Associations Between Biomarkers and Age in the Presenilin 1 E280A Autosomal Dominant Alzheimer Disease Kindred: A Cross-sectional Study

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IMPORTANCE Age-associated changes in brain imaging and fluid biomarkers are characterized and compared in presenilin 1 (PSEN1) E280A mutation carriers and noncarriers from the world's largest known autosomal dominant Alzheimer disease (AD) kindred.

OBJECTIVE To characterize and compare age-associated changes in brain imaging and fluid biomarkers in PSEN1 E280A mutation carriers and noncarriers.

DESIGN, SETTING, AND PARTICIPANTS Cross-sectional measures of 18F-florbetapir positron emission tomography, 18F-fludeoxyglucose positron emission tomography, structural magnetic resonance imaging, cerebrospinal fluid (CSF), and plasma biomarkers of AD were assessed from 54 PSEN1 E280A kindred members (age range, 20-59 years).

MAIN OUTCOMES AND MEASURES We used brain mapping algorithms to compare regional cerebral metabolic rates for glucose and gray matter volumes in cognitively unimpaired mutation carriers and noncarriers. We used regression analyses to characterize associations between age and the mean cortical to pontine 18F-florbetapir standard uptake value ratios, precuneous cerebral metabolic rates for glucose, hippocampal gray matter volume, CSF Aβ1-42, total tau and phosphorylated tau181, and plasma Aβ measurements. Age at onset of progressive biomarker changes that distinguish carriers from noncarriers was estimated using best-fitting regression models.

RESULTS Compared with noncarriers, cognitively unimpaired mutation carriers had significantly lower precuneous cerebral metabolic rates for glucose, smaller hippocampal volume, lower CSF Aβ1-42, higher CSF total tau and phosphorylated tau181, and higher plasma Aβ1-42 measurements. Sequential changes in biomarkers were seen at age 20 years (95% CI, 14-24 years) for CSF Aβ1-42, age 16 years (95% CI, 11-24 years) for the mean cortical 18F-florbetapir standard uptake value ratio, age 15 years (95% CI, 10-24 years) for precuneous cerebral metabolic rate for glucose, age 15 years (95% CI, 7-20 years) for CSF total tau, age 13 years (95% CI, 8-19 years) for phosphorylated tau181, and age 6 years (95% CI, 1-10 years) for hippocampal volume, with cognitive decline up to 6 years before the kindred's estimated median age of 44 years (95% CI, 43-45 years) at mild cognitive impairment diagnosis. No age-associated findings were seen in plasma Aβ1-42 or Aβ1-40.

CONCLUSIONS AND RELEVANCE This cross-sectional study provides additional information about the course of different AD biomarkers in the preclinical and clinical stages of autosomal dominant AD.
there is growing interest in biomarker changes associated with the preclinical stages of Alzheimer disease (AD) and in the use of this information to help inform the design and statistical power of preclinical AD trials. Such trials are under way, predicated on an understanding that Alzheimer pathology and physiology begin decades before clinical symptoms of cognitive dysfunction arise.1-2 Hypotheses about the evolution of abnormalities in biomarkers associated with AD led to revision of research and clinical guidelines for the use of biomarkers in the diagnosis of AD at 3 different stages of disease, including preclinical, mild cognitive impairment (MCI), and dementia.3-4 Preclinical biomarker studies5,6 in autosomal dominant AD (ADAD) have helped elucidate the evolution of biomarker abnormalities before onset of clinical symptoms.

In preparation for the recently started Alzheimer’s Prevention Initiative Autosomal Dominant Alzheimer’s Disease treatment trial of an Aβ-modifying agent,2 we conducted an initial cross-sectional biomarker study in presenilin 1 (PSEN1; OMIM 104311) E280A mutation carriers and noncarriers to better understand preclinical biomarker change associated with age.5 This study aimed to characterize associations among brain imaging and fluid AD biomarkers with mutation status and age, as well as to relate onset of progressive biomarker changes to this kindred’s estimated median ages of 44 and 49 years, respectively, at clinical onset of MCI and dementia due to AD.5 Our group previously reported that the mean cortical 18F-flurbetapir positron emission tomography (PET) measurements of fibrillar Aβ burden begin to rise approximately 16 years before the kindred’s respective median ages at MCI.5 Herein, we extend our analyses to include 18F-fluorodeoxyglucose PET, structural magnetic resonance (MR) imaging, cerebrospinal fluid (CSF), and plasma biomarker measurements from this cohort of clinically unaffected and affected research participants from the world’s largest known single-mutation ADAD kindred.

