

# Alzheimer's disease markers, hypertension, and gray matter damage in normal elderly

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## Abstract

It is not well known whether Alzheimer's disease (AD) cerebrospinal fluid (CSF) biomarkers are associated with brain damage in cognitively normal elderly. The combined influence of CSF biomarkers and hypertension (HTN) on the gray matter (GM) is also not well described. One hundred fifteen cognitively healthy subjects (mean age  $62.6 \pm 9.5\%$ , 62% women) received clinical assessment, a high resolution magnetic resonance imaging (MRI), and a lumbar puncture. The CSF levels of total tau (t-tau), hyperphosphorylated tau (p-tau<sub>231</sub>), amyloid beta (A $\beta$ 42/A $\beta$ 40), p-tau<sub>231</sub>/A $\beta$ 42, and t-tau/A $\beta$ 42 were dichotomized as "high" and "low" based on accepted cut off values. Statistical parametric mapping was used to examine MRI scans for regional GM density, studied as a function of the CSF markers, HTN, and combination of both. Global and medial temporal lobe (MTL) GM was also assessed. Voxel based morphometry revealed that higher t-tau was associated with lower GM density in the precuneus. Subjects with higher p-tau<sub>231</sub> and p-tau<sub>231</sub>/A $\beta$ 42 had less GM in temporal lobes. Low A $\beta$ 42/A $\beta$ 40 was related to less GM in the thalamus, caudate, and midbrain. Subjects with hypertension showed more GM atrophy in the cerebellum, occipital, and frontal regions. Simultaneous presence of elevated CSF AD biomarkers and HTN was associated with more GM atrophy than either marker individually, but no interaction effects were identified. In conclusion, in normal elderly CSF markers were associated predominantly with lower GM estimates in structures typically affected early in the AD process. In this presymptomatic stage when no cognitive impairment is present, AD biomarkers and HTN have additive effects on gray matter damage. © 2012 Elsevier Inc. All rights reserved.

**Keywords:** Aging; Biomarkers; MRI; Alzheimer's disease; Cerebrospinal fluid; Hypertension

## 1. Introduction

Alzheimer's disease (AD) is characterized by a long clinically nonsymptomatic period when detectable biological

abnormalities are present, as evidenced by increased cerebrospinal fluid (CSF) levels of total tau (t-tau) and hyperphosphorylated tau (p-tau) and reductions in amyloid beta 1–42 (A $\beta$ 42) levels. These changes are thought to reflect cell loss, progressive neurofibrillary tangle, and senile plaque accumulation (Blennow et al., 2006) and are predictive of cognitive decline already at normal stages of cognition (Fagan et al., 2007; Glodzik et al., 2010; Li et al., 2007).

Volume reductions of the medial temporal lobes (MTL)

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associated with neuronal loss and the intraneuronal accumulation of neurofibrillary tangles (Braak and Braak, 1991), constitute another typical feature of early AD (de Leon et al., 1989). The pathological involvement of other limbic (amygdale, cingulate gyri) and cortical (temporal, parietal) regions follows the MTL changes. This pattern has been observed with both magnetic resonance imaging (MRI) (Glodzik-Sobanska et al., 2005) and positron emission tomography (PET) (Mosconi, 2005).

Despite the growing recognition of CSF biomarkers and imaging in the early AD diagnosis, it is not clearly established whether abnormal concentrations of CSF markers are associated with regional or global brain volume reductions in normal elderly (Fagan et al., 2009; Loew et al., 2009; Sluimer et al., 2010). An evidence of such association would further corroborate the validity of CSF markers as early indexes of AD pathology, particularly if their presence was related to atrophy in AD-specific brain regions.

Adding complexity to this picture is hypertension (HTN), also associated with brain atrophy and cognitive decline (Firbank et al., 2007; Nagai et al., 2008). It is not well known, however, how this highly prevalent condition interacts with abnormal CSF AD biomarkers to affect brain morphology or cognition in normal subjects, and whether AD pathology and HTN contribute in an additive or synergistic way to brain damage.

The current study examined whether: (1) AD CSF biomarkers are associated with gray matter (GM) atrophy in regions known for their vulnerability to AD; and (2) elevated AD CSF biomarkers and hypertension combine to further increase the GM damage. In addition, we also examined how abnormal CSF biomarkers and HTN, separately and combined, influence cognition.

## 2. Methods

### 2.1. Participants

This cross-sectional study included 115 healthy, cognitively healthy individuals (age  $62.6 \pm 9.5$ , range 46–86 years; education  $16.8 \pm 2$ , range 10–20 years; 62% women) studied at the New York University School of Medicine, Center for Brain Health and Alzheimer's Disease Center. All subjects signed an Institutional Review Board (IRB) approved informed consent. They constituted a consecutive group recruited for aging and memory studies, which involved MRI and CSF examinations. Subjects were recruited either through advertisements or they were the caregivers of subjects seeking treatment for cognitive impairments. They received medical, neurological, psychiatric, radiological, and laboratory examinations and underwent lumbar puncture and MRI examination (high resolution T1, T2 and fluid-attenuated inversion recovery [FLAIR]). Patients with confounding brain pathology (e.g., tumor, neocortical infarction) were excluded. The clinical assessment included a semistructured interview based on the Brief Cognitive Rat-

ing Scale (BCRS) from which a Global Deterioration Scale (GDS) score was derived (Reisberg and Ferris, 1988). All subjects were diagnosed as normal: GDS 1 or 2 (Reisberg et al., 1993). GDS = 1 indicated no subjective memory complaint, and GDS = 2 indicated awareness of memory change over the lifespan, in the absence of objective evidence of memory or functional problems on clinical interview. Subjects scoring  $\geq 16$  on the 17-item Hamilton Depression Scale were excluded (Bech et al., 1986).

General cognitive abilities were tested with Mini-Mental State Examination (MMSE) (Folstein et al., 1975). In addition, a neuropsychological test battery was administered in every case. The measures include subtests of the Guild Memory Scale (Gilbert et al., 1968) assessing immediate and delayed recall of orally presented paragraphs (initial: PARI, and delayed: PARD); verbal paired associates (initial: PRDI, and delayed: PRDD); visual/verbal paired associates with numbers (DESN). Subtests of the Wechsler Intelligence Scale Revised (WAIS-R) (Wechsler, 1981) were used to assess working memory (digits forward: WAISDIG-F, and backward: WAISDIG-B), and attention (digit symbol substitution test: DSST).

The presence of hypertension was determined based on current antihypertensive treatment or systolic blood pressure  $\geq 140$  mm Hg, or diastolic blood pressure  $\geq 90$  mm Hg (Chobonian et al., 2003). If the patient was not treated but high blood pressure was identified, we further verified whether it was high only at 1 occasion or also during other visits to our Center.

### 2.2. Lumbar puncture, CSF collection, and assays

Using a 25 gauge needle guided by fluoroscopy, 15 mL of clear CSF was collected into 3 polypropylene tubes. All CSF samples were kept on ice for a maximum of 1 hour until centrifuged for 10 minutes at 1500g at 4 °C. Samples were aliquoted to 0.25 mL polypropylene tubes and stored in at -80 °C until the assay. All samples were blindly analyzed in batch mode. We determined the concentrations of total tau (t-tau) (Blennow et al., 1995), tau phosphorylated at threonine 231 (p-tau<sub>231</sub>) (Kohnken et al., 2000), A $\beta$ 40, and A $\beta$ 42 (Metha et al., 2000). The ratio of these 2 A $\beta$  species A $\beta$ 42/A $\beta$ 40 was used in the analysis. We also analyzed p-tau<sub>231</sub>/A $\beta$ 42 and t-tau/A $\beta$ 42 ratios.

