

Longitudinal brain activity changes in asymptomatic Alzheimer disease

Kari-Elise T. Codispoti¹, Lori L. Beason-Held², Michael A. Kraut³, Richard J. O'Brien⁴, Gay Rudow¹, Olga Pletnikova¹, Barbara Crain¹, Juan C. Troncoso^{1,4} & Susan M. Resnick²

¹Division of Neuropathology, Department of Pathology, Johns Hopkins University, Maryland

²Laboratory of Behavioral Neuroscience, National Institute on Aging, NIH, Maryland

³Department of Radiology, Johns Hopkins University, Maryland

⁴Department of Neurology, Johns Hopkins University, Maryland

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Correspondence

Dr. Kari-Elise T. Codispoti, Department of Pathology, Division of Neuropathology, Johns Hopkins University, 720 Rutland Avenue, 558 Ross Research Building, Baltimore, MD 21205-2196. Tel: 410 955 5632; Fax: 410 955 9777; E-mail: kcodisp1@jhmi.edu

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Abstract

Asymptomatic Alzheimer disease (ASYMAD) is characterized by normal cognition despite substantial AD pathology. To identify factors contributing to cognitive resilience, we compared early changes in regional cerebral blood flow (rCBF) in individuals subsequently diagnosed as ASYMAD with changes in cognitively impaired (CI) and normal older participants from the Baltimore Longitudinal Study of Aging. Participants underwent annual positron emission tomography (PET) rCBF measurements beginning 10.0 (SD 3.6) years before death and while cognitively intact. Based on clinical and autopsy information, subjects were grouped as cognitively normal (CN = 7), ASYMAD ($n = 6$), and CI ($n = 6$). Autopsy material was analyzed using CERAD and Braak scores and quantitative stereologic measures of tau and amyloid. ASYMAD and CI groups had similar CERAD and Braak scores, similar amounts of β -amyloid and tau in middle frontal (MFG), middle temporal (MTG), and inferior parietal (IP) regions, and more β -amyloid than CN in precuneus, MFG, and IP areas. Voxel-based PET analysis identified similarities and differences in longitudinal rCBF change among groups across a 7.2-year interval. Both ASYMAD and CI groups showed similar longitudinal rCBF declines in precuneus, lingual, and MTG regions relative to CN. The CI also showed greater rCBF decreases in anterior and posterior cingulate, cuneus, and brainstem regions relative to ASYMAD and CN, whereas ASYMAD showed greater relative rCBF increases over time in medial temporal and thalamic regions relative to CI and CN. Our findings provide evidence of early functional alterations that may contribute to cognitive resilience in those who accumulate AD pathology but maintain normal cognition.

Introduction

Alzheimer disease (AD) is a neurodegenerative disorder clinically characterized by progressive dementia. The cardinal pathologic features include accumulations of both extracellular $A\beta$ peptide in neuritic plaques (NPs) and intracellular hyperphosphorylated tau in neurofibrillary tangles (NFTs). These two lesions form the basis of current diagnostic criteria of AD (CERAD, Braak), yet both have been demonstrated in cognitively normal (CN) elderly adults (Tomlinson et al.

1968; Crystal et al. 1988; Katzman et al. 1988; Braak and Braak 1991; Mirra et al. 1991; Mochizuki et al. 1996; Troncoso et al. 1996; Price and Morris 1999; Knopman et al. 2003).

This state, which we have termed asymptomatic AD (ASYMAD) (Riudavets et al. 2007; Iacono et al. 2008), and others have called preclinical AD (Schmitt et al. 2000), or high-pathology controls (Benzing et al. 1993), suggests that some individuals are resistant to the effects of AD pathology. Recent investigations of individuals with ASYMAD have begun to reveal possible underlying mechanisms responsible for this

Table 1. Subject characteristics (mean [SD]).

Group	All subjects	CN	ASYMAD	CI
<i>n</i>	19	7	6	6
Sex (M/F)	15/4	7/0	4/2	4/2
Age @ baseline PET	76.0 (7.1)	78.4 (7.6)	75.9 (8.0)	73.2 (5.4)
Age @ death	85.9 (5.3)	87.3 (5.0)	85.5 (6.7)	84.8 (4.8)
First to last PET (years)	7.2 (2.1)	6.3 (3.0)	7.3 (1.3)	8.1 (0.8)
Last PET to death (years)	2.8 (2.1)	2.7 (2.2)	2.3 (2.1)	3.2 (2.3)
APOE 4 positive	4	0	2	2
Hypertension	9	2	3	4
Smokers	2	1	0	1
Diabetes	5	1	2	2
Education	16.4 (3.2)	16.1 (4.1)	17.2 (2.9)	16.0 (2.8)

Mean (SD) values are shown. There were no significant differences among groups regarding age at baseline, age at death, time interval between first and last PET, time interval between last PET scan and death, or other health-related measures examined.

resilience. For example, ASYMAD subjects have neuronal hypertrophy in the CA1 region of the hippocampus and anterior cingulate cortex (Riudavets *et al.* 2007; Iacono *et al.* 2009a). Neuronal nuclear and nucleolar hypertrophy has also been demonstrated in the hippocampus, anterior and posterior cingulate cortices, and primary visual cortex of these individuals (Iacono *et al.* 2008).

