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Toward a multifactorial model of Alzheimer disease

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Abstract

Relations among antecedent biomarkers of Alzheimer disease (AD) were evaluated using causal modeling; although correlation cannot be equated to causation, causation does require correlation. Individuals aged 43 to 89 years ($N = 220$) enrolled as cognitively normal controls in longitudinal studies had clinical and psychometric assessment, structural magnetic resonance imaging (MRI), cerebrospinal fluid (CSF) biomarkers, and brain amyloid imaging via positron emission tomography with Pittsburgh Compound B (PIB) obtained within 1 year. CSF levels of $A\beta_{42}$ and tau were minimally correlated, indicating they represent independent processes. $A\beta_{42}$, tau, and their interaction explained 60% of the variance in PIB. Effects of *APOE* genotype and age on PIB were indirect, operating through CSF markers. Only spurious relations via their common relation with age were found between the biomarkers and regional brain volumes or cognition. Hence, at least 2 independent hypothesized processes, one reflected by CSF $A\beta_{42}$ and one by CSF tau, contribute to the development of fibrillar amyloid plaques preclinically. The lack of correlation between these 2 processes and brain volume in the regions most often affected in AD suggests the operation of a third process related to brain atrophy.

Keywords: Preclinical Alzheimer disease; Amyloid-β; Tau; PIB; Amyloid plaque; *APOE*; Brain volumetry; Memory; Biomarkers; Cerebrospinal fluid

1. Introduction

It is increasingly accepted that the pathologic changes that lead to the eventual diagnosis of symptomatic Alzheimer disease (AD) begin long before there is sufficient cognitive impairment to warrant a clinical diagnosis of the disease (Jack et al., 2009; Price et al., 2009). Recent advances (Klunk et al., 2004) make it possible to image fibrillar amyloid plaques, a pathologic hallmark of AD, providing one avenue to detection of pathology prior to clinical diagnosis.

There is a strong inverse relation between fibrillar amyloid plaque burden as assessed by positron emission tomography (PET) imaging using the amyloid tracer, Pittsburgh Compound-B (PIB), with levels of cerebrospinal fluid (CSF) $A\beta_{42}$ in cognitively healthy individuals (Fagan et al., 2006, 2009; Tolbloom, 2009). This has been interpreted as suggesting that an early step in the process leading to AD is sequestering of $A\beta_{42}$ in plaques (Hong et al., 2011), thereby reducing the level in the CSF. The amount of plaque burden also is associated with increased levels of CSF total tau and phospho-tau_{181} (ptau; Fagan et al., 2009b). This relation has often been interpreted in terms of the amyloid cascade hypothesis (Selkoe, 1991). In its simplest form the hypothesis states that $A\beta_{42}$ peptides aggregate to form amyloid plaques which, in turn, lead to synaptic loss and cell death, reflected in elevated CSF tau, thereby causing dementia. Recent reviews, however, suggest that the process may not be that simple (Holtzman et al., 2011; Hyman, 2011; Pimplikar, 2009; Small and Duff, 2008).

Other variables associated with one or more of the CSF biomarkers and PIB include age and apolipoprotein (*APOE*)

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genotype, the major genetic susceptibility factor associated with late-onset AD (Morris et al., 2010; Rowe et al., 2010; Sunderland et al., 2004; Vemuri et al., 2010). Mixed results have been reported for forebrain structure (Apostolova et al., 2010; Becker et al., 2011; Chetelat et al., 2010; Fagan et al., 2009a; Mormino et al., 2009; Oh et al., 2011; Tosun et al., 2010). Concurrent measures of cognition, however, are uncorrelated with the CSF measures (Fagan et al., 2009) or PIB (Mormino et al., 2009; Oh et al., 2011; Storandt et al., 2009) in cognitively normal individuals.

We examined all of these variables in cognitively normal individuals using causal modeling in an effort to explore theoretical models of their interrelations. To the best of our knowledge there has been no prior attempt to do so. Causal modeling is a statistical procedure using regression analysis that is designed to determine if empirical data are consistent with a theoretical model. It requires that 3 conditions exist if X is a potential cause of Y (Cohen et al., 2003). One, there must be a correlation between X and Y; that is, although correlation cannot be equated to causation, causation does require correlation. Two, X must precede Y in time. Three, the relation between X and Y must not be spurious; a spurious relation is one in which X and Y are related because both are influenced by a third variable, Z. For example, wrinkled skin and slowed reaction times are correlated because both are associated with age, not because either causes the other. Of course, although correlation cannot be equated to causation, causation does require correlation.

