Brain imaging measurements of fibrillar amyloid-β burden, paired helical filament tau burden, and atrophy in cognitively unimpaired persons with two, one, and no copies of the APOE ε4 allele

Valentina Ghisays1,2 | Dhruman D. Goradia1,2 | Hillary Protas1,2 | Robert J. Bauer III1,2 | Vivek Devadas1,2 | Pierre N. Tariot1,2,3 | Val J. Lowe4 | David S. Knopman5 | Ronald C. Petersen5 | Clifford R. Jack Jr.4 | Richard J. Caselli2,6 | Yi Su1,2 | Kewei Chen1,2,3,7 | Eric M. Reiman1,2,3,7,8

1Banner Alzheimer’s Institute, Phoenix, AZ, USA
2Arizona Alzheimer’s Consortium, Phoenix, AZ, USA
3University of Arizona, Tucson, AZ, USA
4Department of Radiology, Mayo Clinic, Rochester, MN, USA
5Department of Neurology, Mayo Clinic, Rochester, MN, USA
6Mayo Clinic, Scottsdale, AZ, USA
7Arizona State University, Tempe, AZ, USA
8Translational Genomics Research Institute, Phoenix, AZ, USA

Correspondence
Eric M. Reiman, Tel: 602-839-6999; Fax: 602-839-6253.
E-mail: Eric.Reiman@bannerhealth.com

Funding information
National Institutes of Health; National Institute on Aging, Grant/Award Numbers: R01 AG031581, R37 AG011378, R01 AG041851, U01 AG006786, P50 AG016574

Abstract
Introduction: We previously characterized associations between brain imaging measurements of amyloid-β (Aβ) plaque burden and apolipoprotein E (APOE) ε4 gene dose in a small number of cognitively unimpaired late-middle-aged APOE ε4 homozygotes (HMs), heterozygotes (HTs), and noncarriers (NCs). We now characterize cross-sectional Aβ plaque, tau tangle, and cortical atrophy (neurodegeneration) measurements, classifications, and associations with age in a larger number of unimpaired HMs, HTs, and NCs over a wider age range.

Methods: We analyzed 11C Pittsburgh compound B (Aβ) positron emission tomography (PET), flortaucipir (tau) PET, and volumetric magnetic resonance imaging data from 164 study participants of age 47–86 years, including 26 APOE ε4 HMs, 48 HTs, and 90 NCs matched for age and sex.

Results: Aβ PET measurements rose, plateaued at the respective ages of 68 and 76, and then declined with age in unimpaired HM and HT groups. Compared with NCs, these two groups began to have significantly higher Aβ PET measurements at ages 62 and 70, respectively, and no longer had significantly higher measurements by ages 71 and 78, respectively. They began to have significantly higher entorhinal cortex tau PET measurements at ages 66 and 70, respectively, and no longer had significantly higher measurements by ages 74 and 78, respectively. Brain atrophy measurements tended to decline slowly with age in all three genetic groups. Their elevated tau PET...
Alzheimer’s disease (AD) is the most common form of dementia at older ages.1,2 Neuropathologically, AD is characterized by neuritic plaques, composed of fibrillar amyloid-β (Aβ); neurofibrillary tangles, composed of paired-helical filament (PHF) tau; and synaptic and neuronal loss.3–5 The apolipoprotein E (APOE) ε4 allele is the major susceptibility gene for late-onset AD.6,7 APOE ε4 gene dose (i.e., the number of ε4 alleles in a person’s APOE genotype) is associated with a higher risk and earlier age at onset of AD biomarker changes, cognitive decline, and dementia.8–13 For more than two decades (starting before the advent of Aβ and tau positron emission tomography [PET] methods), we (E.M.R. and R.J.C.) and our Arizona APOE Cohort study colleagues have been characterizing brain imaging and cognitive measurements from an ε4 homozygote (HM)–enriched, longitudinally assessed cohort of initially 47- to 68-year-old persons with two, one, and no copies of the APOE ε4 allele, representing three levels of genetic risk for the disease.10,14–20 Meantime, our Mayo Clinic Rochester colleagues (R.C.P., C.R.J., and D.K.) have been characterizing brain imaging and cognitive measures in the Mayo Clinic Study of Aging from a population-based, longitudinally assessed cohort of roughly to the respective ages at which Aβ PET measurements rose and declined. The subgroup analysis was intended to provide a foundation for using AD biomarkers as endophenotypes to help clarify mechanisms by which to-be-discovered resilience or resistance factors that permit certain HM and HT groups to remain cognitively unimpaired at older ages.24–27

1 | METHODS

1.1 | Study participants

We analyzed cross-sectional 11C Pittsburgh compound B (PiB) PET, flortaucipir (FTP) PET, and T1-weighted MRI scans; Mini–Mental State Examination (MMSE) scores; and auditory verbal learning test (AVLT) long-term delayed recall scores from 164 cognitively unimpaired volunteers aged 47–86 years, including 26 APOE ε4 HMs, 48 HTs, and 90 NCs (Table 1). Participants included 15, 26, and 43 HMs, HTs, and NCs, 48–85 years old, from the Arizona APOE Cohort and 11, 22, and 47 HMs, HTs, and NCs, 47–86 years old, from the Mayo Clinic Study of Aging cohort, selected to optimize matching to the Arizona APOE Cohort for age, sex, and education. Studies were approved by the Institutional Review Boards of Banner Alzheimer’s Institute and Mayo
Brain imaging

The performance sites’ PiB PET, FTP PET, and volumetric T1-weighted MRI acquisition methods and scanners and study’s image processing platforms, cerebral and reference regions of interest, and image analysis methods are described in Supplementary Material 1. Briefly, the Statistical Parametric Mapping 12 (SPM12) platform was used to preprocess all images, and the Mayo Clinic Adult Lifespan Template (MCALT) and its modified Automated Anatomic Labeling atlas were used to normalize coregistered MRI scans and extract PET data from regions of interest. FreeSurfer 6 was used to extract MRI measurements of cortical thickness and bilateral hippocampal-to-total intracranial volume ratios. Composite cortical-to-cerebellar crus PiB standard uptake value ratios (SUVRs), composite cortical tau, entorhinal cortex (ERC), and inferior temporal cortex (ITC)-to-cerebellar crus FTP SUVRs, and hippocampal volume ratios were used to compare Aβ plaque, tau tangle, and regional brain volumes in each genetic group. ERC FTP SUVRs were used to compare tau tangle measurements and age associations in the three groups because of its sensitivity to detect tau burden in preclinical AD and its association with memory-related cognitive testing in unimpaired participants.