### 2.3. Study groups

#### 2.3.1. Biomarker groups

To examine the effects of CSF biomarkers on brain measures and cognition subjects were classified into high (+) and low (-) biomarkers groups based on the published diagnostic and predictive cut off values: high t-tau > 350 pg/mL (Hansson et al., 2006), high p-tau<sub>231</sub> > 18 pg/mL (Buerger et al., 2003), low A $\beta$ 42/A $\beta$ 40 < 0.11 (Lewczuk et al., 2004). For p-tau<sub>231</sub>/A $\beta$ 42 and t-tau/A $\beta$ 42 we used their respective medians since the cut off values are not well

established. For all the ratios ( $A\beta_{42}/A\beta_{40}$ ,  $p\text{-tau}_{231}/A\beta_{42}$ ,  $t\text{-tau}/A\beta_{42}$ ) data were available for 86 subjects.

### 2.3.2. Hypertension (HTN) groups

As described above, subjects were classified as hypertensive or normotensive.

### 2.3.3. Biomarker-HTN groups

Dichotomization of CSF biomarkers and HTN was used to test the interaction between these 2 factors. To examine the interactions of the CSF biomarkers and HTN on brain measures and cognition, subjects were classified in the following 4 biomarker-HTN groups: normal biomarker level and HTN absent: (B-/H-); normal biomarker level and HTN present: (B-/H+); abnormal biomarker level and HTN absent: (B+/H-); abnormal biomarker level and HTN present: (B+/H+).

## 2.4. MRI and image analysis

T1 weighted MRI scans were uniformly acquired in the coronal orientation (slice thickness: 1.6 mm field of view (FOV) = 25 cm, number of excitations (NEX) = 1, matrix =  $256 \times 192$ , repetition time (TR) = 35 ms, time to echo (TE) = 9 ms, flip angle (FA) =  $60^\circ$ ), using a 1.5 T GE scanner (GE, Milwaukee, WI, USA). Voxel based morphometry (VBM) was performed with MATLAB 7.1 (Mathworks Inc) and statistical parametric mapping (SPM'2, Wellcome Department of Cognitive Neurology, London, UK) procedures (Ashburner and Friston, 2000; Good et al., 2002).

All scans were realigned and spatially normalized. Normalization was done by estimating the optimal 12-parameter affine transformation (Freeborough et al., 1996). The spatially normalized images were resliced using sinc interpolation to a final voxel size of  $1.5 \times 1.5 \times 1.5$  mm, and segmented into GM, white matter (WM), and CSF images (Ashburner and Friston, 2000). The GM images were renormalized to an a priori GM template (Good et al., 2002), by using the high dimensional normalization (optimal 12-parameter affine transformation), followed by an iterative estimate of local alignment based on a family of  $7 \times 8 \times 7$  discrete cosine functions (Freeborough et al., 1996; Good et al., 2002) and smoothed with an 8-mm FWHM isotropic Gaussian kernel. To preserve the volume of a particular tissue compartment within each voxel, the images were modulated by the Jacobian determinants of the transformed matrix (Good et al., 2002). Anatomical location of areas showing GM effects was described using the Talairach and Tournoux coordinates (Talairach and Tournoux, 1988), after conversion of Montreal Neurological Institute (MNI) coordinates to Talairach space (Lancaster et al., 2000). In all analyses of GM density, age, gender, and education were accounted for. Results were examined over the whole brain at  $p < 0.001$  with a minimum cluster size of 75 voxels. In the context of VBM “density” refers to the relative amount of gray matter in a voxel (Ashburner and Friston, 2009) and

does not refer to cell packing density measured by histology or term “density” as used in computed tomography.

To study global gray matter, native images were segmented into GM, WM, and CSF using SPM'2 routines. Global GM volume was normalized to the total intracranial volume (ICV). The ICV was calculated as the integral of all 3 tissue compartments.

Finally, in an exploratory analysis, in addition to unbiased VBM we used automated MTL regions of interest (ROI) (Li et al., 2008) to extract GM density. Estimates were obtained separately for right and left hemisphere and the average was computed. Average GM density was compared across the high and low biomarkers group, subjects with and without HTN, and across biomarker-HTN groups.

Fluid-attenuated inversion recovery (FLAIR) images were acquired to assess the extent of periventricular (PWMH) and deep white matter hyperintensities (DWMH). We used 0–3 Fazekas scale (Fazekas et al., 1987). For the final analyses a total white matter hyperintensity score was used. It was expressed as a sum of PWMH and DWMH scores.

## 2.5. Statistical analysis

Continuous demographic measures were examined using *t* test or analysis of variance (ANOVA). Categorical variables were examined with  $\chi^2$  test. Between biomarker, HTN, or biomarker-HTN groups comparisons were performed using analysis of covariance (ANCOVA) models with age, gender, education, and WMH score accounted for. Linear contrast analyses were performed when group differences appeared to follow a gradual change in diagnostic category. Cognitive variables were compared using multivariate analysis of covariance (MANCOVA) models with age, gender, and educations as covariates.

Normality was checked with the Shapiro-Wilk test. When the data did not meet the assumptions of normality, the Mann-Whitney *U* test was used to compare 2 groups (*Z* values are given) or Kruskal-Wallis analysis of variance (ANOVA), when more than 2 groups were analyzed. For nonnormally distributed data in analysis of covariance (ANCOVA) models statistical results were verified by using rank transformed values. Results are given for models with transformed (rank) variables, when transformation was necessary. Statistical analyses were performed with SPSS 16 (SPSS Inc, Chicago, IL, USA) with *p* values declared statistically significant when  $< 0.05$ .

To assess the impact of CSF biomarkers and HTN on regional GM SPM'2 was used. For the voxel-wise SPM analysis of the MRI scans, GM volumes were examined using the general linear model (GLM)/univariate analysis (*F* contrast) followed by post hoc *t* tests, to compare high and low biomarker groups, and HTN+ versus HTN- groups, at  $p < 0.001$ , with a minimum cluster size of 75 voxels, uncorrected for multiple comparisons. We then examined the interaction between HTN and biomarker groups on GM

Table 1  
Main characteristics of the entire study group

Variable	
t-tau pg/mL, median (interquartile range)	260.0 (192.0)
p-tau <sub>231</sub> pg/mL, median (interquartile range)	7.25 (12.79)
Aβ42/Aβ40, median (interquartile range)	0.1159 (0.0394)
t-tau/Aβ42, median (interquartile range)	0.2839 (0.2067)
p-tau <sub>231</sub> /Aβ42, median (interquartile range)	0.0069 (0.0101)
Total WMH, median (interquartile range)	1.0 (2.0)
Hypertension, n (%)	39 (34)
Age, years, mean (standard deviation)	62.63 (9.48)
Female, n (%)	71 (62)

White matter hyperintensity score expressed as a sum of Fazekas scores for deep and periventricular white matter hyperintensities; the number of subjects with WMH information available  $n = 112$ .

Key: Aβ42/Aβ40, amyloid beta ratio; p-tau<sub>231</sub>, hyperphosphorylated tau; t-tau, total tau; WMH, white matter hyperintensity.

volumes. Biomarker-HTN interaction was defined as GM reduction beyond the one expected from mere summation of both effects. It was examined with  $F$  contrasts after controlling for the main effects of biomarkers and HTN groups on GM measures. In order to do this, the  $F$  contrast for the interaction was computed after inclusive masking ( $p < 0.001$ , uncorrected) with the main effects. With this procedure, SPM tests for interaction effects only within the voxels showing significant main effects of biomarker and HTN. Finally, a linear contrast was used to test whether the interaction was driven by the B+/H+ group (abnormal biomarker level and HTN present) showing GM reductions compared with the other 3 subgroups. For all analyses, results were examined at  $p < 0.001$ , uncorrected.

Because intraindividual variability of biomarkers' concentration could lead to different group assignments and influence the observed associations between GM and AD CSF biomarkers, in addition to comparing high and low biomarker groups, we repeated the SPM analyses using a regression model where CSF biomarkers were used as continuous variables.

In all analyses of global brain volumes and regional GM densities, age, gender, and education were accounted for. Analyses were repeated after accounting for the WMH score.

### 3. Results

#### 3.1. Characteristics of the study groups

Table 1 presents general characteristics for the entire study group.

