Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Minimal levels of amyloid accumulation in the brains of cognitively unimpaired individuals are associated with long-term cognitive impairment up to 20 years later

Chengjie Xiong

chengjie@wustl.edu

Washington University in St. Louis

Jingqin Luo

Washington University School of Medicine https://orcid.org/0000-0003-2759-3072

Sarah Hartz

Washington University School of Medicine - in St. Louis

Suzanne Schindler

Washington University School of Medicine https://orcid.org/0000-0002-1680-1465

Walter Kukull

Washington University School of Medicine - in St. Louis https://orcid.org/0000-0001-8761-9014

Jason Hassenstab

Washington University in St. Louis School of Medicine

David Wolk

Hospital of the University of Pennsylvania

Carey Gleason

University of Wisconsin School of Medicine

Sterling Johnson

wisc https://orcid.org/0000-0002-8501-545X

Reisa Sperling

Harvard Aging Brain Study, Department of Neurology, Massachusetts General Hospital, Boston, MA 02114;Center for Alzheimer Research and Treatment, Department of Neurology, Brigham and Women's https://orcid.org/0000-0003-1535-6133

Keith Johnson

Massachusetts General Hospital, Harvard Medical School

Erik Roberson

University of Alabama at Birmingham https://orcid.org/0000-0002-1810-9763

Krista Moulder

Washington University School of Medicine - in St. Louis

Emily Gremminger
Washington University School of Medicine - in St. Louis
Folasade Agboola
Washington University School of Medicine - in St. Louis
Elizabeth Grant
Washington University School of Medicine - in St. Louis
Russ Hornbeck
Washington University School of Medicine - in St. Louis
Parinaz Massoumzadeh
Washington University School of Medicine - in St. Louis
Sarah Keefe
Washington University School of Medicine - in St. Louis
Donna Dierker
Washington University School of Medicine - in St. Louis
David Holtzman
Washington University in St Louis School of Medicine https://orcid.org/0000-0002-3400-0856
John Morris
Washington University School of Medicine
Tammie Benzinger
Washington University in St. Louis https://orcid.org/0000-0002-8114-0552

Article

Keywords:

Posted Date: March 3rd, 2026

DOI: https://doi.org/10.21203/rs.3.rs-8928856/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: Yes there is potential Competing Interest. SES has not received any financial compensation from pharmaceutical or diagnostics companies since 2024, when she received honoraria for serving on scientific advisory boards on biomarker testing and education for Eisai and Novo Nordisk and speaking fees from Eisai, Eli Lilly, and Novo Nordisk. She has recently received honoraria for educational presentations from Medscape, PeerView, and the Academy for Continued Healthcare Learning. She has provided unpaid scientific advising to Acumen, Biogen, Cognito Therapeutics, Danaher, Eisai, Eli Lilly, Johnson and Johnson Innovative Medicine, Sanofi, and Siemens. DAW has served as a paid consultant for Beckman Coulter and serves on a DSMB for GSK. ER serves on a data monitoring committee for Eli Lilly. TB participates as a site investigator in clinical trials sponsored by Avid

Radiopharmaceuticals, Eli Lilly and Company, Biogen, Eisai, Janssen, and Roche. TB participates as a site investigator in clinical trials sponsored by Avid Radiopharmaceuticals, Eli Lilly and Company, Biogen, Eisai, Janssen, and Roche. RAS has served as a consultant for AbbVie, AC Immune, Acumen, Alector, Apellis, Biohaven, Bristol Myers Squibb, Ionis, Janssen, Oligomerix, Prothena, Roche and Vaxxinity over the past 3 years, and has received research funding from Eisai and Eli Lilly for public-private partnership trials. DMH co-founded, has equity, and is on the scientific advisory board of C2N Diagnostics. DMH is on the scientific advisory boards of Denali, Genentech, and Switch and consults for Novartis and Pfizer. SCJ has served on advisory boards for Lantheus, Lilly, Enigma, Alamar, Alzpath and 331 Trillium bio. JH has served as a paid consultant for Prothena, Abbvie, and Quanterix. The other Authors declare no Competing Financial or Non-Financial Interests. This work was supported in part by funding from the National Institutes of Health (grant #AG067505). WU has a financial interest in C2N Diagnostics and may financially benefit if the company is successful in marketing its product(s) that is/are related to this research.

Minimal levels of amyloid accumulation in the brains of cognitively unimpaired individuals are associated with long-term cognitive impairment up to 20 years later

Chengjie Xiong, PhD[A,B,*], Jinqing Luo, PhD[C,D,*], Sarah Hartz, MD, PhD[B], Suzanne E. Schindler, MD, PhD[B,E], Walter Kukull, PhD[B,E], Jason Hassenstab, PhD[B,E], David A. Wolk, MD[F], Carey E. Gleason, PhD[G,H,I], Sterling C. Johnson, PhD[G, H, I], Reisa Sperling, MD[J], Keith Johnson, MD[K], Erik D. Roberson, MD, PhD[L], Krista L. Moulder, PhD[B,E], Emily Gremminger[A], Folasade Agboola, MPH[A], Elizabeth A. Grant, PhD[A], Russ Hornbeck, MSc[M], Parinaz Massoumzadeh, PhD[B,M], Sarah Keefe[M], Donna Dierker[M], David Holtzman, MD[B,E] , John C. Morris, MD[B,E,#] , Tammie Benzinger, MD, PhD[B,M,#]

A. Institute for Informatics, Data Science, and Biostatistics, Washington University School of Medicine in St. Louis, St. Louis, MO, USA.
B. Knight Alzheimer Disease Research Center, Washington University School of Medicine in St. Louis, St. Louis, MO, USA.
C. Division of Public Health Sciences, Department of Surgery, Washington University School of Medicine, St. Louis, MO, USA
D. Siteman Cancer Center Biostatistics and Qualitative Research Shared Resource, Washington University School of Medicine, St. Louis, MO, USA
E. Department of Neurology, Washington University School of Medicine in St. Louis, St. Louis, MO, USA.
F. Department of Neurology, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA, USA

G. Division of Geriatrics and Gerontology, Department of Medicine, University of Wisconsin School of Medicine and Public Health, Madison, Wisconsin, USA.
H. Wisconsin Alzheimer's Disease Research Center, Madison, Wisconsin, USA.
I. Geriatric Research, Education and Clinical Center, William S. Middleton Memorial Veterans Hospital, Madison, Wisconsin, USA.
J. Department of Neurology, Center for Alzheimer Research and Treatment, Brigham and Women's Hospital, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA.
K. Department of Neurology, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA.
L. Killion Center for Neurodegeneration and Experimental Therapeutics, Alzheimer's Disease Center, Department of Neurology, University of Alabama at Birmingham, Birmingham, AL, USA
M. Mallinckrodt Institute of Radiology, Washington University School of Medicine in St. Louis, St. Louis, MO, USA

*Both CX and JL contributed equally to the manuscript and are co-first authors.

#Both TB and JCM contributed equally to the manuscript and are co-senior authors.

Corresponding author:

Chengjie Xiong (chengjie@wustl.edu)
Division of Biostatistics
Washington University School of Medicine in St. Louis
St. Louis, MO, USA

41

Abstract

The minimal amyloid accumulation (MAA) in cognitively unimpaired individuals that predicts the onset of cognitive impairment remains unknown. In a multi-center amyloid positron emission tomography (PET) study of 1834 cognitively unimpaired participants with longitudinal clinical/cognitive assessments for up to 20.3 years, we found that 48.7% of the amyloid-negative participants with MAA starting from an amyloid PET centiloid of 1.03 had a higher risk of cognitive impairment than those with the lowest centiloid values (<1.03; hazards ratio (HR)=1.40, p=0.0046), although their risk was clearly lower than amyloid-positive participants (centiloid>20; HR=0.47, p<0.0001). Amyloid positivity was also associated with the fastest longitudinal amyloid accumulation and cognitive decline, followed by the MAA, and finally by the lowest amyloid. These findings suggest that current thresholds of amyloid positivity used for Alzheimer disease diagnosis may be overly restrictive, potentially excluding a large portion of amyloid-negative participants at elevated risk of developing cognitive impairment from prevention trials.

Main

Amyloid plaques are a hallmark of Alzheimer disease (AD) pathology. A positive amyloid positron emission tomography (PET) scan is often required to diagnose AD dementia and to enroll individuals into both treatment trials and secondary prevention trials where change in amyloid burden from baseline also serves as a major efficacy endpoint. The definition of a positive amyloid PET scan is operationalized using a single threshold for a composite measure of amyloid burden in the brain, the amyloid PET centiloid. Despite different thresholds used by different studies, it has been consistently reported that a positive amyloid PET scan is sufficient for significant association with worse cognition[1-3] and higher risk of developing cognitive impairment[4-6]. As roughly less than 30% of cognitively unimpaired middle aged and older individuals are amyloid-positive[7-8], a fundamental question remains: what is the minimal or necessary level of amyloid burden in the brains of cognitively unimpaired individuals that predicts the subsequent development of cognitive impairment? The question is important because its answer may facilitate the identification of cognitively unimpaired individuals who may be amyloid-negative by the current thresholds but are at an elevated risk of developing cognitive impairment.

In this study, we harmonized amyloid PET and clinical/cognitive data across six studies of memory and aging conducted by five institutes: Washington University (WashU), University of Pennsylvania (UPenn), University of Wisconsin (Wisconsin), University of Alabama at Birmingham (UAB), and Harvard University, and examined the ability of a wide range of sub-thresholds below the typically used threshold for amyloid positivity (centiloid 20) to predict the subsequent development of cognitive impairment among cognitively unimpaired individuals.

Results

Baseline characteristics and summary of longitudinal follow up

The longitudinal study included a total of 1834 participants who were cognitively unimpaired (1644 CDR 0 and 190 diagnosed with normal cognition) at baseline. Baseline demographics and clinical characteristics, *APOE* genotype, and amyloid PET centiloid, and summary of longitudinal clinical assessments are provided (**Table 1**). The baseline age ranged from 42 to 95 years with a mean of 68.3 years (SD=8.84 years). The mean years of education were 16.1 years (SD=2.55 years). About 61.5% of the participants were female. Most participants (85.1%) were White, and about 12.7% self-identified as Black or African American. About 34% carried an *APOE* ε4 allele. About 40.1% of the participants had a history of at least one vascular comorbidities (hypertension, diabetes, stroke, and obesity). The duration of longitudinal clinical assessments ranged from 0.2 years to 20.3 years with a mean of 7.27 years (SD=4.67 years). The mean interval

between the baseline amyloid PET and baseline clinical assessment was 0.38 years (SD=0.3 years). A total of 477 participants went on to develop cognitive impairment, while the other 1357 participants remained cognitively unimpaired during the follow up.

Distribution of baseline PET centiloid and the number of participants who subsequently developed cognitive impairment

The distribution of the baseline PET centiloid is in **Supplemental Figure 1**, suggesting at least a bi-modal distribution among cognitively unimpaired individuals whose component distributions may differentially predict the risk of clinical progression. The estimated percentiles from the 10^{th} to 70^{th} of amyloid PET centiloid in increment of 5 percentiles are in **Supplemental Table 1**. The median centiloid was 3.21 which split the entire cohort into 50% each. The 78^{th} percentile corresponded to a PET centiloid of 20 which was conventionally used to define amyloid positivity. For each sub-threshold from the 10^{th} to 70^{th} percentile with an increment of 5 percentiles, participants were classified at baseline into three groups by their amyloid burden: the lowest amyloid group (L; <the threshold), the middle/intermediate amyloid group (M; between the threshold and centiloid 20), and the amyloid-positive group (P; centiloid≥20). **Supplemental Table 1** also contains the sample size (N) for each subgroup (L, M, or P) and the number of 'progressors' (NP) who went on to develop cognitive impairment.