Cortical-to-cerebellar crus PiB SUVRs, cortical tau-to-cerebellar crus FTP SUVRs, and cortical gray matter thickness measurements were used to classify each image as positive or negative, as previously described, and based on the proposed A/T(N) framework. Aβ positivity was defined using PiB SUVRs ≥1.42 threshold, which correspond to at least moderately frequent neuritic plaques. Tau and neurodegeneration positivity were defined using cortical tau SUVR and cortical thickness thresholds >1.23 and ≤2.67 mm, respectively; these thresholds were based on their ability to distinguish cognitively impaired patients with a positive Aβ PET scan from cognitively unimpaired young adults.

Statistical analysis

The three genetic groups were compared in terms of their age, sex, educational level, MMSE scores, AVLT long-term memory recall scores, brain imaging measurements and classifications, and associations with age. Statistical analyses were performed using SPSS version 23.0 (IBM-SPSS Inc., Chicago, IL). Analysis of covariance with pairwise Fisher’s least significance difference comparisons or χ² tests were used to compare participant characteristics, clinical ratings, neuropsychological test scores, brain imaging measurements, and the proportions of participants with positive Aβ, tau, and neurodegeneration images in each genetic group. Analyses of covariance included adjustment for
### Table 1: Participant Characteristics in Cognitively Unimpaired APOE ε4 HMs, HTs, and NCs at Ages 47–86, 47–70, and 71–86

|          | (a) Ages 47–86 | (b) Ages 47–70 | (c) Ages 71–86 |
|----------|----------------|----------------|----------------|
|          | HMs, n = 26    | HTs, n = 48    | NCs, n = 91    | HMs, n = 22    | HTs, n = 34    | NCs, n = 74    | HMs, n = 4     | HTs, n = 14    | NCs, n = 16    |
| Age (range) | 62 ± 9 (48–86) | 64 ± 9 (47–82) | 63 ± 8 (50–85) | 59 ± 7 (48–70) | 60 ± 6 (47–69) | 61 ± 5 (50–70) | 77 ± 7 (71–86) | 76 ± 3 (72–82) | 75 ± 4 (71–85) |
| Female (%) | 73%            | 75%            | 74%            | 77%            | 85%            | 75%            | 50%            | 50%            | 69%            |
| Education | 15.5 ± 2.6     | 15.3 ± 2.4     | 15.6 ± 2.0     | 16.0 ± 1.8     | 14.6 ± 2.3     | 15.3 ± 1.9     | 12.8 ± 4.6     | 16.8 ± 2.1     | 16.9 ± 2.2     |
| MMSE     | 29.5 ± 0.8     | 29.4 ± 0.9     | 29.4 ± 0.8     | 29.5 ± 0.6     | 29.4 ± 0.9     | 29.4 ± 0.8     | 30.1 ± 1.4     | 29.4 ± 0.9     | 29.4 ± 1.0     |
| Long-term recall memory | 10.1 ± 3.0 | 9.4 ± 3.4 | 9.5 ± 3.5** | 9.6 ± 2.7 | 10.4 ± 3.0 | 9.9 ± 3.4** | 12.4 ± 4.3 | 7.1 ± 3.0 | 8.0 ± 4.0 |
| Aβ positive | 39%           | 35%           | 11%          | 41%           | 18%           | 58%           | 25%           | 79%           | 38%           |
| Tau positive | 19%           | 27%           | 20%          | 14%           | 12%           | 18%           | 50%           | 64%           | 31%           |
| Neurodegeneration positive | 8%            | 21%           | 14%          | 5%            | 12%           | 8%            | 25%           | 43%           | 44%           |

Note. ANCOVA 2-tailed tests were used to compare continuous variables (MMSE and AVLT long-term recall memory scores adjusted for performance site, age, sex, and education), and χ² test for categorical variables (Aβ, tau, and neurodegeneration positivity) in (a) 47- to 86-year-old, (b) 47- to 70-year-old, and (c) 71- to 86-year-old age range. Mean ± SD, and χ² P values are listed.

Abbreviations: Aβ, amyloid; β, APOE, apolipoprotein E; AVLT, Auditory Verbal Learning Test; HM, homozygote; HT, heterozygote; NC, noncarrier.

1. HM & HT > NC (P ≤ .001)
2. HM > HT
3. HM > HT & NC (P ≤ .06)
4. HM < HT & NC
5. HM > HT & NC
6. HT > HM & NC (P < .05)
7. Aβ, tau, and neurodegeneration-positive images were defined using cortical PiB SUVRs ≥1.42, cortical flortaucipir SUVRs > 1.23, and cortical thicknesses ≤2.67 mm, as previously described.28

### 1.3.1 | Associations with age

Nonparametric local regression (LOESS) curves and 95% confidence intervals26 were used to characterize a) age associations with cortical PiB SUVRs, ERC, ITC, and cortical tau FTP SUVRs, hippocampal gray matter volumes and cortical thickness in each genetic group; and b) ages at which SUVRs in the HM and HT groups began to be significantly higher than in the NCs, plateaued, and were no longer significantly higher than in the NCs (Fig. 1; Supplementary Figs. 1–2). ERC FTP SUVRs were the prioritized tau PET measurements for the reasons noted above. LOESS fitting was performed using the R software (version 3.4.1; www.r-project.org).

### 1.3.2 | Measurements and Classifications in the Overall, Younger, and Older Age Ranges

Participant characteristics, MMSE and AVLT long-term memory recall scores, brain imaging measurements, Aβ, tau, and neurodegeneration positivity percentages, and A/T(N) classifications were compared in the overall 47- to 86-year-old age range. As noted previously, these variables were assessed post hoc in the younger 47- to 70-year-old and older 71- to 86-year-old age ranges, corresponding roughly to the rise and fall of Aβ PET measurements and dementia onset in the HM group.