The current study investigates differences in longitudinal changes in brain activity among groups of individuals who eventually follow divergent clinical and pathological trajectories. This study is unique in that cerebral blood flow (CBF) positron emission tomography (^{15}O -PET) scans were obtained on average 10.0 (SD 3.6) years prior to death when all participants were CN. Thus, we are able to assess antemortem brain changes in individuals who have AD pathology years later at autopsy yet maintained normal cognition (ASYMAD) and compare them to those with AD pathology and cognitive impairment (CI) as well as those who remained CN and histologically normal.

By examining changes in brain activity demonstrated by the PET imaging, we can assess both similarities and differences in premorbid brain function across the groups. Here, we assess similar changes over time in both ASYMAD and CI groups that may reflect the effects of similar levels of developing neuropathology. We also identify differences distinctive to the ASYMAD group that may help maintain cognitive ability in the face of accumulating neuropathology, and we describe changes distinctive to CI group that likely contribute to CI over time in this group.

Materials and Methods

Subjects

In this study we used PET data from 19 older participants in the neuroimaging substudy (Resnick *et al.* 2000) of the

BLSA who underwent postmortem evaluation of the brain (four female, 15 male). Approximately half the BLSA neuroimaging substudy participants have agreed to autopsy, a rate that is similar to that in the BLSA as a whole. These groups have similar ages, male/female distribution, years of education, and number of APOE e4 alleles (data not shown). The mean (SD) age at PET baseline was 76.0 (SD 7.1) years and age at death was 85.9 (SD 5.3) years. Subjects had to have at least two PET scans, although the majority had more than seven scans ($n = 15$). At autopsy, subjects were determined to meet pathologic criteria for one of the three study groups and individuals were not included if they had evidence of a non-Alzheimer neurodegenerative disorder (e.g., non-AD tauopathy, Parkinson disease, or vascular dementia). All individuals remained in good physical and cognitive health during the period of PET data collection with no history of central nervous system disorders, major psychiatric disorders including depression, or severe cardiovascular disease (Table 1).

This study was approved by the local Institutional Review Boards. All participants provided written informed consent prior to each assessment.

Study design

This study examines serial CBF measurements starting many years prior to death. Participants underwent PET scanning sessions at baseline and annually for up to eight follow-up visits (mean interval 7.2 years). Participants died on average 2.8 years after the last PET scan included in this study and underwent autopsy at that time. The study groups were determined based on the combination of antemortem clinical diagnosis and autopsy findings (see below). The imaging analyses were subsequently performed comparing these three groups.

Cognitive assessment

All participants were followed annually and were reviewed at a consensus conference if their Blessed Information Memory Concentration score was ≥ 4 , or if their informant or subject Clinical Dementia Rating (CDR) score was ≥ 0.5 . Dementia diagnosis was determined according to Diagnostic and Statistical Manual of Mental Disorders, 3rd Ed., Revised (DSM-III-R) criteria. Mild CI (MCI) diagnosis was based on the Petersen (Petersen 2004) criteria.

A battery of neuropsychological tests was administered annually and performance levels were used to determine clinical diagnosis (see Kawas *et al.* 2000 for detailed description). These tests included the CDR scale, Blessed Mental Status (BMS) test, Mini-Mental State Examination, Buschke Immediate and Delayed Cued Recall, Boston Naming Test, Controlled Verbal Fluency, Trail Making Tests A and B, Clock Drawing, and the Center for Epidemiologic Studies Depression Scale.

The annual rate of change in cognitive performance on each test was calculated using linear mixed models. Overall differences in baseline (year 1) and annual rates of change were compared across all groups, followed by pairwise group comparisons.

Neuropathologic assessment

At autopsy, the right hemibrain was coronally sliced and frozen, and the whole left hemibrain was fixed in 10% buffered formaldehyde for at least 2 weeks and subsequently sectioned in the coronal plane. Routine diagnostic sections were obtained from the middle frontal gyrus (MFG), the superior and middle temporal gyri (SMTG), the inferior parietal (IP) lobule, the primary visual cortex, the anterior cingulate, the amygdala, the hippocampus and entorhinal cortex, basal ganglia and basal forebrain, the thalamus, midbrain including the substantia nigra, pons, medulla, spinal cord, and cerebellum. Tissues were processed, embedded in paraffin, cut at 10 μm , and stained with hematoxylin and eosin and with silver Hirano method (Yamamoto and Hirano 1986). Lewy body (LB) pathology was assessed in the brain stem and anterior cingulate cortex with alpha-synuclein immunohistochemistry (Synuclein 1 Transduction Laboratories, Palo Alto, CA, USA; dilution, 1:500).

