Cholinergic depletion and basal forebrain volume in primary progressive aphasia

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A B S T R A C T

Primary progressive aphasia (PPA) is a heterogeneous syndrome with various neuropathological causes for which no medical treatment with proven efficacy exists. Basal forebrain (BF) volume loss has been reported in PPA but its relation to cholinergic depletion is still unclear. The primary objective of this study was to investigate whether cholinergic alterations occur in PPA variants and how this relates to BF volume loss. An academic memory clinic based consecutive series of 11 PPA patients (five with the semantic variant (SV), four with the logopenic variant (LV) and two with the nonfluent variant (NFV)) participated in this cross-sectional in vivo PET imaging study together with 10 healthy control subjects. Acetylcholinesterase (AChE) activity was quantitatively measured in the neo- and allocortex using N-[11C]-Methylpiperidin-4-yl propionate (PMP)-PET with arterial sampling and metabolite correction. Whole brain and BF volumes were quantified using voxel-based morphometry on high-resolution magnetic resonance imaging (MRI) scans.

In the PPA group, only LV cases showed decreases in AChE activity levels compared to controls. Surprisingly, a substantial number of SV cases showed significant AChE activity increases compared to controls. BF volume did not correlate with AChE activity levels in PPA. To conclude, in our sample of PPA patients, LV but not SV was associated with cholinergic depletion. BF atrophy in PPA does not imply cholinergic depletion.

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1. Introduction

Primary progressive aphasia (PPA) is characterized by an isolated language impairment and is divided clinically into the subtypes of logopenic (LV), nonfluent (NFV) and semantic variant (SV) aphasia (Gorno-Tempini et al., 2011; Vandenberghe, 2016). Of the LV cases, 50–60% have underlying Alzheimer’s disease (AD) pathology. In 50–70% of NFV cases the underlying cause is frontotemporal lobar degeneration (FTLD)-tauopathy (i.e. corticobasal degeneration (CBD), progressive supranuclear palsy (PSP) or Pick’s disease pathology) and 69–83% of SV cases have FTLD-Transactive response DNA binding Protein 43 kDa (TDP-43) type C pathology (Grossman, 2010; Vandenberghe, 2016). Cholinergic depletion has been documented in typical AD (Bohnen et al., 2005, 2003; Kuhl et al., 1999), but it is currently unknown whether a cholinergic deficit also occurs in atypical AD presenting as PPA or in PPA due to a tauopathy or TDP-43 proteinopathy.

According to MRI volumetric studies, the basal forebrain (BF) is atrophic in PPA SV and NFV (Teipel et al., 2016, 2014), and mainly so the posterior part of the nucleus basalis (Ch4) and nucleus subparaventricularis (NSP). The BF is the primary source of cholinergic transmission to the neo- and allocortex (Zaborszky et al., 2015), hence the hypothesis that a cholinergic deficit may also exist in non-AD cases of PPA.

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Post mortem morphometric studies of the BF have mainly focused on AD or on FTLD-tau. In AD, severe degeneration of the BF, mainly the posterior part of Ch4, has been reported (Baker-Nigh et al., 2015; Grothe et al., 2014; Kerbler et al., 2015; Teipel et al., 2005). In FTLD-tau, Ch4 degeneration has been shown in PSP (Kasashima and Oda, 2003; Tagliavini et al., 1984), whereas both degeneration (Kasashima and Oda, 2003) and preservation (Dickson, 1999) of Ch4 have been reported in CBD.

Cholinergic denervation resulting from BF volume loss may be reflected in altered AChE activity levels. AChE activity levels can be mapped in vivo using N-[11C]-Methyl-4-piperidyl acetate ([11C]MP4A)- or N-[11C]-Methylpiperidin-4-yI propionate ([11C]MP)-PET (Irie et al., 1996). The rate-dependent enzyme for acetylcholine (ACh) degradation i.e. acetylcholinesterase (AChE) is present at pre- and postsynaptic neurons (Wevers, 2011) and in a soluble form at the synaptic cleft (Scheff et al., 1992).

Studies using [11C]MP4A and [11C]MP-PET have shown thalamic cholinergic depletion in PSP (Hirano et al., 2010; Shinotoh, 1999) and cortical cholinergic depletion in CBD (Shinotoh, 2007) and typical AD. Reduced AChE levels, as measured with [11C]PMP-PET, have also been found in the neocortex and thalamus in two patients with FTDP-17 mutations (Hirano et al., 2010). To date, no PET study investigated the cholinergic system in PPA exclusively.

