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Age-specific population frequencies of amyloidosis and neurodegeneration among cognitively normal people age 50-89 years: a cross-sectional study

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Summary

Background—As treatment of pre-clinical Alzheimer's disease (AD) becomes a focus of therapeutic intervention, observational research studies should recognize the overlap between imaging abnormalities associated with typical aging vs those associated with AD. Our objective was to characterize how typical aging and pre-clinical AD blend together with advancing age in terms of neurodegeneration and b-amyloidosis.

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Disclosure of Conflicts of Interests

Ms. Wiste, Mr. Weigand, Dr. Mielke, Mr. Senjem, Dr. Gunter, Mr. Preboske, and Dr. Vemuri report no disclosures.

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Methods—We measured age-specific frequencies of amyloidosis and neurodegeneration in 985 cognitively normal subjects age 50 to 89 from a population-based study of cognitive aging. Potential participants were randomly selected from the Olmsted County, Minnesota population by age- and sex-stratification and invited to participate in cognitive evaluations and undergo multimodality imaging. To be eligible for inclusion, subjects must have been judged clinically to have no cognitive impairment and have undergone amyloid PET, FDG PET and MRI. Imaging studies were obtained from March 2006 to December 2013. Amyloid positive/negative status (**A+/A−**) was determined by amyloid PET using Pittsburgh Compound B. Neurodegeneration positive/negative status (**N+/N−**) was determined by an AD-signature FDG PET measure and/or hippocampal volume on MRI. We labeled subjects positive or negative for neurodegeneration (FDG PET or MRI) or amyloidosis by using cutpoints defined such that 90% of 75 clinically diagnosed AD dementia subjects were categorized as abnormal. APOE genotype was assessed using DNA extracted from blood. Every individual was assigned to one of four groups: **A−N−, A+N−, A−N+,** or **A+N+**. Age specific frequencies of the 4 **A/N** groups were determined cross-sectionally using multinomial regression models. Associations with APOE ε4 and sex effects were evaluated by including these covariates in the multinomial models.

Findings—The population frequency of **A−N−** was 100% (n=985) at age 50 and declined thereafter. The frequency of **A+N−** increased to a maximum of 28% (95% CI, 24%-32%) at age 74 then decreased to 17% (95% CI, 11%-25%) by age 89. **A−N+** increased from age 60 onward reaching a frequency of 24% (95% CI, 16%-34%) by age 89**. A+N+** increased from age 65 onward reaching a frequency of 42% (95% CI, 31%-52%) by age 89. **A+N−** and **A+N+** were more frequent in APOE ε4 carriers. **A+N+** was more, and **A+N−** less frequent in men.

Interpretation—Accumulation of **A/N** imaging abnormalities is nearly inevitable by old age yet people are able to remain cognitively normal despite these abnormalities. . The multinomial models suggest the **A/N** frequency trends by age are modified by APOE ε4 , which increases risk for amyloidosis, and male sex, which increases risk for neurodegeneration. Changing **A/N** frequencies with age suggest that individuals may follow different pathophysiological sequences.

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Keywords

Cognitive aging; Brain aging; Amyloid imaging; Alzheimer disease; Brain atrophy and Alzheimer disease; FDG PET and Alzheimer disease

Introduction

Recognition that biomarker evidence of Alzheimer's disease (AD) pathophysiology is present long before clinical symptoms become apparent[1] has motivated the formulation of research criteria for preclinical AD.[2,3] In 2011 the National Institute on Aging – Alzheimer's Association (NIA-AA) criteria first described a method for defining and staging pre-clinical AD, defining Stage 1 as cerebral amyloidosis, Stage 2 as amyloidosis plus neurodegeneration, and Stage 3 as amyloidosis, neurodegeneration, and subtle cognitive decline.[2] While the NIA-AA method likely does accurately reflect the onset and staged

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progression of biomarkers of AD pathophysiology,[1,4-6] AD pathology typically does not occur in isolation in elderly subjects but rather co-occurs with other age related degenerative processes.[7] Structural magnetic resonance imaging (MRI) and ^{18}F-fluorodeoxyglucose positron emission tomography (FDG PET) are sensitive measures of neurodegeneration or brain injury, however even signature AD topographic measures on these modalities (for example hippocampal atrophy on MRI) are not etiologically specific for AD.[8-10]

A two-feature biomarker classification system based on both amyloidosis (**A**) and neurodegeneration (**N**), described in [11,12], expands the NIA-AA staging of pre-clinical AD.[2] This two-feature **A/N** system classifies all subjects, rather than only those who are exclusively in the AD pathophysiological pathway, thereby accommodating the facts that; AD and non-AD degenerative processes occur with aging; and, neurodegenerative imaging studies are sensitive to a variety of degenerative processes. Every individual is assigned to one of four groups in this scheme [11,12]: amyloid negative and neurodegeneration negative (**A−N−);** amyloid positive and neurodegeneration negative (**A+N−**); amyloid negative and neurodegeneration positive (**A−N+**); or amyloid positive and neurodegeneration positive (**A +N+). A−N−** corresponds to NIA-AA stage 0; **A+N−** to NIA-AA stage 1; **A−N+** corresponds to suspected non-Alzheimer's pathophysiology (SNAP) as first described in [13]; and **A+N+** to NIA-AA stages 2 and 3.

Our **A/N** classification system [11,12] also operationalizes the new (2014) International working Group (IWG) research criteria for asymptomatic at risk for AD [3]. Asymptomatic at risk for AD is defined by the absence of a clinical phenotype consistent with typical or atypical AD and the presence of a pathophysiological biomarker consistent with the presence of AD pathophysiology. A positive amyloid PET scan is the only currently available imaging study that is diagnostic of AD pathophysiology [11]. Structural MRI and FDG PET abnormalities in topographic areas characteristic of AD are employed to stage disease severity, not as diagnostic measures [3]. Thus, framed in terms of the new IWG criteria, cognitively normal **A+N−** and **A+N+** subjects would be designated as asymptomatic at risk for AD with the latter more severely involved. **A−N−** and **AN+** would not be considered to have evidence of AD pathophysiology.

