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Research report

Atrophy and structural covariance of the cholinergic basal forebrain in primary progressive aphasia

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ABSTRACT

Primary progressive aphasia (PPA) is characterized by profound destruction of cortical language areas. Anatomical studies suggest an involvement of cholinergic basal forebrain (BF) in PPA syndromes, particularly in the area of the nucleus subputaminalis (NSP). Here we aimed to determine the pattern of atrophy and structural covariance as a proxy of structural connectivity of BF nuclei in PPA variants. We studied 62 prospectively recruited cases with the clinical diagnosis of PPA and 31 healthy older control participants from the

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cohort study of the German consortium for frontotemporal lobar degeneration (FTLD). We determined cortical and BF atrophy based on high-resolution magnetic resonance imaging (MRI) scans. Patterns of structural covariance of BF with cortical regions were determined using voxel-based partial least square analysis. We found significant atrophy of total BF and BF subregions in PPA patients compared with controls [$F(1, 82) = 20.2$, $p < .001$]. Atrophy was most pronounced in the NSP and the posterior BF, and most severe in the semantic variant and the nonfluent variant of PPA. Structural covariance analysis in healthy controls revealed associations of the BF nuclei, particularly the NSP, with left hemispheric predominant prefrontal, lateral temporal, and parietal cortical areas, including Broca's speech area ($p < .001$, permutation test). In contrast, the PPA patients showed preserved structural covariance of the BF nuclei mostly with right but not with left hemispheric cortical areas ($p < .001$, permutation test). Our findings agree with the neuroanatomically proposed involvement of the cholinergic BF, particularly the NSP, in PPA syndromes. We found a shift from a structural covariance of the BF with left hemispheric cortical areas in healthy aging towards right hemispheric cortical areas in PPA, possibly reflecting a consequence of the profound and early destruction of cortical language areas in PPA.

1. Introduction

In 1892 Arnold Pick first described the syndrome of progressive aphasia in a patient with frontal and temporal lobe atrophy at autopsy (Pick, 1892). Almost 100 years later Marsel Mesulam defined the clinical entity of primary progressive aphasia (PPA) (Mesulam, 1982) which only recently has been stratified according to clinical phenomenology into the subtypes of semantic variant, logopenic variant and nonfluent/agrammatic aphasia (Gorno-Tempini et al., 2011). The relationship between the clinical syndromes of PPA and underlying neuropathology is not consistent. Tau pathology is most often found in nonfluent PPA, whereas the semantic variant frequently presents with Transactive response DNA binding protein 43 kDa (TDP-43) pathology (Bigio et al., 2010; M. Mesulam, 2013; Mesulam et al., 2008); Alzheimer's disease is the underlying pathology in more than 50% of cases with logopenic variant PPA, but is also found in 15–30% of cases with other PPA variants (Chare et al., 2014; Harris et al., 2013).

The clinical variants of PPA show distinct patterns of cortical atrophy (Bisenius, Neumann, & Schroeter, 2016; Gorno-Tempini et al., 2011; Rogalski et al., 2011; Schroeter, Raczka, Neumann, & Yves von Cramon, 2007). In addition to cortical atrophy, subcortical atrophy involving striatal and thalamic grey matter (GM) has been described in frontotemporal lobar degeneration (FTLD), including cases with PPA (Chow et al., 2008). In Alzheimer's disease (AD), a large body of evidence suggests early and selective involvement of the cholinergic system including decline of presynaptic markers of cholinergic activity in the cortex (Geula, Nagykery, Nicholas, & Wu, 2008; Henke & Lang, 1983) as well as degeneration and loss of cholinergic neurons in the basal forebrain (BF) nuclei (Baker-Nigh et al., 2015; Boissiere, Faucheux, Ruberg, Agid, & Hirsch, 1997; Strada et al., 1992; Vana et al., 2011). The involvement of the cholinergic system in PPA is still a matter of debate. The notion that a relevant proportion of PPA cases is associated with AD pathology would suggest involvement of the BF at least in subtypes of PPA. In addition, neurobiological evidence suggests that a dysbalance of the temporal lobe cholinergic and the dopaminergic innervation may play a significant role in language integrity (Tanaka & Bachman, 2000). Furthermore, Broca's region, the anterior language area, is characterized by a strong cholinergic innervation (Amunts et al., 2010). The nucleus subputaminalis (NSP), a cholinergic neuron cluster in the lateral extension of the Nucleus basalis Meynert, has first been described by Ayala in 1915 (Ayala, 1915). The nucleus of Ayala has only been described in humans and anthropoid monkeys, but not in other species including non-anthropoid primates. The NSP exhibits a strong left laterality; it is located in close proximity to the external capsule, suggesting a strong connectivity to the cortical language areas (Simic et al., 1999). These findings have raised the question whether the NSP is related to language function in the human brain.