The primary objective of this study was to investigate whether cholinergic alterations occur in PPA and how this depends on the variant. Cholinergic activity is crucial for several cognitive processes (Tagliavini et al., 1984), whereas both degeneration (Kasashima and Oda, 2003; Tagliavini et al., 1984), whereas both degeneration (Kasashima and Oda, 2003) and preservation (Dickson, 1999) of Ch4 have been reported in CBD.

### Table 1

| Case | PPA | **Age (y)** | **Symptom duration (y)** | **Gender** | **CDR** | **BNT** | **PALPA** | **AAT-compr** | **BORB-hard** | **BORB-easy** | **CPM** |
|------|-----|-------------|--------------------------|-----------|---------|-------|---------|------------|-------------|-------------|---------|
| 5<sup>th</sup> LV / | 59 | 2 | m | 2 | / | 15<sup>a</sup> | 83<sup>b</sup> | 21<sup>b</sup> | 27<sup>b</sup> | 20<sup>b</sup> |
| 6<sup>th</sup> LV / | 77 | 2 | m | 1 | 39<sup>a</sup> | 25 | 102<sup>a</sup> | 22<sup>a</sup> | 30 | 29 |
| 7<sup>th</sup> LV / | 66 | 3 | m | 1 | 35<sup>a</sup> | 27 | 94<sup>a</sup> | 23a | 32 | 23<sup>a</sup> |
| 8<sup>th</sup> LV AD | 72 | 2 | f | 1 | 24<sup>c</sup> | 26 | 83<sup>a</sup> | 25 | 29 | 17 |
| 13 LV | 57 | 0.5 | f | 0.5 | 56 | 28 | 120 | 25 | 29 | 35 |
| 17 LV | 64 | 3 | m | 0.5 | 41<sup>b</sup> | 23<sup>b</sup> | 114 | 25 | 31 | 33 |
| 23 LV | 71 | 1 | m | 0 | 53 | 28 | 118 | 26 | 31 | 33 |
| 18 LV | 62 | 1.5 | f | 1 | 53 | 27 | 106 | 24 | 31 | 31 |
| 1<sup>st</sup> NFV CBD | 77 | 3 | m | 1 | 43<sup>a</sup> | 25 | 105 | 21<sup>a</sup> | 30 | 24<sup>a</sup> |
| 11<sup>th</sup> NFV | 71 | 2.5 | m | 1 | 45<sup>a</sup> | 29 | 112 | 27 | 30 | 21<sup>a</sup> |
| 15 NFV | 52 | 2 | f | 2 | 58 | 28 | 120 | 26 | 31 | 31 |
| 22 NFV | 79 | 5 | f | 1 | 48 | 22<sup>a</sup> | 105 | 25 | 30 | 24<sup>a</sup> |
| 26 NFV | 71 | 1.5 | m | 0.5 | 55 | 28 | 102<sup>a</sup> | 26 | 31 | 24<sup>a</sup> |
| 27 NFV | 78 | 2.5 | m | 1 | 48 | 23<sup>c</sup> | 77 | 26 | 29 | 30 |
| 28 NFV | 72 | 2.5 | f | 1 | 30<sup>a</sup> | 19<sup>a</sup> | 85<sup>a</sup> | 21<sup>a</sup> | 18<sup>a</sup> | 12<sup>a</sup> |
| 29 NFV | 71 | 5 | f | 0 | 41<sup>b</sup> | 24<sup>b</sup> | 104<sup>b</sup> | 22<sup>b</sup> | 38 | 32 |
| 2<sup>nd</sup> SV | 59 | 4 | m | 1 | 36<sup>a</sup> | 22<sup>a</sup> | 78<sup>a</sup> | 17<sup>a</sup> | 23<sup>a</sup> | 21<sup>a</sup> |
| 3<sup>rd</sup> SV | 63 | 3 | f | 1 | 14<sup>a</sup> | 24<sup>a</sup> | 95<sup>a</sup> | 22<sup>a</sup> | 25<sup>a</sup> | 23<sup>a</sup> |
| 4<sup>th</sup> SV | 71 | 6 | m | 1 | 13<sup>a</sup> | 22<sup>a</sup> | 95<sup>a</sup> | 24 | 28 | 17<sup>a</sup> |
| 9<sup>th</sup> SV TDP-43 I | 52 | 1.5 | f | 1 | / | 17<sup>a</sup> | 62<sup>a</sup> | 21<sup>a</sup> | 23<sup>a</sup> | 36 |
| 10<sup>th</sup> SV | 57 | 2 | m | 1 | 14<sup>a</sup> | 22<sup>a</sup> | 87<sup>a</sup> | 19<sup>a</sup> | 26<sup>a</sup> | 34 |
| 12 SV | 76 | 6 | m | 1 | 34<sup>a</sup> | 27 | 90<sup>a</sup> | 20<sup>a</sup> | 23<sup>a</sup> | 29 |
| 14 SV | 70 | 1.5 | f | 1 | 22<sup>a</sup> | 17<sup>a</sup> | 74<sup>a</sup> | 20<sup>a</sup> | 18<sup>a</sup> | 24<sup>a</sup> |
| 16 SV | 61 | 3 | m | 2 | 7<sup>a</sup> | 18<sup>a</sup> | 73<sup>a</sup> | 18<sup>a</sup> | 20<sup>a</sup> | 36 |
| 19 SV | 64 | 3 | m | 1 | 20<sup>a</sup> | 14<sup>a</sup> | 74<sup>a</sup> | 18<sup>a</sup> | 26<sup>a</sup> | 29 |
| 21 SV | 48 | 6 | f | 1 | 12<sup>a</sup> | 24<sup>a</sup> | 82<sup>a</sup> | 17<sup>a</sup> | 26<sup>a</sup> | 35 |
| 24 SV | 58 | 2 | f | 1 | 35<sup>a</sup> | 27 | 93<sup>a</sup> | 27 | 31 | 36 |
| 25 SV | 68 | 5 | f | 1 | 16<sup>a</sup> | 23<sup>a</sup> | 114 | 25 | 31 | 34 |