Longitudinal study ultimately is required to verify causality, but those results for preclinical AD may not be available for many years. Similarly, longitudinal study is necessary to determine the temporal order of appearance of the various processes, even if they are independent. In the meantime, models built on cross-sectional data can provide useful suggestions about avenues of investigation of various underlying pathophysiolocal processes.

2. Methods

2.1. Participants

The sample included 220 participants (64% women) aged 45 to 89 years (M = 65.8, SD = 9.7) enrolled in longitudinal studies at the Knight Alzheimer's Disease Research Center, Washington University in St Louis. Their mean years of education was 15.7 years (SD = 2.6). Only participants with PIB imaging and lumbar puncture (LP) to obtain CSF within 1 year of each other (M = 1.7 months, SD = 4.4) between December 2003 and April 2010 were included. Participants were cognitively normal (Clinical Dementia Rating [CDR] = 0; Morris, 1993) at the time of assessment; 14 subsequently progressed to a CDR > 0 indicating cognitive impairment. A subset (n = 164) comparable to the total sample in terms of age, gender, education, and *APOE* allele distribution had structural brain assessment with magnetic resonance imaging (MRI) within 1 year of LP (M = 1.2 months, SD = 3.6) and PIB imaging (M = 0.7 months, SD = 2.9). All procedures were approved by the university's Human Research Protection Office; written informed consent was obtained from participants and their collateral sources. Data from many of these participants have appeared in previous reports from the center.

2.2. Clinical evaluation

Experienced clinicians determined if the person was demented (CDR > 0) or not (CDR = 0) based solely on semistructured interviews with participants and their knowledgeable collateral sources (usually spouse or adult child) followed by a neurological examination of the participant. Clinicians determined if any cognitive problems represented decline from former level of function for that individual and interfered to some degree with the person's ability to carry out accustomed activities. Assessment included a health history, medication inventory, and assessment of depression and aphasia. Clinicians were unaware of the results of previous clinical evaluations and of previous and current psychometric test results. The CDR staging and diagnostic protocol is sensitive to clinical progression and highly predictive (93%) of autopsy-confirmed AD (Berg et al., 1998).

2.3. CSF collection, processing, and biomarker measurement

CSF (20–30 ml) free from blood contamination was collected by LP in polypropylene tubes at 8:00 AM after overnight fasting as described previously (Fagan et al., 2006). Samples were gently inverted to avoid gradient effects, briefly centrifuged at low speed to pellet any cellular elements, and aliquoted (500 μl) into polypropylene tubes before freezing at −84 °C. Analyses for Aβ_{42}, total tau, and ptau were performed using commercial enzyme-linked immunosorbent assay (INNOTEST; Innogenetics, Ghent, Belgium). Samples were continuously kept on ice with only a single thaw after initial freezing before assays.

2.4. PET PIB imaging

In vivo fibrillar amyloid imaging via PET with PIB ([*N*-methyl-[^{11}C]]2-(4′-methylaminophenyl)-6-hydroxybenzothiazole) was performed as described previously (Mintun et al., 2006). Approximately 12 mCi of [^{11}C]PIB was administered intravenously simultaneous with initiation of a 60-minute dynamic PET scan in three-dimensional mode. Measured attenuation factors and a ramp filter were used to reconstruct dynamic PET images. Three-dimensional regions of interest (ROIs) were created for each participant based on their individual MRI scans (T1-weighted 1 × 1 × 1.25 mm MPRAGE). A binding potential for each ROI was calculated (Logan et al., 1996) to express regional binding values in a manner proportional to number of binding sites. Values from prefrontal cortex, gyrus rectus, lateral temporal, and precuneus ROIs were averaged to calculate a mean cortical binding potential value

based on brain regions known to have high PIB uptake among participants with AD.

2.5. APOE genotyping

TaqMan assays (Applied Biosystems, Foster City, CA) for both rs429358 (ABI#C_3084793_20) and rs7412 (ABI#C_904973_10) were used for *APOE* genotyping. Allele calling was performed using the allelic discrimination analysis module of ABI Sequence Detection Software. Positive controls for each of 6 possible *APOE* genotypes were included on the genotyping plate.

2.6. MRI acquisition

One to 4 T1-weighted images were acquired in one scanning session in 168 participants on either a Sonata 1.5 T ($n = 17$), Vision 1.5 T ($n = 23$), or Trio 3.0 T scanner ($n = 128$). Cushions reduced head movement during scanning; a scout image was acquired first in order to center the field of view on the brain.