Together, these findings point to a potential role of the cholinergic BF in the development of PPA syndromes. However, this question has only little been studied so far. While cholinergic markers have been studied post mortem in the behavioural variant of FTLD without evidence of major alterations (Procter, Qurne, & Francis, 1999), we are not aware of a post-mortem study on cholinergic markers in PPA. In a previous magnetic resonance imaging (MRI) study in a small sample of 10 patients with PPA, we showed a significant atrophy of the cholinergic BF nuclei, including Ayala's nucleus, compared to 18 age-matched healthy control individuals (Teipel, Flatz, et al., 2014). The number of participants in this previous study, however, was too small to allow for a comparison of the pattern of BF atrophy between subtypes of PPA.

In the present study, we determined the pattern of atrophy of the cholinergic BF in 62 patients with the diagnosis of PPA stratified into the clinical variants according to Gorno-Tempini et al. (2011) in comparison with 31 cognitively healthy controls. Data were retrieved from a prospective

multicenter study on frontotemporal lobar degeneration. In addition, we used a partial least square (PLS) analysis to assess the pattern of structural covariance as a proxy of structural connectivity (Alexander-Bloch, Giedd, & Bullmore, 2013) of the BF in the PPA individuals and controls. The findings would help us to determine the role of atrophy of the cholinergic BF in the clinical syndrome of PPA.

2. Participants and methods

2.1. Participants

We examined 62 patients with the clinical diagnosis of PPA [mean age 75.3 (standard deviation 7.3) years, 32 women]. Nine patients were diagnosed as logopenic variant, 24 patient as nonfluent/agrammatic variant, 18 patients as semantic variant and 11 patients as PPA not further classified according to Gorno-Tempini et al. (2011). For comparison, we investigated 31 cognitively healthy elderly subjects [mean age 68.2 (standard aviation 8.6) years, 16 women]. The data of the patients and controls were retrieved from the cohort of the German consortium for frontotemporal lobar degeneration (Otto et al., 2011), funded by the German Federal Ministry of Research.

PPA patients had undergone an extensive language and neuropsychological assessment. We applied the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) plus battery that includes the Mini-Mental-State Examination (MMSE) (Folstein, Folstein, & McHugh, 1975), verbal fluency tasks, naming of line drawings from the Boston Naming Test as well as verbal and nonverbal delayed recall tasks (Morris et al., 1989). Further we examined the patients' spontaneous speech, their ability to describe a picture and applied subtests of the Aachener Aphasia Examination (Huber, Poeck, Weniger, & Willmes, 1983) as well as a German version of the Repeat and Point Task.

In addition, patients were tested using measures of executive function (e.g., Stroop test, cognitive estimation test) as well as calculation. The clinical examination also included tests for buccofacial and ideomotor apraxia and for the applause sign.

The total PPA group and controls were not significantly different in age and gender distribution. As expected, PPA patients had lower performance in the Mini-Mental Status Examination compared to controls. The demographic characteristics of the PPA patients and controls are summarized in Table 1.

The assessment of patients and healthy subjects included detailed medical history and examination, as well as laboratory tests (complete blood count, electrolytes, glucose, blood urea nitrogen, creatinine, liver-associated enzymes, cholesterol, high density lipoprotein (HDL), triglycerides, serum B12, folate, thyroid function tests, coagulation, and serum iron).

Ethical approval for conduction of the study has been obtained at the coordinating site at the University of Ulm and all participating centres of the German consortium for frontotemporal lobar degeneration. Written informed consent was obtained in every case before examination, according to the declaration of Helsinki.

Table 1 – Participants' demographics.