##### 3.1.1. Biomarker groups (Table 2)

Subjects expressing more abnormal biomarker concentrations were older than those classified into groups with "normal" levels. Statistics were for t-tau groups ( $Z = -3.2$ ,  $p = 0.001$ ), p-tau<sub>231</sub> ( $Z = -3.4$ ,  $p = 0.001$ ), t-tau/Aβ42 (trend,  $t(84) = -1.8$ ,  $p = 0.07$ ) and p-tau<sub>231</sub>/Aβ42 ( $t(84) = -2.1$ ,  $p = 0.04$ ). There were more men than women among

subjects with high p-tau<sub>231</sub> than in the low p-tau<sub>231</sub> group ( $\chi^2 = 3.2$ ,  $p = 0.07$ ) and the high p-tau<sub>231</sub> group had more years of education ( $Z = -2.3$ ,  $p = 0.02$ ). The prevalence of hypertension did not differ between high and low biomarker groups. After accounting for age and gender the groups did not differ in the total WMH score.

##### 3.1.2. HTN groups (Table 2)

HTN was found in 39 (34%) subjects. Individuals with HTN were older ( $Z = -3.4$ ,  $p = 0.001$ ) and more likely to be men ( $\chi^2 = 10.7$ ,  $p = 0.001$ ). After correction for age and gender the mean systolic ( $134.1 \pm 16.5$  vs.  $118.1 \pm 10.0$ ;  $F(3,111) = 26.7$ ,  $p < 0.001$ ) and diastolic ( $80.2 \pm 12.3$  vs.  $71.6 \pm 8.3$ ;  $F(3,110) = 15.5$ ,  $p < 0.001$ ) blood pressures remained higher in the HTN group. Age and gender adjusted biomarkers concentrations did not differ between hypertensive and normotensive group. The means  $\pm$  standard error were respectively, t-tau:  $299.5 \pm 24.7$  (HTN+) versus  $302.2 \pm 17.3$  (HTN-) pg/mL; p-tau<sub>231</sub>:  $10.3 \pm 2.0 = 1$  versus  $11.7 \pm 1.4$  pg/mL; Aβ42/Aβ40:  $0.12 \pm 0.007$  versus  $0.12 \pm 0.005$ ; tau/Aβ42:  $0.40 \pm .079$  versus  $0.39 \pm 0.054$ ; and p-tau<sub>231</sub>/Aβ42:  $0.016 \pm 0.006$  versus  $0.017 \pm 0.004$ . The groups did differ, however, in total WMH score: hypertensive subjects had more white matter lesions ( $F(3,108) = 10.8$ ,  $p < 0.001$ ). Smoking (8% of subjects) and hyperglycemia (9%) were infrequent in our group.

##### 3.1.3. Biomarker-HTN groups (Table 3)

Across all biomarker-HTN groups age increased from the group without any risk markers (B-/H-) to the group with both risk factors (B+/H+). For the 4 t-tau-HTN groups:  $F(3,111) = 9.04$ ,  $p < 0.001$ ; p-tau<sub>231</sub>-HTN groups:  $F(3,111) = 9.3$ ,  $p < 0.001$ ; Aβ42/Aβ40-HTN groups:  $F(3,82) = 4.1$ ,  $p = 0.009$ ; t-tau/Aβ42-HTN groups:  $F(3,82) = 3.7$ ,  $p = 0.02$ ; and p-tau<sub>231</sub>/Aβ42-HTN groups:  $F(3,82) = 3.9$ ,  $p = 0.01$ . Gender distribution was also different with more women in (B-/H-) groups. Statistics were: t-tau-HTN groups:  $\chi^2 = 11.2$ ,  $p = 0.01$ ; p-tau<sub>231</sub>-HTN:  $\chi^2 = 13.9$ ,  $p = 0.003$ ; Aβ42/Aβ40-HTN:  $\chi^2 = 9.4$ ,  $p = 0.02$ ; t-tau/Aβ42-HTN:  $\chi^2 = 8.5$ ,  $p = 0.04$ ; and p-tau<sub>231</sub>/Aβ42-HTN:  $\chi^2 = 7.4$ ,  $p = 0.06$ , respectively. Subjects with HTN and high p-tau<sub>231</sub> had more years of education than other p-tau<sub>231</sub>-HTN groups (Kruskal-Wallis  $\chi^2 = 11.0$ ,  $p = 0.01$ ). After accounting for age and gender total WMH score differed between the groups: t-tau-HTN groups:  $F(5,106) = 4.90$ ,  $p < 0.003$ ; p-tau<sub>231</sub>-HTN groups:  $F(5,106) = 6.40$ ,  $p < 0.001$ ; Aβ42/Aβ40-HTN groups:  $F(5,79) = 2.6$ ,  $p = 0.06$ ; t-tau/Aβ42-HTN groups:  $F(5,79) = 2.6$ ,  $p = 0.06$  and p-tau<sub>231</sub>/Aβ42-HTN groups:  $F(5,79) = 3.3$ ,  $p = 0.03$ .

#### 3.2. Brain effects

##### 3.2.1. Biomarker groups

3.2.1.1. Global effects. The group with high p-tau<sub>231</sub>/Aβ42 had a smaller global GM volume than the group with low ratio ( $F(4,81) = 8.3$ ,  $p = 0.005$ ). Similar trends were seen

Table 2  
Study variables by biomarkers (high/ low) and HTN (absent/present) groups

	Age (years)	Female (%)	Education (years)	HTN (%)	GM volume (% ICV)	WMH <sup>a</sup>	MTL GM <sup>b</sup>
t-tau							
Low ( <i>n</i> = 80)	60.7 ± 8.9	64	16.7 ± 2.0	31	47 ± 2	1.12 ± 1.08	0.80 ± 0.05
High ( <i>n</i> = 35)	67.1 ± 9.4	57	17.1 ± 2.1	40	46 ± 3	1.13 ± 1.26	0.78 ± 0.05
<i>p</i>	0.001	ns	ns	ns	ns	ns	0.05
p-tau <sub>231</sub>							
Low ( <i>n</i> = 91)	61.0 ± 8.9	66	16.7 ± 1.9	33	47 ± 2	0.98 ± 1.04	0.80 ± 0.05
High ( <i>n</i> = 24)	68.7 ± 9.3	46	17.5 ± 2.4	37	46 ± 2	1.75 ± 1.29	0.78 ± 0.05
<i>p</i>	0.001	0.07	0.02	ns	0.10	ns	ns
Aβ42/Aβ40 <sup>c</sup>							
High ( <i>n</i> = 48)	60.6 ± 7.0	65	16.8 ± 1.9	33	47 ± 2	0.91 ± 0.97	0.79 ± 0.05
Low ( <i>n</i> = 38)	63.8 ± 11.0	53	16.6 ± 2.0	31	47 ± 2	1.26 ± 1.20	0.79 ± 0.05
<i>p</i>	ns	ns	ns	ns	ns	ns	ns
t-tau/Aβ42 <sup>c</sup>							
Low ( <i>n</i> = 43)	60.2 ± 7.3	58	16.7 ± 2.0	33	48 ± 2	0.78 ± 0.90	0.79 ± 0.05
High ( <i>n</i> = 43)	63.8 ± 10.3	60	16.7 ± 1.9	33	47 ± 2	1.35 ± 1.19	0.79 ± 0.05
<i>p</i>	0.07	ns	ns	ns	0.07	ns	ns
p-tau/Aβ42 <sup>c</sup>							
Low ( <i>n</i> = 43)	60.2 ± 7.4	63	16.5 ± 2.0	30	48 ± 2	1.05 ± 1.06	0.80 ± 0.05
High ( <i>n</i> = 43)	64.0 ± 10.2	56	16.9 ± 1.9	35	46 ± 2	1.10 ± 1.13	0.79 ± 0.05
<i>p</i>	0.04	ns	ns	ns	0.005	ns	ns
HTN							
Absent ( <i>n</i> = 76)	60.5 ± 9.1	72	16.7 ± 2.1	NA	47 ± 2	0.81 ± 0.86	0.80 ± 0.05
Present ( <i>n</i> = 39)	66.7 ± 8.9	41	17.2 ± 1.9	NA	46 ± 2	1.80 ± 1.32	0.78 ± 0.05
<i>p</i>	0.001	0.001	ns	NA	0.06	0.001	0.02

Values are presented as mean ± standard deviation. For t-tau the cut off of 350 pg/mL (Hansson et al., 2006) was used, for p-tau<sub>231</sub> the cut off of 18 pg/mL (Buerger et al., 2003), and for Aβ42/Aβ40 the cut off of 0.11 (Lewczuk et al., 2004). High and low p-tau<sub>231</sub>/Aβ42 and t-tau/Aβ42 groups were defined based on the median split. For GM comparisons *p* values are given for *F* tests, corrected for age, gender, and education. For WMH comparisons *p* values are given for *F* tests corrected for age and gender; the number of subjects with WMH information available, *n* = 112. For MTL GM comparisons *p* values are given for *t* tests, as explained in the text

Key: Aβ42/Aβ40, amyloid beta ratio; GM, gray matter; HTN, hypertension; ICV, intracranial volume; MTL, medial temporal lobe; NA, not applicable; ns, not significant; p-tau<sub>231</sub>, hyperphosphorylated tau; t-tau, total tau; WMH, white matter hyperintensities.