Very low levels of amyloid associated with elevated risk of developing cognitive impairment

For each sub-threshold (the x-axis) that binarized PET centiloid distribution, **Figure 1** presents the estimated HR of developing cognitive impairment between the higher (>the threshold) and lower (<= the threshold) amyloid groups of participants without (top panel) and with (lower panel) adjustments for major covariates, along with the corresponding 95% confidence intervals (CI). When the threshold was -3.65 which corresponded to the 20th percentile of the baseline centiloid distribution (**Supplemental Table 1**), the top 80% of the participants with higher amyloid burden had a 41% (hazard ratio (HR)=1.41, , 95% CI=1.09 to 1.82, from the unadjusted analyses, FDR p=0.010) and 35% (HR=1.35, 95% CI=1.04 to 1.75, from the adjusted analyses, FDR p=0.03) increased risk to subsequently develop cognitive impairment than those with lower amyloid burden. This sub-threshold (20th percentile at centiloid -3.65) was the lowest PET centiloid that demonstrated a statistically significant increase in the risk of progression to cognitive impairment for the higher amyloid group (in comparison to the lower amyloid group) from both the unadjusted and adjusted analyses. As expected, when the threshold was centiloid 20, the amyloid-positive participants had 236% (HR=3.36, 95% CI: 2.78 to 4.05, FDR P<0.0001 from the unadjusted analyses) and 147% (HR=2.47, 95% CI: 2.00 to 3.04, FDR P<0.0001 from the adjusted analyses) increased risk in developing cognitive impairment, as compared to amyloid-negative participants. When thresholds from the 25th percentile to the 70th percentile were used to binarize the PET centiloid distribution, the estimated HR showed a generally increasing trend (**Figure 1**, **Supplemental Table 1**), indicating that participants with the higher

baseline amyloid burden continued to demonstrate a significantly higher risk of progression to cognitive impairment than those with the lower amyloid burden from both unadjusted and adjusted analyses. The adjusted HR ranged from 1.49 to 2.49, implying at least 49% and up to 149% increased risk.

The minimal PET centiloid level associated with elevated risk of progression to cognitive impairment

Because the higher amyloid group in the PET centiloid dichotomy by each sub-threshold (from 10^{th} to 70^{th} percentile; **Figure 1**) included amyloid-positive participants as a subset, the elevated risk of clinical progression may be attributable to the amyloid-positive participants. To examine whether a minimal amyloid accumulation (MAA) existed in the continuum of amyloid burden in predicting cognitive impairment, we further binarized the amyloid-negative participants by each possible sub-threshold (from 10^{th} to 70^{th} percentile in **Supplemental Table 1**), leading to the trichotomy of entire cohort: the amyloid-positive group (≥centiloid 20), the lowest amyloid group (<the threshold), and the middle/intermediate amyloid group (between the threshold and centiloid 20). **Table 2** contains, for each sub-threshold (left most column), the covariate-adjusted estimates to HR of developing cognitive impairment between the positive and the lowest amyloid groups, between the positive and middle/intermediate amyloid groups, and between the middle/intermediate and the lowest amyloid groups, along with the corresponding 95% CI and FDR-adjusted p-values. Across all sub-thresholds, in comparison to both the middle/intermediate and the lowest amyloid groups, amyloid-positive participants had an estimated 53% to 196% increased risk of developing cognitive impairment (HR from 1.53 to 2.96, all FDR adjusted p-

values<0.05). Participants in the middle/intermediate amyloid group also had an elevated risk of progression to cognitive impairment than the lowest amyloid group at the threshold of 40th percentile that corresponded to centiloid 1.03 (**Supplemental Table 1**). This threshold was the lowest among all the candidates that demonstrated a statistically significant increase in the risk of clinical progression for the middle/intermediate amyloid group, in comparison to the lowest amyloid group from both the covariate-adjusted analyses (HR=1.40, FDR p=0.018) and the unadjusted analyses (HR=1.28, p=0.035; see **Supplemental Table 2** and **Supplemental Figure 2**). Hence, the entire PET centiloid distribution contained three distinctive risk groups divided by two thresholds: the amyloid-positive group (centiloid≥20) which amounted for the upper 22% of the population, and the lowest amyloid group (centiloid<1.03) that covered the lowest 40% of the population, and the group with minimal amyloid accumulation (MAA; 1.03<centiloid<20) that accounted for the middle 38% of the distribution (or equivalently, 48.7% of the amyloid-negative participants). **Figure 2** presents the estimated survival curves for the three groups: the amyloid-positive group had the highest risk of clinical progression, followed by the MAA group, and finally by the lowest amyloid group. Importantly, although the survival curves of the MAA and the lowest amyloid groups started to separate right after baseline, their differences became much more profound only after ~10 years from baseline.

Absolute levels of PET centiloid within each amyloid group and the risk of progression to cognitive impairment

Given that the baseline amyloid trichotomy defined by centiloid 1.03 and 20 implied three distinct amyloid groups with differential risks of progression to cognitive impairment, we then addressed the question whether, within each group, higher levels of amyloid burden were still associated with higher risk of clinical progression by treating PET centiloid as a continuous predictor. After covariate adjustments, every increase of 1 centiloid in the amyloid-positive and the MAA groups was associated with 1.4% increased risk (HR=1.014, 95% CI: 1.008 to 1.02, $p<0.0001$) and 2.9% increased risk (HR=1.029; 95% CI: 0.997 to 1.063, $p=0.081$) of developing cognitive impairment symptoms, respectively, and the difference in the HR was not significant ($p=0.302$). For the lowest amyloid group, however, an increase of centiloid by 1 was no longer associated with a higher risk of clinical progression (HR=0.983, 95% CI: 0.956 to 1.011, $p=0.226$). Further, the association of PET centiloid levels with the risk of clinical progression in the MAA group was significantly stronger than the lowest amyloid group ($p=0.017$).

Longitudinal amyloid accumulation as a function of baseline amyloid trichotomy

A total of 1094 cognitively unimpaired participants (199 in the amyloid-positive group, 446 in the MAA group, and 449 in the lowest amyloid group) had longitudinal amyloid PET assessments with an average follow up of 6.57 years (SD=3.79 years). We examined how the baseline amyloid categorization into trichotomous levels predicted the subsequent amyloid

accumulation over time. Amyloid-positive participants continued to accumulate amyloid at an estimated rate of 4.16 centiloid/year (SE=0.24 centiloid/year, p<0.0001), significantly faster (p<0.0001) than those with MAA (1.00 centiloid/year, SE=0.11 centiloid/year, p<0.0001). With an estimated baseline mean centiloid of 6.65 in the MAA group, it would take about 13.35 years for the mean level of centiloid in the MAA group to reach the threshold (=20) for amyloid positivity. Participants in the lowest amyloid group, however, showed a much slower but statistically significant accumulation of amyloid over time (0.50 centiloid/year, SE= 0.08 centiloid/year, p<0.0001). **Figure 3** presents the spaghetti plots of the longitudinal PET centiloid for the three amyloid groups.

Baseline level and longitudinal change in cognition as functions of amyloid trichotomy

Based on the baseline amyloid trichotomy as defined by the thresholds of centiloid 1.03 and 20, we further estimated the baseline levels and the annual rates of cognitive change and compared them across the groups. **Figure 4** presents the spaghetti plots of the cognitive composite. At baseline, amyloid-positive group had the lowest cognitive score (mean=0.27, SE=0.023), followed by the lowest amyloid group (mean=0.35, SE=0.017) and the MAA group (mean=0.36, SE=0.017). Over time, amyloid-positive group had the fastest cognitive decline (-0.063/year, SE=0.005, p<0.0001), followed by the MAA group at -0.02/year (SE=0.0019, p<0.0001), and finally by the lowest amyloid group at -0.016/year (SE=0.002, p<0.0001). All differences in the baseline levels and the rates of decline for the cognitive composite were statistically

significant between the amyloid-positive group and each of the other two groups, but not between the MAA and the lowest amyloid groups (baseline level: p=0.37; rate of decline: p=0.15).

The amyloid trichotomy validated by sensitivity analyses

We repeated the analyses after accounting for death as a possible competing risk to the progression to cognitive impairment. **Supplemental Figure 3** presents the estimated cumulative incidence curves for the 3 amyloid groups, demonstrating that the MAA group had a 31% increased risk of clinical progression than the lowest amyloid group (HR=1.31, 95% CI: 1.05 to 1.65, p=0.019), but a reduced risk than the amyloid-positive group (HR=0.35, 95% CI: 0.28 to 0.43, p<0.0001).

Next, we repeated the analyses by defining the survival time as from the baseline to the first occurrence of a CDR ≥0.5 or a diagnosis of cognitive impairment after which no regression back to CDR 0 or normal cognition occurred. These analyses confirmed the existence of the MAA group as defined by centiloid 1.03 and 20. The MAA group had a 63% increased risk of clinical progression than the lowest amyloid group (HR=1.63, 95% CI: 1.20 to 2.21, p=0.002), but a reduced risk than the amyloid-positive group (HR=0.25, 95% CI: 0.20 to 0.33, p<0.0001). **Supplemental Figure 4** presents the estimated survival curves for the three amyloid groups with the revised definition of clinical progression. As another sensitivity analysis to make sure our findings were robust against the assumption of proportionality in the Cox

models, we compared the survival distributions across amyloid trichotomy by Wilcoxon's score test, and the results were consistent.

Discussion

Recent research advancement has established the fact that AD has a long window of latent or preclinical stage during which no clinical symptoms are present but AD-related neuropathology, especially amyloid plaque, is accumulating. Because amyloid burden in the brain measured by amyloid PET is inherently continuous, it is necessary to dichotomize it with appropriate thresholds to facilitate diagnosis of AD and enrollments of participants into treatment and prevention trials. The prevalence of amyloid positivity by PET among living individuals depends on multiple risk factors, such as age, *APOE* genotype, sex, and racial/ethnic identity. On average, only ~20% to ~30% of cognitively unimpaired middle aged and elderly individuals have been reported to be amyloid-positive[7-10]. Importantly, a positive amyloid PET scan has been demonstrated sufficient for significant association with worse cognition[1-3], faster onset of cognitive impairment[4-6], lower cerebrospinal fluid (CSF) levels of β-amyloid (1-42) (Aβ42) to β-amyloid (1-40) (Aβ40) ratio and higher CSF levels of total tau (t-tau) and tau phosphorylated at 181 (p-tau181)[2], and lower blood levels of Aβ42/40 and higher blood levels of p-tau217[11-13]. It remains unknown, however, whether the current thresholds for amyloid PET positivity represent the minimal or necessary level of amyloid burden in the brains of cognitively unimpaired individuals that predicts the subsequent development of cognitive impairment. The question is important because its answer may facilitate the identification of

cognitively unimpaired individuals who may be amyloid-negative by the current thresholds but are already at an elevated risk of developing cognitive impairment.

We tackled this very question by centrally processing amyloid PET scans across six studies of memory and aging and assessed the ability of a wide range of sub-thresholds from PET centiloid to predict the development of cognitive impairment among cognitively unimpaired individuals. We first found that at the sub-threshold of centiloid -3.75, the 80% of the participants with higher levels of centiloid had a significantly higher risk of developing cognitive impairment than the lower 20% of the participants, suggesting a significant portion of the amyloid-negative participants may be subject to elevated risk of cognitive impairment because the amyloid-positive participants accounted for only the top 22% of the cohort. We further sought for other sub-thresholds to risk stratify the amyloid-negative participants and found that at the sub-threshold of centiloid 1.03 which divided the entire amyloid-negative cohort into the lowest 51.3% (centiloid <1.03) and the upper 48.7% with minimal amyloid accumulation (centiloid between 1.03 and 20), the latter had a significantly elevated risk (40% higher) than the former, but a significantly reduced (53% lower) risk than the amyloid-positive group, to develop cognitive impairment. Importantly, we observed that the most profound risk differences between the minimal amyloid accumulation and the lowest amyloid groups (**Figure 2**) occurred after at least ~10 years of longitudinal follow up. We further validated the minimal amyloid accumulation group by comparing its longitudinal trajectory with the other two amyloid groups: amyloid-positive participants had the fastest amyloid accumulation over time, followed by the minimal amyloid accumulation group, and finally by the lowest amyloid group. Specifically, participants with minimal amyloid

accumulation accumulated amyloid at a significant rate of 1 centiloid/year, resulting in an estimated 13.35 years for the mean centiloid in the group to reach the threshold for amyloid positivity. The longitudinal rate of cognitive decline across three amyloid groups also followed the similar ordering, although the difference between the minimal amyloid accumulation and the lowest amyloid groups was not significant.