Methods

Participants and Study Design

Participants provided their informed consent before study entry under guidelines approved by local institutional review boards. In those participants who were unable to provide consent because of cognitive impairment, a legal representative provided assent in accord with local laws and institutional regulations. This included agreement that information would not be provided about their PSEN1 or apolipoprotein E (APOE) genotypes, which were obtained as previously described.7,8

The study was performed between September 1, 2011, and July 31, 2012. All biomarkers were collected within a 6-month time frame from clinical evaluations. Participant recruitment and enrollment, clinical and neuropsychological evaluations, and lumbar punctures were performed at the Universidad de Antioquia, Medellin, Colombia. The 18F-fluorodeoxyglucose PET and MR imaging were performed at Hospital Pablo Tobón Uribe in Medellin. Plasma and CSF samples were shipped to and assayed by the D confusion MCI to whole-brain cerebral metabolic rate for glucose (CMRgl) ratios were characterized from a bilateral region of interest (ROI) in each participant’s 18F-fluorodeoxyglucose PET image using an automated brain mapping algorithm (SPM8; http://www.fil.ion.ucl.ac.uk/spm/software/spm8) and the automatic anatomical labeling toolbox.16 Hippocampal to total intracranial volume ratios were characterized from bilateral ROIs in each participant’s T1-weighted MR image using a software package...
SPM8 was used to deform each participant’s 18F-fludeoxyglucose PET image into the coordinates of a brain atlas, normalize the data for whole-brain measurements, and generate a statistical map of CMRgl differences between the cognitively unimpaired mutation carrier and noncarrier groups (P < .005, uncorrected for multiple comparisons). For MR imaging, SPM8 was used in conjunction with the voxel-based morphometry toolbox and diffeomorphic anatomical registration using exponential Lie algebra to generate a statistical map of gray matter volume differences between groups (P < .005, uncorrected for multiple comparisons). The false discovery rate was used to assess significance after correction for multiple comparisons.

CSF and Plasma Biomarkers
The lumbar punctures were performed before noon by a qualified physician at the Universidad de Antioquia after a minimum of 4 hours of fasting. In a seated position, an atarumatic 24G Sprotte needle and sterile polypropylene tubes were used for sample collection. In total, 6 to 12 mL of CSF was acquired in 0.3-mL aliquots, frozen at −70°C, shipped on dry ice, and assayed in a single batch. Luminex xMAP bead-based assays (INNO-BIA AlzBio3; Innogenetics) were used to quantify CSF Aβ42, total tau, and phosphorylated tau181, as well as plasma Aβ42 and Aβ40 (INNO-BIA Plasma Aβ Forms Multiplex Assay; Innogenetics). Sample aliquots were stored for longitudinal comparisons, future analyses, and data sharing.

Analysis of Associations Between Biomarker Measurements and Age
Curvilinear regression models were used to assess associations between prespecified biomarker measurements and age in the mutation carrier and noncarrier groups. Linear, quadratic, or sigmoidal regression curves were fitted to each biomarker within groups. Best-fitting models were selected based on goodness of fit to data (R²) and Akaike information criterion as previously described in detail. Models were compared using a software package (GraphPad Prism; GraphPad Software, Inc). This approach determines how well the data support each model, taking into account goodness of fit (sum of squares) and the number of parameters in the model.

Best-fitting curves were used to determine the age at which biomarker measurements in carriers and noncarriers began to diverge based on an approximate t test (P ≤ .05), the same approach used in the DIAN study.2 As complementary measures, to be consistent with other approaches, we also present 2 alternative methods for determining the age at biomarker change. First, when the initial part of the biomarker curve was found to be flat (ie, for 18F-florbetapir PET and CSF phosphorylated tau181 measurements), the point on the mutation carrier group regression curve at which the biomarker showed a significant slope inflection was used to estimate the age at significant biomarker initial change, consistent with our group’s previous study of cortical 18F-florbetapir standardized uptake value ratio changes. Second, using a more conservative approach, the age at which the 95% CI zones for the carrier and noncarrier group curves became separated is also presented. Results for these 2 alternative estimate methods are presented in table form for comparison with our primary measures. For all estimates of the age at biomarker change, 95% CIs were established using iterative Monte Carlo simulations (MATLAB; The MathWorks, Inc).