<sup>a</sup> White matter hyperintensity score expressed as a sum of Fazekas scores for deep and periventricular white matter hyperintensities.

<sup>b</sup> Values represent estimates (densities) extracted from processed GM images using MTL regions of interest (ROI) (Li et al., 2008).

<sup>c</sup> Results presented for 86 subjects and for WMH for 85 subjects.

for tau/Aβ42 ( $F(4,81) = 3.4$ ,  $p = 0.07$ ) and p-tau<sub>231</sub> groups ( $F(4, 110) = 2.3$ ,  $p = 0.1$ ) (Table 2). Results remained the same after accounting for the total WMH score.

**3.2.1.2. Regional effects.** The high t-tau group had less GM in the right hippocampus, left precuneus, anterior cingulate, and insula than the low t-tau group. The high p-tau<sub>231</sub> group had less GM bilaterally in the temporal lobes: in the right limbic lobe (entorhinal cortex) and the left inferior temporal gyrus. The low Aβ42/Aβ40 group had less GM in the midbrain. The high t-tau/Aβ42 had less GM in the right parietal lobule, left temporal cortex, and anterior cingulate. The high p-tau<sub>231</sub>/Aβ42 group had less GM bilaterally in the temporal gyri (Fig. 1A–E, Table 4). After accounting for a total WMH score the results remained similar: the high t-tau group had less GM in the precuneus and insula. The high p-tau<sub>231</sub> group had less GM in the right limbic lobe and the temporal cortex. The high t-tau/Aβ42 had less GM in the right parietal cortex and left cingulate. The high p-tau<sub>231</sub>/Aβ42 group had less GM bilaterally in the temporal cortex. Aβ42/Aβ40 groups did not differ from each other.

In the regression analyses (Fig. 2, Table 5) the regions of negative correlations between GM and t-tau were found in both precuneus, left insula, right caudate, and thalamus as

well as the right inferior temporal gyrus. Thus precuneus and insula were consistently identified in both analyses, but in the regression model the clusters were considerably larger. Similarly, for p-tau<sub>231</sub> the regions consistently identified in both analyses, but with larger clusters in the regression model were located in the inferior and medial temporal lobe (comprising entorhinal cortex: Brodmann area [BA] 28). For Aβ42/Aβ40 the region showing positive correlation and identified in both analyses comprised left midbrain, but in the regression model the cluster extended to both thalami and caudate. No regions of positive correlation between GM and t-tau or p-tau<sub>231</sub> and no regions of negative correlation between GM and Aβ42/Aβ40 were found. For t-tau/Aβ42 no region was identified in the regression model. For p-tau<sub>231</sub>/Aβ42 both analyses pointed to the temporal cortex. After accounting for a total WMH score the results remained comparable.

### 3.2.2. HTN groups

**3.2.2.1. Global effects.** Subjects with HTN tended to have smaller global GM volumes than subjects without HTN

Table 3  
Study variables by biomarker-HTN groups

	Age	Female (%)	Education (years)	GM volume (% ICV)	WMH <sup>a</sup>	MTL GM <sup>b</sup>
<b>t-tau-HTN</b>						
B-/H- ( <i>n</i> = 55)	58.3 ± 7.5	75	16.6 ± 2.0	48 ± 2	0.75 ± 0.81	0.80 ± 0.05
B-/H+ ( <i>n</i> = 25)	65.9 ± 9.6	40	17.1 ± 2.0	46 ± 2	1.90 ± 1.20	0.79 ± 0.04
B+/H- ( <i>n</i> = 21)	66.4 ± 10.5	66	16.9 ± 2.3	47 ± 3	0.95 ± 0.97	0.79 ± 0.05
B+/H+ ( <i>n</i> = 14)	68.1 ± 7.7	43	17.4 ± 1.9	46 ± 3	1.64 ± 1.55	0.77 ± 0.06
<i>p</i>	< 0.001	0.01	ns	ns	0.003	0.04
<b>p-tau<sub>231</sub>-HTN</b>						
B-/H- ( <i>n</i> = 61)	58.9 ± 7.8	77	16.6 ± 1.9	48 ± 2	0.74 ± 0.81	0.80 ± 0.05
B-/H+ ( <i>n</i> = 30)	65.3 ± 9.6	43	16.7 ± 1.9	46 ± 2	1.47 ± 1.28	0.78 ± 0.04
B+/H- ( <i>n</i> = 15)	67.2 ± 11.3	53	16.8 ± 2.7	46 ± 2	1.07 ± 1.03	0.79 ± 0.06
B+/H+ ( <i>n</i> = 9)	71.3 ± 4.1	33	18.8 ± 0.9	46 ± 2	2.89 ± 0.78	0.77 ± 0.05
<i>p</i>	< 0.001	0.003	0.01	0.04	0.001	0.07
<b>Aβ42/Aβ40-HTN<sup>c</sup></b>						
B-/H- ( <i>n</i> = 32)	58.0 ± 6.0	78	16.5 ± 1.8	48 ± 2	0.61 ± 0.62	0.80 ± 0.04
B-/H+ ( <i>n</i> = 16)	66.0 ± 5.8	37	17.4 ± 2.1	46 ± 3	1.50 ± 1.26	0.78 ± 0.05
B+/H- ( <i>n</i> = 26)	63.3 ± 11.2	58	16.5 ± 2.2	47 ± 2	1.04 ± 1.04	0.80 ± 0.05
B+/H+ ( <i>n</i> = 12)	64.7 ± 10.7	42	16.7 ± 1.6	47 ± 3	1.75 ± 1.42	0.77 ± 0.04
<i>p</i>	0.009	0.02	ns	ns	0.06	ns
<b>t-tau/Aβ42-HTN<sup>c</sup></b>						
B-/H- ( <i>n</i> = 29)	58.0 ± 6.4	72	16.3 ± 2.0	48 ± 2	0.71 ± 0.76	0.80 ± 0.05
B-/H+ ( <i>n</i> = 14)	64.9 ± 7.0	29	17.5 ± 1.9	46 ± 3	1.71 ± 1.27	0.78 ± 0.04
B+/H- ( <i>n</i> = 29)	62.7 ± 10.7	66	16.7 ± 2.0	47 ± 2	0.89 ± 0.94	0.80 ± 0.05
B+/H+ ( <i>n</i> = 14)	66.0 ± 9.0	50	16.7 ± 1.8	47 ± 2	1.50 ± 1.40	0.77 ± 0.05
<i>p</i>	0.02	0.04	ns	ns	0.06	ns
<b>p-tau/Aβ42-HTN<sup>c</sup></b>						
B-/H- ( <i>n</i> = 30)	57.9 ± 6.5	70	16.3 ± 1.9	48 ± 2	0.55 ± 0.57	0.80 ± 0.05
B-/H+ ( <i>n</i> = 13)	64.9 ± 7.1	46	17.0 ± 2.2	47 ± 3	1.30 ± 1.25	0.78 ± 0.05
B+/H- ( <i>n</i> = 28)	63.0 ± 10.7	68	16.7 ± 2.1	47 ± 2	1.07 ± 1.01	0.80 ± 0.05
B+/H+ ( <i>n</i> = 15)	65.9 ± 9.2	33	17.2 ± 1.6	46 ± 2	1.87 ± 1.36	0.77 ± 0.04
<i>p</i>	0.01	0.06	ns	0.02	0.03	ns

Values are presented as mean ± standard deviation. For GM volume comparisons *p* values are given for *F* tests, corrected for age, gender, and education. For WMH comparisons *p* values are given for *F* tests corrected for age and gender; the number of subjects with WMH information available, *n* = 112. For MTL GM comparisons *p* values are given for *F* tests not corrected, as explained in the text.