Our findings are important in better understanding the disease mechanism, optimally designing early prevention trials of AD, and accurately diagnosing the disease at its earliest stages. First, the field's current understanding of amyloid accumulation in cognitively unimpaired individuals has been mostly limited to the dichotomous approach that classified individuals into positive and negative categories. The well-established A/T/N biomarker network of AD stages has been the crescendo of the approach[14-15]. Whereas the A+ individuals have justifiably been the focus of diagnosis and prevention effort in the field during the past decades, the identification of minimal amyloid accumulation among the negative individuals challenges the amyloid dichotomy, which, coupled with the fact that amyloid accumulation is a very early event in the decades-long and continuous neuropathological process of AD[16-17], may allow the field to appreciate the much earlier or perhaps the earliest possible stage of amyloid accumulation that occurs well before reaching the threshold for amyloid positivity. In fact, our findings are consistent with multiple recent longitudinal studies that aimed to establish an amyloid clock for predicting the onset of amyloid positivity and then the symptoms among cognitively unimpaired individuals[4,18]. One of the major requirements for such an amyloid clock to work is that participants must be showing early signs of amyloid accumulation by reaching a much lower sub-threshold, consistent with our findings that a large portion of

amyloid-negative participants are at an elevated risk of developing cognitive impairment. Second, the minimal amyloid accumulation must be appropriately diagnosed. Whereas individuals with minimal amyloid accumulation are classified by the current amyloid dichotomy as negative and hence do not receive a diagnosis of AD, they are different from the lowest amyloid group whose individual levels of centiloid no longer predicted the clinical progression. In fact, albeit at different levels of PET centiloid, the minimal amyloid accumulation group and the amyloid-positive group shared similar within-group HR of clinical progression for every increase of 1 centiloid. Importantly, our findings also indicated that participants with minimal amyloid accumulation continued to longitudinally accumulate amyloid at 1 centiloid/year, much faster than the lowest amyloid group, implying that it is just a matter of time that people with minimal amyloid accumulation will become amyloid-positive (in an estimated 13.35 years) and then on the way to develop cognitive impairment. The minimal amyloid accumulation hence represents a transitional stage from the lowest amyloid or perhaps the amyloid-free stage whose levels did not predict the clinical progression to the fully accumulated amyloid stage (the positive stage) in the brain, and must be adequately recognized in diagnosing AD. Third, prevention of AD is crucial to truly solve the public health crisis associated with AD[19], and the minimal amyloid accumulation may provide a unique target for prevention trials of AD. Secondary prevention therapies have the potential to stop or delay cognitive impairment in people with biomarker evidence of the disease. Primary prevention therapies are designed to avert the disease itself in people without cognitive impairment. Current secondary prevention trials enroll only amyloid-positive individuals which may have excluded a large group of cognitively unimpaired amyloid-negative individuals whose amyloid levels already put them at an elevated risk of

AD over a longer timeframe. For example, even with a relatively lower threshold, about 70.5% of the cognitively unimpaired participants who underwent amyloid imaging were excluded from a recently completed large prevention trial because they did not meet the threshold for amyloid positivity[20]. Our findings suggest that individuals with minimal amyloid accumulation may be considered for inclusion into future secondary prevention trials, which may reduce the large number of PET assessments needed to screen individuals for amyloid-based eligibility to achieve adequate statistical power. However, because the statistical power in the secondary prevention trials depends on not only the sample size but also the rate of clinical progression in the placebo arm, the slower rate of clinical progression and cognitive decline in the minimal amyloid accumulation group as compared to the amyloid-positive group must also be considered in designing future prevention trials. In fact, the slower rate of clinical progression may imply a much longer prevention trial that will then lead to increased cost. More longitudinal studies are needed to fully understand the implications of minimal amyloid accumulation in statistical power and cost of future trials, and most importantly, more research is needed for biomarker surrogate outcomes in prevention trials for this very early stage of AD. Whereas current primary prevention trials of AD enroll amyloid-negative individuals with the current thresholds[21], they may have included participants with minimal amyloid accumulation who are already on their way to accumulate amyloid and develop cognitive impairment. Our findings hence may have important implications to the design of future prevention trials, especially with respect to the inclusion/exclusion of individuals, statistical power, and cost. At minimum, incorporating screening methods emphasizing gradations of amyloid elevation may improve trial design.

Our study has several major strengths. First, both imaging biomarker data and clinical/cognitive data were rigorously harmonized across six major studies of aging and AD, optimizing the quality of the database and improving the representativeness of the study cohort with a reasonable representation of individuals self-identified as Black or African Americans. Second, the sample size of 1834 cognitively unimpaired participants with amyloid PET who underwent comprehensive longitudinal clinical and cognitive assessments represents one of the largest studies in the association of imaging biomarkers with clinical progression. Third, the longitudinal assessments were up to 20.3 years with a large number of participants developing cognitive impairment, providing adequate statistical power to identify the MAA group whose risk of clinical progression falls in between the amyloid-positive group and the lowest amyloid group, especially when the differences in survival curves between the minimal amyloid accumulation and the lowest amyloid groups required at least ~10 years of follow up to fully manifest (**Figure 2**). Fourth, the rigorous control of FDR over repeated testing of many sub-thresholds and the adjustments of all main AD covariates in the primary analyses as well as multiple sensitivity analyses implied that the identified minimal amyloid accumulation group is unlikely a false positive or confounded by main AD risk factors. Despite multiple strengths of the study, a major limitation is the fact that the study cohort is primarily a collection of multiple research cohorts which unlikely captured the true demographics of the elderly American population and hence may affect the generalizability of our findings. Further, it is possible there existed more distinct risk sub-groups within the minimal amyloid accumulation group (**Figure 2**) and even within the amyloid-positive group[22] that the current study failed to detect due to lack of statistical power. More powerful longitudinal studies are

needed to fully explain the amyloid risk stratification. Finally, even with rigorous data harmonizations across studies, measurement errors and noise from amyloid PET data may still exist and have affected our analyses.

In summary, this study assembled a large longitudinal cohort of cognitively unimpaired individuals by rigorously harmonizing imaging biomarker and clinical data from six studies and identified a large portion of amyloid-negative individuals who were cognitively unimpaired but already at an elevated risk of developing cognitive impairment over a longer timeframe. This minimal amyloid accumulation group had its own unique risk profile which was significantly different from the amyloid-positive and the lowest amyloid groups. The minimal amyloid accumulation may enable the field to better understand the earliest possible stage of amyloid accumulation during the decades-long preclinical stage of AD and has the potential to improve the design and analyses of future prevention trials of the disease. Future longitudinal studies with at least a decade of follow up are needed to replicate the long-term risk profile of the minimal amyloid accumulation and generalize these findings to the more representative elderly population, as well as with more scalable modalities of amyloid biomarkers, e.g., plasma p-tau217.

Acknowledgements

The work is partly supported by National Institute on Aging (NIA) grants R01 AG067505 and R01 AG053550 (Dr. Xiong), P30 AG066444 (Dr. Holtzman), P01 AG026276 and P01 AG03991 (Dr. Morris), P30 AG072979 (Dr. Wolk), Pennsylvania Department of Health (2019NF4100087335; Dr. Wolk), P01 AG036694 (Drs. Reisa Sperling and Keith Johnson), R01 AG054059 (Dr. Gleason), and P30 AG086401 and P20 AG068024 (Dr. Roberson). This material is the result of work supported with resources and the use of facilities at the William S. Middleton Memorial VA Hospital in Madison, WI. We

want to thank all the participants of WashU, UPenn, Wisconsin and UAB ADRCs, and WRAP and HABS who contributed to this study and their families.

Potential Conflict of Interest

SES has not received any financial compensation from pharmaceutical or diagnostics companies since 2024, when she received honoraria for serving on scientific advisory boards on biomarker testing and education for Eisai and Novo Nordisk and speaking fees from Eisai, Eli Lilly, and Novo Nordisk. She has recently received honoraria for educational presentations from Medscape, PeerView, and the Academy for Continued Healthcare Learning. She has provided unpaid scientific advising to Acumen, Biogen, Cognito Therapeutics, Danaher, Eisai, Eli Lilly, Johnson and Johnson Innovative Medicine, Sanofi, and Siemens. DAW has served as a paid consultant for Beckman Coulter and serves on a DSMB for GSK. ER serves on a data monitoring committee for Eli Lilly. TB participates as a site investigator in clinical trials sponsored by Avid Radiopharmaceuticals, Eli Lilly and Company, Biogen, Eisai, Janssen, and Roche. TB participates as a site investigator in clinical trials sponsored by Avid Radiopharmaceuticals, Eli Lilly and Company, Biogen, Eisai, Janssen, and Roche. RAS has served as a consultant for AbbVie, AC Immune, Acumen, Alector, Apellis, Biohaven, Bristol Myers Squibb, Ionis, Janssen, Oligomerix, Prothena, Roche and Vaxxinity over the past 3 years, and has received research funding from Eisai and Eli Lilly for public-private partnership trials. DMH co-founded, has equity, and is on the scientific advisory board of C2N Diagnostics. DMH is on the scientific advisory boards of Denali, Genentech, and Switch and consults for Novartis and Pfizer. SCJ has served on advisory boards for Lantheus, Lilly, Enigma, Alamar, Alzpath and Trillium bio. JH has served as a paid consultant for Prothena, Abbvie, and Quanterix. The other Authors declare no Competing Financial or Non-Financial Interests. This work was supported in part by funding from the National Institutes of Health (grant #AG067505). WU has a financial interest in C2N Diagnostics and may financially benefit if the company is successful in marketing its product(s) that is/are related to this research.

Author contributions

CX, JL, SH, SS, WK, JH, DW, CG, SJ, RS, KJ, ER, KM, DH, JM, and TB contributed to the conception and design of the study; CX, JL, SS, WK, JH, DW, CG, SJ, RS, KJ, ER, KM, DH, JM, TB, EG, FA, RH, PM, SK, and DD contributed to the acquisition and/or analysis of data; CX, JL, SH, SS, WK, JH, DW, CG, SJ, RS, KJ, ER, KM, DH, JM, and TB contributed to drafting the text and/or preparing figures and/or reviewing the draft.

Data availability

Anonymized data that support the findings of this study are available from the corresponding author, upon reasonable request from any qualified investigator.

References

1. Xiong C, Jasielec MS, Weng H, Fagan AM, Benzinger TL, Head D, Hassenstab J, Grant E, Sutphen CL, Buckles V, Moulder KL, Morris JC. Longitudinal relationships among biomarkers for Alzheimer disease in the Adult Children Study. Neurology. 2016 Apr 19;86(16):1499-506. doi: 10.1212/WNL.0000000000002593. Epub 2016 Mar 23. PubMed PMID: 27009258; PubMed Central PMCID: PMC4836885.

2. Bonomi S, Lu R, Schindler SE, Bui Q, Lah JJ, Wolk D, Gleason CE, Sperling R, Roberson ED, Levey AI, Shaw L, Van Hulle C, Benzinger T, Adams M, Manzanares C, Qiu D, Hassenstab J, Moulder KL, Balls-Berry JE, Johnson K, Johnson SC, Murchison CF, Luo J, Gremminger E, Agboola F, Grant EA, Hornbeck R, Massoumzadeh P, Keefe S, Dierker D, Gray J, Henson RL, Streitz M, Mechanic-Hamilton D, Morris JC, Xiong C. Relationships of Cognitive Measures with Cerebrospinal Fluid but Not Imaging Biomarkers of Alzheimer Disease Vary between Black and White Individuals. Ann Neurol. 2023 Dec 1. doi: 10.1002/ana.26838. Epub ahead of print. PMID: 38038976; PMCID: PMC10922199.

3. Farrell ME, Jiang S, Schultz AP, Properzi MJ, Price JC, Becker JA, Jacobs HIL, Hanseeuw BJ, Rentz DM, Villemagne VL, Papp KV, Mormino EC, Betensky RA, Johnson KA, Sperling RA, Buckley RF; Alzheimer's Disease Neuroimaging Initiative and the Harvard Aging Brain Study. Defining the Lowest Threshold for Amyloid-PET to Predict Future Cognitive Decline and Amyloid Accumulation. Neurology. 2021 Jan 26;96(4):e619-e631. doi: 10.1212/WNL.0000000000011214. Epub 2020 Nov 16. PMID: 33199430; PMCID: PMC7905788.