**Controls**

| Groupdata | PPA | Mean (SD) | **Symptom duration (y)** | **Gender** | **CDR** | **BNT** | **PALPA** | **AAT-compr** | **BORB-hard** | **BORB-easy** | **CPM** |
|-----------|-----|-----------|--------------------------|-----------|---------|-------|---------|------------|-------------|-------------|---------|
| Controls | 65.8(6.7) | 2.56(1.5) | 13(15) | 0.89 | 34.3(15.7) | 23.4(4.2) | 94.7(16.1) | 22.6(3.1) | 27.4(4.1) | 27.6(4.7) | 32.2(3.2) |

**Abbreviations:** AAT = Aachen aphasia test, comprehension (120); BORB-hard/easy = Birmingham object recognition battery (212); BNT = Boston Naming test (60); CBD = corticobasal degeneration, CDR = Clinical dementia rating scale, CPM = colored progressive matrices (76); PALPA = Psycholinguistic assessment of language in aphasia, subtest 49: associative-semantic task for words (70). PPA variants: LV = logopenic variant, NFV = nonfluent variant, SV = semantic variant PPA patients.

<sup>a</sup> Scores which are significantly different compared to controls based on a modified t-test.

<sup>b</sup> Patients who received [11C]PMP-PET.
All subjects underwent extensive neuropsychological testing. Global cognitive functioning was assessed by the clinical dementia rating scale (CDR). Language was assessed by means of the 60-item version of the Boston Naming test (BNT), the validated Dutch version of the Aachen Aphasia test (AAT) and the verbal semantic association test of the psycholinguistic assessment of language processing in aphasia (PALPA subtest 49). Visual recognition of objects was assessed by the object decision test of the Birmingham object recognition battery (BORB) hard and easy. Fluid intelligence and reasoning ability were quantified by the Raven’s colored progressive matrices (CPM).

2.2. PET acquisition and analysis

[11C]PMP was synthesized as described previously (Snyder et al., 1998). The injected activity was 261.3 ± 28.31 MBq in control subjects and 290.3 ± 62.6 MBq in PPA patients. Dynamic PET data were acquired in 3D mode during 60 min (rebinned in 4 × 15 s, 4 × 1 min, 2 × 2.5 min, 10 × 5 min) on a Siemens/ECAT EXACT HR+ PET scanner (CTI PET System, Inc., Knoxville, TN 37932, USA). Data were reconstructed using 3D-filtered back-projection with a Hanning filter with cut-off frequency of 0.5 of the Nyquist frequency, corrected for random scatter, attenuation and decay and smoothed with a 6 mm isotropic full-width at half-maximum (FWHM) Gaussian 3D kernel.

For each subject 19 arterial samples were collected during the scan period to determine the individual plasma time-activity curve (TAC). Seven additional samples were used for metabolite correction. One LV PPA patient (case 6) (Table 1) and one control subject had to be excluded due to arterial sampling problems. One control subject was excluded based on excessive movement during PET scan acquisition. The remaining eight control subjects and 10 PPA patients were included in our PET-based analyses.