From a clinical standpoint typical aging blends imperceptibly with pre-clinical AD in the population. Our objective was to characterize amyloidosis and neurodegeneration in cognitively normal subjects, which includes typical aging and pre-clinical AD (asymptomatic at risk for AD). We estimated age specific frequencies of the four **A/N** groups described above in a large sample of cognitively normal subjects from a population based cohort age 50 to 89.

Methods

Subjects

We studied cognitively normal participants in the Mayo Clinic Study of Aging (MCSA). The MCSA is a population-based study of cognitive aging among Olmsted County, MN residents.[14] The Rochester Epidemiology Project [15] medical records linkage system was used to enumerate all Olmsted County residents aged 50 to 89.. All residents from the

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population enumeration were randomly ordered in lists based on age- and sex-stratification; potential participants were selected from those ordered lists until the target enrollment in each strata was achieved. Approximately 50% of the randomly identified subjects agree to in-person participation. Men and women are equally represented in each age category. All subjects without a medical contraindication are invited to participate in imaging studies. Since 2004, the MCSA has enrolled non-demented individuals aged 70 to 89 years, and in 2012 started to enroll subjects 50 plus years of age.

To be eligible for inclusion in the current analysis, subjects must have been judged clinically to have no cognitive impairment based on psychometric testing and evaluations by a study coordinator and a physician. Subjects also had to have undergone amyloid PET, FDG PET, and MRI within 7 months of their index clinical visit. A total of 985 subjects met these criteria. The imaging studies were obtained from March 2006 to December 2013. PET and MRI protocols were identical for all subjects. APOE genotype was assessed using standard laboratory procedures using DNA extracted from blood. [16]

Standard protocol approvals, registrations, and patient consents

These studies were approved by the Mayo Clinic and Olmsted Medical Center Institutional Review Boards and written informed consent was obtained from all participants.

Imaging Methods

Amyloid PET imaging was performed with ^{11}C Pittsburgh Compound B (PIB) and consisted of four 5-minute dynamic frames acquired from 40–60 minutes after injection. FDG PET was obtained on the same day as the PIB scan and consisted of four 2-minute dynamic frames acquired from 30–38 minutes after injection. CT was obtained for attenuation correction. Amyloid PET and FDG PET were analyzed with our in-house fully automated image processing pipeline[12] where image voxel values are extracted from automatically labeled regions of interest (ROIs). An amyloid PET standardized uptake value ratio (SUVR) was formed from the prefrontal, orbitofrontal, parietal, temporal, anterior cingulate, and posterior cingulate/precuneus ROIs normalized to the whole cerebellum. An AD-signature FDG PET SUVR was formed from the angular gyrus, posterior cingulate, and inferior temporal cortical ROIs normalized to pons and vermis [17].

MR scanning was performed on three 3 Tesla scanners from the same manufacturer. Hippocampal volume was measured with FreeSurfer (v5.3). Total intracranial volume (TIV) was measured using an in-house method. Tissue class segmentation maps were created by SPM12 with custom priors and passed through a series of morphological opening, erosion, dilation and thresholding steps. The hippocampal masks and TIV masks were manually inspected for quality control by a trained analyst. Each subject's raw hippocampal volume was adjusted for TIV to create a TIV-adjusted hippocampal volume (HVa) by calculating the residual from a linear regression of hippocampal volume (y) versus TIV (x) among 133 CN subjects aged 30 to 59 (as in [12]). HVa can be interpreted as the deviation in cm^3 in a subject's hippocampal volume from what is expected given their head size.

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Cut Points and Subject Classification

We defined the negative/positive threshold for amyloid PET, FDG PET and HVa such that 90% of a group of 75 clinically diagnosed AD dementia subjects from the Mayo Clinic Alzheimer Disease Research Center and Mayo Clinic Study of Aging, were categorized as abnormal using the same approach as in. [12] . Abnormal amyloid PET was defined as ≥1·40 SUVR. Abnormal neurodegeneration was defined as either HVa ≤2·40 cm^3 or FDG ≤1.32 SUVR. Participants were classified into one of four groups defined by the combination of abnormality for amyloid and neurodegeneration: **A−N−, A+N−, A−N+,** or**, A+N+** as in.[12]

Statistical Methods

Pairwise differences in characteristics among the four **A/N** groups were assessed with Wilcoxon rank sum tests or chi-squared tests as appropriate.

We used multinomial regression models that included terms for both age and sex to estimate frequencies (percentages) of each **A/N** group as a function of age. As a generalization of binary logistic regression, multinomial regression is appropriate because each subject could be classified into one of four unordered **A/N** categories. To allow for flexible age-dependent trends in these frequencies, we modeled age trends using restricted cubic splines with knots at 60, 70, and 80 years. We calculated 95% confidence intervals on the probability scale using a parametric bootstrap. This procedure was preferred because linear approximations using the delta method were found to be inadequate. To carry out the parametric bootstrap, we sampled 10,000 multivariate normal deviates with means equal to the parameter estimates and variance structure equal to the variance-covariance matrix of the fitted model. These samples represent plausible realizations of the model coefficients while allowing for statistical uncertainty in their estimated values. Each realization was used as the set of parameter estimates in the multinomial regression equation, and predictions from each resampled model were used to calculate frequency estimates as a function of age for each **A/N** group while controlling for sex differences.

