 (8.5)*#¶	80.2 (9.0)*#	84.6 (7.8)
Caucasian, N (%)	173 (98)	63 (98)	56 (100)	427 (97)	1736 (96)	171 (98)
Hispanic, N (%)	4 (2)	0 (0)	1 (2)	7 (2)	30 (2)	1 (1)
Education, y	15.4 (2.8)	15.1 (3.0)	15.6 (3.3)	15.0 (3.2)	14.3 (3.3)*‡§	14.8 (3.6)
Final Clinical Evaluation						
Time Before Death, y	2.6 (3.0)	3.0 (3.0)	2.9 (3.5)	3.0 (3.3)	2.5 (2.8)§#	3.2 (3.1)
Primary Clinical Diagnosis						
AD, N (%)	0 (0)	22 (34)	19 (34)	340 (77)	1574 (87)	159 (91)
LBD, N (%)	0 (0)	2 (3)	24 (43)	79 (18)	41 (2)	4 (2)
FTD, N (%)	0 (0)	23 (36)	1 (2)	9 (2)	64 (4)	5 (3)
Global CDR	0.1 (0.2)	1.6 (1.2)*	1.4 (1.0)*	2.1 (0.9)*†‡	1.9 (1.0)*†‡§#	2.2 (0.8)*†‡
CDR-SB	0.1 (0.4)	9.0 (6.8)*	7.6 (5.8)*	12.5 (5.5)*†‡	10.8 (6.1)*†‡§#	12.9 (4.9)*†‡

Table 3.2 *APOE* genotypes and allele frequencies by pathologically defined groups. *APOE* ε4 allele frequency was different between pathologically defined groups (overall $\chi^2 = 174.5$, $p < .001$). FDR adjusted $p < .05$ for differences in ε4 allele frequency from *Control, † All AD groups (AD, AD+DLB, and AD+MTLS) based on pairwise chi-squared tests.

	N (%)						
	***APOE* Genotype Frequency**						
	2/2	**2/3**	**2/4**	**3/3**	**3/4**	**4/4**	**ε4 Allele Frequency**
Control	1 (1)	25 (14)	7 (4)	119 (67)	24 (14)	1 (1)	33 (9)
FTD	0 (0)	7 (11)	1 (2)	37 (58)	19 (30)	0 (0)	20 (16)†
DLB	0 (0)	8 (14)	4 (7)	28 (50)	16 (29)	0 (0)	20 (18)*†
AD+DLB	2 (0)	11 (2)	5 (1)	136 (31)	224 (51)	64 (14)	357 (40)*
AD	0 (0)	52 (3)	51 (3)	594 (33)	829 (46)	273 (15)	1426 (40)*
AD+MTLS	0 (0)	8 (5)	5 (3)	58 (33)	83 (47)	21 (12)	130 (37)*

Table 3.3 Associations between the polygenic scores and pathological diagnostic categories and variables. Results of multinomial, ordinal, and binary logistic regression models for each polygenic score and pathological diagnosis group and outcome variable are displayed. For ordinal logistic regressions, proportional odds models were used except in the case where the proportional odds assumption was violated, in which cases partial proportional odds models were used. Significance was set to $p < .017$, Bonferroni corrected for comparisons across the three polygenic scores.

	AD PHS		**PD PRS**		**DLB PRS**	
Pathological Diagnosis	*RRR (95% CI)*	*p-value*	*RRR (95% CI)*	*p-value*	*RRR (95% CI)*	*p-value*
MLR, Control as Reference						
FTD	1.60 (1.16–2.21)	.004	1.01 (.60–1.70)	.97	1.24 (.76–2.02)	.40
DLB	1.73 (1.23–2.42)	.002	1.39 (.80–2.41)	.24	3.22 (1.62–6.40)	< .001
AD+DLB	3.05 (2.47–3.77)	< .001	.93 (.67–1.28)	.65	4.47 (2.76–7.22)	< .001
AD	3.06 (2.52–3.71)	< .001	.92 (.69–1.22)	.57	3.08 (2.33–4.09)	< .001
AD+MTLS	3.14 (2.45–4.02)	< .001	.95 (.65–1.39)	.78	2.94 (2.07–4.18)	< .001
	OR (95% CI)	*p-value*	*OR (95% CI)*	*p-value*	*OR (95% CI)*	*p-value*
Braak Stage						
OLR, POM	-	-	0.93 (0.82–1.05)	.24	-	-
OLR, PPOM						
I	2.34 (1.60–3.42)	< .001	-	-	1.89 (1.06–3.39)	.03
II	2.10 (1.76–2.50)	< .001	-	-	2.05(1.52–2.76)	< .001
III	2.28 (1.99–2.62)	< .001	-	-	2.33 (1.88–2.89)	< .001
IV	1.88 (1.68–2.10)	< .001	-	-	1.95 (1.63–2.33)	< .001
V	1.53 (1.40–1.68)	< .001	-	-	1.50 (1.30–1.72)	< .001
VI	1.33 (1.23–1.44)	< .001	-	-	1.34 (1.18–1.51)	< .001
Neuritic Plaque Density						
OLR, POM	-	-	0.85 (0.73–0.99)	.04	-	-
OLR, PPOM						
Sparse	2.69 (2.21–3.26)	< .001	-	-	2.79 (1.95–3.98)	< .001
Moderate	2.42 (2.07–2.84)	< .001	-	-	2.42 (1.88–3.12)	< .001
Frequent	1.71 (1.55–1.88)	< .001	-	-	1.63 (1.41–1.88)	< .001
Lewy Pathology Stage						
BLR	1.03 (0.93–1.14)	.52	1.04 (0.87–1.24)	.66	1.45 (1.04–2.03)	.03