2.7. Regional volumetry

Regional volumes were obtained using Freesurfer software (Desikan et al., 2006; Fischl et al., 2002). During processing each voxel is assigned a neuroanatomical label based on probabilistic information derived from a manually labeled training set, which included healthy young and older adults. ROIs included lateral parietal (combined inferior parietal and supramarginal regions), temporal neocortical (combined superior, middle, and inferior temporal gyri), anterior cingulate, posterior cingulate, precuneus, hippocampal, entorhinal cortex and parahippocampal cortex. Previous work indicates that this technique generates volumes with a high correspondence to manually generated volumes (Desikan et al., 2006; Fischl et al., 2002). As there were no hypotheses regarding laterality effects, volumes were summed across the left and right hemispheres. Total intracranial volume (ICV) was used to adjust volumes used in the analyses for body size differences via a formula based on the analyses of covariance approach: Adjusted volume = raw volume—($b\times$ [ICV—mean ICV]), where b is the slope of the regression of the ROI volume on ICV. There is evidence of reliability of Freesurfer-derived estimates of cortical thickness and volumes across scanner upgrades, different manufacturers, and number of MP-RAGE acquisitions (Fennema-Notestine et al., 2007; Jovicich et al., 2009); cross-scanner aggregation has been successfully used previously (McEvoy et al., 2009; Storandt et al., 2009).

2.8. Psychometric assessment

Participants received 1 of 2 batteries of cognitive measures a few weeks after the clinical assessment. The 4 measures common to the 2 batteries were examined here. Selective Reminding Test free recall (Grober et al., 1988) measures episodic memory; Animal Naming for 1 minute (Goodglass and Kaplan, 1983) assesses semantic memory. Trailmaking A and B (Armitage, 1945) are speeded visuospatial tests; the score for each was the number of connections per second.

2.9. Statistical analyses

Analyses were conducted using SPSS 18.0 with alpha set at 0.05. The distributions of the quantitative variables were assessed for normality using the Kolmogorov-Smirnov one-sample tests. Pearson product-moment correlations among the variables as well as partial correlations controlling for age were computed. Scatter plots were examined and tests for curvilinear relations were conducted using hierarchical regression, after converting quantitative variables to z scores; *APOE*ε4 and ε2 were scored 1 if present, otherwise 0. Additional hierarchical regression analyses were conducted first regressing PIB on Aβ_{42} and tau including a curvilinear component for Aβ_{42} as well as interactions between the 2 CSF measures. The analysis was repeated substituting ptau for tau. Analysis was then conducted entering the organismic variables (APOE and age) prior to the CSF measures. Partial correlations among all the variables including 4 cognitive measures and brain volume in 8 ROIs controlling for age were then examined. Finally, regression analyses examining the linear and quadratic relation between age and each of the 8 brain volume ROIs were conducted.

3. Results

3.1. Distributions of variables

Kolmogrov-Smirnov one-sample tests revealed that the quantitative variables were normally distributed with the exception of PIB, tau, and ptau. Histograms for PIB, A$\beta_{42,}$ tau, and ptau are shown in Fig. 1. The APOE genotypes were as follows: 22, 1%; 23, 9%, 24, 1%, 33, 55%, 34, 28%, 44, 6%.

3.2. Correlations among variables

Table 1 shows the zero-order correlations among the measures above the diagonal. All the variables except *APOE* were significantly correlated with age. Therefore the partial correlations among the variables controlling for age are shown below the diagonal. Only one regional brain volume (hippocampus) and one memory measure (SRT free recall) are included here; the others are reported in Supplemental Tables 1 and 2.

As previously reported (Fagan, 2009; Tolboom, 2009), PIB was strongly correlated with CSF A$\beta_{42,}$ tau, and ptau; the correlations changed very little when controlled for age (Table 1, Fig. 2). In addition to a linear relation between PIB and Aβ_{42}, there was also a significant ($p < .0001$) quadratic component; this was not the case for the relation between PIB and tau (quadratic $p = .53$).

As shown in Table 1, CSF Aβ_{42} and tau were uncorrelated linearly ($r = -.06$), although there was a small qua-