4. Schindler SE, Li Y, Buckles VD, Gordon BA, Benzinger TLS, Wang G, Coble D, Klunk WE, Fagan AM, Holtzman DM, Bateman RJ, Morris JC, Xiong C. Predicting Symptom Onset in Sporadic Alzheimer Disease with Amyloid PET. Neurology. 2021 Nov 2;97(18):e1823-e1834. doi: 10.1212/WNL.0000000000012775. Epub 2021 Sep 9. PMID: 34504028; PMCID: PMC8610624.

5. Ossenkoppele R, Pichet Binette A, Groot C, Smith R, Strandberg O, Palmqvist S, Stomrud E, Tideman P, Ohlsson T, Jögi J, Johnson K, Sperling R, Dore V, Masters CL, Rowe C, Visser D, van Berckel BNM, van der Flier WM, Baker S, Jagust WJ, Wiste HJ, Petersen RC, Jack CR Jr, Hansson O. Amyloid and tau PET-positive cognitively unimpaired individuals are at high risk for future cognitive decline. Nat Med. 2022 Nov;28(11):2381-2387. doi: 10.1038/s41591-022-02049-x. Epub 2022 Nov 10. PMID: 36357681; PMCID: PMC9671808.

6. Younes K, Johns E, Young CB, Kennedy G, Mukherjee S, Vossler HA, Winer J, Cody K, Henderson VW, Poston KL, Betthauser TJ, Bevis B, Brooks WM, Burns JM, Coombes SA, DeCarli C, DiFilippo FP, Duara R, Fan AP, Gibbons LE, Golde T, Johnson SC, Lepping RJ, Leverenz J, McDougall S, Rogalski E, Sanders E, Pasaye J, Sridhar J, Saykin AJ, Sridharan A, Swerdlow R, Trittschuh EH, Vaillancourt D, Vidoni E, Wang WE, Mez J, Hohman TJ, Tosun D, Biber S, Kukull WA, Crane PK, Mormino EC. Amyloid PET predicts longitudinal functional and cognitive trajectories in a heterogeneous cohort. Alzheimers Dement. 2025 Mar;21(3):e70075. doi: 10.1002/alz.70075. PMID: 40145384; PMCID: PMC11947745.

7. Roberts RO, Aakre JA, Kremers WK, Vassilaki M, Knopman DS, Mielke MM, Alhurani R, Geda YE, Machulda MM, Coloma P, Schauble B, Lowe VJ, Jack CR Jr, Petersen RC. Prevalence and Outcomes of Amyloid Positivity Among Persons Without Dementia in a Longitudinal, Population-Based Setting. JAMA Neurol. 2018 Aug 1;75(8):970-979. doi: 10.1001/jamaneurol.2018.0629. PMID: 29710225; PMCID: PMC6142936.

8. Landau SM, Mintun MA, Joshi AD, et al. ; Alzheimer's Disease Neuroimaging Initiative . Amyloid deposition, hypometabolism, and longitudinal cognitive decline. Ann Neurol. 2012;72(4):578-586.

9. Parnetti L, Chipi E, Salvadori N, D'Andrea K, Eusebi P. Prevalence and risk of progression of preclinical Alzheimer's disease stages: a systematic review and meta-analysis. Alzheimers Res Ther. 2019 Jan 15;11(1):7. doi: 10.1186/s13195-018-0459-7. PMID: 30646955; PMCID: PMC6334406.

10. Kern S, Zetterberg H, Kern J, Zettergren A, Waern M, Höglund K, Andreasson U, Wetterberg H, Börjesson-Hanson A, Blennow K, Skoog I. Prevalence of preclinical Alzheimer disease: Comparison of current classification systems. Neurology. 2018 May 8;90(19):e1682-e1691. doi: 10.1212/WNL.0000000000005476. Epub 2018 Apr 13. PMID: 29653987; PMCID: PMC5952969

11. Jack CR Jr, Wiste HJ, Algeciras-Schimnich A, Weigand SD, Figdore DJ, Lowe VJ, Vemuri P, Graff-Radford J, Ramanan VK, Knopman DS, Mielke MM, Machulda MM, Fields J, Schwarz CG, Cogswell PM, Senjem ML, Therneau TM, Petersen RC. Comparison of plasma biomarkers and amyloid PET for predicting memory decline in

cognitively unimpaired individuals. Alzheimers Dement. 2024 Mar;20(3):2143-2154. doi: 10.1002/alz.13651. Epub 2024 Jan 24. PMID: 38265198; PMCID: PMC10984437.

12. de Rojas I, Romero J, Rodríguez-Gomez O, Pesini P, Sanabria A, Pérez-Cordon A, Abdelnour C, Hernández I, Rosende-Roca M, Mauleón A, Vargas L, Alegret M, Espinosa A, Ortega G, Gil S, Guitart M, Gailhajanet A, Santos-Santos MA, Moreno-Grau S, Sotolongo-Grau O, Ruiz S, Montrreal L, Martín E, Pelejà E, Lomeña F, Campos F, Vivas A, Gómez-Chiari M, Tejero MA, Giménez J, Pérez-Grijalba V, Marquié GM, Monté-Rubio G, Valero S, Orellana A, Tárraga L, Sarasa M, Ruiz A, Boada M; FACEHBI study. Correlations between plasma and PET beta-amyloid levels in individuals with subjective cognitive decline: the Fundació ACE Healthy Brain Initiative (FACEHBI). Alzheimers Res Ther. 2018 Nov 29;10(1):119. doi: 10.1186/s13195-018-0444-1. PMID: 30497535; PMCID: PMC6267075.

13. Jack CR, Wiste HJ, Algeciras-Schimnich A, Figdore DJ, Schwarz CG, Lowe VJ, Ramanan VK, Vemuri P, Mielke MM, Knopman DS, Graff-Radford J, Boeve BF, Kantarci K, Cogswell PM, Senjem ML, Gunter JL, Therneau TM, Petersen RC. Predicting amyloid PET and tau PET stages with plasma biomarkers. Brain. 2023 May 2;146(5):2029-2044. doi: 10.1093/brain/awad042. PMID: 36789483; PMCID: PMC10151195.

14. Jack CR Jr, Bennett DA, Blennow K, Carrillo MC, Feldman HH, Frisoni GB, Hampel H, Jagust WJ, Johnson KA, Knopman DS, Petersen RC, Scheltens P, Sperling RA, Dubois B. A/T/N: An unbiased descriptive classification scheme for Alzheimer disease biomarkers. Neurology. 2016 Aug 2;87(5):539-47. doi: 10.1212/WNL.0000000000002923. Epub 2016 Jul 1. PMID: 27371494; PMCID: PMC4970664.

15. Jack CR Jr, Andrews JS, Beach TG, Buracchio T, Dunn B, Graf A, Hansson O, Ho C, Jagust W, McDade E, Molinuevo JL, Okonkwo OC, Pani L, Rafii MS, Scheltens P, Siemers E, Snyder HM, Sperling R, Teunissen CE, Carrillo MC. Revised criteria for diagnosis and staging of Alzheimer's disease: Alzheimer's Association Workgroup. Alzheimers Dement. 2024 Aug;20(8):5143-5169. doi: 10.1002/alz.13859. Epub 2024 Jun 27. PMID: 38934362; PMCID: PMC11350039.

16. Luo J, Agboola F, Grant E, et al. Accelerated longitudinal changes and ordering of Alzheimer disease biomarkers across the adult lifespan. Brain. 2022 Dec 19;145(12):4459-73.
17. Luo J, Agboola F, Grant E, et al. Sequence of Alzheimer disease biomarker changes in cognitively unimpaired adults: A cross-sectional study. Neurology. 2020 Dec 8;95(23):e3104-e16.
18. Li Y, Yen D, Hendrix RD, Gordon BA, Dlamini S, Barthélemy NR, Aschenbrenner AJ, Henson RL, Herries EM, Volluz K, Kirmess K, Eastwood S, Meyer M, Heller M, Jarrett L, McDade E, Holtzman DM, Benzinger TLS, Morris

JC, Bateman RJ, Xiong C, Schindler SE. Timing of Biomarker Changes in Sporadic Alzheimer's Disease in Estimated Years from Symptom Onset. Ann Neurol. 2024 May;95(5):951-965. doi: 10.1002/ana.26891. Epub 2024 Feb 24. PMID: 38400792; PMCID: PMC11060905.

19. Dementia prevention needs clinical trials. *Nat Med* **31**, 353 (2025). https://doi.org/10.1038/s41591-025-03552-7
20. Sperling RA, Donohue MC, Raman R, Rafii MS, Johnson K, Masters CL, van Dyck CH, Iwatsubo T, Marshall GA, Yaari R, Mancini M, Holdridge KC, Case M, Sims JR, Aisen PS; A4 Study Team. Trial of Solanezumab in Preclinical Alzheimer's Disease. N Engl J Med. 2023 Sep 21;389(12):1096-1107. doi: 10.1056/NEJMoa2305032. Epub 2023 Jul 17. PMID: 37458272; PMCID: PMC10559996.
21. Bateman RJ, Benzinger TL, Berry S, Clifford DB, Duggan C, Fagan AM, Fanning K, Farlow MR, Hassenstab J, McDade EM, Mills S, Paumier K, Quintana M, Salloway SP, Santacruz A, Schneider LS, Wang G, Xiong C; DIAN-TU Pharma Consortium for the Dominantly Inherited Alzheimer Network. The DIAN-TU Next Generation Alzheimer's prevention trial: Adaptive design and disease progression model. Alzheimers Dement. 2017 Jan;13(1):8-19. doi: 10.1016/j.jalz.2016.07.005. Epub 2016 Aug 29. PMID: 27583651; PMCID: PMC5218895.
22. Sperling RA, Donohue MC, Rissman RA, Johnson KA, Rentz DM, Grill JD, Heidebrink JL, Jenkins C, Jimenez-Maggiora G, Langford O, Liu A, Raman R, Yaari R, Holdridge KC, Sims JR, Aisen PS. Amyloid and Tau Prediction of Cognitive and Functional Decline in Unimpaired Older Individuals: Longitudinal Data from the A4 and LEARN Studies. J Prev Alzheimers Dis. 2024;11(4):802-813. doi: 10.14283/jpad.2024.122. PMID: 39044488; PMCID: PMC11266444.
23. Morris JC, Schindler SE, McCue LM, et al. Assessment of Racial Disparities in Biomarkers for Alzheimer Disease. JAMA Neurol. 2019 Mar 1;76(3):264-73.
24. Howell JC, Watts KD, Parker MW, et al. Race modifies the relationship between cognition and Alzheimer's disease cerebrospinal fluid biomarkers. Alzheimers Res Ther. 2017 Nov 2;9(1):88.
25. Hanseeuw BJ, Betensky RA, Jacobs HIL, et al. Association of Amyloid and Tau With Cognition in Preclinical Alzheimer Disease: A Longitudinal Study. JAMA Neurol. 2019 Aug 1;76(8):915-24.
26. Dagley A, LaPoint M, Huijbers W, et al. Harvard Aging Brain Study: Dataset and accessibility. Neuroimage. 2017 Jan;144(Pt B):255-8.
27. Wolk DA, Mancuso L, Kliot D, Arnold SE, Dickerson BC. Familiarity-based memory as an early cognitive marker of preclinical and prodromal AD. Neuropsychologia. 2013 May;51(6):1094-102.
28. Morris JC, Weintraub S, Chui HC, et al. The Uniform Data Set (UDS): clinical and cognitive variables and descriptive data from Alzheimer Disease Centers. Alzheimer Dis Assoc Disord. 2006 Oct-Dec;20(4):210-6.
29. Weintraub S, Salmon D, Mercaldo N, et al. The Alzheimer's Disease Centers' Uniform Data Set (UDS): the neuropsychologic test battery. Alzheimer Dis Assoc Disord. 2009 Apr-Jun;23(2):91-101.

