Original Investigation

Age, Sex, and APOE ε4 Effects on Memory, Brain Structure, and β-Amyloid Across the Adult Life Span

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IMPORTANCE Typical cognitive aging may be defined as age-associated changes in cognitive performance in individuals who remain free of dementia. Ideally, the full adult age spectrum should be included to assess brain imaging findings associated with typical aging.

OBJECTIVE To compare age, sex, and APOE ε4 effects on memory, brain structure (adjusted hippocampal volume [HVa]), and amyloid positron emission tomography (PET) in cognitively normal individuals aged 30 to 95 years old.

DESIGN, SETTING, AND PARTICIPANTS Cross-sectional observational study (March 2006 to October 2014) at an academic medical center. We studied 1246 cognitively normal individuals, including 1209 participants aged 50 to 95 years old enrolled in a population-based study of cognitive aging and 37 self-selected volunteers aged 30 to 49 years old.

MAIN OUTCOMES AND MEASURES Memory, HVa, and amyloid PET.

RESULTS Overall, memory worsened from age 30 years through the 90s. The HVa worsened gradually from age 30 years to the mid-60s and more steeply beyond that age. The median amyloid PET was low until age 70 years and increased thereafter. Memory was worse in men than in women overall (P < .001) and more specifically beyond age 40 years. The HVa was lower in men than in women overall (P < .001) and more specifically beyond age 60 years. There was no sex difference in amyloid PET at any age. Within each sex, memory performance and HVa were not different by APOE ε4 status at any age. From age 70 years onward, APOE ε4 carriers had significantly greater median amyloid PET than noncarriers. However, the ages at which 10% of the population were amyloid PET positive were 57 years for APOE ε4 carriers and 64 years for noncarriers.

CONCLUSIONS AND RELEVANCE Male sex is associated with worse memory and HVa among cognitively normal individuals, while APOE ε4 is not. In contrast, APOE ε4 is associated with greater amyloid PET (from age 70 years onward), while sex is not. Worsening memory and HVa occur at earlier ages than abnormal amyloid PET. Therefore, neuropathological processes other than β-amyloidosis must underlie declines in brain structure and memory function in middle age. Our findings are consistent with a model of late-onset Alzheimer disease in which β-amyloidosis arises in later life on a background of preexisting structural and cognitive decline that is associated with aging and not with β-amyloid deposits.

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Typical cognitive aging may be defined as age-associated changes in cognitive performance in individuals who remain free of dementia. Interrelationships among biomarkers of β-amyloid, neurodegeneration, and cognitive performance have been the focus of much recent literature. However, studies that include all of these variables have focused predominantly on elderly individuals, typically included few (if any) individuals younger than 60 years, and tended to be composed of selected volunteers rather than population-based samples. We measured memory performance, hippocampal volume, and β-amyloidosis as a function of age using cross-sectional data from a large sample of cognitively normal individuals aged 30 to 95 years old. Individuals were grouped by sex and APOE ε4 status. The present study differs from a recent publication in which our group examined neither memory performance nor individuals younger than 50 years and in which our independent variables were not continuous measures. Differentiating features of the present study compared with other multimodality imaging studies in aging are (1) inclusion of the full adult age spectrum (30-90 years), (2) the population-based nature of 97.0% of our participants, (3), our transformation of the imaging and cognitive measures to a common scale to facilitate comparison across different modalities, and (4) the large sample size. Our objectives were to compare age, sex, and APOE ε4 effects on memory performance, hippocampal volume, and amyloid positron emission tomography (PET) across the adult life span.

Methods

Standard Protocol Approvals, Registrations, and Patien Consents

This study was approved by the Mayo Clinic and Olmsted Medical Center (Rochester, Minnesota) institutional review boards. Written informed consent was obtained from all participants.

Study Participants

We studied 1246 cognitively normal individuals from 2 different cohorts. The largest group (n = 1209) was 50 to 95 years old and comprised participants enrolled in the Mayo Clinic Study of Aging (MCSA). The MCSA is a population-based study of cognitive aging among Olmsted County, Minnesota, residents. The Olmsted County population is enumerated in the eligible age strata. From this enumeration, we selected individuals for recruitment using an age-stratified and sex-stratified random sampling strategy. These individuals were then invited to participate. The second group (n = 37) was 30 to 49 years old, equally stratified by 5-year age groups and sex (referred to as young normal). These individuals were self-selected volunteers and were recruited via word of mouth and were not population based. The study dates were March 2006 to October 2014.