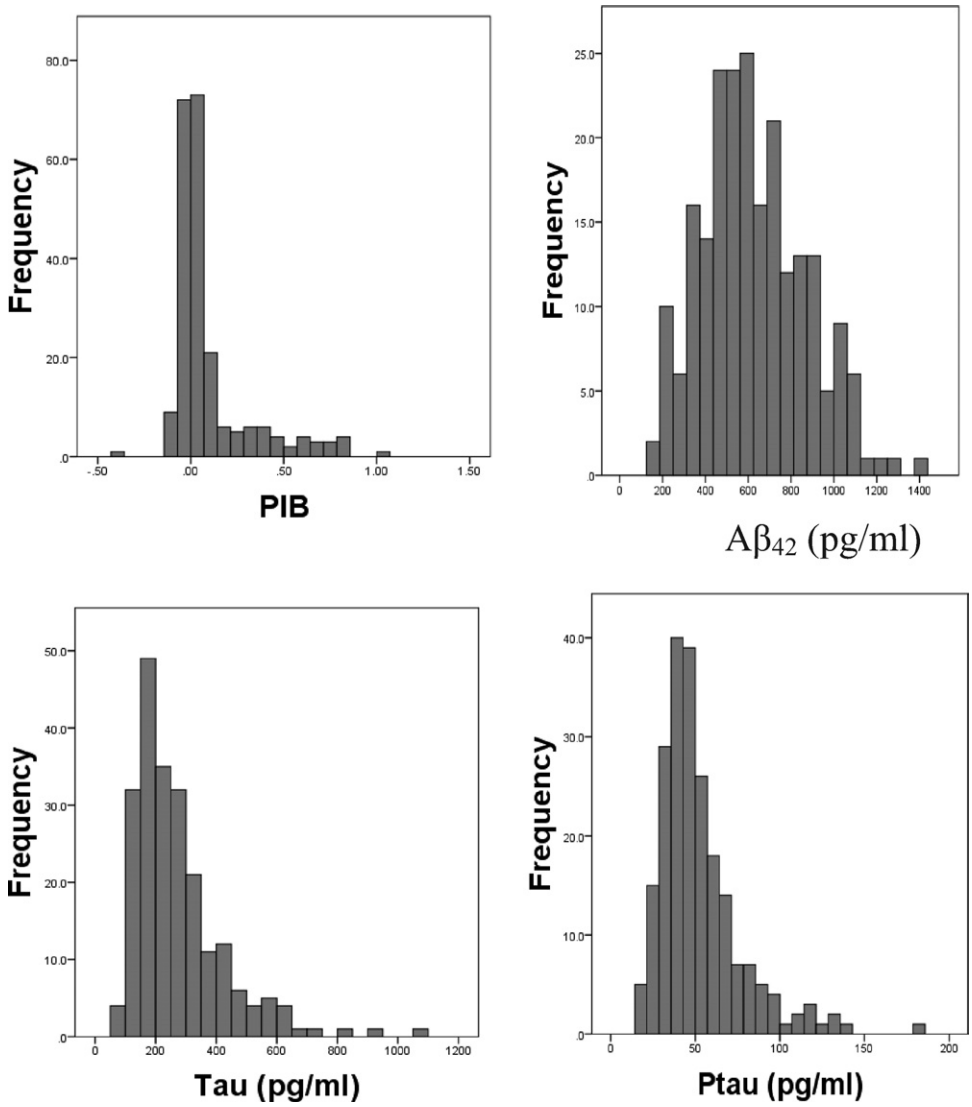

Fig. 1. Histograms showing distributions of PIB and the CSF biomarkers ($A\beta_{42}$, tau, ptau).

dratic relation ($p = .001$, Fig. 2) indicating 4% shared variance between the two variables after controlling for age. Using a break point of 500 pg/mL on $A\beta_{42}$ (Fagan et al., 2009), piecewise regression revealed a significant difference in slopes before and after that point. The partial r controlling for age between $A\beta_{42}$ and tau for those with lower values of $A\beta_{42}$ was -0.20 ($p = 0.10$) compared with $+0.25$ ($p = 0.003$) for those with higher values. Analogous results were obtained substituting ptau for tau ($r = -0.15$ for $A\beta_{42}$ values < 500 pg/mL compared with $r = +0.27$ for $A\beta_{42}$ values > 500 pg/mL).

Given the pattern of observed relations (minimal relation between $A\beta_{42}$ and tau but strong relations of each with PIB) the next analysis determined how much of the total variance in fibrillar amyloid plaque formation was explained by the two biomarkers in combination. A hierarchical regression analysis was conducted with PIB as the dependent variable and the independent variables entered in the following order: $A\beta_{42}$ linear, $A\beta_{42}$ quadratic, tau, $A\beta_{42}$ linear x tau interaction, and $A\beta_{42}$ quadratic x tau interaction. Each of the first four predictors produced a significant increment in the R^2, producing a total R^2 of 0.60 (Table 2). Unique contributions of significant predictors (i.e., beta weights from the model at the fourth step) were as follows: $A\beta_{42}$ linear $= -0.47$, $A\beta_{42}$ quadratic $= 0.20$, tau $= 0.36$, $A\beta_{42}$ linear x tau interaction $= 0.24$. When the analysis was repeated using ptau instead of tau the R^2 was 0.55.