Key: Aβ42/Aβ40, amyloid beta ratio; B-/H-, normal biomarker level and HTN absent; B-/H+, normal biomarker level and HTN present; B+/H-, abnormal biomarker level and HTN absent; B+/H+, abnormal biomarker level and HTN present; GM, gray matter; HTN, hypertension; ICV, intracranial volume; MTL, medial temporal lobe; NA, not applicable; ns, not significant; p-tau<sub>231</sub>, hyperphosphorylated tau; t-tau, total tau; WMH, white matter hyperintensity.

<sup>a</sup> White matter hyperintensity score expressed as a sum of Fazekas scores for deep and periventricular white matter hyperintensities.

<sup>b</sup> Values represent estimates (densities) extracted from processed GM images using MTL regions of interest (ROI) (Li et al., 2008).

<sup>c</sup> Results presented for 86 subjects and for WMH for 85 subjects.

( $F(4,110) = 3.6, p = 0.06$ ) (Table 2). After accounting for total WMH score the results were:  $F(5,106) = 1.9, p = 0.17$ .

**3.2.2.2. Regional effects.** Subjects with HTN showed significantly less GM in the cerebellum, occipital, and frontal regions (Fig. 1F, Table 4). After accounting for a total WMH score GM reductions were still seen in the cerebellum.

### 3.2.3. Biomarker-HTN groups

**3.2.3.1. Global effects.** Global GM volumes were significantly different across p-tau<sub>231</sub>-HTN groups ( $F(6,108) = 2.8, p = 0.04$ ), and p-tau<sub>231</sub>/Aβ42-HTN groups ( $F(6,79) = 3.4, p = 0.02$ ) (Table 3). Linear contrasts analyses revealed decreasing GM volumes from the group without any risk markers (B-/H-) to the group with both risk factors (B+/H+) both for p-tau<sub>231</sub>-HTN (trend:  $p = 0.06$ ) and p-tau<sub>231</sub>/Aβ42-HTN groups ( $p = 0.02$ ). The groups expressing only 1 risk marker had intermediate values (Fig. 3). Accounting for the WMH did not change the results substantially:

p-tau<sub>231</sub>-HTN groups ( $F(7,104) = 2.2, p = 0.09$ ); p-tau<sub>231</sub>/Aβ42-HTN groups ( $F(7,77) = 2.7, p = 0.05$ ).

**3.2.3.2. Regional effects.** No interactions at the regional level were found with VBM.

### 3.2.4. Medial temporal lobe ROIs analyses

**3.2.4.1. Biomarker groups (Table 2).** Subjects with high t-tau tended to have lower MTL GM density ( $t(113) = 1.9, p = 0.05$ ). A similar trend was found for p-tau<sub>231</sub> ( $t(113) = 2.3, p = 0.1$ ).

**3.2.4.2. HTN groups (Table 2).** Subjects with HTN had lower MTL GM density ( $t(113) = 2.3, p = 0.02$ ) than normotensive individuals.

**3.2.4.3. Biomarker-HTN groups (Table 3).** Significant differences were found between t-tau-HTN groups ( $F(3,111) = 2.9, p = 0.04$ ) and a trend for p-tau<sub>231</sub>-HTN groups ( $F(3,111) = 2.4, p = 0.07$ ) groups. Linear contrasts analyses both for t-tau-

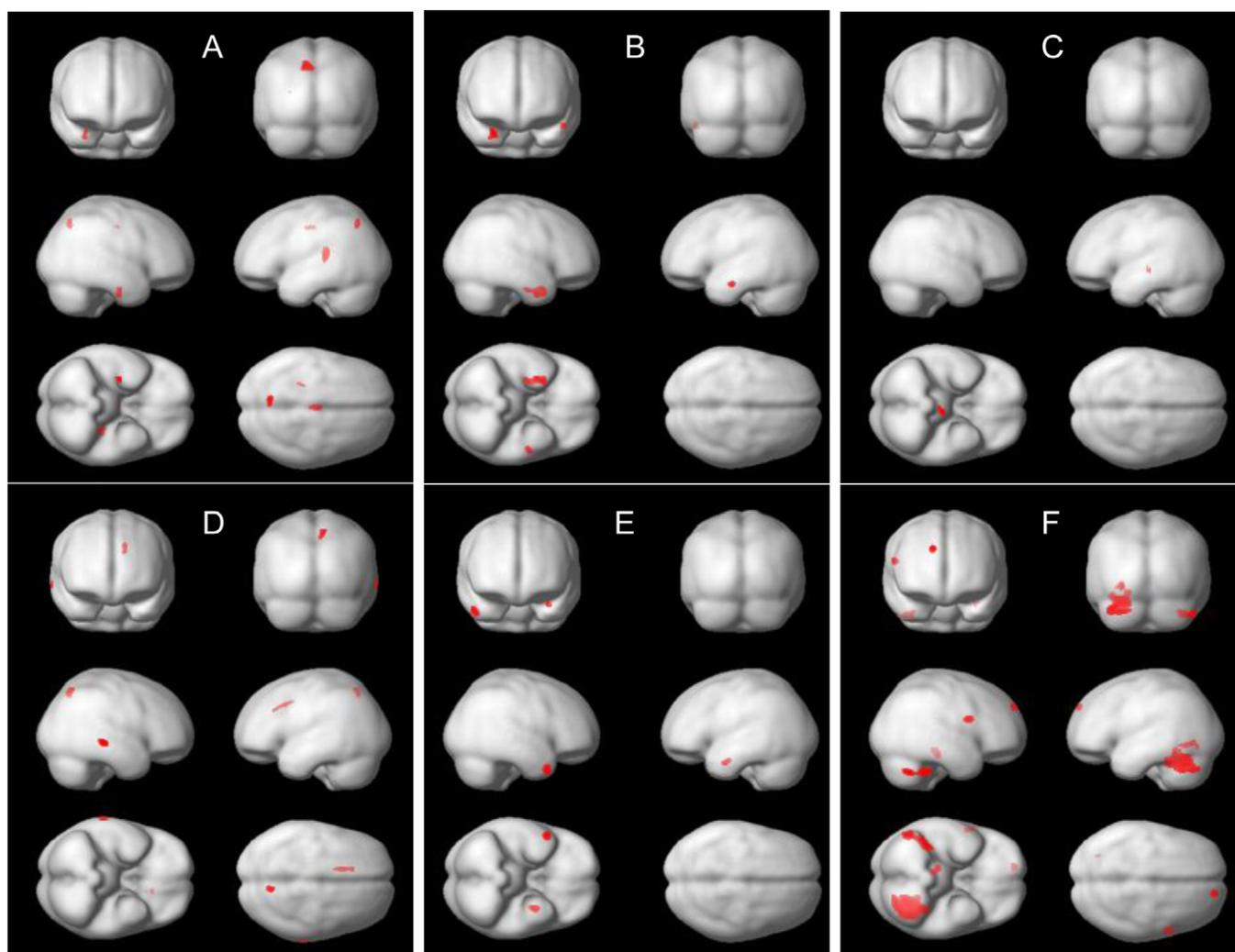


Fig. 1. Regions showing significant gray matter density differences (as detected with statistical parametric mapping [SPM]) between groups with: (A) high and low total tau (t-tau), (B) high and low hyperphosphorylated tau (p-tau<sub>231</sub>), (C) high and low amyloid beta ratio (Aβ<sub>42</sub>/Aβ<sub>40</sub>), (D) high and low t-tau/Aβ<sub>42</sub> ratio, (E) high and low p-tau<sub>231</sub>/Aβ<sub>42</sub> ratio, and (F) with and without hypertension. All analyses accounted for age, gender, and education.