Blood samples were centrifuged, and the radioactive plasma was subsequently eluted on high-performance liquid chromatography and counted in a gamma counter. The intact fractions of tracer over time and decay and smoothed with a 6 mm isotropic full-width at half-maximum (FWHM) Gaussian 3D kernel.

PET images were processed using Statistical Parametric Mapping 8 (SPM8, Wellcome Trust Centre for Neuroimaging, London, UK). For each subject, subject-specific volumes of interest (VOI) were calculated by intersecting the subjects normalized gray matter (GM) map (thresholded at 0.25) with each automatic anatomic labeling (AAL) (Tzourio-Mazoyer et al., 2002) VOI. With the metabolite-corrected plasma TACs as input, regional brain TACs were fitted to the theoretical two-tissue three-rate constant compartment model including a cerebral blood volume fraction. This model characterizes rate constants for influx ($K_1$, min⁻¹) and efflux ($K_2$, min⁻¹) across the blood-brain barrier (first tissue compartment) and the rate of hydrolysis ($K_3$, min⁻¹) of $[11C]PMP$ (second tissue compartment) (Koepp et al., 1999). The $K_3$ values were calculated for each subject-specific VOI (Fig. A.1). These $K_3$ values are a proxy for AChE activity levels in the brain (Koepp et al., 1999). Left and right hemispheres were analyzed separately. To reduce spillover from high uptake regions to regions with low or moderate uptake, we additionally applied a mask in VOIs with low or moderate uptake. This mask contained all voxels with high uptake values across all control subjects and as a result, it excluded these voxels within VOIs with low or moderate uptake (spill-over correction). We then calculated a weighted mean $k_v$ value by taking into account the size of each subject-specific VOI of the left and right neocortex (including frontal, parietal, temporal and occipital neocortical VOIs) and left and right allocortex (including olfactory gyri, amygdala, hippocampus and parahippocampal gyrus VOIs) to obtain weighted mean $k_v$ values in known projection regions of the BF (Mesulam, 2013).

2.3. MRI acquisition and analysis

The 11 PPA and 10 HC received a T1-weighted structural MRI on a 1.5T Siemens Sonata system (Siemens Medical Solutions, Erlangen, Germany) and eight-channel head coil (coronal inversion recovery prepared 3D gradient-echo images; inversion time (TI) 800 milliseconds (ms), echo time (TE) = 3.93 ms, voxel size (1 × 1 × 1) mm³).

Preprocessing of the T1-weighted images was performed with Voxel-Based Morphometry 8 (VBM8) (Ashburner and Friston, 2000), implemented in Matlab R2012b as described previously (Gillebert et al., 2015). This procedure generated non-linear only modulated GM maps, corrected for total intracranial volumetric differences. These GM maps were used to obtain mean BF GM volumes by averaging the unsmoothed modulated warped GM voxel values within the BF mask (Kiliman et al., 2014). All BF volumes have been adjusted for overall brain size by using only the nonlinear component of the spatial normalization for modulation of GM voxel intensities.

In order to determine whether any AChE changes occurred specifically in regions that were typically affected in a particular subtype, an extended sample of PPA and controls were analyzed to determine the regions of subtype-specific atrophy with high sensitivity. In addition to the initial sample of 11 PPA and 10 control subjects, this extended sample contained 24 additional controls who had undergone a volumetric research MRI on the same scanner using the same sequence as mentioned above as well as 17 additional PPA (4 LV, 6 NFV and 7 SV) (Grube et al., 2016) and 77 additional controls who had undergone a T1-weighted structural MRI on a 3T Philips Intera system and an eight-channel head coil (coronal inversion recovery prepared 3D gradient-echo images; TI 900 ms, TE = 4.5 ms, voxel size (0.98 × 0.98 × 1.2) mm³).

To identify subtype-specific atrophy, we contrasted the group of 28 PPA cases to the 111 controls in a voxelwise ANCOVA in SPM8 with smoothed modulated GM volume maps as within-subject factor, group (4 levels: LV, SV, NFV, healthy controls) as between-subject factor and age, gender and scanner as nuisance variables. Results were considered significant at voxel-level uncorrected p < 0.001 combined with cluster-level family-wise error (FWE)-corrected p < 0.05 (Poline et al., 1997). For voxelwise statistical comparisons, GM maps were smoothed with an 8 mm isotropic FWHM Gaussian 3D kernel.

2.4. Statistics

Standard statistical analyses were performed in Statistics Software Package for the Social Sciences (version 23, IBM Statistics, Armonk, USA).