The significant interaction between $A\beta_{42}$ and tau indicates that the magnitude of the relation between tau and PIB depends on the level of $A\beta_{42}$, even though tau and $A\beta_{42}$ are largely independent; this is illustrated in Fig. 3. The simple regression lines relating tau and PIB are plotted at low, medium, and high values of $A\beta_{42}$ as defined by -1 SD (373 pg/mL), mean (610 pg/mL), $+1$ SD (983 pg/mL). The relation between tau and PIB was stronger as $A\beta_{42}$ decreased. Because of the curvilinear nature of the relationship

Table 1
Zero-order (above diagonal) and partial correlations controlling for age (below diagonal)

	PIB	$A\beta_{42}$	Tau	Ptau	*APOEε4*	*APOEε2*	Hippocampus	Memory
Age	0.26	−0.17	0.37	0.37	−0.13	0.12	−0.54	−0.38
PIB		−0.49	0.54	0.44	0.22	−0.17	−0.20	−0.18
$A\beta_{42}$	−0.46		−0.06	0.01	−0.22	0.22	0.07	0.11
Tau	0.51	−0.01		0.85	0.03	−0.01	0.13	−0.24
Ptau	0.41	0.06	0.83		0.03	−0.04	−0.14	−0.24
APOEε4	0.26	−0.24	0.09	0.08		−0.18	−0.01	0.03
APOEε2	−0.20	0.24	−0.04	−0.07	−0.16		.02	−0.07
Hippocampus	−0.09	0.01	0.05	0.02	−0.10	0.07		−0.21
Memory	−0.09	0.05	−0.10	−0.14	−0.02	−0.03	−0.02	
Mean	0.10	626	274	52.4			7654	31.3
Median	0.02	595	235	46.1			7675	32.0
SD	0.22	241	150	24.4			875	6.0

Note: Correlations with an absolute value ≥ .16 are significant at $p < .05$. The memory measure is the Selective Reminding Test. Means, medians, and SDs for quantitative variables are shown in the last Three-rows.

between $A\beta_{42}$ and PIB, the rate of increase in PIB as tau increased was especially dramatic at low values of $A\beta_{42}$.

Both *APOE* variables and age were modestly correlated with PIB and $A\beta_{42}$; only age was correlated with tau (Table 1). Because both *APOE* variables exist from conception and therefore precede the biomarkers, the hierarchical regression analysis was repeated entering them at Steps 1 and 2. Similar logic applies to age in a cross-sectional analysis; it precedes measurement of the biomarkers; therefore, it was entered at Step 3. The Age x ε4 interaction (Morris et al., 2010) was added at Step 4. Order of entry thereafter was as in the previous regression analysis: $A\beta_{42}$ linear, $A\beta_{42}$ qua-