All participants in this study were judged to have no cognitive impairment according to published criteria. All 1246 individuals (MCSA and young normal) underwent identical PET, magnetic resonance imaging, and memory testing protocols, which included the Auditory Verbal Learning Test. The sum of trials 1 through 5 plus the immediate and delayed recall trials (possible total score, 105) was the learning and memory performance measure (referred to as memory) used in our analyses.

Imaging

Amyloid PET was performed with Pittsburgh Compound B C 11 tracer. Standardized uptake value ratios were formed from the prefrontal, orbitofrontal, parietal, temporal, anterior cingulate, posterior cingulate, and precuneus regions of interest normalized to the whole cerebellum. Magnetic resonance imaging was performed at 3 T, and hippocampal volume was measured with publicly available software (FreeSurfer, version 5.3.0; https://surfer.nmr.mgh.harvard.edu/). Total intracranial volume was measured using an in-house method.

Statistical Analysis

Some individuals were enrolled in the MCSA before availability of amyloid PET and received prior cognitive testing. To eliminate confounding due to the well-established learning effect on serial Auditory Verbal Learning Test performance in cognitively normal individuals, we created a partial residual that adjusted for educational level and the number of times an individual had taken the test before baseline, which for this study was the date of the imaging studies. This adjusted Auditory Verbal Learning Test measure can be interpreted as the difference (in the number of words correctly recalled) from the expected number for a person given his or her educational level and the number of previous exposures to the test. To adjust hippocampal volume for total intracranial volume, we fit a regression model among 133 individuals aged 30 to 59 years old of hippocampal volume vs total intracranial volume. The adjusted hippocampal volume (HVa) was defined as the residual from this model and can be interpreted as the difference (in cubic centimeters) compared with the expected hippocampal volume given a person’s head size.

Memory performance, HVa, and amyloid PET are reported in modality-specific native units and in centiloid-like units (scale, 0-100). This process is similar to scaling biomarkers from normal to maximum abnormal levels (as described by Jack et al). To create reference points for scaling, we defined 0 (normal) for the scaled units as the 95th percentile for memory and HVa and the 5th percentile for amyloid PET among the young normal study participants 30 to 49 years old. We defined 100 (abnormal) for the scaled units as the 5th percentile for HVa and the 95th percentile for amyloid PET among a group of 42 individuals with moderately demented Alzheimer disease (AD) (Clinical Dementia Rating, 1-3). We defined 100 (abnormal) for memory based on the 5th percentile among a larger group of 382 individuals with moderately demented AD (Clinical Dementia Rating, 1-3) who underwent memory testing but not necessarily magnetic resonance imaging and PET. These individuals with AD were participants in the MCSA or the Mayo Clinic Alzheimer’s Disease Research Center and had undergone the same battery of evaluations as our study participants. An individual’s memory, HVa, or amyloid PET in native units was scaled linearly to centiloid-like units (eFigure 1 in the Supplement).
We used quantile regression to estimate the median (rather than the mean) memory, HVa, and amyloid PET vs age by sex and APOE ε4 status. Quantile regression is particularly appropriate for modeling amyloid PET because its distribution is highly skewed and not conditionally normal even after log or other parametric transformations. For each response variable, we fit a single model that included age, sex, and APOE ε4 status along with all 2-way interactions. To allow for nonlinear associations with age, we modeled age with restricted cubic splines using knots at ages 50, 75, and 80 years. We recommended by Harrell, we specified the knot locations based on the distribution of ages in our data set, as well as to serve as reference points to support a broad class of flexible nonlinear curves. We used the percentile bootstrap based on 5000 replicates to report 95% CIs for differences in medians between 2 measures or between 2 groups.

We also reported the P values for a general sex effect for each outcome from a 4-df Wald test, which tests the additive and interaction terms involving sex. Similarly, we report the P values for a general APOE ε4 effect for each outcome.

We assessed the influence of individuals younger than 50 years on model fit and our conclusions. This assessment was performed with a sensitivity analysis limited to individuals aged 50 years or older.