We used this same procedure to also calculate 95% confidence intervals (CIs) for the differences in frequency between **A/N** groups by age. These CIs were defined as the $2{\cdot}5^{th}$ and $97{\cdot}5^{th}$ quantiles of the resampled distribution. We interpreted CIs that did not include zero as significant.

We also examined how the age-dependent **A/N** group frequencies varied by combinations of sex and APOE ε4 carrier status. We used likelihood ratio tests to evaluate the significance of additive effects for each of these patient characteristic groupings, as well as two- and three-way interactions among age, sex, and APOE. Our analysis examines age, sex, and APOE associations across four groups and arguably raises the question of multiple comparisons. However, since our inference is primarily based on just two models, one with 12 parameters and one with 15 parameters—relatively few parameters given the sample size of 985 subjects—we do not believe classical multiple testing problems, or the interrelated issue of spare data bias, are applicable. We used SAS version 9.3 (SAS Institute Inc, Cary, NC, USA) and R version 3.0.2 (R Foundation for Statistical Computing, Vienna) with the “multinom” function from the “nnet” add-on package.

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Results

Demographics by A/N group

The median age increased by **A/N** group in the following order: **A−N−** then **A+N−** then **A−N+** then**A+N+** ($p < 0{\cdot}0001$ for all pairwise comparisons except A−N+ vs. A+N+ ($p=0{\cdot}013$), **Table 1**). Men were more common than women in **A−N+** compared to **A+N−** ($p=0{\cdot}006$). Men were more common in **A+N+** compared to both **A−N−** ($p=0{\cdot}010$) and **A+N−** ($p=0{\cdot}0007$). APOE ε4 carriers were more common than non-carriers in **A+N−** and **A+N+** compared to **A−N−** and **A−N+** ($p \leq 0{\cdot}0001$ for all). Of the 269 neurodegeneration positive subjects, 63% (n=170) were classified as such because of abnormalities on FDG alone; 19% (n=51) because of abnormal HVa alone; and 18% (n=48) because of both abnormal FDG and HVa.

Overall A/N group frequencies by age

Frequencies of **A/N** groups by five-year age strata vary markedly with age (**Fig S1, Table 2**). Abnormal imaging values first appeared at age 60. The model-based estimates and 95% CIs adjusted for sex (**Fig 1**) are largely in agreement with the empirical values in **Fig S1** and **Table 2.** The model generates "smoothed" or "de-noised" estimated frequencies, which are more realistic, because jumps with successive 5-year age bracket are smoothed. We therefore base our inferences on the model-based estimates (**Fig 1**). At age 50, all subjects are **A−N−**. The frequency of **A−N−** falls monotonically with age, reaching approximately 17% (95% CI, 11%-24%) by age 89. The frequency of **A+N−** is zero at age 50 and increases to a maximum of 28% (95% CI, 24%-32%) by age 74. Thereafter the frequency decreases to 17% (95% CI, 11%-25%) by age 89. The frequency of **A−N+** is close to zero before age 60 and increases monotonically thereafter, reaching a frequency of 24% (95% CI, 16%-34%) by age 89. The frequency of **A+N+** is close to zero before age 65 and increases monotonically thereafter, reaching about 42% (95% CI, 31%-52%) by age 89.

Fig 2 shows pairwise differences in **A/N** group frequencies by age and indicates approximate ages where significant differences in frequencies of the 4 **A/N** groups were present. We interpret differences as significant when the 95% confidence interval (CI) around the estimated mean difference does not include zero. **A−N−** is more frequent than any of the other 3 groups from age 50 until around 80. The frequency of **A+N−** exceeds that of **A−N+** and **A+N+** from around 60 through the late 70s. **A+N+** is more frequent than **A+N−** and **A−N−** in the mid-80s. The frequencies of **A+N+** and **A−N+** do not differ significantly at any age.

A/N group frequencies by age: associations with sex and APOE ε4

Age, sex, and APOE were each independently associated with **A/N** group frequencies ($p<0{\cdot}0001$, $p=0{\cdot}004$, $p<0{\cdot}0001$ respectively). We did not find evidence of an interaction between age and APOE ($p=0{\cdot}63$), sex and APOE ($p=0{\cdot}78$), or of a three-way interaction ($p=0{\cdot}47$) on **A/N** group frequencies. The age-by-sex interaction was nearly significant ($p=0{\cdot}06$). However, a model incorporating this interaction provided **A/N** frequency estimates that were strikingly similar (but with wider confidence intervals at old and younger ages) to those where age, sex, and APOE were treated as additive effects (i.e. no interaction). We

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therefore chose to report **A/N** frequencies from the model with additive age, sex, and APOE effects.

The **A/N** group frequencies by age among groups defined by sex and APOE ε4 status are shown in **Fig 3** while differences in frequencies comparing men versus women within APOE ε4 status, or ε4 carriers versus non-carriers within sex are plotted in **Fig S2. A−N−** was more frequent in APOE ε4 non-carriers than carriers at all ages (**Fig S2**, column A). **A+N−** was more frequent in APOE ε4 carriers than non-carriers until age 80 (**Fig S2**, column B). **A+N−** was more frequent in women than men from about age 75 onward. **A−N+** was less frequent in APOE ε4 carriers than non-carriers from about age 75 onward (**Fig S2**, column C). **A+N+** was more frequent in APOE ε4 carriers than non-carriers from about age 65 onward (**Fig S2**, column D). **A+N+** was more frequent in men than women from late 60s onward. In the discussion we focus on sex and APOE findings that are of particular scientific interest.