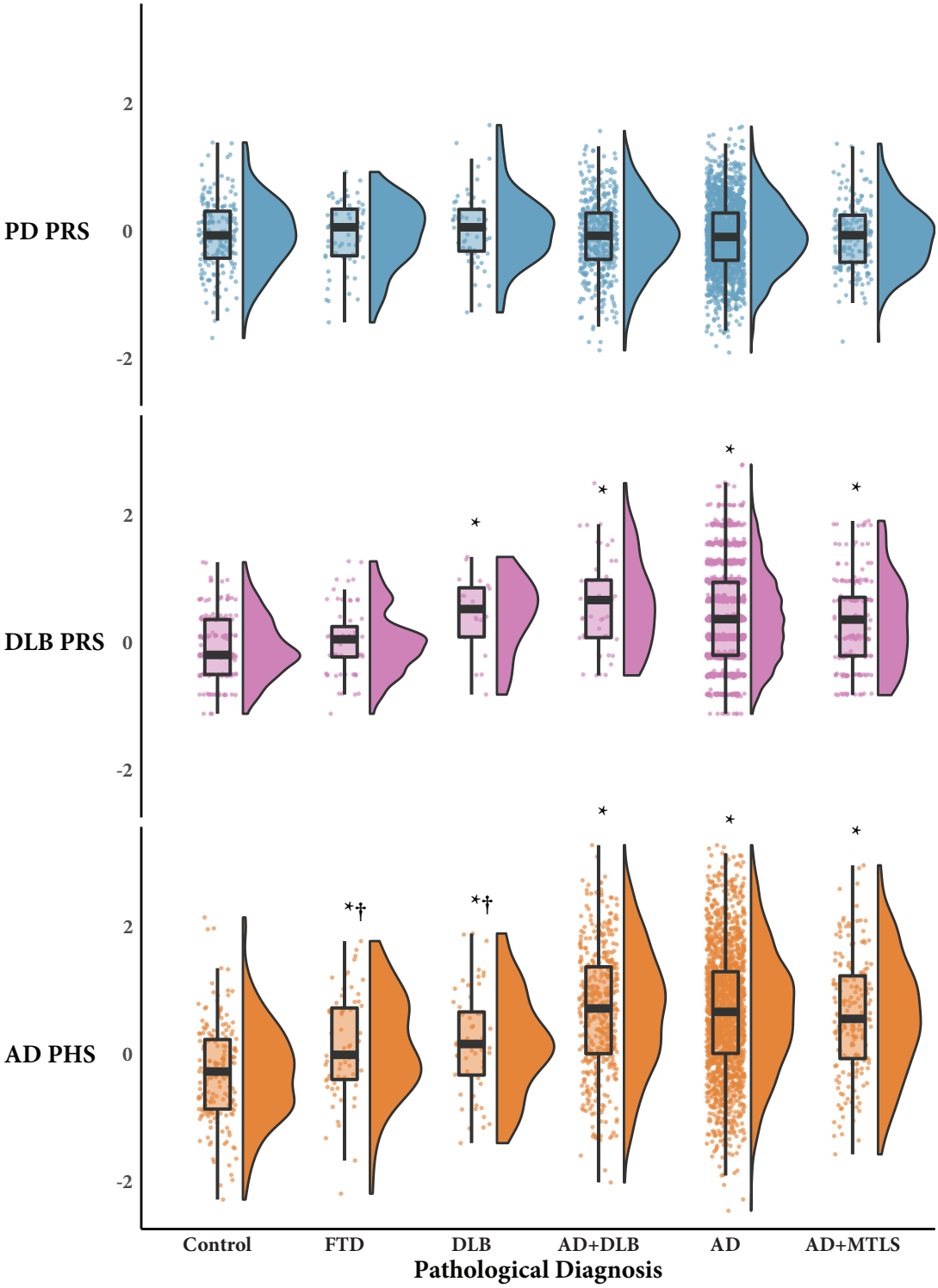

Figure 3.1 Relationship between polygenic scores and pathological diagnostic categories. Significant difference from: *Control, †All AD groups (AD, AD+DLB, and AD+MTLS) based on Bonferroni corrected multinomial regression models.