Results

Participant Characteristics
Fifty-four research participants were successfully screened and enrolled into the study. 18F-florbetapir PET, 18F-fludeoxyglucose PET, MR imaging, CSF, and plasma measurements were acquired in each participant with only the following exceptions: 4 participants did not travel to Arizona for 18F-florbetapir PET, and 2 participants declined to have 18F-fludeoxyglucose PET. Participant characteristics, including representative clinical ratings and neuropsychological test scores, are summarized in Table 1. Seven of the cognitively impaired mutation carriers met diagnostic criteria for MCI due to AD, and 5 met criteria for mild dementia due to AD. The cognitively unimpaired mutation carrier and noncarrier groups did not differ significantly in their age, sex, education, APOE carrier proportion, clinical ratings, or neuropsychological test scores. Compared with unimpaired mutation carriers, cognitively impaired mutation carriers were older and had significantly lower education, clinical ratings, and neuropsychological test scores. Correlation analyses between years of education and each biomarker measurement revealed no significant associations. Therefore, years of education were not corrected for in group analyses.

Cognitive Test Performance
Cognitive test performance for global measures, attention, executive function, and memory was evaluated for age-related onset and change, revealing evidence of cognitive decline up to 6 years before the kindred’s estimated median age of 44 years at MCI diagnosis (95% CI, 43-45 years).7 Cognitive changes were approximated to begin at age 38 years for CDR sum of boxes (95% CI, 33-42 years), MMSE (95% CI, 33-41 years), and Consortium to Establish a Registry for Alzheimer’s Disease word list delayed recall (95% CI, 34-41 years) (Figure 1). Cognitive changes were approximated to begin at age 40 years (95% CI, 35-45 years) for letter fluency and at age 42 years for Trail Making Test A (95% CI, 33-47 years) and category (animal) fluency (95% CI, 36-45 years).

18F-fludeoxyglucose PET
Unimpaired carriers had lower CMRgl in the prespecified precuneus ROI.23-25 than noncarriers (P = .02). Lower CMRgl was associated with older age in the carrier group (P = .001) but not in the noncarrier group (P = .30). Regression modeling revealed a linear relationship between lower precuneus CMRgl and older age among the mutation carriers (R² = 0.31). Carrier precuneus CMRgl began to diverge from noncarrier CMRgl at approximately age 29 years (95% CI, 20-34 years), about 15 years before the kindred’s estimated median age at MCI onset (Figure 1 and Table 2). Voxel-based analyses demonstrated
### Table 1. Characteristics of Research Participants

<table>
<thead>
<tr>
<th>Variable</th>
<th>Noncarriers (n = 22)</th>
<th>Unimpaired Carriers (n = 20)</th>
<th>Impaired Carriers (n = 12)</th>
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<tr>
<td>Age, mean (SD) [range], y</td>
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<td>32 (9) [20-44]</td>
<td>49 (5) [42-59]</td>
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<td>8</td>
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<tr>
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<td>8</td>
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<tr>
<td>Educational level, mean (SD), y</td>
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<td>12 (3)</td>
<td>8 (4)</td>
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<td>3.5 (2.4)</td>
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<td>29.8 (0.4)</td>
<td>22.3 (4.4)</td>
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<td>CERAD word list delayed recall</td>
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<td>20.5 (3.0)</td>
<td>5.9 (3.3)</td>
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<td>Trail Making Test Ad</td>
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<td>39.6 (11.3)</td>
<td>143.5 (94.5)</td>
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<td>Category fluency, animals</td>
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<td>21.5 (5.4)</td>
<td>10.3 (4.3)</td>
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<td>Verbal fluency, FAS</td>
<td>33.4 (6.3)</td>
<td>36.2 (11.9)</td>
<td>16.2 (8.8)</td>
</tr>
</tbody>
</table>

Abbreviations: APOE, apolipoprotein E; CDR, Clinical Dementia Rating (range, 0-18, with 0 a perfect score); CERAD, Consortium to Establish a Registry for Alzheimer’s Disease; MMSE, Mini-Mental State Examination (range, 0-30, with 30 a perfect score).

* P < .05 compared with noncarriers.

* P < .05 compared with unimpaired carriers.

APOE information is missing for one impaired carrier for whom assent was not provided for APOE testing.

No data were available for 2 impaired carriers.