30. Morris JC. The Clinical Dementia Rating (CDR): current version and scoring rules. Neurology. 1993 Nov;43(11):2412-4.
31. McKhann GM, Knopman DS, Chertkow H, et al. The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimer's & dementia : the journal of the Alzheimer's Association. 2011 May;7(3):263-9.
32. Monsell SE, Dodge HH, Zhou XH, et al. Results From the NACC Uniform Data Set Neuropsychological Battery Crosswalk Study. Alzheimer Dis Assoc Disord. 2016 Apr-Jun;30(2):134-9.
33. Xiong C, Luo J, Agboola F, Li Y, Albert M, Johnson SC, Koscik RL, Masters CL, Soldan A, Villemagne VL, Li Q-X, McDade EM, Fagan AM, Massoumzadeh P, Benzinger T, Hassenstab J, Bateman RJ, Morris JC, the Dominantly Inherited Alzheimer Network. A harmonized longitudinal biomarkers and cognition database for assessing the natural history of preclinical Alzheimer disease from young adulthood and for designing prevention trials. Alzheimer's & Dementia. 2019 Nov;15(11):1448-1457. PubMed PMID: 31506247; PubMed Central PMCID: PMC6874758.

34. Landau SM, Ward TJ, Murphy A, Iaccarino L, Harrison TM, La Joie R, et al. Quantification of amyloid beta and tau PET without a structural MRI. Alzheimer's & Dementia 2023;19:444–55. https://doi.org/10.1002/alz.12668.
35. Klunk WE, Koeppe RA, Price JC, Benzinger TL, Devous MD, Jagust WJ, et al. The Centiloid Project: Standardizing quantitative amyloid plaque estimation by PET. Alzheimer's & Dementia 2015;11:1-15.e4. https://doi.org/10.1016/j.jalz.2014.07.003.

36. Su Y, Flores S, Hornbeck RC, Speidel B, Vlassenko AG, Gordon BA, et al. Utilizing the Centiloid scale in cross-sectional and longitudinal PiB PET studies. NeuroImage: Clinical 2018;19:406–16. https://doi.org/10.1016/j.nicl.2018.04.022.

37. Su Y, Flores S, Wang G, Hornbeck RC, Speidel B, Joseph-Mathurin N, et al. Comparison of Pittsburgh compound B and florbetapir in cross-sectional and longitudinal studies. Alzheimer's & Dementia: Diagnosis, Assessment & Disease Monitoring 2019;11:180–90.

38. Xiong C, Schindler SE, Henson RL, Wolk DA, Shaw LM, Agboola F, Morris JC, Lu R, Luo J. Correlational analyses of biomarkers that are harmonized through a bridging study due to measurement errors. Stat Methods Med Res. 2024 Feb;33(2):185-202. doi: 10.1177/09622802231215810. Epub 2023 Nov 22. PMID: 37994004; PMCID: PMC10939855.

39. Xiong C, Lu R, Wolk D, Shaw LM, Gleason CE, Johnson SC, Agboola F, Schindler SE, Morris JC, Luo J. A General Linear Mixed Effect Model to Infer Biomarker Correlations by Bridging Retrospectively Measured Data Across

Multiple Studies. Stat Med. 2025 Jul;44(15-17):e70200. doi: 10.1002/sim.70200. PMID: 40689809; PMCID: PMC12621524.

40. Breslow NE (1975). "Analysis of Survival Data under the Proportional Hazards Model". *International Statistical Review / Revue Internationale de Statistique*. **43** (1): 45–57.

41. Vittinghoff E, McCulloch CE. Relaxing the rule of ten events per variable in logistic and Cox regression. Am J Epidemiol. 2007 Mar 15;165(6):710-8. doi: 10.1093/aje/kwk052. Epub 2006 Dec 20. PMID: 17182981.

42. Benjamini Y, Hochberg Y (1995). "Controlling the false discovery rate: a practical and powerful approach to multiple testing". *Journal of the Royal Statistical Society, Series B*. **57** (1): 289–300.

43. Fine JP, Gray RJ. A proportional hazards model for the subdistribution of a competing risk. *J Am Statist Assoc*. 1999; **94**: 496-509.

44. Gray RJ. A class of K-sample tests for comparing the cumulative incidence of a competing risk. *Ann Stat*. 1988; **16**: 1141-1154.

45. Laird NM, Ware JH. Random-effects models for longitudinal data. Biometrics 38:963-74, 1982.

46. Posit team (2025). RStudio: Integrated Development Environment for R. Posit Software, PBC, Boston, MA. URL: http://www.posit.co/.

Legends

Figure 1. Every increase of five percentiles from the 10th to 70th (upper x-axis) of the baseline PET centiloid levels (bottom x-axis) was used as a sub-threshold to binarize participants. Estimated HRs (with 95% pointwise CI in shaded grey) between participants with higher and lower amyloid were plotted as a function of the threshold from the unadjusted (top figure) and covariate-adjusted (bottom figure) analyses. Vertical dashed line corresponded to the threshold for amyloid positivity (centiloid 20).

Figure 2. Estimated survival curves to cognitive impairment for the three distinct amyloid groups identified by baseline PET centiloid 1.03 and 20: amyloid-positive participants had the highest risk of clinical progression, followed by those with the minimal amyloid accumulation, and finally by participants in the lowest amyloid group

Figure 3. Spaghetti plots of the longitudinal PET centiloid for the three amyloid groups defined by centiloid 1.03 and 20. An estimated longitudinal trajectory by locally estimated scatterplot smoothing (LOESS) was superimposed.

Figure 4. Spaghetti plots of the cognitive composite for the amyloid trichotomy as defined by centiloid 1.03 and 20. An estimated longitudinal trajectory by locally estimated scatterplot smoothing (LOESS) was superimposed.

Supplemental Figure 1. Histogram of baseline PET centiloid with a fitted bi-modal distribution as indicated by red and green density curves, suggesting more than one sub-groups of the entire amyloid distribution

Supplemental Figure 2. Unadjusted (top panel) and covariate-adjusted (bottom panel) estimates to the HR of progression to cognitive impairment as functions of centiloid sub-threshold (x-axis) between amyloid-positive group (P), middle/intermediate amyloid group (M), and lowest amyloid group (L). The analyses indicated that participants between centiloid 1.03 and centiloid 20 had a significantly higher risk of progression to cognitive impairment than those whose PET centiloid is below centiloid 1.03, but a significantly lower risk of progression than amyloid-positive participants.

Supplemental Figure 3. Estimated cumulative incidence curves (CIC) for the 3 amyloid groups defined by centiloid 1.03 and 20 after taking death into account as a competing risk, demonstrating that the minimal amyloid group had a 31% increased risk of clinical progression than the lowest amyloid group (HR=1.31, 95% CI: 1.05 to 1.65, p=0.019), but reduced risk than the amyloid-positive group (HR=0.35, 95% CI: 0.28 to 0.43, p<0.0001)

Supplemental Figure 4. Estimated survival curves for the three amyloid groups defined by centiloid 1.03 and 20 with the survival time defined as from the baseline amyloid PET scan collection to the first occurrence of cognitive impairment after which no regression back to CDR 0 or normal cognition occurred---a sensitivity analysis

Tables

Table 1: Baseline characteristics of participants in the longitudinal cohort

	Overall (N=1834)
Age: Mean (SD)	68.3 (8.84)
Race: n (%) for White/Black/Other/Missing)	1561 (85.1%)/233 (12.7%) /28 (1.5%)/12 (0.7%)
Sex: n (%) for M/F/Missing	704 (38.4%)/1127 (61.5%)/3 (0.2%)
Education: n (%) for <=12 yrs />12yrs /Missing	241 (13.1%)/1584 (86.4%)/9 (0.5%)
APOE ε4: n (%) for Positive/Negative/Missing	624 (34.0%)/1187 (64.7%)/23 (1.3%)
Site: n (%) for WashU/UPenn/HABS/Wisconsin ADRC/Wisconsin WRAP/UAB	920 (50.2%)/106 (5.8%)/339 (18.5%)/110 (6.0%)/358 (19.5%)/1 (0.1%)
Interval between amyloid PET and Clinical assessments (y): Mean (SD)	0.377 (0.300)
Length of clinical follow up (y): Mean (SD)	7.37 (4.67)
PET centiloid: Mean (SD)	12.9 (27.1)
Vascular comorbidity: n (%) for Yes/No/Missing	735 (40.1%)/546 (29.8%)/553 (30.2%)
Cognitive composite: Mean (SD)	0.295 (0.487)

Table 2: Covariate-adjusted estimates of HR (95% CI) between the amyloid-positive (P), middle/intermediate amyloid (M), and lowest amyloid (L) groups as a function of threshold (in percentiles; first column). Both FDR adjusted (FDR p) and unadjusted p-values are presented.

Threshold	HR: M vs. L	p-value: M vs. L	FDR p : M vs. L	HR: P vs. L	p-value: P vs. L	FDR p : P vs. L	HR: P vs. M	p-value: P vs. M	FDR p: P vs. M
10%	1.05 (0.73~1.52)	0.7791	0.8072	2.58 (1.76~3.79)	1.25E-06	1.25E-06	2.45 (1.98~3.04)	1.97E-16	1.28E-15
15%	0.95 (0.70~1.28)	0.7297	0.8072	2.36 (1.71~3.26)	1.51E-07	1.64E-07	2.49 (2.01~3.10)	1.69E-16	1.28E-15
20%	1.03 (0.79~1.36)	0.8072	0.8072	2.53 (1.88~3.42)	1.00E-09	1.19E-09	2.45 (1.97~3.05)	1.09E-15	4.73E-15
25%	1.14 (0.88~1.47)	0.3311	0.4305	2.70 (2.04~3.56)	2.61E-12	3.39E-12	2.38 (1.90~2.97)	2.15E-14	6.99E-14
30%	1.19 (0.93~1.52)	0.1672	0.2480	2.77 (2.12~3.61)	8.48E-14	1.23E-13	2.32 (1.86~2.91)	1.92E-13	5.00E-13
35%	1.32 (1.04~1.68)	0.0219	0.0475	2.92 (2.26~3.78)	4.31E-16	7.00E-16	2.21 (1.76~2.77)	9.19E-12	1.99E-11
40%	1.40 (1.11~1.78)	0.0046	0.0183	2.96 (2.31~3.79)	1.24E-17	3.22E-17	2.11 (1.67~2.66)	3.07E-10	4.99E-10
45%	1.22 (0.96~1.54)	0.0983	0.1825	2.71 (2.13~3.44)	2.87E-16	5.32E-16	2.22 (1.74~2.83)	9.58E-11	1.78E-10
50%	1.18 (0.93~1.51)	0.1717	0.2480	2.64 (2.09~3.33)	2.70E-16	5.32E-16	2.23 (1.73~2.87)	4.15E-10	5.99E-10
55%	1.36 (1.06~1.75)	0.0170	0.0442	2.73 (2.17~3.43)	5.30E-18	1.72E-17	2.01 (1.55~2.62)	2.00E-07	2.60E-07
60%	1.45 (1.12~1.90)	0.0056	0.0183	2.73 (2.18~3.41)	1.43E-18	6.18E-18	1.88 (1.42~2.48)	9.75E-06	1.15E-05
65%	1.64 (1.23~2.19)	0.0007	0.0088	2.74 (2.20~3.42)	3.06E-19	3.98E-18	1.67 (1.24~2.25)	0.0008	0.0008
70%	1.73 (1.23~2.42)	0.0015	0.0096	2.65 (2.13~3.29)	9.02E-19	5.87E-18	1.53 (1.08~2.18)	0.0174	0.0174

FIGURES

Figure 1. Every increase of five percentiles from the 10th to 70th (upper x-axis) of the baseline PET centiloid levels (bottom x-axis) was used as a sub-threshold to binarize participants. Estimated HRs (with 95% pointwise CI in shaded grey) between participants with higher and lower amyloid were plotted as a function of the threshold from the unadjusted (top figure) and covariate-adjusted (bottom figure) analyses. Vertical dashed line corresponded to the threshold for amyloid positivity (centiloid 20).