Discussion

A variety of processes which can be detrimental to brain structure and function become more prevalent in the adult population with increasing age. These include AD pathology, non-AD pathologies, and aging without specific pathology.[9,18-21] An idealized system can be envisioned where subjects are classified on the basis of biomarkers of all relevant age-related processes. While such a classification system may evolve in the future if biomarkers of all major processes associated with cognitive aging become available, in the present, the four-class **A/N** system [11,12] we describe seems useful.

The frequency of abnormal amyloid PET scans by age we observed is similar to prior reports [22]. However, classification of subjects by both amyloidosis and neurodegeneration adds an important additional dimension to classification by amyloidosis alone. We estimate that from age 50-60 the frequency of pre-clinical AD (asymptomatic at risk for AD) is close to zero, and by age 89 over half (59%) of the cognitively normal general population will meet these criteria [2,3]. More specifically, by age 89 the frequency of **A+N−,** asymptomatic at risk for AD without neurodegeneration (pre-clinical AD stage 1), is 17%; while the frequency of **A+N+,** asymptomatic at risk for AD with neurodegeneration (pre-clinical AD stage 2 plus 3), is 42%.

A−N+ or SNAP, represents an increasingly significant proportion of the general CN population above age 60. In our initial publication describing SNAP [11], we suggested that imaging evidence of AD-like neurodegeneration without amyloidosis likely represents any combination of non-AD processes including medial temporal tauopathy, cerebrovascular disease, Lewy body disease, grain disease, aging changes without specific pathology, and in older individuals hippocampal sclerosis or TDP-43) [7,19] . Although both our FDG PET and MRI measures capture characteristic topographic patterns of AD and thus are useful for staging AD severity, neither is specific for AD pathophysiology and thus may be abnormal in non-AD conditions. In addition, because FDG PET was not corrected for partial volume effects, this measure captures both the effects of decreased FDG uptake and brain atrophy.

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A/N frequencies in the population change notably with age (**Figs S1, 1, Table 2).** Reasons for this fall into two main categories: selective censoring of individuals in one (or more) **A/N** group relative to others due to death, non-participation, or progression to cognitive impairment; or, transition from one **A/N** group to another while remaining cognitively normal (thus remaining in the eligible study pool). We acknowledge that without longitudinal data in every subject the relative contributions of each effect cannot be disentangled. However, an obvious major overall trend in the 60s is a monotonic decrease in the frequency of **A−N−** with age coupled with increases in the frequencies of other groups. It is highly improbable that selective elimination of subjects from the cognitively normal population in their 60s explains these trends. The better explanation almost certainly is that individuals transition from **A−N−** to more advanced **A/N** stages while remaining cognitively normal. Beyond age 70s the most likely cause of selective elimination of subjects from the study pool is progression to cognitive impairment. However, this is most probable in **A+N+** [23,24] which would decrease the frequency of **A+N+** with age. Yet we see the opposite, a dramatic increase in the frequency of **A+N+** beyond age 65. Therefore transitions from less to more severe **A/N** groups while subjects remain cognitively normal must be a dominant explanation for the changing cross-sectional **A/N** frequencies with age that we observed. Interpretive parallels might therefore be drawn between our data and studies of Braak and colleagues [25] and Duckyaerts [26] who infer the natural history of progression of tauopathy and β-amyloidosis within individuals, based on the observation that population frequencies of lower stages decline and higher stages increase with advancing age.

Our recent theoretical modeling studies [27,28] provide a possible integrated explanation for the patterns of change in **A/N** frequency with age observed in the present study. These theoretical modeling studies [27,28] predict that cognitively normal subjects may follow different pathophysiological sequences denoted by amyloid and neurodegenerative biomarkers. The first sequence is **A−N−** to **A+N−** to **A+N+ (Fig 4a)**. This is the AD biomarker sequence of pre-clinical AD without major comorbid non-AD pathology[2,29]. The second pathophysiological sequence is **A−N−** to **A−N+** to **A+N+ (Fig 4b)**. We have reported the **A−N+** to **A+N+** transition part of this sequence in an earlier study of incident amyloid PET positivity among CN subjects [13] and proposed that it indicates someone who first develops SNAP neurodegeneration, then later enters the AD pathophysiological pathway, denoted by a positive amyloid PET study. The assumption that **A−N+** (SNAP) represents non-AD neurodegenerative pathology is supported by the fact that the proportion of APOE ε4 carriers in **A−N+** is low (18%) compared to around 40% in **A+N−** and **A+N+ (Table 1)**. The third sequence is **A−N− to A−N+ (Fig 4c)** which we propose represents someone who develops SNAP neurodegeneration without progressing into the AD pathophysiological pathway. We do not discuss **A−N−** to **A+N+** because we believe that it is unlikely that an individual would simultaneously progress **to A+** and to **N+** if it were possible to sample these imaging findings in real time.

We believe that it is biologically meaningful that of the 3 **A/N** groups with abnormal values (**A+N−, A−N+**, and **A+N+**), only **A+N−** falls in frequency with age. (**Figs S1, 1, Table 2**). While the decrease in **A+N−** frequency above age 74 could be due to higher rates of progression to cognitive impairment than in other **A/N** groups, the fact that **A+N+** are most

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likely to become impaired [23,24] yet increase in frequency monotonically with age argues against this possibility. A possible explanation for our observation is that **A+N−** is an inherently unstable state and that above age mid-70s transition out of **A+N−** while remaining cognitively normal for some period of time, is likely. Because we believe that **A+N-** is not a natural biomarker end state, it is not included as a possible pathway endpoint in **Fig 4**.