Silver stained sections were used in the standard assessment of AD pathology according to CERAD guidelines (Mirra *et al.* 1993). NP density was determined in the MFG, SMTG, and IP lobule and a CERAD age-related plaque score was assigned: 0 = none, A = sparse, B = moderate, C = frequent (Mirra *et al.* 1993). In combination with the clinical data, a pathological diagnosis of normal with respect to AD, possible AD, probable AD, or definite AD was rendered according to CERAD guidelines. As an additional approach to assessing the severity of neurodegeneration, the distribution of NFTs was assessed

and graded on a scale of 0–VI according to Braak (Braak and Braak 1991).

As an adjunct to the standard means of assessing and diagnosing AD, stereologic analysis was performed using 10 μm sections of the MFG, middle temporal gyrus (MTG), IP, and precuneus (PreCu) were stained with an antibody for A β -amyloid (6E10, Covance, Emeryville, CA, USA, dilution 1:500) and phosphorylated tau (PHF-1 antibody; gift of Dr. P. Davies, dilution, 1:100). Stereological measurements were performed using a Zeiss light microscope equipped with a 100 \times , NA 1.30, oil Plan neofluor $\infty/0.10$ objective, and interfaced with a Stereo-Investigator system (MBF bioscience, Williston, VT, USA). The fractional area of immunoreactivity was measured utilizing the area fraction fractionator probe. The gray matter was outlined and an array of 100 points (150 $\mu\text{m} \times 150 \mu\text{m}$) was placed in a systematic random position spaced at 800 μm (MFG 6E10, MFG PHF-1, PreCu 6E10, PreCu PHF-1, IP PHF-1), 900 μm (MTG 6E10), or 1000 μm (MTG PHF-1) intervals. The area fraction was calculated as the number of points falling over amyloid (plaques) or tau (NFTs, NP, or neurites) immunoreactivity divided by the total number of points. Data from the three groups were compared using the nonparametric exact Kruskal–Wallis test. Pairwise comparisons used the exact Wilcoxon two-sample test to determine the relationship between each group.

PET scanning conditions

Participants underwent PET scanning sessions at baseline (year 1) and annually for up to eight follow-up visits (mean interval 7.2 years). During each imaging session, a resting state PET scan was performed. During rest, participants were instructed to keep their eyes open and focused on a computer screen covered by a black cloth. For the analyses, scans were censored at the time of clinical diagnosis of dementia onset, documented transient ischemic attack/cerebral infarction, or development of seizures.

PET scanning parameters

PET measures of regional CBF (rCBF) were obtained using [^{15}O] water. For each scan, 75 mCi of [^{15}O] water were injected as a bolus. Scans were performed on a GE 4096+ scanner, which provides 15 slices of 6.5-mm thickness. Images were acquired for 60 sec from the time the total radioactivity counts in brain reached threshold level. Attenuation correction was performed using a transmission scan acquired prior to the emission scans.

PET data analysis

For each subject, the PET scans were realigned, resliced to a voxel size of $2 \times 2 \times 2$ mm, spatially normalized into standard stereotactic, and smoothed to a full width at half maximum of 12, 12, and 12 mm in the x , y , and z planes. To control for variability in global flow, rCBF values at each voxel were ratio

Table 2. Cognitively impaired subjects.

Subject	Clinical diagnosis	CERAD diagnosis	No. of PET scans included	Year of last PET	Year of diagnosis	Year of death	APOE
1	Probable AD	Probable AD	8	2001	2002	2002	3/3
2	Probable AD	Probable AD	9	2002	2007	2008	3/4
3	Possible AD	Probable AD	8	2001	2004	2004	3/4
4	Possible AD + vascular dementia	Probable AD	8	2001	2002	2004	3/3
5	Dementia ¹	Probable AD	8	2001	2004	2007	3/3
6	Amnesic MCI	Possible AD ²	9	2003	2007	2008	2/3

The last PET included in analysis was prior to the diagnosis of dementia.

¹This individual exhibited a pattern of cognitive decline consistent with AD, but had one episode of hallucinatory events.

²By CERAD criteria, a subject with an age-related plaque score of B must have clinically diagnosed dementia to be considered probable AD. A clinical diagnosis of MCI, although included in our cognitively impaired subject group, is only considered possible AD using CERAD criteria.

adjusted and scaled to a mean global flow of 50 mL/100 g/min for each image. The image data were analyzed using Statistical Parametric Mapping (SPM5; Wellcome Department of Cognitive Neurology, London, England), where whole brain voxel by voxel comparisons determined significant similarities and differences in longitudinal rCBF change between the groups. Group \times time linear comparisons were performed to assess (1) similar changes over time in both ASYMAD and CI groups relative to CN, (2) changes in the ASYMAD group relative to CI and CN, and (3) changes in the CI group relative to ASYMAD and CN groups. All contrasts were adjusted for sex and baseline age at year 1. Significant effects for each contrast were based on the magnitude ($P \leq 0.005$) and spatial extent (> 50 voxels) of activity. To examine the direction and patterns of change in significant regions, rCBF values were extracted from 6 mm spherical regions centered on the local maxima of each region. The rCBF values were then used to calculate differences in baseline (year 1) levels between the groups, and to calculate and compare mean trajectories of change over time for each group using linear mixed models.