BF volumes and AChE activity levels were checked for outliers using the Grubbs’ test: Z scores higher than three standard deviations were classified as outliers. The individuals’ Neuropsychological test scores were compared to controls using a modified t-test (Crawford and Garthwaite, 2012) (Table 1). Differences in age between PPA patients and control subjects were assessed using Kruskal-Wallis and with Pearson chi² tests for gender.

2.4.1. Primary outcome analyses

Given the relatively limited sample size, the primary analysis was based on a multiple single case series methodology. Differences of AChE activity levels in global neo- and the global allocortical VOIs in the left and right hemisphere were assessed in each individual patient in comparison to the eight controls using a modified t-test (Crawford and Garthwaite, 2012).

As a second primary outcome analysis, a Spearman or Pearson correlational analysis was performed between AChE activity levels averaged over left and right allo- and neocortex, and BF volume, depending on normality of the data.
2.4.2. Secondary outcome analyses

Secondary outcome analyses aimed to evaluate effects at the overall group and at the subgroup level, to examine the relationship between the AChE activity levels and structural changes, and to test the replicability of the BF atrophy in PPA reported previously (Teipel et al., 2016, 2014).

1. Differences in AChE activity were assessed at the group level (PPA versus controls) using Kruskal-Wallis and with post hoc Mann-Witney U tests for differences between subgroups (uncorrected, two-tailed p values).

2. In order to evaluate the relationship between AChE activity levels and structural atrophy, AChE activity levels were calculated in each individual patient compared to controls in the regions that were characteristically atrophic for the subtype to which the case belonged (LV, NFV or SV) (VOI shown in Fig. 3). In order to determine whether AChE activity levels were specifically lower in the atrophic regions, AChE activity levels were also evaluated in cortical regions falling outside subtype-specific regions of atrophy. The latter region was determined by subtracting the region of characteristic atrophy from the subjects normalized GM map (thresholded at $\alpha=0.25$). For each individual patient, differences in AChE levels compared to controls were assessed using a modified $t$-test.

3. In order to evaluate whether BF volume decreases that have previously been reported (Teipel et al., 2016, 2014) could be replicated, a BF volume analysis was performed in the extended sample of 28 PPA and 111 control subjects. By means of an ANCOVA model, BF volumes were assessed at the group level in controls, LV, NFV and SV PPA with scanner type as nuisance variable, and post hoc Bonferroni correction for the number of comparisons at $\alpha=0.05$.

4. Finally, we determined the effects of symptom duration, age, and gender on AChE activity levels by means of Spearman or Pearson correlation analyses or Mann Witney U tests (two-tailed).

3. Results

3.1. Primary outcome analyses

3.1.1. AChE activity levels in PPA patients

Two out of three LV patients showed significantly lower neocortical AChE activity levels than controls in the left (case 7: $t = -2.68, p = 0.016$ and case 8: $t = -4.12, p = 0.002$) (Fig. 1A) and in the right hemisphere (case 7: $t = -3.17, p = 0.008$ and case 8: $t = -5.59, p < 0.001$) (Fig. 1B). No differences were found in NFV or SV cases compared to controls (all $p > 0.13$) (Fig. 1A, B).

In two out of three LV patients, left allocortical AChE activity levels were significantly lower than in controls (case 7: $t = -1.93, p = 0.047$ and case 8: $t = -2.70, p = 0.015$) (Fig. 1C). In one of these cases, right-hemispheric allocortical AChE activity levels were also lower compared to controls (case 8: $t = -4.20, p = 0.002$) (Fig. 1D).

Four out of five SV patients showed significantly elevated right allocortical AChE activity levels (case 3: $t = 3.39, p = 0.006$; case 4: $t = 4.57, p = 0.001$; case 9: $t = 2.26, p = 0.029$; case 10: $t = 3.28$, ...)
In one of these cases, left allocortical AChE activity levels were significantly higher than in controls (case 10: \( t = 2.0, p = 0.042 \)) (Fig. 1C). No significant differences compared to controls were found in NFV patients (all \( p > 0.37 \)).

In order to gain further insight in the elevation of allocortical AChE activity levels in SV cases, we analyzed each of the four subVOIs of which the allocortical VOI was composed. The elevation of AChE activity was mainly seen in the subVOI corresponding to the right parahippocampal gyrus (case 3: \( t = 3.72, p = 0.004 \); case 4: \( t = 5.22, p < 0.001 \); case 9: \( t = 2.11, p = 0.036 \) and case 10: \( t = 3.93, p = 0.003 \)) and least so in the subVOI corresponding to the olfactory gyrus (case 9: \( t = 1.95, p = 0.046 \)) (Fig. 1C and D).