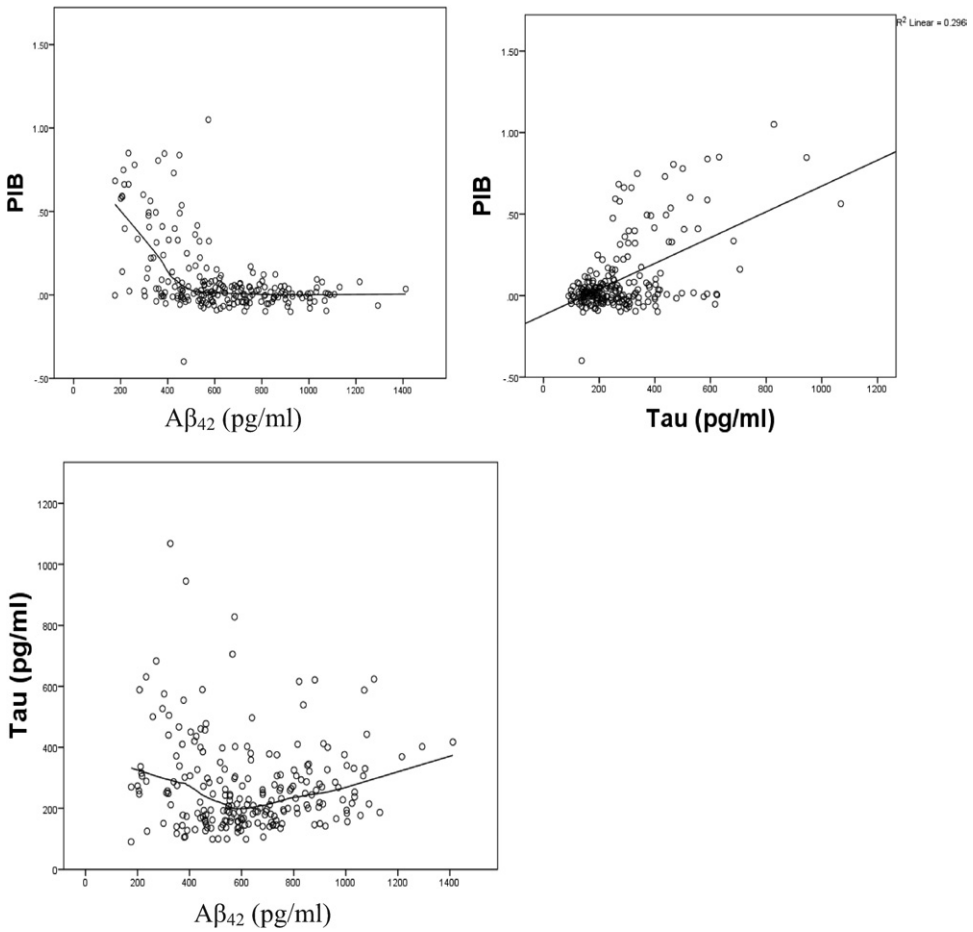

Fig. 2. Scatter plots of relations among PIB, $A\beta_{42,}$ and tau.

Table 2
Hierarchical regression model of PIB on $A\beta_{42}$ (linear and quadratic), tau, and their interactions ($N = 220$)

Step	Predictor added	R^2 increment	F	*d.f.* error	P
1	$A\beta_{42}$ (linear)	0.24	67.48	218	< 0.0001
2	$A\beta_{42}$ (quadratic)	0.11	36.17	217	< 0.0001
3	Tau	0.20	93.29	216	< 0.0001
4	$A\beta_{42}$ (linear) x tau	0.05	27.15	215	< 0.0001
5	$A\beta_{42}$ (quadratic) x tau	0.00	2.29	214	0.13

Note. $R^2 = .60$.

dratic, tau, $A\beta_{42}$ linear x Tau interaction. The $A\beta_{42}$ quadratic x Tau interaction was not included because it was not significant in the previous analysis.

The increment in the R^2 was significant at each step. The R^2 was 0.18 after the first 4 steps, but the beta weights for these 4 variables were no longer significant after inclusion of the remaining terms representing the 2 CSF markers: *APOE* ε4 $\beta = 0.07$, *APOE* ε2 $\beta = -0.05$, age $\beta = 0.02$, Age x ε4 $\beta = 0.10$. These results indicate that the effects of *APOE* and age on plaque formation are *indirect*, operating through their influence on $A\beta_{42}$ and tau. They may have a causal effect on the 2 CSF biomarkers, but they have no additional causal effect on fibular amyloid plaque burden as detected by PIB. The demographic variables of gender and education were not correlated with any of the biomarkers and, therefore, were not included in the model.

The sample was randomly split in half and the model was tested on each half with similar results in terms of the pattern of increments in the R^2 and significant beta weights in the final step. The R^2 was 0.62 for Sample 1 and 0.59 for Sample 2.

As shown in Table 1, the significant zero-order correlations (above the diagonal) of episodic memory, hippocampus volume, tau, and PIB were spurious; they disappeared when controlled for age (below the diagonal). The partial rs between memory and the other brain regions ranged from −0.06 to 0.06. None of the partial rs controlling for age between the other 3 cognitive measures and $A\beta_{42}$, tau, ptau, PIB, or any of the brain regions were significant (all $ps > 0.05$).

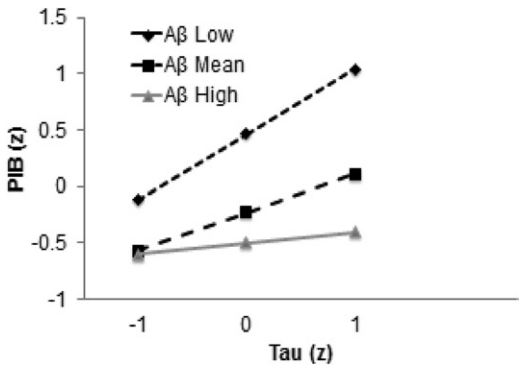

Fig. 3. Interaction between CSF $A\beta_{42}$ and tau as related to amyloid plaque burden (PIB).