In a secondary analysis, we fit a logistic regression model with age and APOE ε4 genotype to predict the probability of abnormal amyloid PET and used the estimates from this model to identify the age at which the probability reached 10% for both APOE ε4 carriers and noncarriers. To be consistent with our group’s recent publications, we defined abnormal as a standardized uptake value ratio of 1.4 or greater. Sex was not included in the model because it was not significantly associated with the probability of abnormal amyloid PET.

**Results**

Demographic features and imaging and memory performance data by age group are listed in the Table. There was no significant difference in age by sex, but APOE ε4 carriers were on average 1 year younger than noncarriers (median, 71 vs 72 years; P = .04). The proportion of APOE ε4 carriers did not differ by sex. Educational level was not different by APOE ε4 status, but men were slightly more educated than women (median, 16 vs 14 years of education; P < .001). We show the young normal volunteers separately from the MCSA participants, who are grouped into 15-year age strata to illustrate the effects of advancing age (Table and eFigure 1 in the Supplement). Associations of memory, HVa, and amyloid PET vs age by sex and APOE ε4 status are shown in Figure 1, Figure 2, and eFigure 2 in the Supplement and are interpreted descriptively. Plots comparing differences in the outcomes among APOE ε4 carriers vs noncarriers within men and within women isolate the effect of APOE ε4 within sex (Figure 3). Plots comparing differences in the outcomes among men vs women within carriers and within noncarriers isolate the effect of sex within APOE ε4 genotype. These difference plots illustrate approximate ages at which significant differences were present in the outcomes by sex and APOE ε4 status. That is, when the 95% bootstrap CI around the median is above 0, we interpret this result as a significant difference in the outcomes by the group of interest at those ages. Therefore, plots in Figures 1 and
Figure 1. Memory, Adjusted Hippocampal Volume (HVa), and Amyloid Positron Emission Tomography (PET) in Modality-Specific Units by Age, With Participants Categorized Into 4 Groups by Sex and APOE ε4 Genotype (Carriers vs Noncarriers)

2 and eFigure 2 in the Supplement are interpreted qualitatively, while plots in Figure 3 are interpreted statistically.

**Age, Sex, and APOE ε4 Group Effects on Memory**
In all 4 groups, the median memory performance worsened from age 30 years through the 90s (Figures 1 and 2 and eFigure 2 in the Supplement), with a steeper decline after age 70 years in male APOE ε4 carriers and in women. Memory was worse in men than in women overall (P < .001) and more specifically beyond age 60 years (Figure 3). Within each sex, HVa was not different by APOE ε4 status (P = .15). Individual values within each group followed a gaussian distribution around the median for age (Figure 1 and eFigure 2 in the Supplement).

**Age, Sex, and APOE ε4 Group Effects on HVa**
In all 4 groups, HVa worsened gradually from age 30 years to the mid-60s and more steeply beyond that age (Figures 1 and 2 and eFigure 2 in the Supplement). The HVa was lower in men than in women overall (P < .001) and more specifically beyond age 60 years (Figure 3). Within each sex, HVa was not different by APOE ε4 status (P = .15). Individual values within each group followed a gaussian distribution around the median for age (Figure 1 and eFigure 2 in the Supplement).

**Age, Sex, and APOE ε4 Group Effects on Amyloid PET**
Unlike memory or HVa, the distribution of amyloid PET standardized uptake value ratios by age is highly skewed above age 65 years (Figure 1 and eFigure 2 in the Supplement). Amyloid PET was different by APOE ε4 status (P < .001). The median amyloid PET has a slight upward trend from age 30 years through the 90s among APOE ε4 noncarriers. In APOE ε4 carriers, there was a slight upward trend until age 70 years and then a steeper increase in the median after that (Figures 1 and 2 and eFigure 2 in the Supplement). While the median amyloid PET was greater in APOE ε4 carriers compared with noncarriers older than age 70 years (Figure 3), the ages at which 10% of the population were amyloid PET positive were 57 years (95% CI, 53–59 years) for APOE ε4 carriers and 64 years (95% CI, 60–68 years) for APOE ε4 noncarriers.
CI, 62-66 years) for noncarriers (Figure 4). Sex differences in amyloid PET were not significant ($P = .25$); however, women trended toward greater $\beta$-amyloid beyond age 70 years (Figure 3).