By age 89 about 83% of cognitively normal subjects have mild AD-like levels of amyloidosis, neurodegeneration or both (**Figs S1, 1, Table 2**). Thus these abnormalities seem almost an inevitable consequence of aging yet people are able to remain cognitively normal despite these abnormalities. Thus typical cognitive aging, defined as remaining free of dementia, is most often characterized by the presence rather than the absence of these imaging abnormalities. The fact that some elderly individuals retain normal cognitive function in the presence of significant brain pathology while others do not has become an area of high research interest. [30,31] .

We found important APOE- and sex-specific variations in the frequencies of the four **A/N** groups. APOE ε4 is over represented in **A+N−** and **A+N+** (**Table 1, Fig S2**). This suggests APOE ε4 selectively increases risk for amyloidosis among CN subjects which is consistent with prior literature[32,33].

Men are over represented in **A+N+** and to a lesser degree in **A−N+** and underrepresented in **A+N−** (**Table 1**). This can be interpreted as men being better able to tolerate neurodegeneration and still remain cognitively normal. Alternatively, men may be at increased risk for neurodegeneration, perhaps due to greater life style risk exposures for cerebro-vascular disease[34] or greater risk of Lewy body disease compared to women.[35]. The effect of male sex on neurodegeneration appears from the late 60s onward (**Fig S2**) which is consistent with an acquired rather than a developmental effect.

Because effects of both sex and APOE were seen on **A/N** group frequencies, one might expect sex and APOE interactions as previously reported.[36,37] However, we were unable to detect any sex and APOE interactions on **A/N** group frequencies by age among CN subjects.

A few comments clarifying study methods and limitations seem worthwhile. We grouped subjects by imaging abnormalities that were of sufficient severity to be on par with the mild end (10^{th} percentile) of those found in AD dementia subjects; therefore, we did not capture subtle amyloidosis or neurodegeneration below this threshold. Amyloidosis approaches a plateau by moderate AD dementia while FDG PET and MRI continue to progress. [1,38,39] As we have pointed out previously [28], selecting cut points in an identical way for all imaging measures, as we did here, seems rational. However, this will place the cut point for amyloid PET at a more advanced stage in in its full dynamic range than the cut points for FDG PET or MRI. Unfortunately, it is not feasible to scan individuals in end stage dementia and thus the maximum abnormal values for FDG PET and MRI cannot realistically be ascertained. Another caveat is that our sample includes only cognitively normal subjects. Certainly for ages over 80 the frequency of **A−N−** would be lower and **A+N+** higher if our sample included the entire cognitive spectrum. Finally, our data is cross-sectional and a more

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complete understanding of changing **A/N** frequencies with age, including how subjects transition between the different biomarker groups, will require longitudinal data acquired uniformly in all subjects across the entire age spectrum.

Systematic Review

We searched PubMed for reports published in English up to May 29, 2014 with the search terms “aging and brain volume”, “amyloid PET”, “aging and amyloid PET”, and “aging and FDG PET”. Reference lists of found papers were included in the search approach. We found that studies that have acquired MRI, FDG PET and amyloid PET in all study subjects have included few individuals less than 60 years of age and also are composed of selected volunteers rather than population-based samples. Samples composed of large numbers of cognitively normal subjects, all thoroughly studied with an array of imaging biomarkers from middle age onward, are needed to understand the background on which dementia arises in old age.

Interpretation

We describe a system that classifies all cognitively normal subjects on the basis of both amyloidosis and neurodegeneration, and provide estimates of the frequency of these **A/N** groups by age from age 50-89 in a population-based sample. These age trends are modified by APOE ε4, which increases risk for amyloidosis, and male sex, which increases risk for neurodegeneration. This classification system can be used to operationalize the new IWG and NIA-AA criteria for pre-clinical/asymptomatic at risk for AD. The high frequency of **A/N** imaging abnormalities in old age among cognitively normal subjects illustrates that typical cognitive aging, defined as remaining free of dementia, is most often characterized by the presence rather than the absence of these imaging abnormalities.. We provide a theoretical framework to interpret changing **A/N** frequencies based on the idea that people may follow several different possible pathophysiological sequences while remaining cognitively normal.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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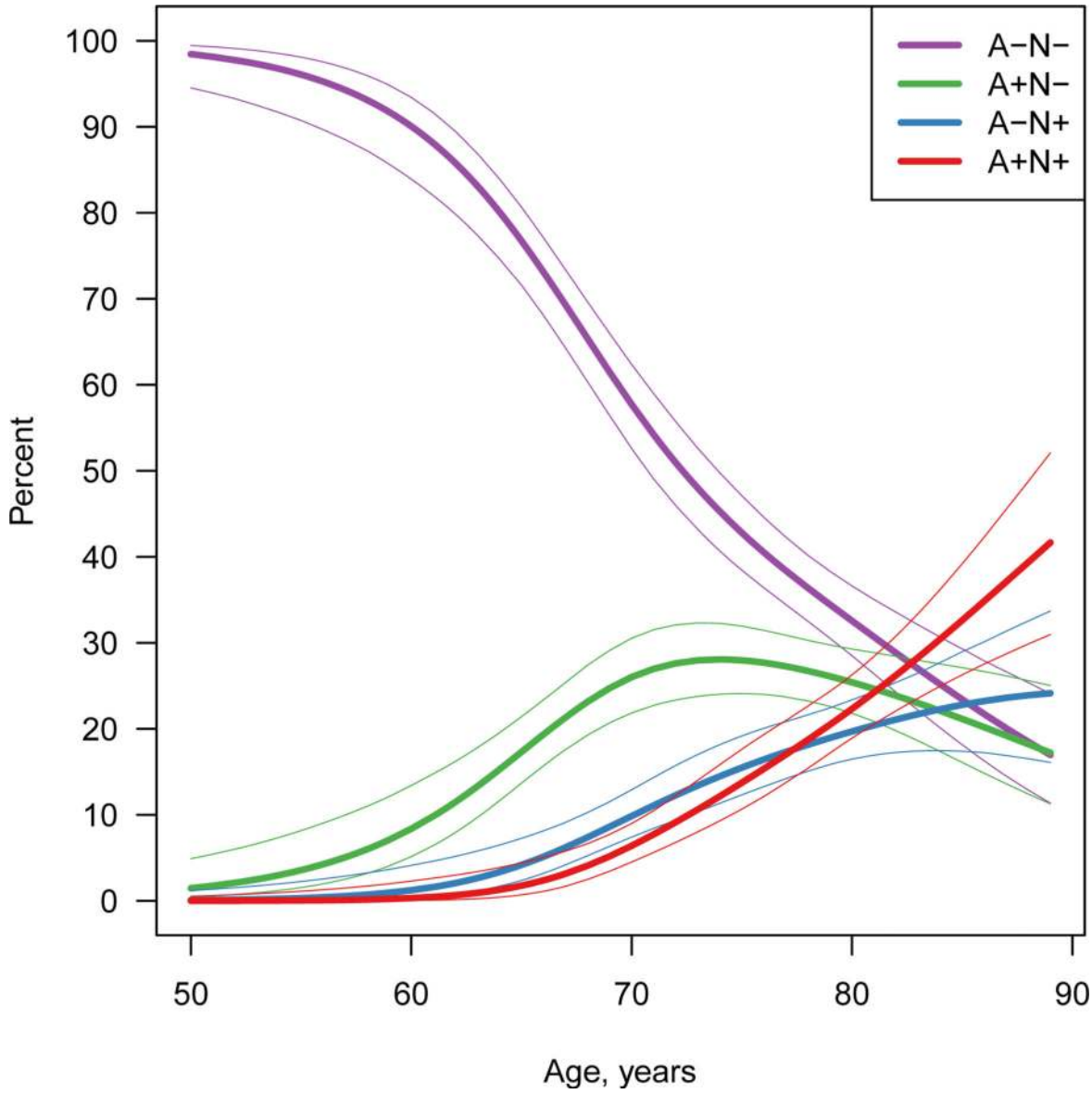