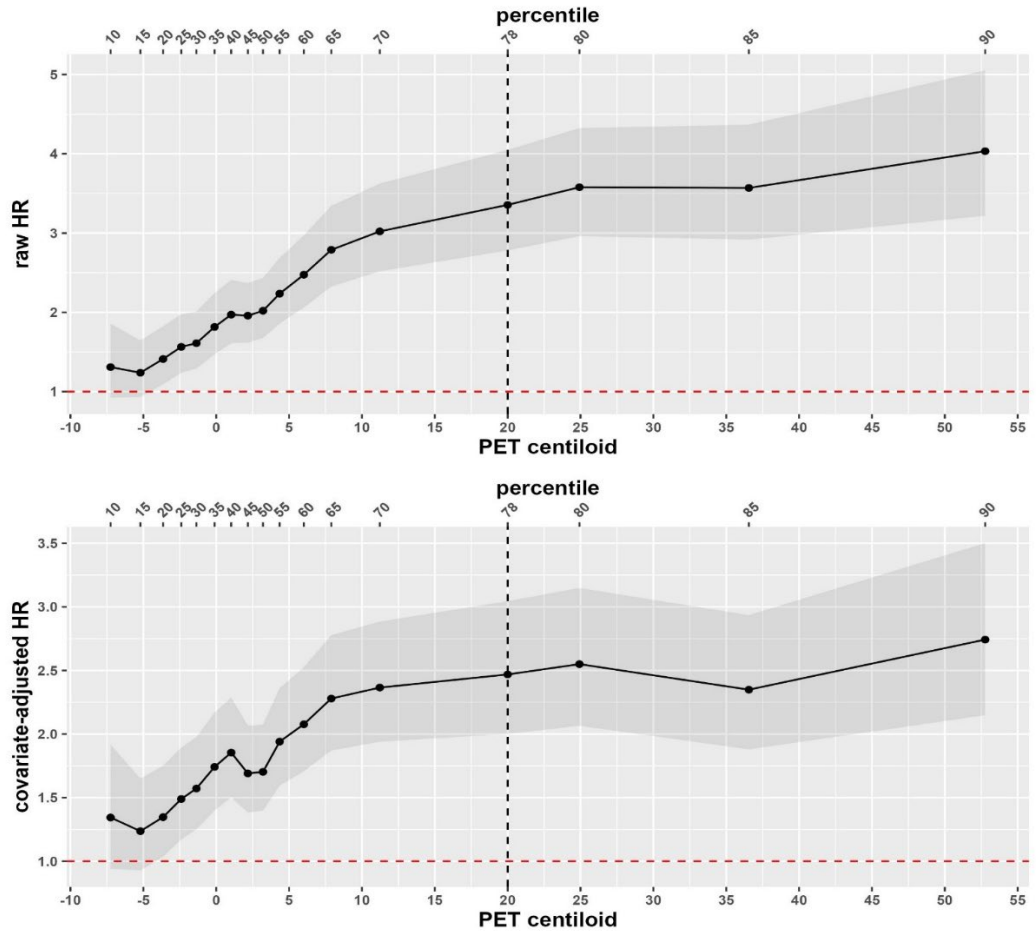


Figure 2. Estimated survival curves to cognitive impairment for the three distinct amyloid groups identified by baseline PET centiloid 1.03 and 20: amyloid-positive participants had the highest risk of clinical progression, followed by those with the minimal amyloid accumulation, and finally by participants in the lowest amyloid group.

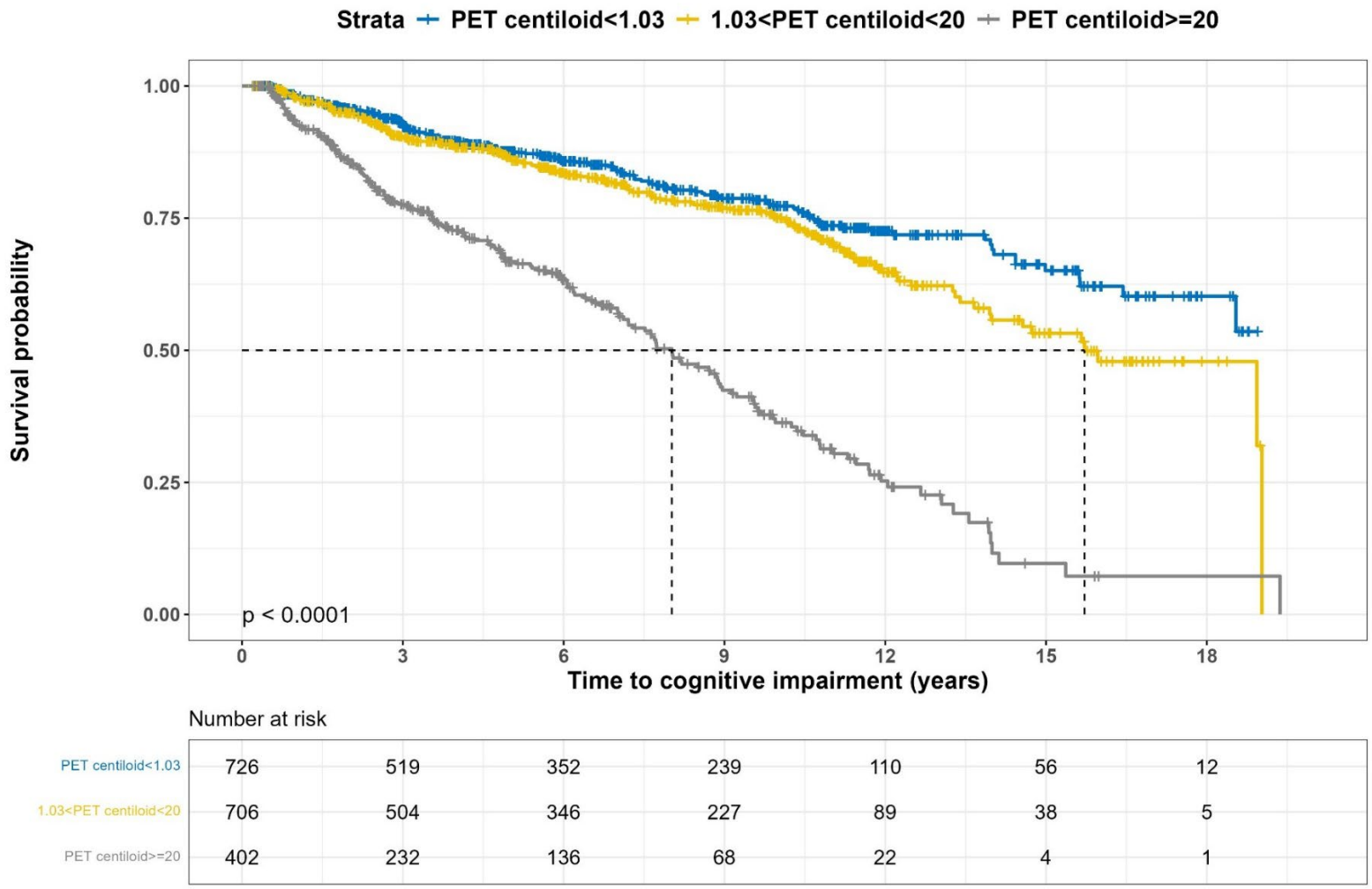


Figure 3. Spaghetti plots of the longitudinal PET centiloid for the three amyloid groups defined by baseline centiloid 1.03 and 20. An estimated longitudinal trajectory by locally estimated scatterplot smoothing (LOESS; in black curve) was superimposed.

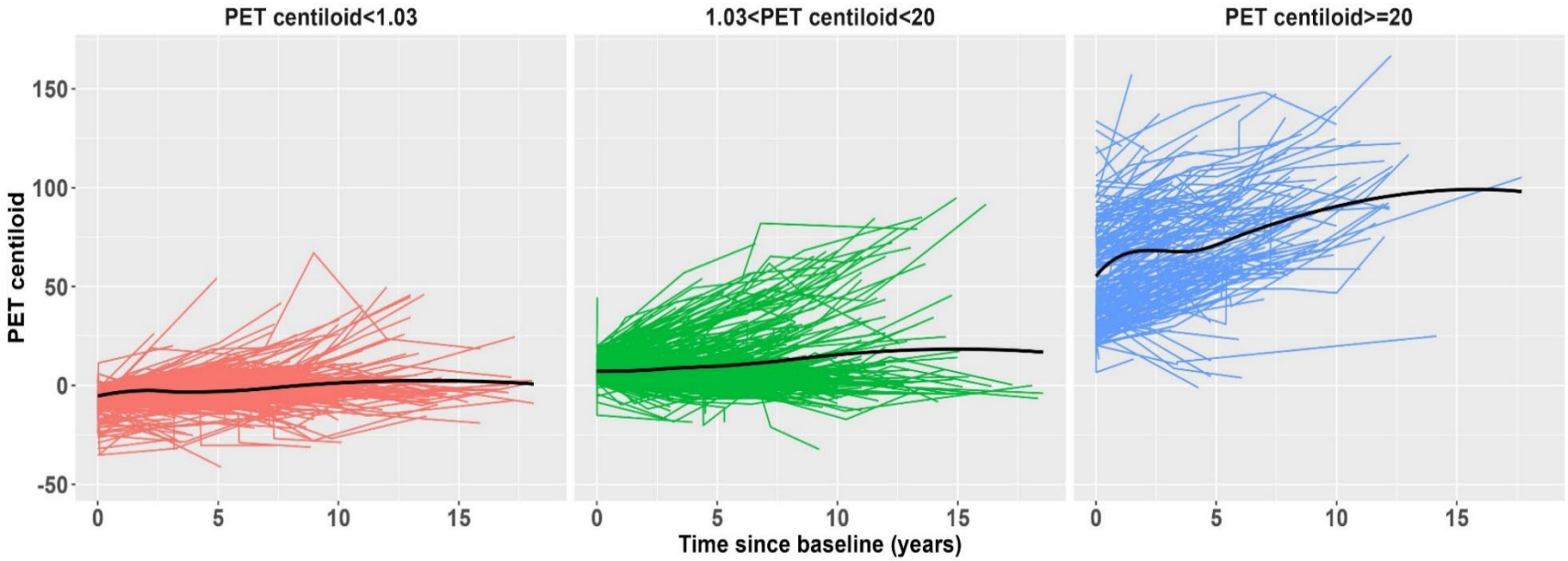
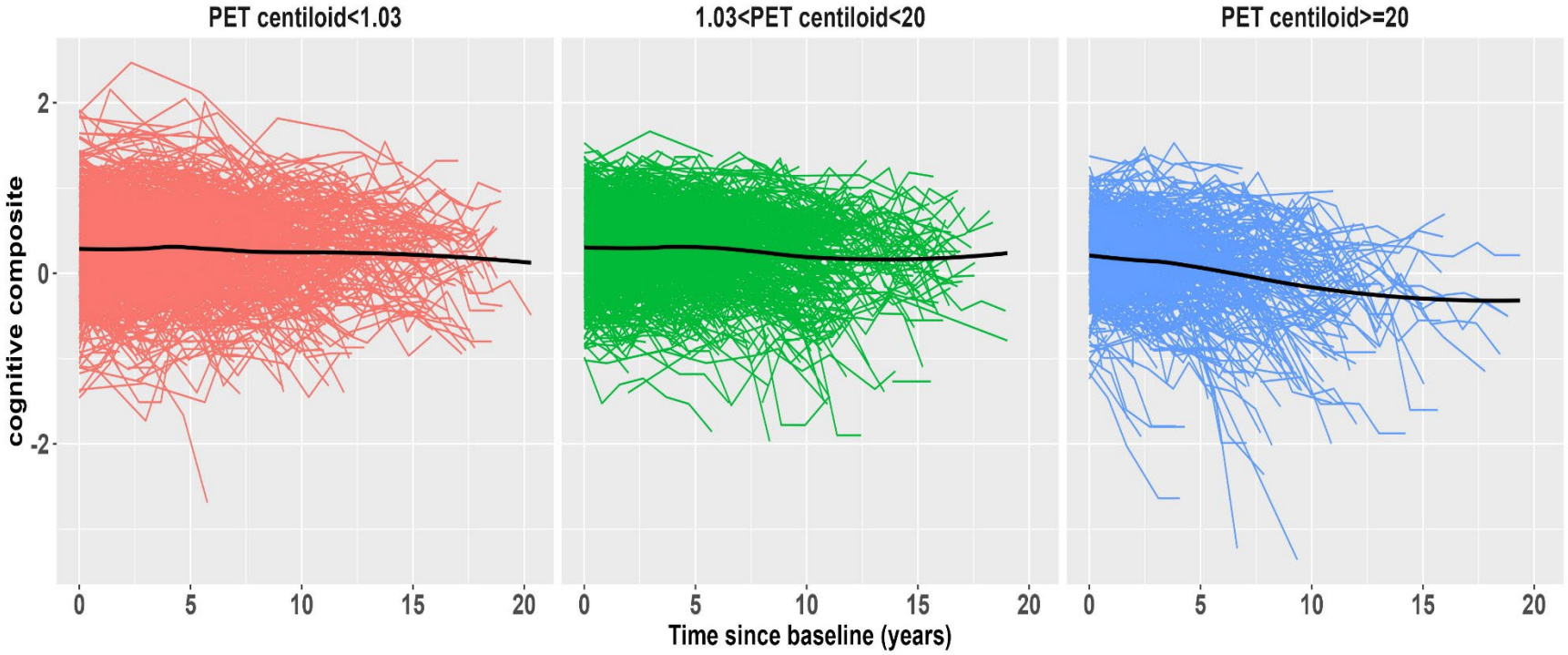


Figure 4. Spaghetti plots of longitudinal cognitive composite for the amyloid trichotomy as defined by baseline centiloid 1.03 and 20. An estimated longitudinal trajectory by locally estimated scatterplot smoothing (LOESS; in black curve) was superimposed.