## 2 | RESULTS

### 2.1 | Participant Characteristics

As shown in Table 1, APOE ε4 HM, HT, and NC groups did not differ significantly in age, sex, or MMSE scores in the overall, younger, and older age ranges. Although they did not differ significantly in educational level or AVLT long-term recall memory scores in the overall age range, HMs had significantly higher educational levels than HT groups in the younger age range and significantly lower educational levels but higher recall memory scores than HT and NC groups in the older age range (P < .05, adjusted for age, sex, performance site, education, Aβ positivity, and ERC tau PET measurements, uncorrected for multiple comparisons).

There were only four HMs in the unimpaired older group, which could be due to our exclusion of participants who had already become
impaired and a historical emphasis on the enrollment of younger participants in the Arizona APOE Cohort, some of whom have not reached older ages. Supplementary Table 1 illustrates the potential impact of differential survivor bias on our findings: 24.0%, 9.5%, and 3.7% of initially enrolled late-middle-aged cognitively unimpaired HM, HT, and NC groups in the Arizona APOE Cohort who subsequently progressed to MCI due to possible AD, respectively (P < .001), met MCI criteria at the respective ages of 69, 75, and 77 (P < .001), and were excluded from this analysis.

### 2.2 Associations with age

Associations between respective Aβ plaque, tau tangle, and brain atrophy measurements with age, in the APOE ε4 HM, HT, and NC groups are shown using LOESS curves and 95% confidence intervals in Fig. 1 and Supplementary Figures 1 and 2 (wider 95% confidence intervals at the youngest and oldest ages are at least partly due to fewer participants at those ages). In general, Aβ and tau PET measurements rose, plateaued, and declined with age in the HM and HT groups and rose more slowly in the NC group, whereas brain atrophy measurements tended to decline slowly with age in all three genetic groups.

Ages at which Aβ and tau PET measurements in the HM and HT groups began to be significantly different from NCs ("rise"), plateaued, and were no longer significantly different from NCs ("decline") and are also shown in Table 2. In HMs, cortical Aβ PET measurements began to rise at age 62, plateaued at age 68, and declined by age 71. ERC tau PET measurements began to rise at age 66, plateaued at age 71, and declined by age 74. ITC and cortical tau PET measurements plateaued at age 76 but were not significantly different from NCs at any age. HTs generally demonstrated a similar pattern, but with most rises, plateaus, and declines occurring about 4-8 years later. Their cortical Aβ and ERC...
TABLE 2  Aged at brain imaging AD biomarker onset, plateau, and decline in cognitively unimpaired APOE ε4 HMs and HTs compared with NCs

| Biomarker Measurement | HMs, n = 26 | | HTs, n = 48 | |
|-----------------------|-------------|------------|-------------|------------|
|                       | Onset age   | Plateau age| Decline age | Onset age   | Plateau age| Decline age |
| Aβ                    |             |            |             |             |            |             |
| Cortical PiB          | 62          | 68         | 71          | 70          | 76         | 78          |
| Tau                   |             |            |             |             |            |             |
| Entorhinal FTP        | 66          | 71         | 74          | 70          | 76         | 78          |
| Inferior temporal FTP | -           | 76         | -           | 70          | 74         | 77          |
| Cortical tau          | -           | 76         | -           | 71          | 74         | 77          |
| Atrophy               |             |            |             |             |            |             |
| Hippocampal volume    | -           | -          | -           | -           | -          | -           |
| Cortical thickness    | -           | -          | -           | -           | -          | -           |

NOTE. Onset is defined by the age at which measurements began to be significantly higher than those in the NC group. Decline is defined by the age at which measurements were no longer significantly higher than in the NC groups. Estimated ages at onset, plateau and decline are likely to be influenced by differential survivor bias (i.e., the exclusion of carriers who became cognitive impaired at younger ages) and sample size (e.g., the impact of sample size on statistical significance).

Abbreviations: Aβ, amyloid β; APOE, apolipoprotein E; FTP, flortaucipir; HM, homozygote; HT, heterozygote; NC, noncarrier.

tau PET measurements began to rise at age 70, plateaued at age 76, and declined by age 78. ITC and cortical tau PET measurements began to rise at the respective ages of 70 and 71, plateaued at age 74, and declined by age 77.

2.3 | Brain imaging biomarker findings

2.3.1 | Classifications

Table 1 shows the percentages of cognitively unimpaired HM, HT, and NC groups in the overall (47–86), younger (47–70), and older (71–86 year-old) age range who were found to be Aβ+, tau, and neurodegeneration-positive using their Aβ PET, tau PET, and MRI scans with the previously described criteria.28

2.3.2 | Aβ positivity

As shown in Table 1 and Fig. 2 (top), 39%, 35%, and 11% of HM, HT, and NC groups in the overall age range, 41%, 18%, and 5% in the younger age range, and 25%, 79%, and 38% of those in the older age range had positive Aβ PET scans, respectively. The percentage of participants with a positive Aβ PET scan was significantly associated with APOE ε4 gene dose (HM > HT > NC) in the overall and younger age ranges (linear trend, P < .05), but not in the older age range. Conversely, the percent-age of A–T–(N–) participants was lower in HMs than in NCs, inversely associated with APOE ε4 gene dose (HM < HT < NC) in the younger age range (linear trend, P < .05), and significantly lower in older than younger participants (attributable to HTs and NCs, P < .01, but did not reach significance for HMs).

2.3.3 | Tau and neurodegeneration positivity

As shown in Table 1, the percentage of tau-positive and cortical gray matter atrophy/neurodegeneration-positive participants was not significantly different in HM, HT, and NC groups or associated with APOE ε4 gene dose in the overall, younger, and older age range.