Results

Clinical diagnosis

All participants remained cognitively intact during the period in which PET scans were performed. Thirteen participants re-

mained CN by consensus criteria (Kawas *et al.* 2000) through the last available cognitive evaluation (last evaluation on average was 14.8 months prior to death, range 3–30 months). The remaining six individuals developed some degree of CI (last evaluation on average 2.8 months prior to death, range 1–5 months) by consensus criteria. One declined to amnesic MCI (Petersen 2004), one to possible AD ($n = 1$), two to probable AD, one to dementia of undetermined etiology that was consistent with probable AD on pathology ($n = 1$), and one to mixed vascular dementia and AD ($n = 1$) (Table 2).

Autopsy diagnostic evaluation

All brains from subjects with CI had NP CERAD age-related plaque scores of B and NFT Braak scores of III ($n = 2$), IV ($n = 3$), and V ($n = 1$). Among the 13 subjects that remained CN, six had substantial AD pathology, that is, NP age-related scores of B and NFT Braak scores of II ($n = 1$), III ($n = 2$), or IV ($n = 3$). The remaining seven CN subjects had NP CERAD age-adjusted plaque scores of 0 ($n = 6$) or A ($n = 1$), and NFT Braak scores of II ($n = 3$), III ($n = 1$), or IV ($n = 3$) (Table 3; Braak and Braak 1991; Mirra *et al.* 1991).

Ten (4/6 CI, 1/6 ASYMAD, 5/7 CN) participants had evidence of remote microinfarcts or lacunes at the time of autopsy. The majority of these were located in the basal ganglia

Table 3. CERAD and Braak scores.

Group	CERAD age-adjusted plaque score				Braak neurofibrillary tangle score					
	0	A	B	C	I	II	III	IV	V	VI
CN	6	1	0	0	0	3	1	3	0	0
ASYMAD	0	0	6	0	0	1	2	3	0	0
CI	0	0	6	0	0	0	1	3	2	0

Number of individuals per group with each plaque or NFT score. The CERAD scores represent: 0 = no neuritic plaques A = sparse neuritic plaques, B = moderate neuritic plaques, C = frequent neuritic plaques. Braak score is determined as per Braak and Braak (1991). Low Braak stages (I–II) have NFTs in the entorhinal cortex (I) and hippocampus (II), mid Braak stages (III–IV) have NFTs extending to the neocortical association areas, and high Braak stages (IV–V) have NFTs extending to the parastriate (V) and striate (VI) cortices.

or cerebellum. One subject in the CI group had an acute infarct in the distribution of the right middle cerebral artery. A number of other pathologies were found in subjects in the CN, each occurring in one subject. These included: LB pathology limited to rare LB in the substantia nigra, the amygdala, and temporal cortex corresponding to a brainstem distribution of α -synuclein pathology (McKeith et al. 2005); inactive demyelinating lesions consistent with multiple sclerosis, which were incidentally discovered; focal perivascular cuffing with mononuclear cells in a few vessels in the basal ganglia; and focal areas of tau positive astrocytes in the amygdala and substantia nigra. In all cases, NP and NFT evaluations and quantitative stereology were performed in tissue sections not affected by these non-AD-related findings.

Definition of groups according to clinical/cognitive-pathological correlations

Based on the clinical and neuropathologic diagnoses, we divided the 19 participants into three groups: CN ($n = 7$, 7 males, 0 females), asymptomatic Alzheimer's disease (ASYMAD; $n = 6$, 4 males, 2 females), or cognitively impaired (CI; $n = 6$, 4 males, 2 females). CN were CN individuals by clinical consensus criteria (Kawas et al. 2000) and also had none to sparse numbers of NP (CERAD age-related plaque score 0 or A), Braak scores ranging from II to IV, and received a neuropathologic diagnosis of normal with respect to AD by CERAD criteria (Mirra et al. 1991). ASYMAD were CN by consensus criteria and had moderate numbers of NP (CERAD age-related plaque score B), Braak scores ranging from II to IV, and received a neuropathologic diagnosis of possible AD by CERAD criteria. CI showed variable degrees of CI, which included the clinical diagnoses of amnesic MCI ($n = 1$) (Petersen 2004), possible AD ($n = 1$), probable AD ($n = 2$), demented ($n = 1$), and mixed vascular dementia and AD ($n = 1$) at the time of death. All individuals in the CI group had moderate NP (CERAD score B), Braak scores ranging from II to V, and received a neuropathologic diagnosis of possible ($N = 1$, subject with MCI) or probable AD ($n = 6$) by CERAD criteria.