Online Methods

Participants

Participants were from a multi-center study that harmonized imaging data and clinical data collected by multiple biomarker studies of memory and aging in middle-aged and older individuals: the WashU Knight Alzheimer Disease Research Center (ADRC), the UPenn ADRC, the Wisconsin ADRC, the UAB ADRC, the Wisconsin Registry of Alzheimer Prevention (WRAP), and the Harvard Aging Brain Study (HABS). Recruitment details for these studies have been described previously[23-27]. Inclusion criteria for the current study included categorization as cognitively unimpaired at study baseline (the time of initial amyloid PET assessments), and availability of longitudinal clinical and cognitive assessments since baseline. Participants who had conditions that could prevent participation in neuroimaging (e.g., a pacemaker), or that could affect long-term participation (e.g., metastatic cancer), were excluded. All participants provided written informed consent at recruitment from their parent studies. The WashU Human Research Protection Office approved the current study.

Clinical and cognitive assessments

Clinical and cognitive assessment protocols across WashU, UPenn, Wisconsin, and UAB ADRCs included common elements from the National Alzheimer Coordinating Center (NACC) Uniform Data Set (UDS)[28-29]. The Clinical Dementia Rating® (CDR®)[30] was performed at each clinical assessment to determine the presence or absence of cognitive impairment, and when present, its severity. Standard criteria were applied to diagnose the likely etiology of cognitive impairment[31]. HABS and WRAP also followed assessment protocols largely consistent with the NACC UDS. Age, sex, years of education, self-reported race, and medical history were collected at study entry and updated at each visit. In this study, we defined cognitively unimpaired as CDR 0, or if CDR was missing, a diagnosis of normal cognition. Development of cognitive impairment over time was defined as CDR>0, or if CDR was missing, a diagnosis of cognitive impairment.

The cognitive batteries across the studies were also similar to the NACC UDS, which included measures of episodic memory, working memory, semantic knowledge, executive function and attention, and visuospatial ability. Cognitive measures were harmonized as necessary across UDS version 2 and 3[32] using approaches that have been previously described[33]: the Mini-Mental State Examination (MMSE, harmonized with Montreal Cognitive Assessment [MoCA]), Animal Fluency (60 seconds), Vegetable Fluency, Wechsler Adult Intelligence Scale (WAIS-R) Digit Symbol, Digit Span, Logical Memory Immediate Recall (harmonized with Craft Story Immediate Recall), Logical Memory Delayed Recall (harmonized with Craft Story Delayed Recall), Boston Naming Test (BNT; harmonized with Multilingual Naming Test),

Free and Cued Selective Reminding, and Trail Making Test A and B. All scales were oriented such that a higher score indicates better cognition. Z-scores were computed by subtracting the mean from the individual test scores across all subjects and times and dividing the difference by the standard deviation (SD). A global cognitive composite score was constructed by averaging Z-scores across all available tests. A minimum of two tests were required to compute the cognitive composite score.

Apolipoprotein E genotypes

Details of the apolipoprotein E (*APOE*) genotyping protocols have been described previously[2]. The *APOE ε*4 carrier status was dichotomized as positive if an individual carried either one or two *APOE ε*4 alleles, or negative if no *APOE ε*4 alleles.

Amyloid PET collection and processing

Amyloid PET was performed with either ^{11}C-Pittsburgh Compound B (PiB), ^{18}F-florbetapir (FBP), or ^{18}F-florbetaben (FBB), and the quantification was based on the post-injection windows of 30-60, 50-70, or 90-110 minutes, respectively. All amyloid PET scans from WashU, UPenn, Wisconsin, UAB, and most of the scans from HABS were centrally processed at the Knight Alzheimer Research Imaging (KARI) Program at WashU using a protocol similar to that used by the Alzheimer Disease Neuroimaging Initiative (ADNI). Details of the amyloid PET protocols are provided elsewhere[2]. The centralized processing was MR-free[34], and PET data were corrected for inter-frame motion and summed over frames covering the quantitative window using PET Unified Pipeline (https://github.com/ysu001/PUP). Preprocessed output was fed into a local implementation of the MR-free PET pipeline[35] where it was resampled and aligned to the MNI-152 atlas using the SPM12 (Wellcome Centre for Human Neuroimaging, London, UK) *Coregister* module. The MNI-aligned PET data was smoothed to a full-width-half-max (FWHM) of 8mm and spatially normalized to a tracer-specific template image using the *Normalise* module. To harmonize data from different tracers (PiB, Florbetapir or Florbetaben) used in amyloid PET imaging scans across studies, we calculated a global cortical standardized uptake value ratio (SUVr) using the standard centiloid [35] cortical summary regions of interest with whole cerebellum as the reference and then converted to centiloid using equations derived from a Level 2-calibration of the WashU-implemented MR-free PET pipeline, similar to our MR-based centiloid equations[36-37]. A total of 687 amyloid PET scans from 282 participants of HABS were also centrally processed by WashU with the identical protocol, which served as the bridging data to harmonize the amyloid PET distribution volume ratio (DVR) values from the rest of 71 HABS participants into the centiloid scale, following published methodologies[38-39].

Statistical analysis

The baseline of this study was defined as the time of the first amyloid PET assessments. Baseline demographics, *APOE ε*4 status (carrier or non-carrier), and presence of select medical comorbidities (hypertension, diabetes, stroke, obesity) were summarized with the mean and standard deviation (SD) for continuous variables and count (percentage) for categorical variables.

The primary analyses were conducted to detect a minimal level of PET centiloid among cognitively unimpaired individuals that predicted the subsequent onset of cognitive impairment, defined as the first occurrence after baseline of a CDR ≥0.5 or a diagnosis of cognitive impairment. The 'survival' time from the baseline to the onset of cognitive impairment was analyzed by Cox proportional hazards models[40]. The survival time for participants who never received a CDR ≥0.5 or a diagnosis of cognitive impairment during the entire follow-up period was treated as statistically right-censored, using the time the participants had stayed in the study[40]. To locate the minimal amyloid burden that predicted the onset of cognitive impairment, a wide range of possible thresholds lower than a commonly used amyloid PET positivity threshold of centiloid 20[4] were considered. Because amyloid positivity have been associated with faster cognitive decline[1-3] and elevated risk of developing cognitive imapirment[4-6], we designed the analyses to focus on the lowest possible threshold starting from the 10th percentile of the baseline centiloid distribution. Specifically, for every increase of 5 percentiles from the 10th to 70th percentile of PET centiloid (a centiloid of 20 corresponded to the 78th percentile), we first binarized participants into higher (≥the threshold) and lower (<the threshold) amyloid groups and located the minimum threshold where the corresponding higher amyloid group had a significantly higher risk of progression to cognitive impairment than the lower amyloid group by estimating and testing the associated hazards ratio (HR). Next, because the higher amyloid group contained amyloid-positive participants as a subset, in order to demonstrate that the elevated risk of clinical progression for the higher amyloid group was not solely attributable to the amyloid-positive participants, we searched for the second minimum threshold such that the resulting middle/intermediate amyloid group of participants (with centiloid from the threshold to centiloid 20) had a significantly increased risk of clinical progression than the lowest amyloid group (<the threshold) but a significantly reduced risk than the amyloid-positive group. The 10th and 70th percentiles were chosen because of the recommended minimum number of 'events' (e.g., individuals who progressed to cognitive impairment) necessary to reliably estimate the risk of clinical progression[41] in the lowest and middle/intermediate amyloid groups. To control for the possible inflation of false-positives in the search of the minimal sub-threshold, we adjusted statistical significance for a False Discovery Rate (FDR)[42] of 5%. Both unadjusted and adjusted analyses were conducted, with the latter including major AD risk factors, baseline age, *APOE ε4* carrier status, sex, education (>12 yrs vs. ≤12 yrs), race, and presence of medical comorbidities in the models. Because death may be a competing event to dementia onset, we conducted sensitivity analyses by using the Fine and Gray semiparametric proportional hazards model[43-44] to estimate the cumulative

incidence curve of cognitive impairment for amyloid groups of participants (defined by the thresholds), and tested the difference between the groups using Gray's *K*-sample statistic[44]. For another sensitivity assessment, we repeated the analyses by defining the survival time as the time to the first occurrence of a CDR ≥0.5 or cognitive impairment after which no regression back to a cognitive unimpaired status (CDR 0 or normal cognition) occurred.

Once we identified the minimal sub-threshold of PET centiloid that predicted clinical progression to cognitive impairment, we validated it by estimating and comparing the annual rates of longitudinal change in PET centiloid and cognition between the resulting three groups of participants with the lowest (centiloid<the minimal sub-threshold), minimal (centiloid from the minimal sub-threshold to 20), and positive (centiloid≥20) amyloid accumulation. These analyses were based on the random intercept and slope models[45], and the statistical comparisons of the rate of change were based on the approximate t tests with the degrees of freedom estimated by the Satterthwaite method. All analyses were implemented in R/Rstudio (Version. 2025.9.2.418)[46].