Because brain volume was available for only a subset of the sample, its role was examined in separate analyses. Hippocampus volume was associated with PIB ($r = -0.20$), but not with either CSF measure (Table 1) this single significant correlation was spurious, however, in that it resulted from the correlation of each variable with age. As shown below the diagonal in Table 1 their partial r controlling for age was −0.09 ($p = 0.27$). A similar pattern was observed in the other brain regions examined. Although some modest zero-order correlations were significant, partial rs controlling for age were not (range = −0.16 to 0.16). There was a significant curvilinear component to the relation between age and brain region volume (Table 3), which has been reported previously (Raz and Rodrigue, 2006) for the hippocampus and some, but not all, of the other brain regions that were examined.

Figure 4 provides a summary of the relations among the domains examined in this report using the results for the hippocampus and SRT to represent brain structure and cognition.

4. Discussion

The distributions of both fibrillar amyloid plaques and tau were skewed, as would be expected if these 2 biomarkers reflect underlying pathology in some of these cognitively healthy participants. Only a small number of people are in the beginning stage of the disease and therefore have higher values of these 2 indexes. In contrast, the distribution of CSF $A\beta_{42}$ was not skewed. Instead, it was not significantly different from a normal distribution. Normal distributions are more often seen in individual difference variables such as height. Perhaps there are 2 influences on CSF $A\beta_{42}$ level. One may reflect normal human biological variability, whereas the other indicates onset of pathology. The median value of CSF $A\beta_{42}$ was slightly less than the mean (594 vs. 626; index of skewness = 0.40, see Fig. 1). Whether this is due to sampling variability or indicates that CSF $A\beta_{42}$ levels have dropped from prior levels for a small portion of the participants can only be determined by future studies, particularly longitudinal ones.

Table 3
Results of hierarchical regression analyses of regional brain volumes on age linear and quadratic (n = 164)

		Age (linear)			Age (quadratic)	
Region	R^2	F(1,162)	p	R^2 increment	F(1,161)	p
Hippocampus	0.28	62.90	< 0.0001	0.09	22.04	< 0.0001
Temporal	0.25	52.88	< 0.0001	0.07	16.00	< 0.0001
Lateral parietal	0.21	44.11	< 0.0001	0.06	13.10	< 0.0001
Precuneus	0.21	43.42	< 0.0001	0.05	10.79	0.001
Posterior cingulate	0.09	16.03	< 0.0001	0.03	5.34	0.02
Anterior cingulate	0.00	0.08	0.77	0.03	5.07	0.03
Entorhinal	0.00	0.37	0.54	0.05	8.29	0.005
Parahipppocampus	0.01	1.22	0.27	0.02	3.91	0.05

CSF Aβ_{42} and tau were not correlated linearly. There was a very small nonlinear relation between these 2 biomarkers indicating only 4% shared variation. Although we cannot find any report in the literature referring to this correlation in cognitively normal people, we expected it to be negative (low CSF Aβ_{42} associated with high CSF tau) if both CSF markers occurred as described by the amyloid cascade hypothesis. As indicated in the introduction, that hypothesis suggests that Aβ_{42} peptides aggregate to form amyloid plaques which, in turn, lead to synaptic loss and cell death, reflected in elevated CSF tau, thereby causing cognitive impairment.

The model observed in the analyses reported here indicates, as suggested previously (Holtzman et al., 2011; Hyman, 2011; Pimplikar, 2009; Small and Duff, 2008), that there are at least 2 independent processes, one represented by CSFAβ_{42} and the other by CSF tau, related to fibrillar amyloid plaque burden. These 2 biomarkers, and the processes they represent, were associated with an impressive amount of the variance in PIB binding (R^2 = 0.60) in this cognitively normal sample, 17% of whom had MCBP values ≥ 0.18. This value was used previously to describe individuals as PIB positive (Fagan et al., 2009; Mintun et al., 2006) or having fibrillar amyloid plaque burden similar to those with AD. Similarly, Shaw et al. (2009) reported that these 2 biomarkers made independent contributions to the differentiation of autopsy-verified AD cases from normal controls. There may be, however, still more processes contributing to plaque formation or, perhaps more important, to brain atrophy and dementia (Rowe et al., 2010). In this cross-sectional sample fibrillar amyloid plaque formation had only a spurious relation with brain atrophy via their mutual association with age.