### 3.1.2. Correlation between BF volume and AChE activity levels

BF volume in PPA patients did not correlate with AChE activity levels in the left (Spearman \( \rho(8) = 0.16, p = 0.65 \)) or right neocortex (Pearson \( \rho(8) = 0.37, p = 0.29 \)) (Fig. 2A and B), nor in the left (Pearson \( \rho(8) = -0.24, p = 0.51 \)) or right allocortex (Pearson \( \rho(8) = 0.28, p = 0.43 \)) (Fig. 2C and D).

### 3.2. Secondary outcome analyses

#### 3.2.1. Group-based comparisons of AChE activity levels in neo- and allocortex

Left neocortical AChE activity differed significantly between groups (chi\(^2\)(3) = 9.5, \( p = 0.024 \)). Only LV patients showed significantly lower left neocortical AChE activity levels compared to controls (U = 2, \( p = 0.041 \)) and compared to SV patients (U = 0, \( p = 0.025 \)). No differences were found between other subgroups (all \( p > 0.053 \)) (Fig. 2A, B).

Right allocortical AChE activity levels were significantly different between groups (chi\(^2\)(3) = 11.3, \( p = 0.010 \)) (Fig. 2D). SV patients had significantly elevated right allocortical AChE activity levels compared to controls (U = 0, \( p = 0.003 \)) and compared to LV (U = 0, \( p = 0.025 \)). No significant differences were found between other subgroups (all \( p > 0.053 \)).

#### 3.2.2. Topography of AChE activity changes versus topography of cortical volume loss in PPA

##### 3.2.2.1. Definition of PPA atrophic regions

In the extended sample of PPA patients and controls, LV patients showed atrophy restricted to the left posterior third of the left middle temporal gyrus, extending towards the temporoparietal junction as well as bilateral atrophy of the precuneus (Fig. 3A). NFV patients exhibited atrophy of the bilateral supplementary motor area and cingulum, together with left inferior and middle frontal lobe atrophy extending towards the left insula (Fig. 3B). SV patients showed severe atrophy of the bilateral anterior temporal lobe, including the anterior fusiform gyri, as well as atrophy of the amygdalae, hippocampi and insulae (Fig. 3C).

Overall, there was a predominant left lateralization of atrophy independently of PPA subtype.

##### 3.2.2.2. AChE activity levels in the PPA atrophic and preserved neocortical regions

At the individual patient level, two out of three LV patients showed lower AChE activity levels compared to controls in brain regions typically atrophic in LV PPA (case 7: \( t = -2.65, p = 0.016 \) and case 8: \( t = -6.20, p < 0.001 \)) (Fig. 4A). These two LV patients showed also lower AChE activity levels (case 7: \( t = -3.04, p = 0.009 \) and case 8: \( t = -4.73, p = 0.001 \)) compared to controls outside the regions that were typically atrophic in LV PPA (Fig. 4B). This suggests that the reduction in AChE activity levels is not restricted to the regions of characteristic atrophy in LV but is more diffuse.

Compared to controls, two of the five SV patients showed elevated AChE activity levels in regions typically atrophic in SV PPA (case 9: \( t = 2.09, p = 0.038 \); case 10: \( t = 1.88, p = 0.05 \)) (Fig. 4A). SV patients did not show abnormal AChE activity levels outside the SV atrophic...
regions (Fig. 4B). This may suggest that the increase in AChE activity levels in SV could potentially be most pronounced in those regions that are also most affected structurally. No significant differences were found for NFV patients compared to controls (all p > 0.31).

3.2.3. BF volumetry in the extended PPA sample

In the entire group of PPA patients, BF volume was reduced in three out of eight LV (case 5: \( t = -1.75, p = 0.042 \), case 7: \( t = -2.41, p = 0.009 \) and case 17: \( t = -2.00, p = 0.023 \)), one out of eight NFV (case 11: \( t = -1.91, p = 0.029 \)) and five out of 12 SV patients (case 2, \( t = -3.42, p < 0.001 \), case 9: \( t = -1.91, p = 0.029 \), case 14: \( t = -2.45, p = 0.008 \), case 19: \( t = -2.56, p = 0.006 \) and case 25: \( t = -3.07, p = 0.001 \)) compared to controls (Fig. 5).

There was a significant overall effect of group on BF volume (\( F(3134) = 11.8, p < 0.001 \)). Post hoc tests revealed that BF volume was most reduced in SV compared to controls (−17.2 ± 11%, \( p < 0.001 \)). No significant differences were found in LV (−7.3 ± 13%, \( p = 0.40 \)) or NFV (−9.6 ± 7.8%, \( p = 0.07 \)) patients compared to controls. No significant differences of BF volume were found between PPA variants (\( p > 0.1 \)) (Fig. 5).