There were no significant differences in the number of years of education (CI 16.0 SD 2.4, ASYMAD 17.7 SD 2.9, and CN 16.1 SD 4.1) or baseline Primary Mental Ability Test (PMA) Vocabulary Test score (CI 33.7 SD 11.0, ASYMAD 39.9 SD 2.9, and CN 38.6 SD 7.0) among the three groups. On the tests used to determine clinical diagnosis, there were no baseline (year 1) differences between the groups. When examining the annual rates of change in performance, the BMS ($P = 0.006$), Boston Naming ($P = 0.03$), and Trails B ($P = 0.006$) tests showed overall group differences. Follow-up comparisons revealed that the CI group showed greater decline than the CN group on the Boston Naming task ($P = 0.01$), and greater decline than both CN and ASYMAD

groups on the Trails B test (P 's < 0.03). The ASYMAD group, although still considered CN, showed greater decline than the CI group on the BMS exam ($P = 0.002$). The last available CDR scale scores were obtained on average 11 months (range 1–30 months) prior to death and were significantly greater in the CI group than the ASYMAD ($P < 0.005$) and CN groups ($P < 0.05$).

There were no significant differences between the groups in the number of individuals who were positive for the APOE e4 allele, as both CI and ASYMAD groups had two individuals with an APOE genotype of 3/4. There were also no differences in the number of individuals with history of hypertension, smoking, or diabetes during the study.

Table 3 shows the semiquantitative neuropathologic assessment of CERAD and Braak scores. The CI and ASYMAD groups had identical CERAD NP scores (all subjects CERAD age-related plaque score B). The CN group had less NP pathology than both the ASYMAD and CN groups. The majority of CN subjects ($n = 6$) had no NP, while one subject had sparse NP (CERAD age related plaque score A). There was no difference in Braak scores among the three groups.

Stereology

Two subjects in the CN group did not have adequate material to perform quantitative stereology on the MTG. For all other areas (MFG, IP, precuneus) material was available from all 19 subjects.

The CI and ASYMAD groups showed no significant difference in amyloid (both neuritic and diffuse plaques) in the MFG and IP. CI had greater β -amyloid in the PreCu and MTG ($P < 0.05$) than both CN and ASYMAD. ASYMAD and CI had more β -amyloid than CN in MFG, PreCu, and IP ($P \leq 0.05$). Additionally, CI had greater β -amyloid than CN in the MTG ($P \leq 0.05$). Table 4 shows the mean area fraction of β -amyloid immunoreactivity in the MFG, MTG, IP, and PreCu in the three groups.

There was no significant difference between CI and ASYMAD or between ASYMAD and CN in the mean area fraction of tau in any of the four regions. CI had significantly greater tau than CN in all four regions ($P < 0.05$). ASYMAD and CN did not show significant differences in the amount of tau, yet the ASYMAD group showed a trend toward greater tau in the MFG as compared with CN ($P = 0.07$). Table 4 shows the mean area fraction of tau immunoreactivity in the MFG, MTG, IP, and PreCu in the three groups.

PET imaging

In terms of longitudinal change, some regions showed similar declines in rCBF over time in both ASYMAD and CI groups relative to CN. These declines were observed in bilateral precuneus (Brodmann Area 7) [stereotactic coordinate: $-6 -48 42$], lingual gyrus (BA 18) [$0 -60 4$], and superior aspects of

Table 4. Fractional areas of β -amyloid and tau.

Group	Mean (SD) fractional area tau				Mean (SD) fractional area amyloid			
	MFG	MTG	PreCu	IP	MFG	MTG	PreCu	IP
CN	0 (0)	0.0017 (0.0041)	0 (0)	0.0043 (0.011)	0.73 (1.9)	0.52 (1.3)	0.40 (1.1)	0.45 (1.2)
ASYMAD	0.74 (1.8)	0.050 (0.087)	0.0067 (0.016)	0.033 (0.072)	2.6 (1.3)	1.5 (0.18)	0.91 (0.75)	1.1 (1.3)
CI	0.23 (0.33)	0.33 (0.29)	0.018 (0.026)	0.29 (0.33)	4.0 (1.3)	3.1 (1.3)	2.4 (1.4)	2.3 (1.0)

All values have been divided by a factor of 10^2 . There are no significant differences ($P \geq 0.05$) in the mean area fractions of amyloid between ASYMAD and CI in the MFG, MTG, and IP.

There are no significant differences ($P \geq 0.05$) in the mean area fractions of tau between ASYMAD and CI in any of the four regions.

CI has significantly more tau and amyloid ($P \leq 0.05$) than CN in all four regions.