**Comparisons Between Memory, HVa, and Amyloid PET vs Age Within Group**

Both memory and HVa were more abnormal than amyloid PET beyond age 30 to 40 years in all 4 groups. These results are shown in eFigure 3 and eFigure 4 in the Supplement.

**Discussion**

Our major findings are that the median amyloid PET is greater in cognitively normal $APOE \epsilon 4$ carriers compared with noncarriers older than age 70 years and that the age at which 10% of the carriers are classified as amyloid PET positive is 7 years younger compared with noncarriers. Male sex is associated with worse memory and HVa among cognitively normal individuals, while $APOE \epsilon 4$ is not. Declining memory performance and HVa occur at earlier ages than abnormal amyloid PET.

The estimated age at which 10% of our $APOE \epsilon 4$ carriers were amyloid PET positive was 57 years compared with 64 years for noncarriers. These ages depend on the cut point used for amyloid PET positivity as well as the threshold chosen for the proportion who are positive. However, we wanted to make a concrete statement about the age at which abnormal amyloid PET first appears in the population and operationalized this finding as the age when the frequency of abnormality in the population reaches 10%. This is more robust than reporting the age when an abnormal scan first occurs in a single individual, which is very sensitive to outliers. In addition, our data show that, from age 70 years onward, $APOE \epsilon 4$ carriers had significantly greater median amyloid PET than noncarriers. These results are consistent with the well-established link between $APOE \epsilon 4$ and increased risk of $\beta$-amyloidosis.\textsuperscript{2,3,14-17} In turn, $\beta$-amyloidosis increases the risk of cognitive impairment and dementia.\textsuperscript{2,18-24}

Overall age-dependent trends in our data are largely consistent with prior studies that show progressive declines in memory\textsuperscript{25,26} and brain volumes\textsuperscript{27,28} with age. Recognition that AD pathology, particularly amyloid plaques, can exist in situ for a decade or longer without producing overt cognitive symptoms\textsuperscript{39-33} has raised the idea that subclinical declines in brain structure and cognitive function in middle age are often due to underlying $\beta$-amyloid deposition. However, we found that memory and HVa worsen continuously from age 30 years onward and that these trends are established before obviously abnormal amyloid PET appears in the population (Figures 1 and 2 and eFigure 2 in the Supplement). Memory and HVa values are symmetrically distributed around the population median, which implies that declines in brain structure and memory are a fundamental characteristic of typical aging. In contrast, amyloid PET is skewed above age 65 years (Figure 1 and eFigure 2 in the Supplement) such that some individuals accumulate high amyloid loads, while many survive to old age without developing significant $\beta$-amyloidosis. The differing distributions of memory and HVa vs amyloid PET around the population medians with age imply that declining memory and HVa must have some mechanistic independence from $\beta$-amyloid accumulation. Also, direct comparisons of memory, HVa, and amyloid PET among individuals show that memory and HVa were consistently more abnormal than amyloid PET across the age range 30 to 40 years. We acknowledge that amyloid PET measures only fibrillar amyloid deposits and that potential effects of soluble $\beta$-amyloid cannot be assessed. Given this caveat, our data are nonetheless consistent with the concept that age-related degenerative processes affecting brain structure and cognitive function that are unrelated to fibrillar $\beta$-amyloid deposition\textsuperscript{8,13,32-37} exist from at least age 30 years onward and are characteristic of typical aging. Reasonable candidates for non-AD processes associated with structural and functional decline in middle age are cerebrovascular disease and its risk factors, including primary age-related tauopathy.\textsuperscript{38,39} Brain aging in the absence of any specific pathophysiological process\textsuperscript{36,40} or combinations of these. Our data...
are consistent with models of late-onset AD in which β-amyloidosis, which defines preclinical AD, typically arises in later life on a background of preexisting age-related cognitive and structural decline.\textsuperscript{12,13,34,38,42,46}

With regard to sex effects, we found that men perform worse than women on memory beginning in their 40s, as has been shown previously.\textsuperscript{47} We also found that HVa was smaller in men than in women beyond age 60 years. This sex effect on memory and HVa was likely not due to sex differences in age or APOE ε4 because differences in age by sex or by APOE ε4 were small (median, approximately 1 year different) and because there were no differences in APOE ε4 prevalence by sex. Men were slightly more educated than women (median, 16 vs 14 years of education). However, if anything, this factor would tend to enhance memory performance in men compared with women, which is opposite from what we found. Moreover, we adjusted memory for educational level and practice effects.