2.3.4 | A/T(N) classifications

Table 3 shows the percentage of cognitively unimpaired participants with each of the eight proposed A/T(N) research framework classifications. Although none of the HMs in any age range were A+T+(N+), 12%, 10%, and 2% of HM, HT, and NC groups in the overall age range and 9%, 3%, and 0% in the younger age range met A/T(N) criteria for AD (i.e., A+T+ irrespective of their N classification), and 27%, 13%, and 6% (overall age range) and 32%, 12%, and 3% (younger age range) met criteria for "AD pathologic change" (i.e., A+T–(N–), respectively. Thus, the percentage of participants who met A/T(N) criteria for AD and AD pathologic change were each associated with APOE ε4 gene dose (HM > HT > NC) in the overall and younger age ranges (linear trend, P < .05), but not in the older age range. Conversely, the percent-age of A–T–(N–) participants was lower in HMs than in NCs, inversely associated with APOE ε4 gene dose (HM < HT < NC) in the younger age range (linear trend, P < .05), and significantly lower in older than younger participants (attributable to HTs and NCs, P < .01, but did not reach significance for HMs).

2.3.5 | Measurements

Table 4 shows PiB PET measurements of cortical Aβ plaque burden, FTP PET measurements of the ERC, ITC, and cortical tau/tangle burden, and MRI measurements of cortical gray matter and hippocampal atrophy in HMs, HTs, and NCs from the overall, younger, and older age ranges.

2.3.6 | PiB PET measurements of Aβ plaque burden

As shown in Table 4 and Fig. 2 (bottom), HM and HT groups had significantly higher cortical PiB SUVRs than NCs in the overall age range.
**FIGURE 2**  Relationships between Aβ plaque classification, measurements, and APOE ε4 gene dose in cognitively unimpaired participants age 47–86, 47–70, and 71–86 years. χ² tests were used to compare the proportions of Aβ positivity (top), and ANCOVA 2-tailed tests (adjusted for age, performance site, sex, and education) were used to compare cortical PiB SUVRs (bottom) in APOE ε4 HM, HT, and NCs. * * * * * * is P < .05, .01, and .001, respectively, for post hoc pairwise differences with Fisher’s LSD. Means ± SD for cortical PiB SUVRs are shown (Aβ positivity threshold ≥1.42 is indicated with the red dotted line). Abbreviations: Aβ, amyloid β; ANCOVA, analysis of covariance; APOE, apolipoprotein E; HM, homozygote; HT, heterozygote; NC, noncarrier; SUVR, standard uptake value ratio.

PiB SUVRs were significantly higher in HM than in HT and NC, slightly higher in HT than in NC groups (P = .06), and were associated with APOE ε4 gene dose (linear trend, P < .05) in the younger age range. There were significantly higher cortical PiB SUVRs in HT than HM (who had relatively low SUVRs) and NC groups in the older age range.

### 2.3.7 | FTP PET measurements of tau/tangle burden

As shown in Table 4 and Fig. 3, ERC FTP SUVRs were significantly higher in HM and HT than NC groups in the overall age range; they were significantly higher in HM than HT (trend) and NC groups and associated with APOE ε4 gene dose in the younger age range (linear trend, P < .05); and they were significantly higher in HT than NC groups in the older age range. As shown in Fig. 3 and Supplementary Table 2, ERC FTP SUVRs were significantly higher in those with a positive Aβ PET scan and elevations in the HM and HT groups were attributable to those carriers with a positive Aβ PET scan.

As shown in Table 4 and Supplementary Tables 2, 3a and 3b, HT groups had significantly higher ITC and cortical tau SUVRs than NCs and were attributable to those HTs with a positive Aβ PET scan in the overall age range (P < .05). They were not significantly different in HM, HT, and NC groups or associated with APOE ε4 gene dose in the younger age range. They were significantly higher in HT than NC groups in the older age range, but not solely attributable to the older HT group with a positive Aβ PET scan.

### 2.3.8 | MRI measurements of brain atrophy/neurodegeneration

As shown in Table 4, MRI measurements of cortical thickness and hippocampal gray matter volumes were not significantly different in the unimpaired HM, HT, and NC groups or associated with APOE ε4 gene dose in the overall, younger, or older age ranges and were significantly lower in older than younger participants, irrespective of APOE ε4 gene dose (P ≤ .01, adjusted for site, sex, education, and APOE status).

### 3 | DISCUSSION

This study provides information about Aβ PET, tau PET, and volumetric MRI measurements, their associations with age, and A/T(N) classifications in a relatively large number of APOE ε4 HM, HT, and NCs,
TABLE 3  Classification of amyloid-β burden (A), neurofibrillary tau burden (T), and neurodegeneration (N) in cognitively unimpaired APOE ε4 HMs, HTs, and NCs

|                    | (a) Ages 47–86 | (b) Ages 47–70 | (c) Ages 71–86 |
|--------------------|----------------|----------------|----------------|
| HMs, n = 26        | HMs, n = 22    | HMs, n = 4     |
| A+T+(N)+           | 0              | 0              | 0              |
| A+T+(N)−           | 12             | 9              | 25             |
| A+T−(N)+           | 27             | 32             | 0              |
| A+T−(N)−           | 0              | 0              | 0              |
| A−T+(N)+           | 8              | 5              | 25             |
| A−T−(N)+           | 46             | 50             | 25             |

NOTE. Proportions were compared with a χ² test. (a) In the overall 47- to 86-year-old age range, higher proportion of HTs were A+T+(N)+ compared with NCs. A+T+(N)− nonsignificant trend, indicated significantly higher proportions of HMs and HTs compared with NCs. Post hoc pairwise comparisons of the nonsignificant trend in the A+T+(N)− indicated significantly higher proportions of HMs and HTs compared with NCs. Higher proportion of HMs were A+T−(N)− compared with NCs but not compared with HTs or between HTs and NCs. (b) In the younger 47- to 70-year-old age range, higher proportion of HMs were A+T+(N)− and A+T−(N)− compared with NCs. Proportion of A+T−(N)− HMs were also slightly higher than in HTs and slightly higher in HTs than in NCs (nonsignificant). In the A−T+(N)−, differences in the proportions did not reach significance but the linear trend did (ManTEL-Haenszel, P = .03), indicating an inverse association with APOE ε4 genotype dose (HM < HT < NC); post hoc pairwise comparisons were also significant with lower proportions of A−T−(N)− HMs compared with NCs but not with HTs or between HTs and NCs. (c) In the older 71- to 86-year-old age range, higher proportion of HTs were A+T+(N)+ compared with NCs but did not reach significance compared with HMs. χ² P values and percent listed.