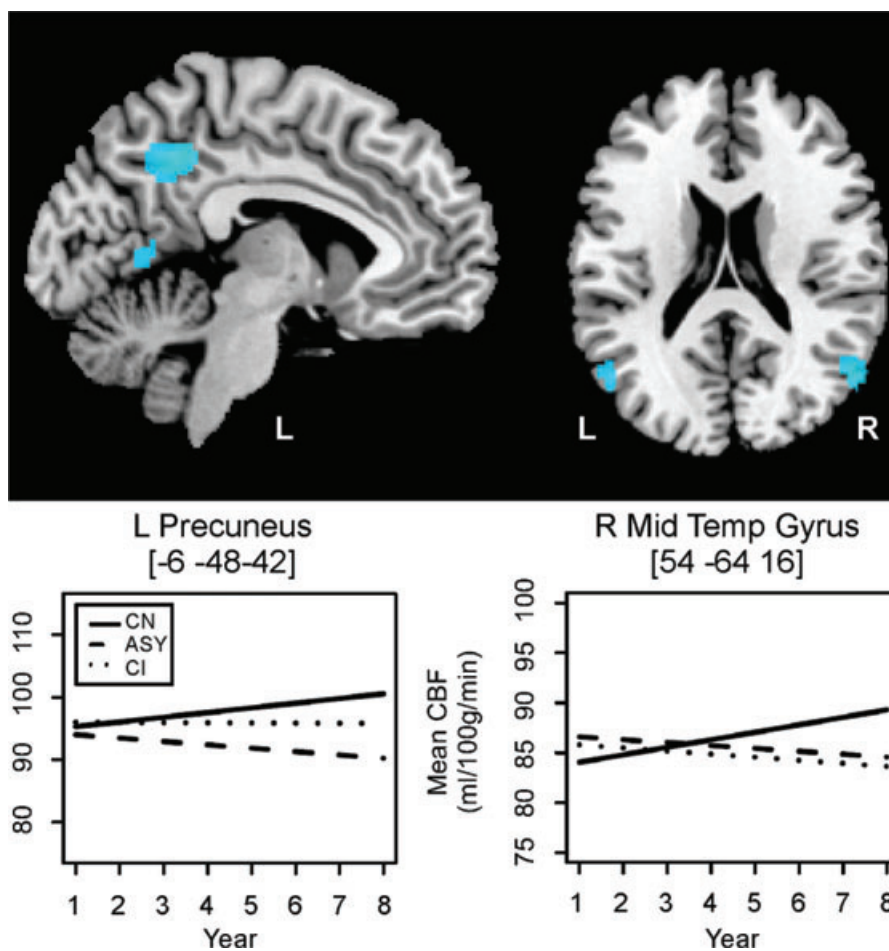


Figure 1. Common areas of rCBF decline in ASYMAD and CI groups. Regions that show similar rCBF decline over time in ASYMAD and CI groups. Precuneus, lingual gyrus, and bilateral middle temporal regions bordering on inferior parietal cortex are shown. Trajectories of CBF change over time are shown for precuneus and middle temporal regions. All regions show a rate of decline that is significantly greater than CN ($P_s \leq 0.001$).

the MTG bordering on IP cortex (BA 39) [54 –64 16; –50 –68 –22] (Fig. 1).

The analyses also showed significant differences in rCBF change among the ASYMAD, CI, and CN groups (Fig. 2).

These differences are described in terms of the direction and pattern of rCBF change among groups. In ASYMAD, several regions showed increases in CBF over time relative to both the CI and CN groups. These regions included the right anterior