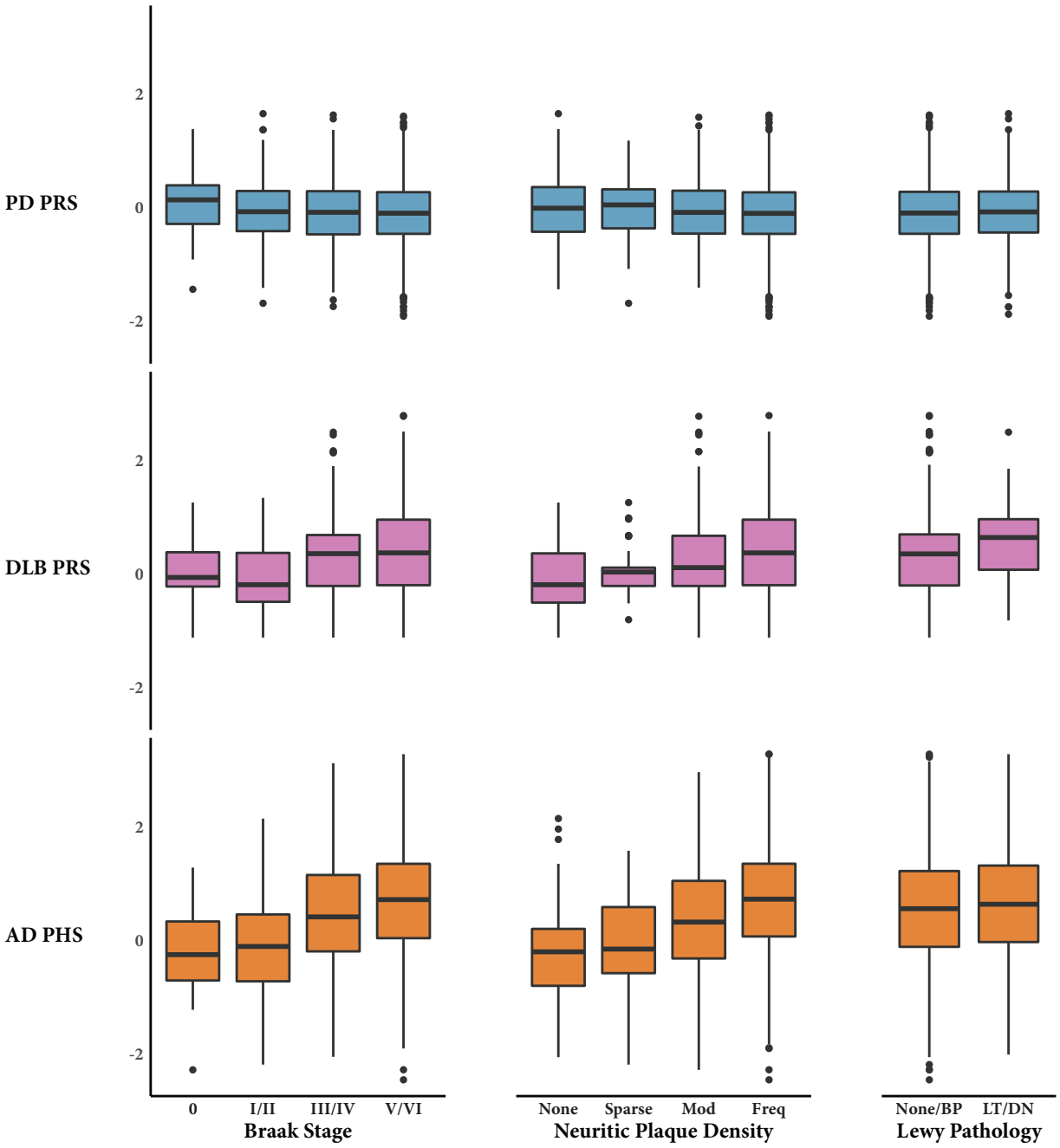

Figure 3.2 Relationship between polygenic scores and pathological variables. Graphical visualization of the relationship between the PD, DLB, and AD polygenic scores and measures of AD (Braak stage and neuritic plaque density) and Lewy pathology. Results of ordinal and binary logistic regression models for each polygenic score and pathological outcome variable are included in Table 3.3.

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