Fig. 3. Cortical atrophy patterns in PPA. T-maps, generated by contrasting each PPA subgroup (total N = 28) versus controls (N = 111) in SPM8, are projected onto a surface rendering of a template brain and on coronal slices with corresponding MNI coordinates. Threshold: voxel-level uncorrected \( p < 0.001 \) combined with cluster-level FWE-corrected \( p < 0.05 \). T-maps depict brain regions of reduced gray matter volume in (A) logopenic variant (LV), (B) nonfluent variant (NFV), and (C) semantic variant (SV) PPA patients.

Fig. 4. AChE activity levels in subtype-specific PPA atrophic regions and in the associative-semantic network. AChE activity based on a modified t-test with a threshold of \( \alpha = 0.05 \) in (A) subtype-specific atrophy in PPA and (B) complementary non-atrophic brain regions. Note that the subtype-specific regions differed depending on the variant and that the values plotted for each subtype are obtained in the regions that are atrophic for that particular subtype. Accordingly, the corresponding values in the controls were obtained in that same subtype-specific region. Patients that significantly differed from controls are indicated with an asterisk on the color bar. Abbreviations: LV = logopenic variant, HC = healthy controls, NFV = nonfluent variant, SV = semantic variant. Case numbers correspond to Table 1.
3.2.4. Effects of age, gender and symptom duration on AChE activity

In the current PPA sample, there were no effects of symptom duration on AChE activity levels in the left or right neo- or allocortical region (p > 0.30) and no effects of age (p > 0.058) or gender (p > 0.57) on AChE activity levels.

4. Discussion

The current findings demonstrate that BF atrophy does not imply cholinergic depletion. The presence of BF atrophy in SV was replicated, however this was not associated with a cholinergic deficit. Instead AChE activity in SV was increased. Cholinergic depletion was only seen in LV cases, both within and outside the neocortical regions of characteristic atrophy.

4.1. Cholinergic alterations

The LV with pathologically definite AD (case 8) showed the strongest reductions in AChE activity levels. LV PPA can occur as an atypical variant of AD and 50–60% of cases show AD pathology (Matías-Guiu et al., 2015). Loss of the cholinergic systems integrity is among the earliest events in the pathogenesis of typical AD: several studies have shown reduced AChE activity in typical AD patients using [11C]PMP-PET (Bohnen et al., 2005, 2003; Kuhl et al., 1999). The current study shows that this finding can be extended to a subset of LV PPA cases.

The reduction in AChE activity did not appear to be limited to regions typically affected by volume loss in LV PPA. AChE activity reductions were seen also outside regions of typical atrophy. If AChE activity reductions in LV are a consequence of neuronal loss in diffusely projecting subcortical nuclei such as Ch4, one would expect that the PET measure would not strictly overlap with the measure of regional volume loss.

Unexpectedly, despite the BF volume loss, SV PPA was associated with an increase in AChE activity levels. This increase was most pronounced in regions of maximal volume loss, both in the neocortical and in the allocortical regions such as the parahippocampal gyrus. The colocalisation between AChE activity increases and structural volume loss could possibly suggest that AChE activity increases are a compensatory mechanism and a reaction to neuronal loss in SV PPA. This increase was only observed in SV cases and the vast majority of SV PPA is caused by a TDP-43 proteinopathy (Grossman, 2010; Josephs et al., 2009). In the current study, SV case 9 received a diagnosis of pathologically definite FTLD with TDP-43 inclusions subtype 1. A previous expression study in transgenic mice (Tg-VLW) expressing human tau mutations, suggested that tauopathy may be associated with AChE activity increases (Silveira et al., 2012). This is the first study which showed AChE increases in a case with proven TDP-43 proteinopathy. It may be worth investigating how specific types of proteinopathy differentially affect neurotransmitter systems and the regional specificity of such effects.

Neuropathological examinations of other cholinergic biomarkers in semantic dementia showed that there is an increase in the proportion of cholinergic M2 muscarinic receptors compared to controls, with a decrease in the proportion of cholinergic M1 muscarinic receptors (Odawara et al., 2003). The increase in M2 receptors found by Odawara as well as the increase in AChE activity levels in the current study are possibly suggesting a compensatory reaction to the neurodegenerative neuronal loss.

Elevated AChE activity levels as measured with PET have previously been reported in FTD, although non-significantly and restricted to the thalamus (Hirano et al., 2010). However, most studies on FTD failed to show any abnormalities in AChE, be it post mortem (Procter et al., 1999) or at the level of cerebrospinal fluid (CSF) (Wallin et al., 2003).