Supplemental Figures

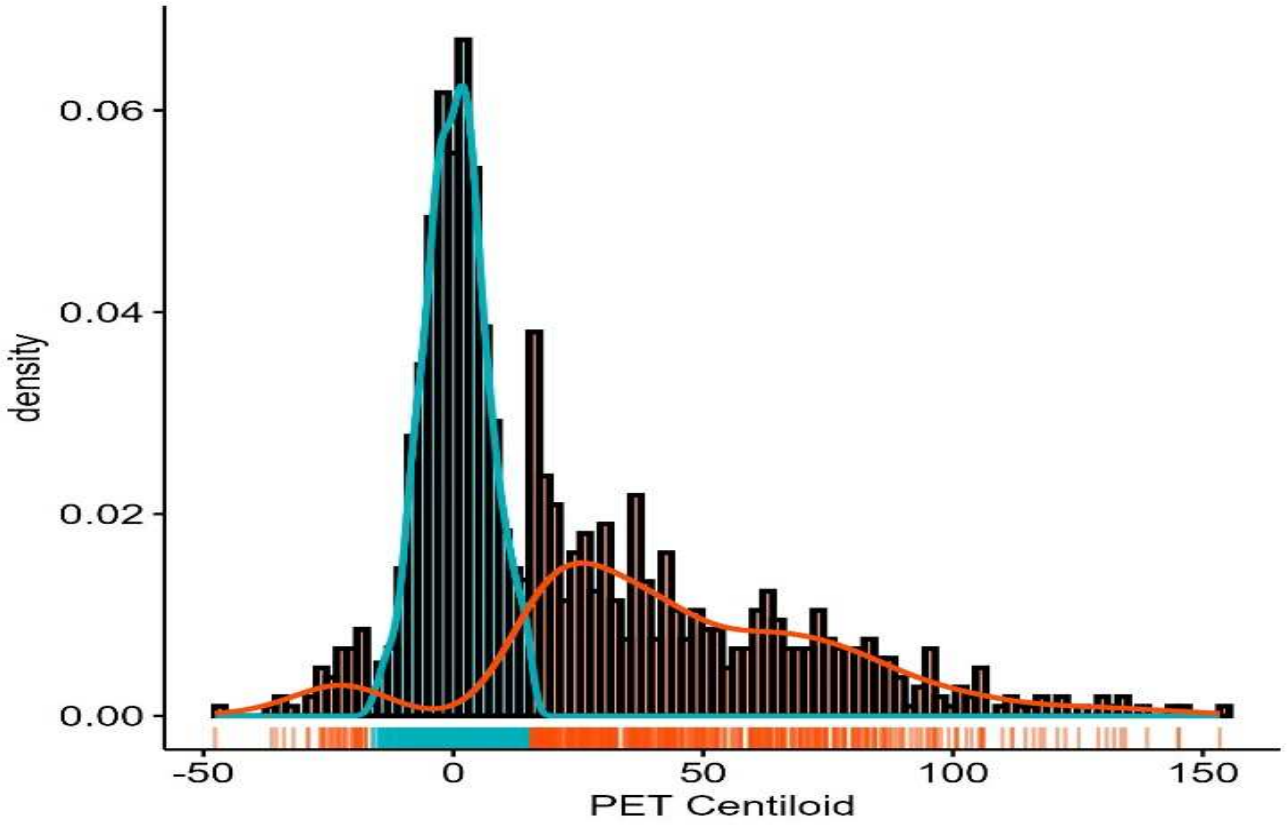
Supplemental Table 1. For each sub-threshold from the 10th to 70th percentile (first column) of the baseline amyloid PET centiloid (second column), participants are divided into 3 groups: L= lowest amyloid (centiloid<the threshold), P=positive amyloid (centiloid ≥20), and M=middle/intermediate amyloid (centiloid from the threshold to 20). The sample size (N) of each group and the number of participants who progressed to cognitive impairment (progressors) during the longitudinal follow up (NP) are presented.

		L		M		P	
Threshold in Percentile	PET centiloid	N	NP	N	NP	N	NP
10%	-7.25	184	34	1248	258	402	185
15%	-5.20	271	54	1161	238	402	185
20%	-3.65	372	69	1060	223	402	185
25%	-2.40	468	86	964	206	402	185
30%	-1.36	543	100	889	192	402	185
35%	-0.11	636	114	796	178	402	185
40%	1.03	726	133	706	159	402	185
45%	2.17	829	162	603	130	402	185
50%	3.21	921	182	511	110	402	185
55%	4.35	1005	198	427	94	402	185
60%	6.01	1100	215	332	77	402	185
65%	7.90	1192	230	240	62	402	185
70%	11.22	1284	251	148	41	402	185
78%	20	1432	292	0	0	407	185

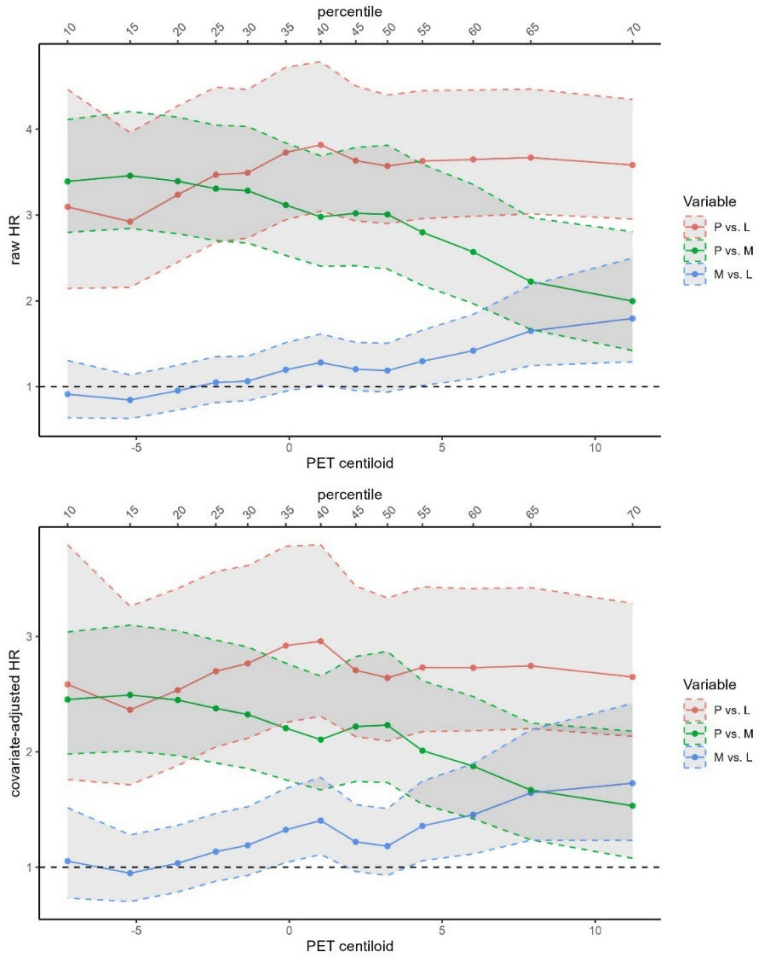
Supplemental Table 2. Unadjusted estimates to HR (95% CI) between amyloid-positive group (P), middle/intermediate amyloid group (M), and lowest amyloid group (L) as a function of sub-threshold (first column) along with the p-values for statistical significance

Threshold (percentile)	HR: M vs. L	p-value: M vs. L	HR: P vs. L	p-value: P vs. L	HR: P vs. M	p-value: P vs. M
10%	0.91 (0.64~1.31)	0.6157	3.09 (2.15~4.46)	1.49E-09	3.39 (2.80~4.11)	1.68E-35
15%	0.85 (0.63~1.14)	0.2659	2.92 (2.16~3.96)	4.62E-12	3.46 (2.84~4.21)	2.13E-35
20%	0.95 (0.73~1.25)	0.7306	3.24 (2.45~4.27)	1.10E-16	3.39 (2.78~4.14)	1.88E-33
25%	1.05 (0.82~1.35)	0.7085	3.47 (2.68~4.49)	2.88E-21	3.31 (2.70~4.047)	3.94E-31
30%	1.06 (0.83~1.35)	0.6183	3.49 (2.73~4.46)	1.64E-23	3.28 (2.67~4.03)	7.65E-30
35%	1.20 (0.95~1.51)	0.1351	3.73 (2.94~4.72)	8.26E-28	3.12 (2.534~3.84)	1.38E-26
40%	1.28 (1.02~1.61)	0.0350	3.82 (3.04~4.79)	4.19E-31	2.98 (2.40~3.69)	1.72E-23
45%	1.20 (0.95~1.52)	0.1172	3.63 (2.93~4.50)	5.16E-32	3.02 (2.41~3.79)	9.53E-22
50%	1.19 (0.94~1.51)	0.1548	3.57 (2.90~4.40)	4.34E-33	3.01 (2.37~3.81)	1.13E-19
55%	1.30 (1.01~1.66)	0.0385	3.63 (2.96~4.45)	3.41E-35	2.80 (2.18~3.59)	6.62E-16
60%	1.42 (1.09~1.84)	0.0085	3.65 (2.99~4.46)	8.89E-37	2.57 (1.97~3.36)	4.23E-12
65%	1.65 (1.25~2.18)	0.0005	3.67 (3.01~4.47)	2.85E-38	2.22 (1.67~2.97)	5.97E-08
70%	1.79 (1.29~2.50)	0.0005	3.58 (2.95~4.35)	2.50E-38	2.00 (1.42~2.80)	6.69E-05

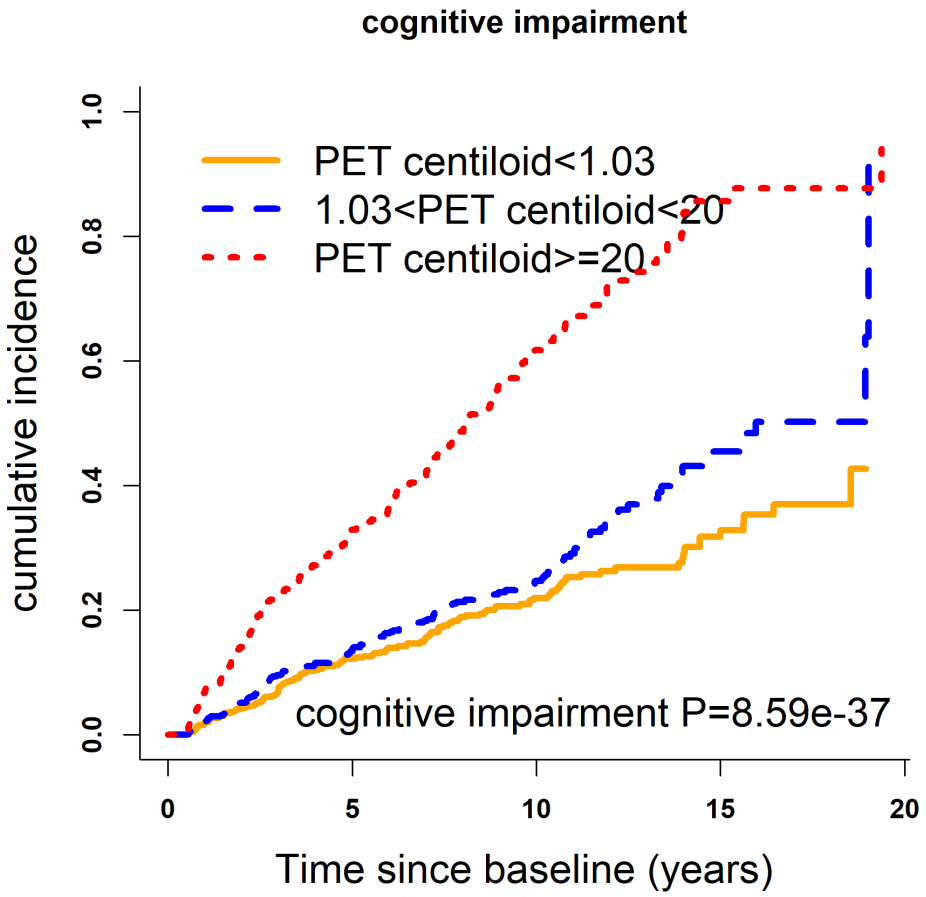
Supplemental Figure 1. Histogram of baseline PET centiloid with a fitted bi-modal distribution as indicated by red and green density curves, suggesting more than one sub-group of the entire amyloid distribution



Supplemental Figure 2. Unadjusted (top panel) and covariate-adjusted (bottom panel) estimates to the HR of progression to cognitive impairment as functions of centiloid sub-threshold (x-axis) between amyloid-positive group (P), middle/intermediate amyloid group (M), and lowest amyloid group (L). The analyses indicated that participants between centiloid 1.03 and centiloid 20 had a significantly higher risk of progression to cognitive impairment than those whose PET centiloid is below centiloid 1.03, but a significantly lower risk of progression than amyloid-positive participants.



Supplemental Figure 3. Estimated cumulative incidence curves (CIC) for the 3 amyloid groups defined by centiloid 1.03 and 20 after taking death into account as a competing risk, demonstrating that the minimal amyloid group had a 31% increased risk of clinical progression than the lowest amyloid group (HR=1.31, 95% CI: 1.05 to 1.65, p=0.019), but reduced risk than the amyloid-positive group (HR=0.35, 95% CI: 0.28 to 0.43, p<0.0001)



Supplemental Figure 4. Estimated survival curves for the three amyloid groups defined by centiloid 1.03 and 20 with the survival time defined as from the baseline amyloid PET scan collection to the first occurrence of cognitive impairment after which no regression back to CDR 0 or normal cognition occurred---a sensitivity analysis