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**Figure 1. Biomarker Measure Associations With Age**

Shown are age-associated biomarker curves for mutation noncarriers and carriers. Some data points were withheld to protect individual identities associated with age. CDR indicates Clinical Dementia Rating; CERAD, Consortium to Establish a Registry for Alzheimer’s Disease; CSF, cerebrospinal fluid; MMSE, Mini-Mental State Examination; PET CMRgl, positron emission tomography precuneous cerebral metabolic rate for glucose; and SUVR, mean standardized uptake value ratio (previously reported).
lower CMRgl among cognitively unimpaired mutation carriers than noncarriers in bilateral precuneus and occipital locations ($P < .005$, uncorrected for multiple comparisons) (Figure 2A).

**Volumetric MR Imaging Measurements**

There were no overall group differences in prespecified hippocampal ROI1,26 volume between unimpaired carriers and noncarriers ($P = .77$) (Figure 3). However, smaller hippocampal volume was associated with older age in the mutation carrier group ($P < .001$) but not in the noncarrier group ($P = .41$). Regression models revealed that a quadratic curve best fit the association between age and hippocampal volume in the mutation carriers ($R^2 = 0.48$). Mutation carrier hippocampal volume began to diverge from noncarrier volume at approximately age 38 years (95% CI, 33-43 years), 6 years before the kindred’s median age at MCI onset (Figure 1 and Table 2). Post hoc evaluation of standardized whole-brain volume (smaller) and ventricular volume (larger) revealed similar age-related changes and estimated age at onset of divergence compared with hippocampal volume.

Cognitively unimpaired mutation carriers had significantly less gray matter than noncarriers in bilateral precuneus, posterior cingulate, lateral parietotemporal cortex, medial temporal lobe, and thalamus ($P < .005$, uncorrected for multiple comparisons) (Figure 2B). Based on these results, we performed post hoc age association ROI analyses of gray matter volume within the precuneus and posterior cingulate cortex.
24-37 years), and phosphorylated tau181 levels began to diverge from noncarrier levels at approximately age 29 years (95% CI, 20-30 years) (Figure 1 and Table 2). Carrier ratios of total tau to Aβ1-42 and phosphorylated tau181 to Aβ1-42 in mutation carriers began to diverge from noncarrier levels at approximately age 31 years (95% CI, 25-36 years for both), best fitting sigmoidal-shaped curves ($R^2 = 0.4$ for both).

The CSF Aβ1-42 levels were reduced with age in the mutation carrier group ($R = -0.62, P < .001$) but not in the noncarrier group ($R = -0.092, P = .86$). Comparing linear, quadratic, and sigmoidal regression models revealed that a linear relationship between CSF Aβ1-42 levels and age was the best fit in the mutation carriers. Using the approximate $t$ test, mutation carrier CSF Aβ1-42 began to diverge from noncarrier levels at approximately age 24 years (95% CI, 20-30 years) (Figure 1 and Table 2), about 20 years before the kindred’s median age at MCI onset. Best fitting to sigmoidal curve models, CSF total tau and phosphorylated tau181 levels were higher with age in the mutation carrier group ($R^2 = 0.39, P < .003$ and $R^2 = 0.40, P < .002$, respectively) but not in the noncarrier group ($R^2 = 0.071, P = .21$ and $R^2 = 0.037, P = .39$, respectively). Carrier CSF total tau levels began to diverge from noncarrier levels at approximately age 29 years (95% CI, 24-37 years), and phosphorylated tau181 levels began to diverge at approximately age 31 years (95% CI, 25-36 years) (Figure 1 and Table 2). Carrier ratios of total tau to Aβ1-42 and phosphorylated tau181 to Aβ1-42 in mutation carriers began to diverge from noncarrier levels at approximately age 31 years (95% CI, 25-36 years for both), best fitting sigmoidal-shaped curves ($R^2 = 0.4$ for both).

Figure 3. Biomarker Comparisons Between Unimpaired Carriers and Noncarriers

Shown are between-group cross-sectional comparisons between unimpaired PSEN1 E280A mutation carriers vs noncarriers. Significant differences are seen in cerebrospinal fluid (CSF) Aβ1-42, amyloid positron emission tomography (PET) ($^{18}$F-florbetapir standardized uptake value ratio [SUVR]), CSF total tau, and $^{18}$F-fludeoxyglucose PET precuneus cerebral metabolic rate for glucose (CMRgl) but not in bilateral standardized hippocampal volume ($P < .05$).