Abbreviations: Aβ, amyloid β; APOE, apolipoprotein E; HM, homozygote; HT, heterozygote; NC, noncarrier.

† HT > NC
‡ HM & HT > NC
§ HM > NC (P < .05)
¶ HM > HT & NC
∫ HM < NC (P < .06)
¶¶ HT > NC (P < .01)

TABLE 4  Brain imaging measurements of Aβ plaque burden, tau/tangle burden, and atrophy/neurodegeneration in cognitively unimpaired APOE ε4 HMs, HTs, and NCs

|                    | (a) Ages 47–86 | (b) Ages 47–70 | (c) Ages 71–86 |
|--------------------|----------------|----------------|----------------|
| HMs, n = 26        | HMs, n = 22    | HMs, n = 4     |
| Cortical PiB SUVR  | 1.50 ± .06     | 1.51 ± .04     | 1.09 ± .28     |
| ERC FTP SUVR       | 1.12 ± .02     | 1.10 ± .02     | 1.07 ± 1.0     |
| ITC FTP SUVR       | 1.19 ± .02     | 1.17 ± .02     | 1.17 ± .02     |
| Cortical thickness | 2.78 ± .02     | 2.80 ± .02     | 2.68 ± .08     |
| Hippocampal volume | 0.53 ± .01     | 0.54 ± .01     | 0.48 ± .04     |

NOTE. ANCOVA 2-tailed tests, adjusted for age, performance site, sex, and education were used to compare brain imaging measurements in (a) 47–86, (b) 47–70, and (c) 71–86 year-old age ranges. Aβ positivity was used as an interaction term in the overall 47–86 age range [APOE ε4 group*Aβ status (Aβ+/Aβ−)] for tau PET and MRI measures. Mean SUVR ± SE, and significant (P < .05) and nonsignificant trends (P values between .05 and .10) with pairwise differences using Fisher’s LSD, are listed.

Abbreviations: Aβ, amyloid β; ANCOVA, analysis of covariance; APOE, apolipoprotein E; ERC, entorhinal cortex; FTP, flortaucipir; HM, homozygote; HT, heterozygote; ITC, inferior temporal cortex; NC, noncarrier; SUVR, standard uptake value ratio.

†† HM & HT > NC
††† HT > NC (P < .05)
‡‡ HM > HT & NC
‡‡‡ HM > HT & NC (P < .06)
†††† HT > HM & NC
FIGURE 3  Relationships between entorhinal tau deposition, Aβ positivity, and APOE ε4 gene dose in cognitively unimpaired participants at age 47–86, 47–70, and 71–86 years. ANCOVA 2-tailed tests (adjusted for age, performance site, sex, and education) were used to compare (a) entorhinal FTP SUVRs in APOE ε4 HM, HT, and NCs, and in Aβ-positive (Aβ+) vs Aβ-negative (Aβ-) participants. (b-c) Aβ positivity was used as an interaction term in the overall 47–86 age range [APOE ε4 group*Aβ status (Aβ+/Aβ−)] and ran separately in the Aβ+ and Aβ− 47- to 70-year-old and 71- to 86-year-old subgroups. * is P < .05 for post hoc pairwise differences with Fisher’s LSD and means ± SD are shown.

Abbreviations: Aβ, amyloid β; ANCOVA, analysis of covariance; APOE, apolipoprotein E; HM, homozygote; HT, heterozygote; NC, noncarrier; FTP, flortaucipir; SUVR, standard uptake value ratio.

and over a relatively large middle-to-older age range. Aβ and ERC tau PET measurements rose, plateaued, and declined with age in the unimpaired HM and HT groups and did so earlier in HMs than HTs. ERC tau PET measurements were significantly greater in the HM and HT groups, and these elevations were attributable to those carriers with a positive Aβ PET scan. Together, our findings suggest that cognitively unimpaired HMs can be studied before their 70s to evaluate biomarker changes, risk factors, pathophysiological changes, and interventions involved in the predisposition to and potential prevention of AD. Although our study included only four unimpaired HMs aged over 70 years, our findings suggest that HMs who remain cognitively unimpaired after their 70s could be used to evaluate biomarker changes, risk factors, pathophysiological changes, and interventions involved in the resilience or resistance to and prevention of AD.

We hypothesize that subsequent biomarker declines are attributable to resistance factors that permit HMs and HTs to remain cognitively unimpaired at older ages. We reason that the decline in prevalence among HM and HT groups is not due to decline in levels of protein deposition in the brains of individuals over time, but rather it is a drop in the group mean due to selective survival of resilient low amyloid and low tau individuals at older ages.

In the cognitively unimpaired APOE ε4 HM group, cortical Aβ PET measurements rose with age, were significantly different from those in NC group by age 62, plateaued at age 71, declined, and were no longer significantly different from NC by age 71. ERC tau PET measurements rose with age, were significantly different from those in the NC groups by age 66, plateaued at age 71, declined, and were no longer significantly different from NCs by age 74. The HT group demonstrated a similar pattern of Aβ and ERC tau PET increases, plateaus, and declines occurring about 4-8 years later. We reason that the decline in prevalence among HM and HT groups is not due to decline in levels of protein deposition in the brains of individuals over time, but rather it is a drop in the group mean due to selective survival of resilient low amyloid and low tau individuals at older ages.
about when the risk of AD dementia begins to rise, plateau, and decline in HM and HT groups, our study provides information about the ages at which brain imaging biomarkers of Aβ plaque and tau/tangle deposition begins to rise, plateau, and decline.

We hypothesize that cognitively unimpaired HM and HT groups could be studied before their 70s, when their biomarker changes are associated with three levels of genetic risk, for the following purposes: 1) to clarify the impact of genetic and nongenetic risk factors and their interaction with APOE ε4 gene dose, on AD biomarkers (or “endophenotypes”), as we have done in the past using a more limited number of biomarker measurements and 2) to evaluate promising prevention therapies before AD is extensive, including those who have or have not yet have biomarker evidence of amyloid burden, as we are doing in the Alzheimer’s Prevention Initiative Generation Program.