HTN and p-tau<sub>231</sub>-HTN groups revealed decreasing MTL GM volumes from the group without any risk markers (B-/H-) to the group with both risk factors (B+/H+) ( $p < 0.05$  for both). Originally all the ROIs analyses were performed after accounting for age, gender, and education. However more parsimonious models (without covariates) had the best fit and are presented here.

### 3.3. Cognitive effects

No differences were found in cognitive measures between high and low biomarker groups, nor between subjects with and without HTN. Similarly, biomarkers-HTN groups were not different.

## 4. Discussion

In VBM analyses positive AD CSF biomarkers and HTN were associated with different patterns of regional GM atrophy. Elevated p-tau<sub>231</sub> and t-tau were related to lower

GM estimates in regions implicated in AD pathology. Hypertension was associated with GM loss in the cerebellum and frontal regions. No regional biomarker-HTN interactions, defined as GM reduction beyond the one expected from mere summation of both effects, were found.

Higher p-tau<sub>231</sub> levels were associated with lower GM density in temporal lobes, encompassing entorhinal regions (BA 28). High t-tau correlated with atrophy in the precunei. This, we believe, concurs with the observation that the medial temporal region is an early site of neurofibrillary pathology (Braak and Braak, 1991). The atrophy of the cingulate (Pennanen et al., 2005) and precunei (Fennema-Notestine et al., 2009) has been shown in subjects with mild cognitive impairment (MCI), and hypometabolism in limbic and parietal/precuneus areas occurs early in the disease (Mosconi, 2005). We are not aware of any previous report showing the inverse relationship between t-tau and GM density in the precunei in normal individuals, but we believe

Table 4  
SPM analysis

	Cluster extent	Brain region	Talaraich coordinates (x,y,z)	F value
t-tau+ < t-tau-	75	R hippocampus	30, -12, -24	3.52
	131	L parietal lobe, precuneus, BA 7	-9, -64, 45	4.16
	112	L insula, BA 13	-26, -30, 14	4.37
	105	L anterior cingulate, BA 24	0, -13, 40	3.82
p-tau <sub>231</sub> + < p-tau <sub>231</sub> -	317	R limbic lobe, BA 28	29, -10, -25	3.84
	75	R inferior temporal gyrus, BA 20	33, 0, -32	3.54
Aβ42/Aβ40- < Aβ42/Aβ40+ <sup>a</sup>	108	L inferior temporal gyrus, BA 20	-50, -10, -20	3.84
	75	L midbrain	-6, -23, -4	3.59
t-tau/Aβ42+ < t-tau/Aβ42- <sup>a</sup>	86	R superior parietal lobule, BA 7	11, -61, 53	3.95
	94	L middle temporal gyrus, BA 21	71, -30, -4	4.02
	85	L anterior cingulate, BA 32	-14, 15, 34	3.77
	166	R middle temporal gyrus, BA 21	53, 10, -31	4.51
p-tau <sub>231</sub> /Aβ42+ < p-tau <sub>231</sub> /Aβ42- <sup>a</sup>	96	L middle temporal gyrus, BA 21	-35, -4, -25	3.58
	447	R cerebellum, posterior lobe	53, -60, -30	4.32
HTN+ < HTN-	83	R superior frontal gyrus, BA 9	15, 57, 30	4.26
	142	R cerebellum, anterior lobe	12, -29, -15	4.17
	88	R inferior frontal gyrus, BA 44	57, 7, 19	4.15
	2435	L cerebellum, posterior lobe	-27, -61, -21	4.46
	131	L lingual gyrus, BA 19	-30, -61, -4	3.66

Regions showing significant differences between groups with high and low biomarkers levels. For t-tau the cut off of 350 pg/mL (Hansson et al., 2006) was used, for p-tau<sub>231</sub> the cut off of 18 pg/mL (Buerger et al., 2003), for Aβ42/Aβ40 the cut off of 0.11 (Lewczuk et al., 2004). High and low t-tau/Aβ42 and p-tau<sub>231</sub>/Aβ42 groups were defined based on the median split. All analyses accounted for age, gender, and education.  $p < 0.001$ , minimal cluster size = 75. Key: Aβ42/Aβ40, amyloid beta ratio; Aβ42/Aβ40+, high Aβ42/Aβ40 group; Aβ42/Aβ40-, low Aβ42/Aβ40 group; BA, Brodmann area; HTN, hypertension; HTN+, group with hypertension; HTN-, group without hypertension; L, left; p-tau<sub>231</sub>, hyperphosphorylated tau; p-tau<sub>231</sub>+, high p-tau<sub>231</sub> group; p-tau<sub>231</sub>-, low p-tau<sub>231</sub> group; p-tau<sub>231</sub>/Aβ42+, high p-tau<sub>231</sub>/Aβ42 group; p-tau<sub>231</sub>/Aβ42-, low p-tau<sub>231</sub>/Aβ42 group; R, right; SPM, statistical parametric mapping; t-tau, total tau; t-tau+, high t-tau group; t-tau-, low t-tau group; t-tau/Aβ42+, high t-tau/Aβ42 group; t-tau/Aβ42-, low t-tau/Aβ42 group.

<sup>a</sup> Results presented for 86 subjects.

this is consistent with the known progression of the disease. It is also in agreement with a recent study of healthy controls, showing a negative correlation between t-tau and p-tau and glucose metabolism in posterior cingulate/precuneus and parahippocampal regions (Petrie et al., 2009). The above regions were identified both by between group comparisons and regression analyses. Importantly, no regions of positive correlation between GM and t-tau or p-tau<sub>231</sub> were found. This, we believe, increases the reliability of our findings. Our results conflict with earlier negative reports for the association between tau markers and MRI atrophy in cognitively healthy subjects (Loew et al., 2009; Sluimer et al., 2010). Because our sample was considerably larger than the others, we offer that they might have been underpowered to detect associations which among normal subjects are most likely subtle.

The lack of association between global or MTL GM and Aβ42/Aβ40 is in contrast to an earlier study where normal subjects with low Aβ42 had lower whole brain and hippocampal volumes (Fagan et al., 2009). This discrepancy might be due to different levels of AD pathology in the groups studied. CSF Aβ42 has been found to correlate with fibrillar amyloid in the brain (Fagan et al., 2006). However, in the very early stages of AD diffuse plaques, which are not associated with marked neuronal damage or loss, dominate. Second, at autopsy the neurofibrillary stage but not the amyloid burden correlates better with brain atrophy (Josephs et al., 2008). Aβ42/Aβ40 positively correlated with

GM density in both thalami, caudate, and left midbrain. Interestingly, Pittsburgh Compound B (PiB) retention was found in thalami and striatum both by us (Mosconi et al., 2010) and others (Klunk et al., 2007; Koivunen et al., 2008; Schöll et al., 2009) in subjects at risk or with AD. Even though these amyloid deposits are not believed to be related to cell loss (Klunk et al., 2007) and the relationships between amyloid burden and atrophy are less consistent than relationships between neurofibrillary pathology and atrophy (Driscoll et al., 2010; Fagan et al., 2009; Glodzik et al., 2010; Josephs et al., 2008), a recent report demonstrated an association between PiB retention and gray matter atrophy on a region by region basis (Chetelat et al., 2010). Because CSF Aβ42 correlates with PiB retention in subjects without dementia (Fagan et al., 2006), we speculate that the observed correlations between CSF Aβ42/Aβ40 and GM may reflect a subtle regional atrophy. This explanation is, however, only tentative.