Figure 1. Estimated percentage of participants in each biomarker group by age

Estimates are from a multinomial model adjusted for sex. Nonlinearity in age was allowed in the model by fitting age as a spline with knots at 60, 70, and 80 years. 95% parametric bootstrap confidence intervals are also shown.

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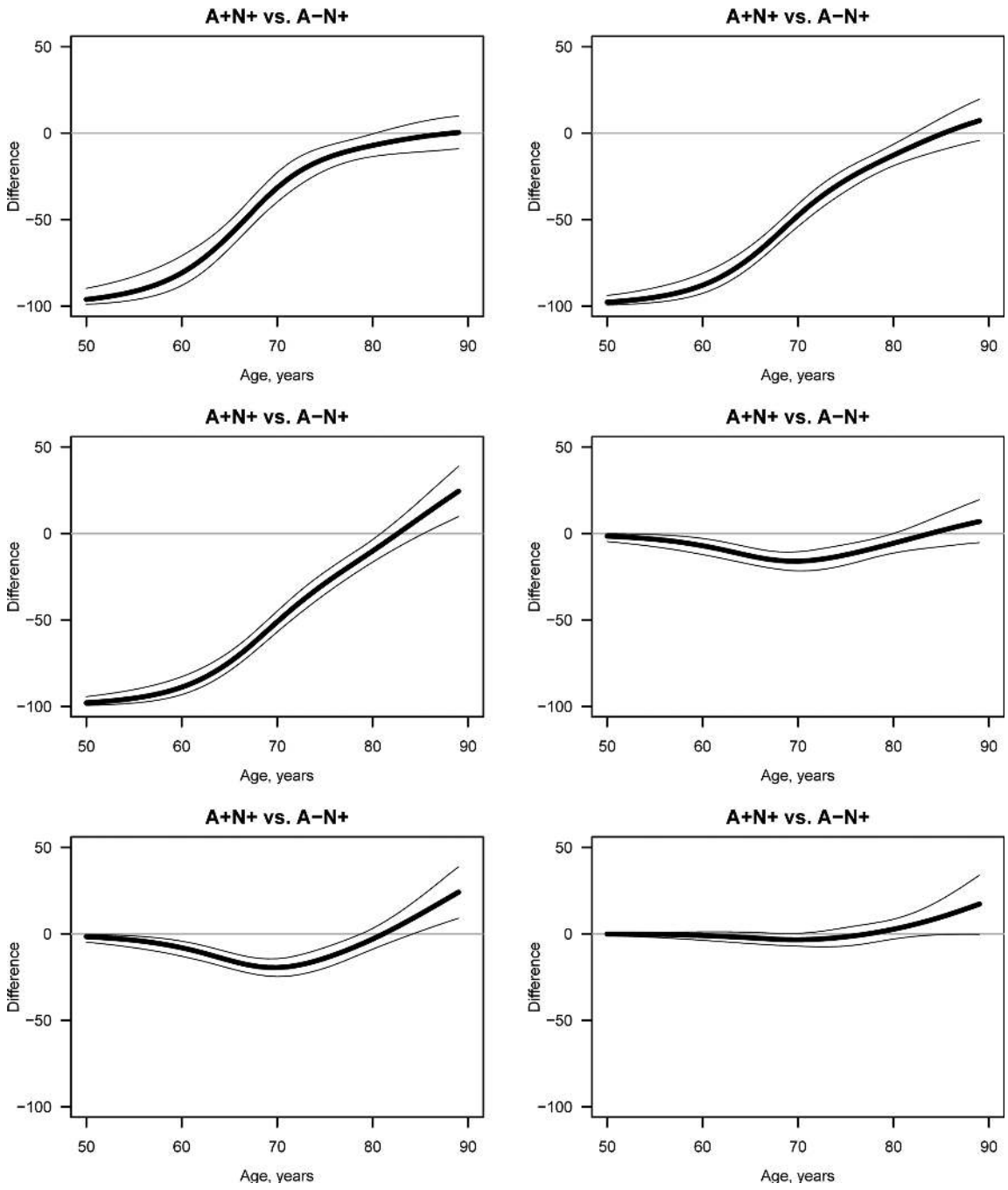