We hypothesize that cognitively unimpaired HM and HT groups who remain unimpaired at older ages could be used to investigate the impact of putative protective factors and clarify differential effects of these protective factors on Aβ or downstream neuroinflammatory, tau, or neurodegeneration biomarkers. This information could help provide new targets at which to aim promising prevention therapies.

In addition to the associations of biomarker changes with age in unimpaired HMs, HTs, and NCs, our study provides other insights about biomarker changes and classifications in these groups. For instance, it suggests that ERC tau PET elevations in HM and HT groups are attributable to those with a positive Aβ PET scan. Although one cannot draw strong conclusions about the causal connection between Aβ and tau PET measurements in these at risk groups, this finding does support the possibility that treatments that prevent the initial accumulation of neuritic plaques might reduce the development of downstream neuropathological changes and ensuing cognitive decline—a possibility that is now being explored in the subset of cognitively unimpaired 60- to 75-year-old HMs that are being evaluated using an anti-amyloid immunotherapy in the Alzheimer’s Prevention Initiative Generation Program.

In our effort to classify the three genetic groups based on criteria for a “positive” or “negative” Aβ PET, tau PET, and volumetric MRI findings, we found a surprisingly low percentage of HMs who were neurodegeneration positive, and we did not see a strong association between tau PET measurements and APOE ε4 gene dose in ITC and cortical regions that have been suggested to help distinguish between AD cases and controls. These “negative” findings could be attributable to several factors, such as a relatively brief interval between neurodegeneration positivity and clinical progression, exclusion of those who had already progressed, resilience or resistance factors in the small number of those HMs who remained unimpaired at older ages, and/or cortical tau and cortical atrophy thresholds used to define positivity. Additional image analysis techniques and thresholds may be needed to define tau and neurodegeneration positivity in the preclinical stages of AD. These thresholds could then be evaluated in terms of their prognostic value, their diagnostic value (including their correspondence to postmortem neuropathology), and their predictive value (i.e., their ability to inform the differential response to treatment). We do note, however, that there is an APOE ε4 gene dose effect (i.e., HM > HT > NC, linear trend) when we compare the proportions of A+T+ (irrespective of N) or A+T–N– HMs, HT, and NC groups in the overall and younger age ranges.

Finally, recent studies report biomarker effects on memory performance, particularly with ERC tau deposition in cognitively unimpaired participants however, APOE ε4 effects on memory performance in the present study are not as clear and will likely require more than one memory measurement and a larger sample to properly assess. Despite some of these limitations, we note significantly higher long-term recall memory scores in the older HMs than in HTs and NC groups (Table 1), suggesting that resilience or resistance to cognitive decline in the small older HM group may not be solely attributable to education-related cognitive reserve. We also see lower MMSE and long-term recall scores in Aβ-positive participants relative to Aβ negative for the overall and younger age ranges (Supplementary Table 2).

Strengths of this study include the relatively large number of cognitively unimpaired APOE ε4 HMs and age-, sex-, and education-matched HTs and NCs with PiB PET, FTP PET, and volumetric MRI, and the nearly 40-year age range, which permitted us to characterize associations with age, some information from the longitudinal Arizona APOE Cohort to help inform the impact of differential survivor bias on our findings, and the opportunity afforded by differential survivor bias to help in the study of resilience or resistance at older ages. Limitations include the size of the APOE ε4 HM group, particularly at older ages, the differential survivor bias described previously (including likely affected age estimates and the absence of Aβ PET, tau PET, and MRI data from participants after their clinical progression), differences between the participants and measurements included in the two cohorts, and the absence of longitudinal data to go beyond the study of age associations to the characterization of trajectories.

4 | CONCLUSIONS

This study provides information about Aβ plaque burden, tau-tangle burden, and neurodegeneration in cognitively unimpaired persons at three levels of genetic risk for AD. We suggest that unimpaired APOE ε4 HMs can be studied before their 70s to clarify the biomarker changes, risk factors, pathophysiological processes, and interventions involved in the predisposition to and prevention of AD and after their 70s to clarify the biomarker factors, risk modifiers, pathophysiological processes, therapeutic targets, and interventions involved in the resilience or resistance to and prevention of AD.

ACKNOWLEDGMENTS

This study is supported by the National Institute of Health and National Institute on Aging (R01 AG031581, R37 AG011378, R01 AG041851, U01 AG006786, and P50 AG016574), State of Arizona Department of Health Services (CTR040636, ADHS16-121321), Arizona Alzheimer’s Consortium, Mayo Clinic Foundation, the Alexander Family Professorship of Alzheimer’s Disease Research, GHR Foundation, The W. Garfield Weston Foundation/Weston Brain Institute Fellowship Grant, and Women Inspiring Scientific Progress (WISP) grant. The authors
thank the clinical research volunteers and teams at the participating institutions and Dr. Khachaturian for his helpful feedback and advice.