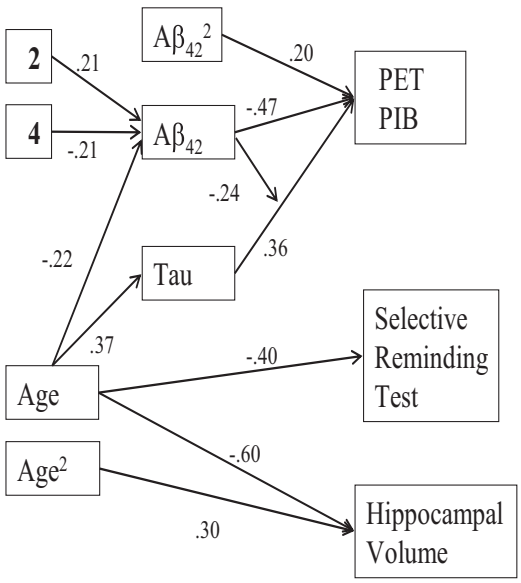

Fig. 4. Model showing relations among all of the variables. The beta weights relating APOE and age to Aβ_{42} are from the simultaneous regression of those 3 variables on Aβ_{42}. The beta weights relating Aβ_{42} and tau to PIB are from the final step of the hierarchical regression analysis reported in Table 2. The beta weights relating age to hippocampus volume are from the second step of the analysis reported on the first line of Table 3.

In addition to their main effects on PIB, there was also an interaction between CSF tau and Aβ_{42}. The correlation between CSF tau and PIB was much stronger when Aβ_{42} was low than when Aβ_{42} was moderate or high. This is analogous to the textbook example of an interaction provided by Cohen et al. (2002) showing a strong negative correlation between age and endurance on a treadmill that is much weaker in people with a history of aerobic exercise.

Multiple previous studies of these 2 CSF biomarkers in cognitively normal samples with a wide age range have alluded to the possibility of their interaction when they examined how the ratio of tau to Aβ_{42} was related to other variables. Long ignored by researchers in many fields, Pearson (1897) pointed out over a century ago that the ratio of 2 variables can produce spurious results. The ratio actually represents a combination of the main effects of each of the variables in the ratio as well as their interaction. The analysis reported here used the appropriate procedure by first entering the main effects of Aβ_{42} and tau followed by their product, representing the interaction, in a subsequent step of a hierarchical regression analysis (Kronmal, 1993). Each CSF biomarker was associated with unique, unrelated portions of the variance in PIB, and their interaction was associated with an additional, unrelated portion. This result,

along with the minimal association between the 2 CSF biomarkers, again suggests the operation of at least 2 independent processes, one associated with lower levels of CSF $A\beta_{42}$ and one associated with increased levels of CSF tau. One does not cause the other, but when both are present the effect is enhanced. A recent study (Desikan et al., 2011) identified an interaction between $A\beta_{42}$ and ptau in the prediction of longitudinal changes in the volume of the entorhinal cortex in healthy controls and individuals with amnestic mild cognitive impairment.

The effects of *APOE* and age on plaque burden were indirect through CSF $A\beta_{42}$ and tau. It should be noted, however, that *APOE* and age together accounted for only 13% of the variance in $A\beta_{42}$ and 14% of tau. There clearly are other important variables to be identified in explaining the processes associated with CSF levels of $A\beta_{42}$ and tau, and their inclusion may change the model.

Brain volumes in the 8 ROIs were uncorrelated with CSF measures or with PIB after controlling for age. As noted in the introduction, previous studies have reported mixed results in efforts to relate PIB to brain structure. There are likely numerous reasons for the differences. One involves the adjustment for age, which has not always been done. Others probably relate to brain regions examined, the measure of structural integrity (e.g., cortical thickness vs. volume), age range of the sample, and inclusion criteria. One study (Chetelat et al., 2010), for example, found no correlation when people with subjective memory complaints were excluded. Similarly, in a study based on neuropathological data (Price et al., 2001) little atrophy was observed in those without a diagnosis of at least very mild symptomatic AD.

The strengths of the current study include the wide age range and large sample size of carefully characterized cognitively normal individuals for whom a number of the biomarkers shown previously to be present prior to clinical diagnosis of AD were obtained in relatively close temporal proximity. A primary limitation is its cross-sectional nature, which allows only modeling of potential causative relations. These relations may be affected if individual biomarkers become abnormal at different times in the AD process. Further, reliability of some of the measures used in the analyses has not been well studied; failure to detect relations may reflect measurement error. A similar limitation is that PIB measures only fibrillar $A\beta$ deposits; the model does not address earlier potential processes such as $A\beta$ oligomerization and aggregation into diffuse plaques. Because the biology of AD undoubtedly is complex and may not be well-represented by the assumptions of our model, any potential causative relations must be confirmed by longitudinal studies. Our findings do, however, highlight the importance of considering multiple contributors to AD pathogenesis as that longitudinal research is pursued.

Disclosure statement

The authors have no actual or potential conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found ,in the online version, at 10.1016/j.neurobiolaging.2011.11.029.

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