Figure 2. Estimated differences in percentage of participants in each biomarker group by age

Probabilities in each group were estimated from a multinomial model adjusted for sex. Nonlinearity in age was allowed in the model by fitting age as a spline with knots at 60, 70, and 80 years. Differences in probabilities are plotted with 95% parametric bootstrap confidence intervals.

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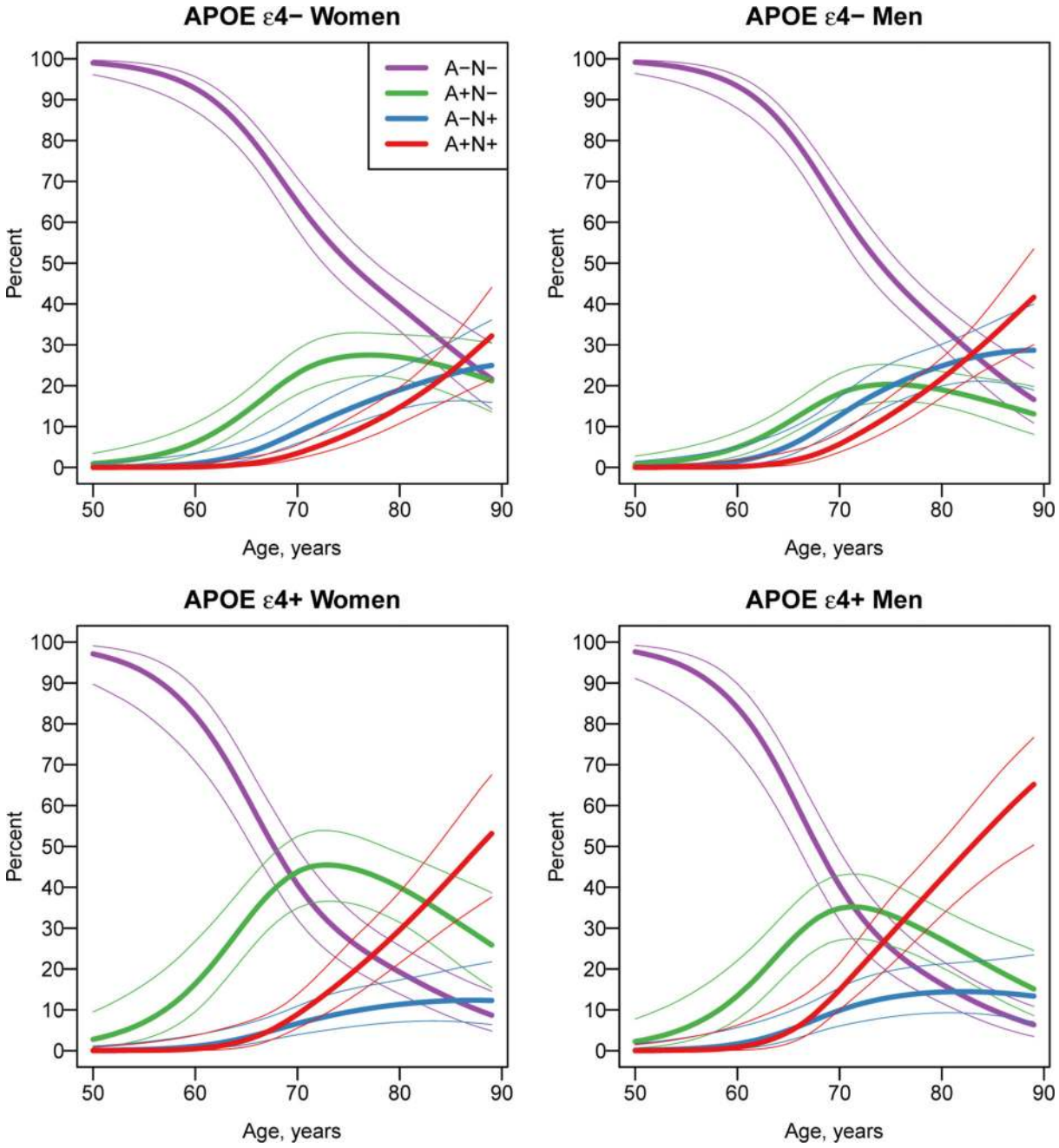

Figure 3. Estimated percentage in each biomarker group by age, sex, and APOE genotype

Percentages are estimated from a multinomial model with age, sex, and APOE genotype. Nonlinearity in age was allowed in the model by fitting age as a spline with knots at 60, 70, and 80 years. 95% parametric bootstrap confidence intervals are also shown.