As for other ratios: high p-tau<sub>231</sub>/Aβ42 effects were seen in temporal lobes, high t-tau/Aβ42 was associated with GM damage in parietal and cingulate areas, although because regression analysis did not confirm these last results, they have to be treated with caution. High ratios of t-tau or p-tau<sub>231</sub> to Aβ42 were also associated with less global GM. This, we speculate, corroborates the utility of tau to Aβ42 ratio concept. As in our previous work we used Aβ42/Aβ40 ratio instead of Aβ42 alone (Brys et al., 2008; Glodzik et al., 2010). In our experience ratio better reflects possible

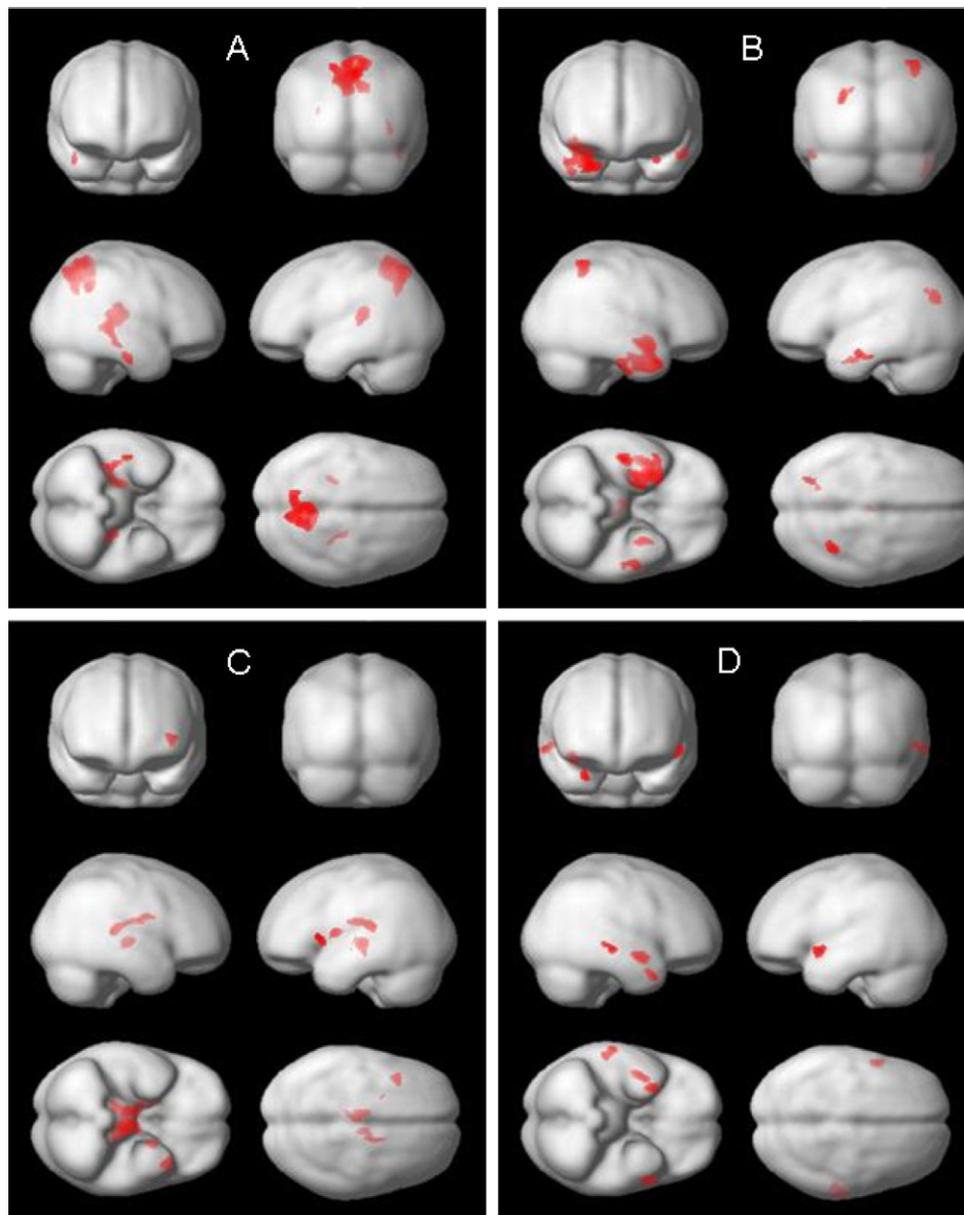


Fig. 2. Regions showing significant correlations (as detected with statistical parametric mapping [SPM]) between gray matter density and cerebrospinal fluid (CSF) Alzheimer's disease (AD) biomarkers: (A) total tau (t-tau) (negative correlation), (B) hyperphosphorylated tau (p-tau<sub>231</sub>) (negative correlation), (C) amyloid beta ratio (A $\beta$ 42/A $\beta$ 40) (positive correlation), (D) p-tau<sub>231</sub>/A $\beta$ 42 ratio (negative correlation). All analyses accounted for age, gender, and education.

AD, because the concentration of A $\beta$ 42 is determined not only by ongoing pathology but also by the total CSF A $\beta$  peptide concentration which is individually different (Wiltfang et al., 2007).

In agreement with previous reports (Firbank et al., 2007; Nagai et al., 2008), hypertension was related to global GM atrophy. Similarly, cerebellar involvement was not surprising, based on earlier volumetric study (Strassburger et al., 1997) and, we believe, also supports the notion that cerebellar Purkinje cells are particularly susceptible to ischemia (Cervos-Navarro and Diemer, 1991). Yet again, this observation requires replication because signal differences in the

cerebellum are often observed with SPM due to the registration difficulties in this region. We also found regional effects of HTN in both occipital and frontal regions. This replicates former findings of an increased atrophy in these areas in hypertensive subjects (den Heijer et al., 2005; Gianaros et al., 2006; Strassburger et al., 1997). We did not find an evidence for MTL atrophy with VBM approach, possibly it can be seen only in more advanced HTN. White matter hyperintensities are common in aging and are associated with brain atrophy (Godin et al., 2009); not surprisingly they were strongly related to HTN in our study. We repeated our analyses after accounting for their burden. It did not

Table 5  
SPM analysis

	Cluster extent	Brain region	Talaraich coordinates (x,y,z)	F value	
t-tau	2349	R parietal lobe, precuneus, BA 7	8, -65, 48	5.31	
		L parietal lobe, precuneus, BA 7	-8, -64, 44	4.92	
	230	L insula, BA 13	-26, -31, 14	4.84	
	611	R caudate tail	36, -34, 2	4.18	
		R thalamus	27, -31, 9	3.84	
92	R inferior temporal gyrus, BA 20	47, -18, -24	3.70		
p-tau <sub>231</sub>	1788	R superior temporal gyrus, BA 38	30, 8, -26	5.56	
		R superior temporal gyrus, BA 38	39, 1, -15	5.01	
		R limbic lobe, BA 36	23, -4, -30	4.39	
	201	R limbic lobe, BA 28	29, -10, -25	4.02	
		R inferior temporal gyrus, BA 20	47, -19, -27	4.13	
		L parietal lobe, precuneus, BA 7	-26, -69, 28	4.12	
	249	R inferior parietal lobule, BA 40	36, -49, 55	3.99	
	A $\beta$ 42/A $\beta$ 40 <sup>a</sup>	151	L inferior temporal gyrus, BA 20	-51, -9, -20	3.87
			R thalamus	3, -19, 15	3.70
		91	L amygdala	-30, -4, -24	3.62
			L thalamus	-6, -22, -1	4.37
		685	R thalamus	8, -17, 1	3.75
			L midbrain	-3, -15, -6	3.70
386		L thalamus	-11, -28, 14	4.26	
293		R thalamus	12, -29, 12	3.98	
		R caudate	15, -8, 17	3.53	
121		L insula, BA 13	-41, 17, 2	3.87	
77	L lentiform nucleus	-20, 0, 7	3.73		
t-tau/A $\beta$ 42 <sup>a</sup>		None			
p-tau <sub>231</sub> /A $\beta$ 42 <sup>a</sup>	111	L superior temporal gyrus, BA 38	-54, 5, -8	5.52	
		R temporal lobe, BA 21	41, -3, -12	4.53	
	102	R superior temporal gyrus, BA 38	30, 9, -27	4.10	
		R middle temporal gyrus, BA 21	64, -24, -6	3.68	

Regions showing significant correlations between CSF Alzheimer's disease (AD) biomarkers and gray matter density. All correlations were negative except for these presented for A $\beta$ 42/A $\beta$ 40, which were positive. All analyses accounted for age, gender and education.  $p < 0.001$ , minimal cluster size = 75.