4.2. BF atrophy and relation to cholinergic alterations

The BF receives afferents from the amygdala and hippocampus (Aggleton et al., 1987). On their turn, cholinergic pathways arising from the BF project among others to the temporal lobe and the parahippocampal gyrus (Selden et al., 1998). These regions are typically affected in SV PPA in cross-sectional (Mummery et al., 2000; Rosen et al., 2002; Whitwell et al., 2005) and longitudinal studies of the disease (Brambati et al., 2007).

BF volume loss in SV however was not associated with cholinergic depletion. The neuronal, glial and interstitial compartments that contribute to MRI measures of BF are far broader than the cholinergic neuronal cell population. For instance, cholinergic neurons are typically found intermingled with inhibitory interneurons, the second most important BF cell types (Mesulam, 2013).
Our data caution against inferences based upon MRI BF volume measures with regards to cholinergic status in PPA.

4.3. A cholinergic therapy for PPA?

There are currently no medications approved by the US Food and Drug Administration (FDA) to treat PPA. Language regions contain a relatively high density of AChE-containing neurons (Husler and Gazzaniga, 1991), which may suggest a rational basis for cholinergic treatment in PPA. Cholinergic therapy improves language in various disorders affecting the language network (Bertheit et al., 2006; Ferris and Farlow, 2013; Tanaka et al., 1997; Yoon et al., 2015), although regulatory approval is restricted to clinically probable AD.

To date, only one study with an open-label period of 18 weeks and a randomized, placebo-controlled phase for eight weeks with galantamine included PPA patients (Kertesz et al., 2008). The overall language performance showed a trend towards stabilization over a total duration of eight weeks. A randomized, placebo-controlled study with memantine showed general good tolerance, however there were more frequent cognitive adverse events (Boxer et al., 2013). A therapeutic trial of donepezil in FTD failed to show any efficacy and a subgroup of patients experienced worsening of symptoms (Mendez et al., 2007).

Currently, cholinergic medication may be prescribed off-label in clinical practice for PPA patients who are amyloid positive (amyloid-PET or CSF amyloid/tau). AChE-PET allows one to directly discriminate PPA cases with a cholinergic deficit from those without a cholinergic deficit. This distinction could possibly predict a therapeutic response, but this remains to be demonstrated. The cholinergic deficit in LV in the current study provides a potential rationale for off-label use of AChE inhibitors (AChEi) in LV PPA due to underlying AD pathology. However our findings do not provide direct support for the use of AChEi in SV PPA, since AChE activity appears to be already naturally increased in this subtype compared to healthy controls.

4.4. Potential study limitations

The number of PPA patients included is small. This is inherent to the relatively high demands of the scanning procedures, in particular for dynamic PET acquisition with arterial sampling, and the relatively low prevalence of PPA (Gorno-Tempini et al., 2011). We calculated $k_3$ as index for AChE activity based on the well-established theoretical two-tissue three-rate constant compartment model with a cerebral blood volume fraction (Koepp et al., 1999). Cerebral atrophy does not influence the rate constants including $k_3$, nor does $k_3$ depend on blood flow or aging (Kuhl et al., 1999). Based on the compartment model used, partial volume effects on $k_3$ are negligible if spillover from neighboring regions can be neglected (Koepp et al., 1999; Kuhl et al., 1999). The effects of butyrylcholinesterase (BuChE), which has been shown to increase in the course of neurodegenerative diseases (Nordberg et al., 2013), on the $k_3$ values are also negligible (Kuhl et al., 1999).

The diffuse structure of the BF itself may cause difficulties to reliably delineate its borders on MRI. We therefore used the BF mask obtained with a procedure of Kilimann (2014) based on histological staining of post mortem brain sections which were subsequently transferred to MNI space (Kilimann et al., 2014). Given the small volume of each subnucleus, we decided to assess the BF volume as one single volume to as provide a more robust estimation. The distribution of the current BF mask used in this study was compared to two previous masks, one based on a single subject (Teipel et al., 2005), and one probabilistic map based on 10 subjects (Zaborszky et al., 2008) in a study of Teipel and colleagues (Teipel et al., 2016). There was a high general agreement between all three masks. These findings suggest that the anatomy of the BF nuclei delineated by means of this procedure is relatively stable across subjects.

5. Conclusion

We established involvement of the cholinergic system in PPA, both at the structural and functional level. The observed cholinergic deficit further encourages development of cholinergic PPA treatments in LV, possibly in NFP but not in SV PPA cases. Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.nicl.2016.11.027.

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