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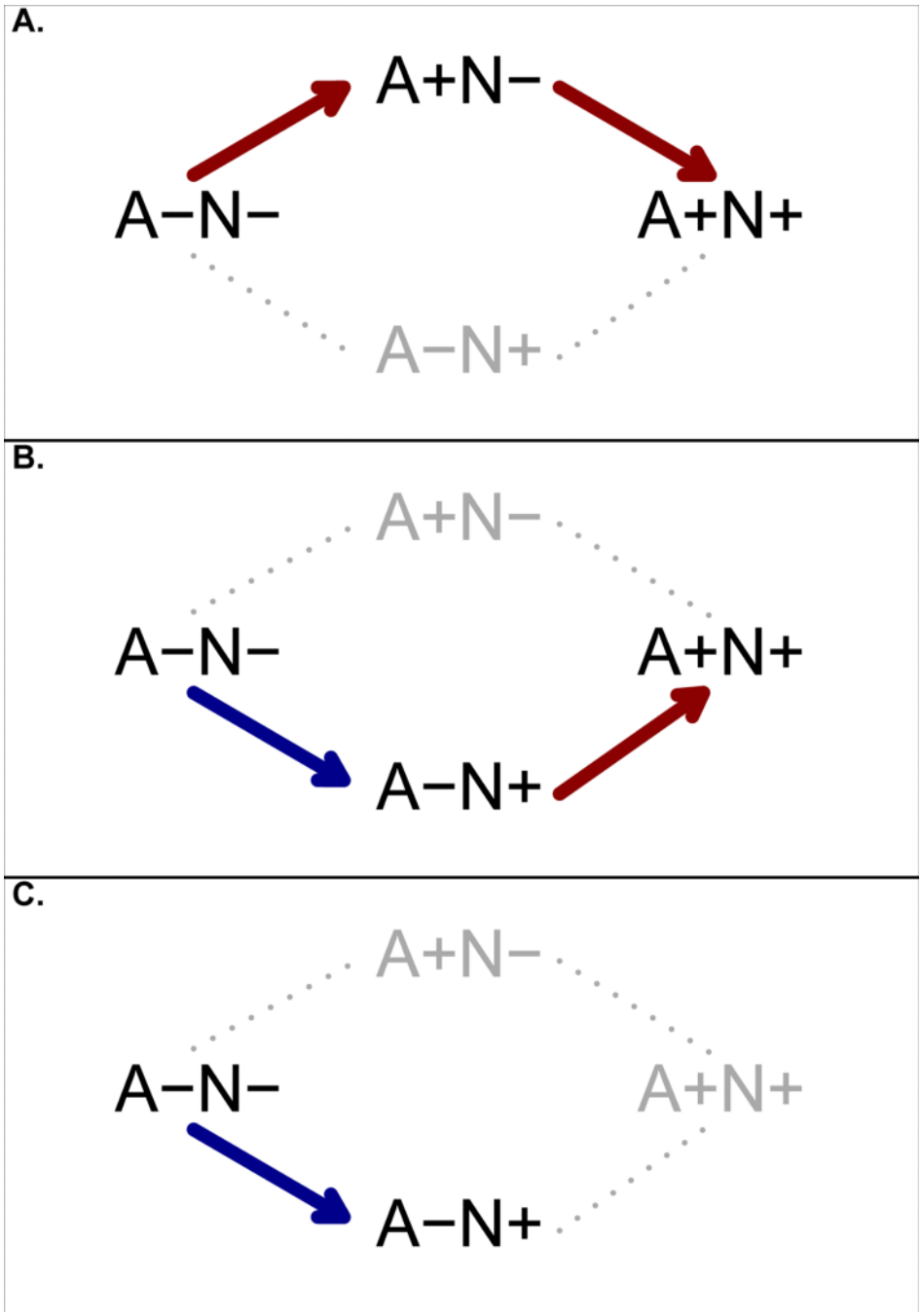

Figure 4. Possible transitions from one A/N group to another conditioned on remaining cognitively normal

Fig 4a illustrates the pathophysiological sequence of pre-clinical AD without major comorbid non-AD pathology, **A−N−** to **A+N−** to **A+N+. Fig 4b** illustrates the pathophysiological sequence someone who first develops SNAP neurodegeneration, then later enters the AD pathophysiological pathway, denoted by a positive amyloid PET study, **A−N−** to **A N+** to **A+N+. Fig 4c** illustrates the pathophysiological sequence of someone

who develops SNAP neurodegeneration without progressing into the AD pathophysiological pathway, **A−N−** to **A−N+.**

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Table 1

Characteristics of All Participants

Characteristic	Overall (N = 985)	A-N- (N = 503)	A+N- (N = 213)	A-N+ (N = 130)	A+N+ (N = 139)
Age, years, Median (IQR)	74 (67, 80)	70 (63, 76)	74 (70, 80)	77 (74, 83)	80 (77, 83)
Male gender, no. (%)	540 (55)	268 (53)	100 (47)	81 (62)	91 (65)
Education, years, Median (IQR)	14 (12, 16)	15 (12, 17)	14 (12, 16)	14 (12, 16)	14 (12, 16)
APOE ε4 positive, no. (%)	255 (26)	98 (20)	79 (37)	23 (18)	55 (40)
AVLT sum of trials, Median (IQR)*	59 (47, 70)	62 (52, 73)	59 (50, 70)	51 (42, 64)	50 (39, 61)

*Value in the table represents the sum of trials 1 through 5 plus the immediate and delayed recall trials (possible total score of 105).

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Table 2

Number (percentage) of participants in each A/N biomarker group by 5-year age strata

5-year age bin	A-N- (N = 503)	A+N- (N = 213)	A-N+ (N = 130)	A+N+ (N = 139)
50-54	35 (100)	0 (0)	0 (0)	0 (0)
55-59	37 (100)	0 (0)	0 (0)	0 (0)
60-64	93 (85)	13 (12)	3 (3)	1 (1)
65-69	80 (61)	37 (28)	10 (8)	5 (4)
70-74	110 (52)	58 (27)	28 (13)	16 (8)
75-79	91 (43)	44 (21)	36 (17)	40 (19)
80-84	40 (24)	45 (27)	33 (20)	50 (30)
85-89	17 (21)	16 (20)	20 (25)	27 (34)