Key: A $\beta$ 42/A $\beta$ 40, amyloid beta ratio; BA, Brodmann area; L, left; p-tau<sub>231</sub>, hyperphosphorylated tau; R, right; SPM, statistical parametric mapping; t-tau, total tau;

<sup>a</sup> Results presented for 86 subjects.

change the association between AD biomarkers and GM, but somewhat lessened the relationship between HTN and brain atrophy, confirming that they can mediate HTN effects on the brain.

In addition to VBM analyses we performed direct comparisons of MTL GM density extracted with automated MTL ROI (Li et al., 2008). Both high tau markers and HTN were related to lower MTL GM density. This last observation supports the results published by Korf et al. (2004). The discrepancies between VBM and ROI approach came from different significance threshold. In unbiased VBM it was set up to 0.001 while for ROIs analyses we used a more liberal, 50 times higher threshold of 0.05. Altogether ROI analyses confirmed that markers of tau pathology are related to MTL damage, pointed out to HTN as another factor possibly implicated in MTL atrophy and indicated additive effects of abnormal CSF tau markers and HTN on MTL GM. None-

theless, due to less stringent statistical criteria they require further replication.

AD CSF biomarkers and HTN seemed to have additive effects on the brain. The simultaneous presence of both high p-tau<sub>231</sub> or high p-tau<sub>231</sub>/A $\beta$ 42 and HTN were associated with more global GM atrophy than either marker individually. Similar results were seen in MTL ROIs analyses. The presence of both high t-tau or p-tau<sub>231</sub> and HTN was associated with more MTL GM atrophy than either marker alone. In all analyses group differences followed a gradual change in global GM volume or MTL GM density, suggesting rather additive than interaction effects.

The prevalence of HTN across biomarker groups was not different and HTN groups did not differ in their biomarker levels. Pathology reports are contradictory. Some studies, consistent with our results, show a lack of association between AD neuropathology and HTN (Wang

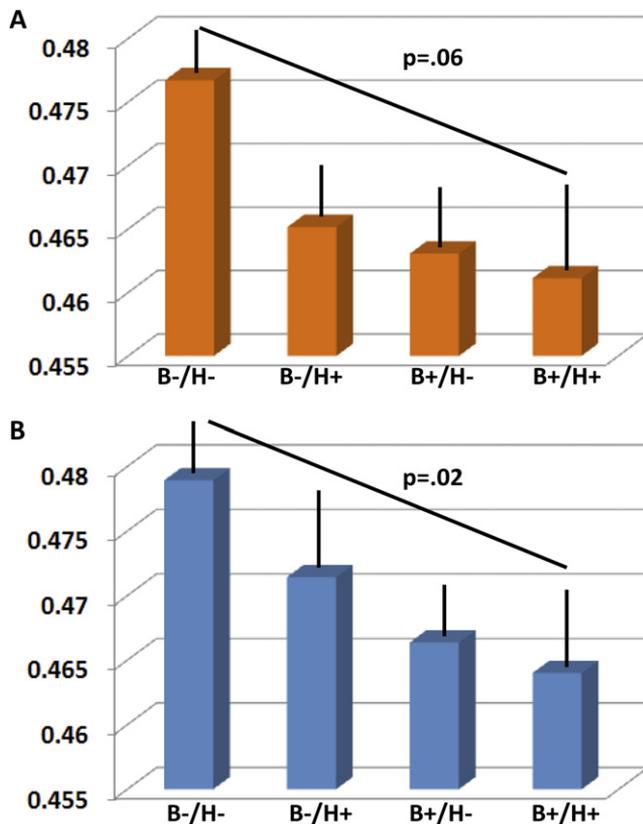


Fig. 3. Estimated means for global gray matter (GM) volume (% of intracranial volume [ICV]) after accounting for age, gender, and education, as a function of hyperphosphorylated tau (p-tau<sub>231</sub>)-hypertension (HTN) groups (A), and p-tau<sub>231</sub>/amyloid beta (Aβ<sub>42</sub>)-HTN groups (B). Please note a linear decrease in GM volumes from the group without any risk markers (B-/H-) to the group with both risk factors (B+/H+). The groups expressing only 1 risk marker show intermediate values. Bars represent standard errors. B-/H-, normal biomarker level and HTN absent; B-/H+, normal biomarker level and HTN present; B+/H-, abnormal biomarker level and HTN absent; B+/H+, abnormal biomarker level and HTN present.

et al., 2009) or atherosclerosis (Luoto et al., 2009), while others report increased numbers of hippocampal neurofibrillary tangles and senile plaques in HTN patients (Petrovitch et al., 2000; Sparks et al., 1995). Possibly in our group HTN impact was not yet severe enough to cause AD-related abnormalities, detectable in CSF. The eligibility criteria precluded inclusion of subjects with substantial vascular pathology (i.e., uncontrolled hypertension). Notably, the mean systolic and diastolic blood pressures, although different, were within normal ranges for both subjects with and without HTN. Also other risk factors like smoking or hyperglycemia were uncommon. This relatively low HTN burden may also help explain the lack of regional biomarker and HTN interactions. Possibly, these interactions can be seen only with more severe hypertension and/or in later stages of cognitive deterioration, or in cognitively normal population only with functional but not structural imaging.

We did not find differences in cognitive performance between the biomarker groups, the HTN groups, or biomarker-HTN groups, which may weaken the importance of our results. This unexpected finding is perhaps due to the entry criteria for the study. All the participants were cognitively healthy, and subjects with mild cognitive impairment were rigorously excluded. Thus, the differences in performance must have been subtle or else the subject would not have been included. Finally, it is possible that MRI changes are more sensitive and precede changes in cognitive measures. Only longitudinal study can confirm risk for decline among these biomarker and HTN groups.

The prevalence of “abnormal” biomarker’s levels in clinically healthy subjects merits a discussion. Thirty percent of our subjects were classified as having high t-tau, 21% as having high p-tau<sub>231</sub>, and more than 40% as having low Aβ<sub>42</sub>/Aβ<sub>40</sub>. Arguably, such high prevalence can put in question their validity as indicators of pathology. Alternatively, maybe they should be seen more as trait not state dependent. Finally, the characteristic of our cohort can explain these numbers: despite all efforts, a selection bias associated with recruitment to memory and aging studies cannot be avoided. Many participants, although healthy, come because of the concern related to subjective memory impairment or having a family member with AD. Thus our sample may suffer from “self-enrichment”.

The study had several limitations. Our population was highly educated, predominantly Caucasian, with moderate levels of vascular risk, thus further studies of more heterogeneous populations are needed. The present VBM and automated ROI findings need to be replicated with manually traced regions of interest. We believe that evaluation of brain amyloid deposits with PiB, would be also a better indicator of pathology than CSF amyloid levels. Also perfusion studies may be warranted for better assessment of HTN influence on brain. It would be interesting to examine how both HTN and CSF AD biomarkers affect cerebral blood flow, because functional changes associated with them may be more prominent than structural changes.

In conclusion, in cognitively healthy subjects CSF tau markers were associated predominantly with lower GM estimates in structures typically affected in early AD, highlighting their validity as indicators of pathology at the presymptomatic stage. These associations seem to be present early, when no clear cognitive abnormalities can be detected. The simultaneous presence of hypertension and abnormal biomarkers was associated with more pronounced GM atrophy. Neurofibrillary pathology and HTN both seem to contribute to brain damage before cognitive impairment is present. Lack of regional interactions in VBM and gradual increase in atrophy across biomarker-HTN groups indicates rather additive than in-

teraction effects. Longitudinal studies are warranted to assess the risk for cognitive decline.

### Disclosure statement

Dr. Zinkowski is an employee of Applied NeuroSolutions, Inc. and has stock options and owns stock in Applied NeuroSolutions, Inc. Dr. Blennow has served at an Advisory Board for Innogenetics, Ghent, Belgium.

Other authors have nothing to disclose in connection with this manuscript.

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