Plasma Aβ1-42 levels were significantly higher in mutation carriers than in noncarriers (mean [SD], 45 [10] vs 39 [6] pg/mL; $P = .03$) but were not significantly associated with age. Plasma Aβ1-40 levels were not significantly different between carriers and noncarriers (mean [SD], 130 [30] vs 138 [20] pg/mL; $P = .35$).

Age-Related Biomarker Changes

To directly compare age-related biomarker changes, previously reported mean cortical to pontine $^{18}$F-florbetapir standard uptake value ratios, $^5$ CSF Aβ1-42 and total tau levels, precuneus CMRgl, and hippocampal volume were transformed to a minimum-maximum standard scale from zero to one, with increasing values representing higher abnormalities in the mutation carriers (Figure 4). Best-fitting regression models were used. The CSF and PET measures of Aβ pathology began to diverge in the carrier and noncarrier groups at the youngest ages, followed soon by changes in CSF total tau and $^{18}$F-fludeoxyglucose PET, with hippocampal volume and notable cognitive changes closer to the age at MCI onset.

Discussion

This cross-sectional study characterized and compared age-associated brain imaging and CSF AD biomarker changes in PSEN1 E280A mutation carriers and noncarriers, estimating the number of years these changes occur before the kindred’s estimated median ages at MCI and dementia onset. Findings from this study are largely consistent with previous cross-sectional studies $^5$-$^7$ comparing ADAD mutation carrier biomarkers with the age at symptom onset. Furthermore, our results are con-
consistent with the recently updated hypothetical models by Jack and colleagues, regarding the sequence of progressive preclinical AD pathology, namely, CSF and PET measures of Aβ pathology followed by CSF measures of tau pathology and regional CMRgl decline, followed by hippocampal atrophy and clinical progression. In addition, this time line of pathophysiological events is consistent with a recent longitudinal biomarker study in late-onset sporadic AD that demonstrated amyloid PET changes 17 years before and hippocampal changes 4 years before a CDR of 1, suggesting similarities in preclinical biomarker progression between ADAD and late-onset sporadic AD.

Most previous biomarker studies in ADAD have focused on individuals close to the age at clinical onset, with smaller cohorts (often from families with mixed mutations), and that largely reported on a single-biomarker modality. Advantages of the present analysis include having a cohort not only with a single genetic variant but also from the same race/ethnicity and general geographic location and with similar cultural influences. Controlling for these genetic and demographic variations may result in reduced variability of data and greater accuracy for predicting estimated ages at symptomatic onset and biomarker trajectories than is possible in studies of families with mixed mutations. The DIAN study assessed the age at biomarker change in 88 mutation carriers, combining 51 different mutation pedigrees. Consistent with the DIAN and other ADAD studies, cognitively unimpaired mutation carrier standardized z score curves from zero to one for cerebral metabolic rate for glucose (CMRgl), bilateral standardized hippocampal volume, and memory (Consortium to Establish a Registry for Alzheimer's Disease [CERAD] word list delayed recall). The age at significant difference from mutation noncarriers is marked with a circle for each respective biomarker. MCI indicates mild cognitive impairment.

Figure 4. Age and Biomarker Associations and Comparison of Age at Onset of Biomarker Changes

Shown are cognitively unimpaired mutation carrier standardized z score curves from zero to one for cerebral metabolic rate for glucose (CMRgl), bilateral standardized hippocampal volume, and memory (Consortium to Establish a Registry for Alzheimer's Disease [CERAD] word list delayed recall). The age at significant difference from mutation noncarriers is marked with a circle for each respective biomarker. MCI indicates mild cognitive impairment.
sitive measures of preclinical and early clinical cognitive decline, such as composite cognitive tests.\textsuperscript{34}

**Conclusions**

Longitudinal studies are needed to further characterize and confirm the findings presented herein. This study and the recently started Alzheimer’s Prevention Initiative Autosomal Dominant Alzheimer’s Disease treatment trial aim to clarify the extent to which AD biomarkers change in association with age. These studies and others in presymptomatic AD provide guidance to predict subsequent clinical progression and differential treatment response and to accelerate the evaluation of putative preclinical AD treatments.

**REFERENCES**


9. Folstein MF, Folstein SE, McHugh PR. "Mini-Mental State": a practical method for grading