CONFLICT OF INTERESTS

E.M.R. receives research support from the NIH/NIA, Alzheimer’s Association, Banner Alzheimer’s Foundation, FBRI, GHR, NOMIS Foundation, and the Flinn Foundation; compensated consultation services to Aural Analytics, Alkahest, Alzheon, Denali, Green Valley, Roche (expenses only), and Zinfandel Pharma; research contracts from Avid, Eli Lilly, Genentech/Roche, Novartis, and Amgen; and grants from NIH/NIA, Novartis, Amgen, and Banner Alzheimer’s Foundation. P.T. receives personal compensation for consulting, serving on scientific advisory board, speaking, or other activities with AbbVie, AC Immune, Acadia, Auspex, Boehringer Ingelheim, Chase Pharmaceuticals, Corium, Eisai, GlaxoSmithKline, INSYS Therapeutics, Pfizer, T3D, AstraZeneca, Avanir, Biogen, Brian Test Inc., Cognoptix, Eli Lilly, H. Lundbeck A/S, Merck and Company, and Roche; receives royalty, license fees, or contractual rights payments from the University of Rochester; holds stock and/or stock options in Adamas; receives support from AstraZeneca, Avanir, Biogen, Brian Test Inc., Cognoptix, Eli Lilly, H. Lundbeck A/S, Merck and Company, Roche, Amgen, Avid, Functional Neuromodulation, GE Healthcare, Genentech, Novartis, Takeda, Targacept, the National Institute on Aging, and the Arizona Department of Health Services. R.J.C., an investigator in clinical trials, is sponsored by Merck and Novartis and receives research support from the NIA and the Arizona Alzheimer’s Consortium. K.C. is a full-time employee of Green Valley Pharmaceuticals and is an adjunct Sr. Scientist of Banner Alzheimer’s Institute. Y.S. is a consultant for Green Valley Pharmaceuticals. C.R.J. has consulted for Lilly and serves on an independent data monitoring board for Roche and as a speaker for Eisai, but he receives no personal compensation from any commercial entity. He receives research support from NIH and the Alexander Family Alzheimer’s Disease Research Professorship of the Mayo Clinic. V.J.L. serves on scientific advisory boards for Bayer Schering Pharma, Merck Research, and Piramal Life Sciences and receives research support from GE Healthcare, Siemens Molecular Imaging, AVID Radiopharmaceuticals, and the NIH (NIA, NCI). D.S.K. serves on a Data Safety Monitoring Board for the DIAN study; is an investigator in clinical trials and sponsored by Biogen, Lilly Pharmaceuticals, and the University of Southern California; and receives research support from the NIH. R.C.P. serves on data monitoring committees for Janssen Alzheimer Immunotherapy and is a consultant for Biogen, Roche, Merck, Genentech, Inc.; receives publishing royalties from Mild Cognitive Impairment (Oxford University Press, 2003); and receives research support from the NIH/NIA, V.G., D.D.G., H.P., R.B., and V.D. report no disclosures.