This detrimental effect of male sex on memory and HVa must also be independent of β-amyloid deposition because (1) we found no sex differences in amyloid PET at any age (Figure 3) and (2) sex differences were present in memory (beginning at age 40 years) well before abnormal amyloid PET first appeared in the population (Figures 1, 3, and 4 and eFigure 2 in the Supplement). These sex differences in memory and HVa could be developmental,\textsuperscript{48} a hormonal protective effect,\textsuperscript{49} or attributable to a greater prevalence of adverse lifestyle-related exposures (eg, vascular risk factors) in men.\textsuperscript{47}

Perhaps the most controversial findings from this study come from comparing associations between sex vs APOE ε4 on age-dependent trends in memory and HVa. Some prior studies\textsuperscript{26,35} are consistent with our finding of no association between APOE ε4 and hippocampal volume in cognitively normal individuals. However, other studies have indicated that, among cognitively normal individuals without β-amyloid dep-

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Figure 3. Plots of Groupwise Differences in Scaled Units for Memory, Adjusted Hippocampal Volume (HVa), and Amyloid Positron Emission Tomography (PET)
osition, APOE ε4 carriers have hypometabolism in AD-like regions,\textsuperscript{50} abnormal functional connectivity,\textsuperscript{53,54} worse cognitive performance,\textsuperscript{55} and smaller regional brain volumes.\textsuperscript{56,57} Such findings have been taken as evidence that APOE ε4 exerts harmful effects throughout life on brain structure and function that are independent of its role in promoting β-amyloid deposition.\textsuperscript{58,59} In contrast, we found that, while male sex was associated with smaller hippocampal volume and worse memory, APOE ε4 carriers within each sex did not have worse memory or HVa than noncarriers at any age.\textsuperscript{60} Had we examined other imaging measures (eg, fluorodeoxyglucose F 18 PET or functional magnetic resonance imaging) or perhaps other cognitive indexes, the findings might have been different. Nonetheless, our results paint a different picture than is presented in much of the recent imaging literature, which has focused great attention on the effect of APOE ε4 but little on the effect of sex on brain structure and function.

Our study has limitations. While all individuals 50 years or older in our sample were derived from an epidemiologically defined cohort, the non-population-based nature of those aged 30 to 49 years old and the small sample size in this age range are acknowledged limitations. However, when obvious inflection points exist in plots of memory, HVa, and amyloid PET vs age, they occur well within the age range of 50 years to the 90s of the MCSA cohort (Figures 1 and 2 and Figures 2 and 3 in the Supplement) and not at the age junction of the young normal group and the MCSA cohort. In addition, a sensitivity analysis indicated that plots (among those 50 years or older) of memory, HVa, and amyloid PET vs age did not change when individuals younger than 50 years were excluded. This finding indicates that the imbalance in the numbers of individuals aged 50 years or older vs younger than 50 years did not unduly influence our conclusions. Another limitation is that cross-sectional studies tend to confound age effects with birth cohort effects.\textsuperscript{61} However, cohort effects are unavoidable when examining age-dependent trends covering a range of 60 years. Within-individual longitudinal data typically found in studies with intensive multimodality imaging (1-5 years) will not ameliorate birth cohort effects when the research questions of interest are age trends spanning 60 years. A final methodological point concerns interpretation of the results given that all participants were cognitively normal. Individuals who remain cognitively normal into old age represent a subset of those who were members of their birth cohort at younger ages.

Conclusions

Despite these limitations, we believe that this study of typical aging reveals interesting sex and APOE ε4 effects on age-related trends in brain structure, function, and β-amyloidosis. To date, these effects have not been widely appreciated. Our findings are consistent with a model of late-onset AD in which β-amyloidosis arises in later life on a background of pre-existing structural and cognitive decline that is associated with aging and not with β-amyloid deposits.
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