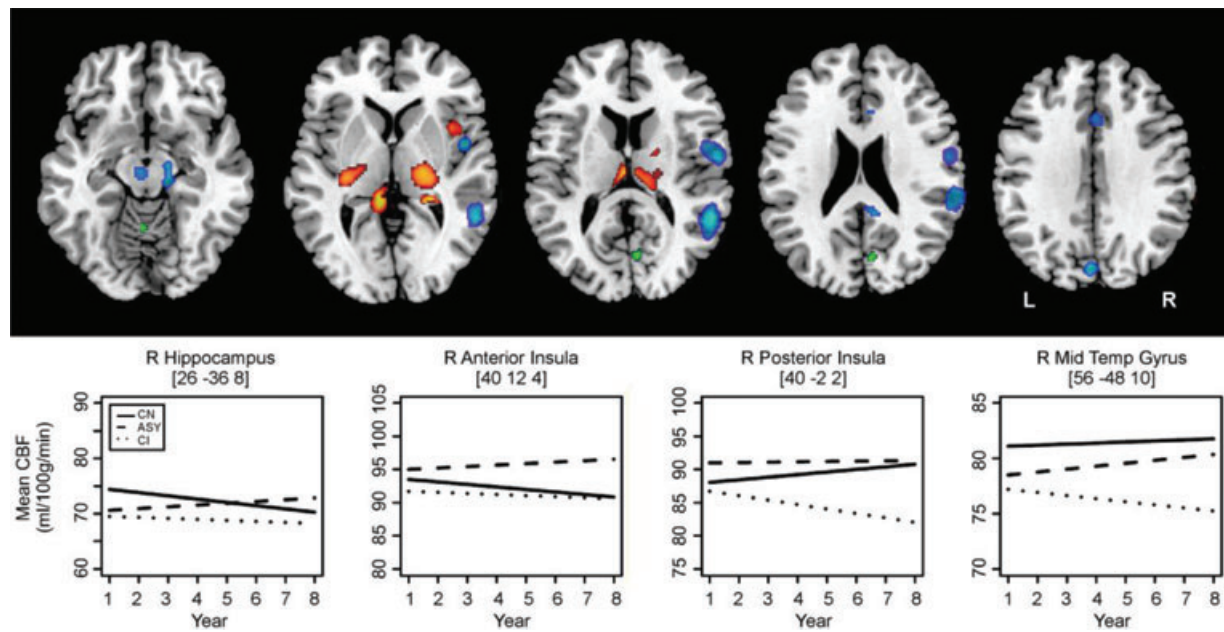


Figure 2. rCBF changes distinctive to ASYMAD and CI groups. Areas where ASYMAD and CI show longitudinal changes in rCBF. Regions in red illustrate areas that increase rCBF over time in ASYMAD relative to CI and CN groups. Regions in blue illustrate regions that decrease rCBF over time in CI relative to ASYMAD and CN groups. Cerebellar and cuneus regions in green are areas that show increased rCBF over time in CN but not CI. Trajectories of CBF change are shown for hippocampal and anterior insular regions, where ASYMADs show increased CBF over time; trajectories of change in posterior insula and middle temporal regions show decreased CBF in the CI group.

insula [40 12 4], right hippocampus and parahippocampal gyrus (Brodmann Area 30) [26 –36 8], and bilateral thalamic regions [20 –18 2; –30 –22 2]. Longitudinal rCBF in the left parahippocampal gyrus (BA 30) [–8 –36 4] was also higher in the ASYMAD group over time, but this was due to stability of CBF in this group in conjunction with a decline over time in the CN group.

The CI group showed greater rCBF declines than ASYMAD and CN in several regions. These included the right anterior cingulate (BA 32) [6 18 28], right posterior cingulate (BA 23) [10 –42 24], right posterior insula [60 –6 16], left cuneus (BA 18) [–2 –80 34], and bilateral brainstem [–2 –18 –8; 14 –26 –14] areas. There was also an area in the right MTG (BA 21) [56 –48 10] that showed both a decrease in CI and an increase in ASYMAD and CN. The CI group also showed effective declines over time in the right cuneus (BA 18) [6 –72 16] and left cerebellum [–2 –56 –6] that were reflected as a failure to increase rCBF over time as observed in the CN group. Because these are relative changes in CBF, the apparent CBF increases in ASYMAD and CN groups could actually reflect stability of CBF change over time in these latter two instances. The differences between CI and other groups, however, still result from greater decline in CBF in the CI group.

Within the regions of longitudinal change, there were no significant differences in baseline (year 1) CBF with one ex-

ception. The anterior cingulate region (BA 32), which showed greater longitudinal decline in the CI group relative to the CN and ASYMAD groups, had baseline CBF levels that were significantly different across groups ($P = 0.04$). This effect was driven by higher initial baseline levels in the CI group than the ASYMAD group ($P = 0.01$).

Discussion

In this study, we compared longitudinal changes in rCBF in BLSA participants classified as CN, ASYMAD, and CI based on clinical data and neuropathological findings at autopsy. Across the groups, we observed significant differences in brain activity over time measured many years before death and while all participants were CN. The ASYMAD and CI groups differed from CN in several areas, suggesting that some regions show similar functional loss due to neuropathologic changes in the brain. Changes distinctive to either ASYMAD or CI groups were also noted. Because these differential patterns of CBF were identified years prior to the development of CI and death, these functional changes may be related to the difference in subsequent cognitive ability between these groups.

The CI and ASYMAD groups exhibited similar amounts of neuropathology at autopsy. The CI and ASYMAD groups not only had identical scores for NPs, which is based on

the current criteria used in the neuropathologic diagnosis of AD, but they also had similar cortical burdens of pathology by quantitative assessments of β -amyloid. Additionally, Braak scores were not significantly different between these groups, indicating a comparable distribution of NFTs, which was strengthened by the demonstration of similar quantitative assessments of cortical tau (NFTs and threads) in the ASYMAD and CI groups.