REFERENCES

1. Hyman BT, Phelps CH, Beach TG, Bigio EH, Cairns NJ, Carrillo MC, et al. National Institute on Aging-Alzheimer’s Association guidelines for the neuropathologic assessment of Alzheimer’s disease. Alzheimer’s Dement. 2012;8:1-13.
2. Montine TJ, Phelps CH, Beach TG, Bigio EH, Cairns NJ, Dickson DW, et al. National Institute on Aging-Alzheimer’s Association guidelines for the neuropathologic assessment of Alzheimer’s disease: a practical approach. Acta Neuropathol. 2012;123:1-11.
3. Braak H, Braak E. Neuropathological staging of Alzheimer-related changes. Acta Neuropathol. 1991;82:239-259.
4. Thal DR, Rüb U, Orantes M, Braak H. Phases of Aβ deposition in the human brain and its relevance for the development of AD. Neurology. 2002;58:1791-1800.
5. Braak H, Alafuzoff I, Arzberger T, Kretzschmar H, Del Tredici K. Staging of Alzheimer disease-associated neurofibrillary pathology using paraffin sections and immunocytochemistry. Acta Neuropathol. 2006;112:389-404.
6. Corder EH, Saunders AM, Strittmatter WJ, Schmechel DE, Gaskell PC, Small GW, et al. Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer’s disease in late onset families. Science. 1993;261:921-923.
7. Coon KD, Myers AJ, Craig DW, Webster JA, Pearson JV, Linke DH, et al. A high-density whole-genome association study reveals that APOE is the major susceptibility gene for sporadic late-onset Alzheimer’s disease. J Clin Psychiatry. 2007;68:613-618.
8. Bondi MW, Salmon DP, Galasko D, Thomas RG, Thal LJ. Neuropsychological function and apolipoprotein E genotype in the preclinical detection of Alzheimer’s disease. Psychol Aging. 1999;14:295-303.
9. Lange KL, Bondi MW, Salmon D, Galasko D, Delis DC, Thomas RG, et al. Decline in verbal memory during preclinical Alzheimer’s disease: examination of the effect of APOE genotype. J Int Neuropsychol Soc. 2002;8:943-955.
10. Reiman EM, Chen K, Liu X, Bandy D, Yu M, Lee W, et al. Fibrillar amyloid-beta burden in cognitively normal people at 3 levels of genetic risk for Alzheimer’s disease. Proc Natl Acad Sci U S A. 2009;106:6820-6825.
11. Caselli RJ, Dueck AC, Osborne D, Sabbagh MN, Connor DJ, Ahern GL, et al. Longitudinal modeling of age-related memory decline and the APOE epsilon4 effect. N Engl J Med. 2009;361:255-263.
12. Jansen WJ, Ossenkoppele R, Knol DL, Tijms BM, Scheltens P, Verhey FR, et al. Prevalence of cerebral amyloid pathology in persons without dementia: a meta-analysis. JAMA. 2015;313:1924-1938.
13. Coughlan G, Coutrot A, Khondoker M, Minihane AM, Spiers H, Hornberger M. Toward personalized cognitive diagnostics of at-genetic-risk Alzheimer’s disease. Proc Natl Acad Sci U S A. 2019;116:9285-9292.
14. Reiman EM, Caselli RJ, Yun LS, Chen K, Bandy D, Minoshima S, et al. Preclinical evidence of Alzheimer’s disease in persons homozygous for the epsilon 4 allele for apolipoprotein E. N Engl J Med. 1996;334:752-758.
15. Reiman EM, Caselli RJ, Alexander GE, Chen K. Tracking the decline in cerebral glucose metabolism in persons and laboratory animals at genetic risk for Alzheimer’s disease. Clin Neurosci Res. 2001;1:194-206.
16. Reiman EM, Caselli RJ, Chen K, Alexander GE, Bandy D, Frost J. Declining brain activity in cognitively normal apolipoprotein E epsilon 4 heterozygotes: A foundation for using positron emission tomography to efficiently test treatments to prevent Alzheimer’s disease. Proc Natl Acad Sci U S A. 2001;98:3334-3339.
17. Reiman EM, Chen K, Alexander GE, Caselli RJ, Bandy D, Osborne D, et al. Functional brain abnormalities in young adults at genetic risk for late-onset Alzheimer’s dementia. Proc Natl Acad Sci U S A. 2004;101:284-289.
18. Reiman EM, Chen K, Alexander GE, Caselli RJ, Bandy D, Osborne D, et al. Correlations between apolipoprotein E epsilon4 gene dose and brain-imaging measurements of regional hypometabolism. Proc Natl Acad Sci U S A. 2005;102:8299-8302.
19. Reiman EM, Chen K, Caselli RJ, Alexander GE, Bandy D, Adamson JL, et al. Cholesterol-related genetic risk scores are associated with hypometabolism in Alzheimer’s-affected brain regions. Neuroimage. 2008;40:1214-1221.
20. Reiman EM, Chen K, Langbaum JB, Lee W, Reschke C, Bandy D, et al. Higher serum total cholesterol levels in late middle age are associated with glucose hypometabolism in brain regions affected by Alzheimer’s disease and normal aging. Neuroimage. 2010;49:169-176.
21. Roberts RO, Geda YE, Knopman DS, Cha RH, Pankratz VS, Boeve BF, et al. The Mayo Clinic Clinic of Aging: design and sampling, participation, baseline measures and sample characteristics. Neuroepidemiology. 2008;30:58-69.
22. Petersen RC, Wiste HJ, Weigand SD, Rocca WA, Roberts RO, Mielke MM, et al. Association of Elevated Amyloid Levels With Cognition and Biomarkers in Cognitively Normal People From the Community. JAMA Neurol. 2016;73:85-92.
23. Jack CR Jr., Wiste HJ, Weigand SD, Therneau TM, Knopman DS, Lowe V, et al. Age-specific and sex-specific prevalence of cerebral β-amyloidosis, tautopathy, and neurodegeneration in cognitively unimpaired individuals aged 50-95 years: a cross-sectional study. The Lancet Neurol. 2017;16:435-444.
24. Farrer LA, Cupples LA, Haines JL, Hyman B, Kukull WA, Mayo R, et al. Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease - A meta-analysis. JAMA 1997;278:1349-1356.
25. Meyer MR, Tschanz JT, Norton MC, Welsh-Bohmer KA, Steffens DC, Wyse BW, et al. APOE genotype predicts when—not whether—one is predisposed to develop Alzheimer disease. Nat Genet. 1998;19:321-322.
26. Breitner JC, Wyse BW, Anthony JC, Welsh-Bohmer KA, Steffens DC, Norton MC, et al. APOE-4 count predicts age when prevalence of AD increases, then declines. Neurology. 2000;55:161-162.
27. Arenaza-Urquijo EM, Vemuri P. Resistance vs resilience to Alzheimer disease: Clarifying terminology for preclinical studies. Neurology. 2018;90:695-703.
28. Jack CR Jr., Wiste HJ, Weigand SD, Therneau TM, Lowe VJ, Knopman DS, et al. Defining imaging biomarker cut points for brain aging and Alzheimer’s disease. Alzheimer’s & dementia. Alzheimer’s Assoc. 2017:13:205-216.
29. Hixson JE, Vernier DT. Restriction isotyping of human apolipoprotein E by gene amplification and cleavage with HhaI. J Lipid Res. 1990;31:545-548.
30. Crook R, Hardy J, Duff K. Single-day apolipoprotein E genotyping. J Neurosci Methods. 1994;53:125-127.
31. Iturria-Medina Y, Sotero RC, Toussaint PJ, Evans AC. Alzheimer’s Disease Neuroimaging Initiative. Epidemic spreading model to characterize misfolded proteins propagation in aging and associated neurodegenerative disorders. Plos Comput Biol. 2014;10:e1003956.
32. Quiroz YT, Sperling RA, Norton DJ, Baena A, Arboleda-Velasquez JF, Cosio D, et al. Association Between Amyloid and Tau Accumulation in Young Adults With Autosomal Dominant Alzheimer Disease. JAMA Neurol. 2018;75:548-556.
33. Knopman DS, Lundt ES, Therneau TM, Vemuri P, Lowe VJ, Kantarci K, et al. Entorhinal cortex tau, amyloid-beta, cortical thickness and memory performance in non-demented subjects. Brain. 2019;142:1148-1160.
34. Lowe VJ, Bruinsma TJ, Wiste HJ, Min HK, Weigand SD, Fang P, et al. Cross-sectional associations of tau-PET signal with cognition in cognitively unimpaired adults. Neurology. 2019;93:e29-e39.
35. Jack CR Jr., Bennett DA, Blennow K, Carrillo MC, Dunn B, Haeberlein SB, et al. NIA-AA Research Framework: Toward a biological definition of Alzheimer’s disease. Alzheimer’s Dement. 2018;14:535-562.
36. Fox J, Weisberg S. An R companion to applied regression. 2018; Sage Publications.
37. Qian J, Wolters FJ, Beiser A, Haan M, Ikram MA, Karlawish J, et al. APOE-related risk of mild cognitive impairment and dementia for prevention trials: An analysis of four cohorts. Plos Med. 2017:14:e1002254.
38. Langbaum JB, Chen K, Launer LJ, Fleisher AS, Lee W, Liu X, et al. Blood pressure is associated with higher brain amyloid burden and lower glucose metabolism in healthy late middle-age persons. Neurobiol Aging. 2012;33:827.e11-827.e19.
39. Reiman EM. Alzheimer disease in 2016: Putting AD treatments and biomarkers to the test. Nat Rev Neurol. 2017;13:74-76.
40. Reiman EM, Langbaum JB, Tariot PN, Lopera F, Bateman RJ, Morris JC, et al. CAP-advancing the evaluation of preclinical Alzheimer disease treatments. Nat Rev Neurol. 2016;12:56-61.
41. Reiman EM, Langbaum JB, Fleisher AS, Caselli RJ, Chen K, Ayutyanont N, et al. Alzheimer’s Prevention Initiative: a plan to accelerate the evaluation of presymptomatic treatments. J Alzheimers Dis. 2011;26:321-329.

SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section at the end of the article.