Based on these results, it could be hypothesized that the CI and ASYMAD groups would show similar differences in brain function when compared to pathologically normal individuals. Indeed, both of the groups with AD pathology showed similar longitudinal declines in rCBF in the precuneus, lingual gyrus, and middle temporal regions. As the precuneus and middle temporal regions demonstrated similar mean area fractions of amyloid and tau in both CI and ASYMAD groups, these results suggest that premorbid function in these regions may decline with the accumulation of the neuropathology over time. However, the functional decline in these regions is likely not the primary contributor to the subsequent differences in cognitive ability, as the declines occur in individuals who maintain cognitive ability as well as those who develop impairments.

The ASYMAD and CI groups also demonstrated distinctive changes in brain function. The ASYMAD group showed longitudinal increases in rCBF in the anterior insula, hippocampus and parahippocampal gyrus relative to both CI and CN groups. These functional differences occurred even though all three groups had similar Braak scores, indicating similar amounts of tau in the medial temporal lobe. The fact that ASYMAD, CI, and CN groups have tau in the medial temporal lobe argues against increased rCBF in response to the presence neuropathology alone. Instead, previous evidence of neuronal plasticity from other cases from the BLSA may support these functional differences. It has been shown that hippocampal neurons exhibit hypertrophy of the cell body, nucleus, and nucleolus in ASYMAD that is not observed in individuals with MCI or AD (Riudavets *et al.* 2007; Iacono *et al.* 2008; Iacono *et al.* 2009a); a finding that has been replicated in cases from the Nun Study (Riudavets *et al.* 2007; Iacono *et al.* 2008; Iacono *et al.* 2009a). Furthermore, in the neurons of ASYMAD there is increased expression of cyclins (M. Riudavets, unpubl. ms.) and of mRNA for multiple synaptic proteins (Iacono *et al.* 2009b). Together, these cellular and brain activity changes suggest the possibility of compensatory processes in ASYMAD subjects that may contribute to cognitive resilience in the face of substantial AD pathology.

The CI group, conversely, showed decreased rCBF in several regions relative to ASYMAD and CN. These included rCBF declines in the anterior and posterior cingulate, the cuneus, and the brainstem. Previous studies lend support to these findings, in that these regions show early metabolic

decreases in AD (Mosconi 2005), and rCBF in the posterior cingulate cortex correlates with Braak NFT scores (Bradley *et al.* 2002). In terms of function, the cingulate regions are thought to participate in higher order cognitive functions (Binder *et al.* 2009; Medford and Critchley 2010) and have also been implicated in the default mode network of resting state brain activity (Shulman *et al.* 1997; Buckner and Vincent 2007), the disruption of which may impact cognitive ability in the aging brain (Lustig *et al.* 2003; Grady *et al.* 2006). Together, these declines in brain activity may be related to the decline in cognitive function that ultimately occurred in the impaired group.

The differential patterns of rCBF between the ASYMAD and CI groups are intriguing since they occurred in groups with similar pathologic features but divergent clinical outcomes. However, due to the small size of the study group, it is possible that we were not able to detect a difference in the amount of tau or amyloid between the CI and ASYMAD subjects. Nevertheless, our observations of differences in rCBF for ASYMAD compared with CN and CI, in conjunction with differences in neuronal size and protein and gene expression, support the concept of a process unique to ASYMAD subjects, which allows them to resist the potentially deleterious effects of accumulating pathology. Had the ASYMAD individuals survived longer, however, it is possible that cognitive decline eventually would have occurred once the pathologic burden reached a critical threshold or when the compensatory events were no longer sufficient. While some investigators argue that variability in cognitive reserve may account for resistance to pathology in ASYMAD (Stern 2009), our groups did not significantly differ with respect to years of education or PMA Vocabulary, both proxies for cognitive reserve.

Although this study has a limited number of subjects, it is distinctive in that subject groups were based on both clinical and pathologic features. As the majority of studies evaluating brain activity classify individuals clinically, there are only limited studies evaluating rCBF in subjects with pathologically confirmed diagnoses (Jobst *et al.* 1992; Bonte *et al.* 1993; Jobst *et al.* 1997; Jagust *et al.* 2001; Bradley *et al.* 2002), and these primarily focused on the utility of rCBF in diagnosis of AD. Our study also offered the unique opportunity to evaluate longitudinal differences in rCBF not only in normal and impaired subjects, but in the group of individuals with Alzheimer pathology who retain normal cognition, that is, ASYMAD individuals, and appear to be resistant to the deleterious effects of AD pathology.

Due to the small sample size of our study, the findings are preliminary and a larger cohort is needed to confirm and extend these results and perhaps uncover additional variations among the groups. However, compared to the full BLSA autopsy cohort, our participants are representative of this larger sample in terms of age, sex ratio, education, and APOE status. Despite the small number of subjects, we were

able to identify differential patterns of activity years prior to death indicating differences in brain function between subjects who will ultimately develop CI in association with AD pathology and those who remain cognitively intact. These changes may represent compensatory processes or perhaps the utilization of alternative brain networks in the face of accumulating pathology. This study provides further evidence that ASYMAD subjects are a unique group of individuals characterized by intact cognitive and brain function despite AD pathology.

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