	N (women)[a]	Age (SD) years[b,c]	MMSE (median + 25th and 75th percentile)[d]
Controls	31 (16)	68.2 (8.6)	29 (29, 30)
PPA	62 (32)	65.3 (7.3)	23 (17, 27)
nfvPPA	24 (10)	69.2 (7.0)	23 (20, 26)
lvPPA	9 (6)	64.7 (5.0)	20 (18, 25)
svPPA	18 (8)	61.9 (7.1)	24 (21, 27)
PPAnc	11 (8)	62.8 (6.7)	20 (15, 29)

nfvPPA = nonfluent/agrammatic variant PPA.
lvPPA = logopenic variant PPA.
svPPA = semantic variant PPA.
PPAnc = PPA not further classified.
SD = standard deviation

[a] Not significantly different between controls and total PPA group ($Chi^2 = .0$, 1 df, $p = 1$), and between controls and PPA variants ($Chi^2 = 4.1$, 4 df, $p = .39$).

[b] Not significantly different between controls and total PPA group ($t = 1.7$, 91 df, $p = .09$).

[c] Significantly different across controls and PPA variants [$F(4, 88) = 3.7$, $p = .008$]; with significant difference between controls and svPPA ($t = 2.6$, 47 df, $p = .012$), but no significant difference between the other PPA variants and controls.

[d] Significantly different between controls and total PPA group (Mann–Whitney U test, $p < .001$), and between controls and PPA variants (Mann–Whitney U test, $p < .001$), but not different between PPA variants.

2.2. Cognitive tests

To limit the number of comparisons on the association between BF volume and cognitive performance, we a priori selected tests from the comprehensive neuropsychological battery that are believed to be sustained by cholinergic system integrity (Bracco, Bessi, Padiglioni, Marini, & Pepeu, 2014): episodic long term memory (CERAD word list delayed free recall), verbal fluency (phonematic and semantic fluency), and executive function (Trail-Making Test – TMT A and B). In addition, as a control, we selected free figure recall, for which we expected no association with BF volume.

2.3. MRI acquisition

MRI acquisitions of the brain were conducted at 3 T scanners with parallel imaging capabilities (Siemens Magnetom Trio and Siemens Magnetom Verio, Siemens Medical Solutions, Erlangen, Germany). All scanners used a quadrature detection head coil, where number of channels ranged between 12 and 32 (transmit–receive circularly polarized – CP-head coil).

For the anatomical study, sagittal high-resolution 3-dimensional gradient-echo sequences were performed (magnetisation-prepared rapid gradient-echo (MPRAGE), field-of-view ranged between 239 and 256 mm, spatial resolution was .9 × .9 × 1.0 – 1.0 × 1.0 × 1.0 mm^3, repetition time ranged between 6.6 and 2500 msec, echo time ranged between 2.98 and 4.82 msec, inversion time ranged between 500 and 1200 msec, flip angle ranged between 7° and 15°, and number of slices ranged between 160 and 208). To identify white matter (WM) lesions, coronal 2-dimensional T2-weighted

sequences were performed (fluid attenuation inversion recovery – FLAIR).

2.4. MRI data processing

The processing of structural MRI scans was implemented through statistical parametric mapping, SPM8 (Wellcome Dept. of Imaging Neuroscience, London) and the Voxel based morphometry (VBM) 8-toolbox (http://dbm.neuro.uni-jena.de/vbm/) implemented in MATLAB 7.1 (Mathworks, Natwick). First, images were segmented into GM, WM and cerebrospinal fluid (CSF) partitions using the tissue prior free segmentation routine of the VBM8-toolbox. The GM and WM partitions of each subject were then high-dimensionally registered to a crisp template of average anatomy in Montreal Neurological Institute (MNI) space (IXI-template) using the Diffeomorphic Anatomic Registration using Exponentiated Lie algebra (DARTEL) algorithm (Ashburner, 2007). The IXI-template is part of the VBM8-toolbox and was derived by DARTEL inter-subject alignment of 550 healthy control subjects of the publicly available IXI-Database (http://www.brain-development.org).

Flow-fields resulting from the DARTEL registration to the IXI-template were used to warp the GM segments and voxel-values were modulated for non-linear effects of the high-dimensional normalization. This preserves the total amount of GM volume after linear effects of global head size and shape differences have been accounted for. Finally, modulated warped GM segments were resliced to an isotropic voxel-size of 1.5 mm^3 and smoothed with a Gaussian smoothing kernel of 8 mm full-width at half maximum (FWHM) for the analysis of cortical atrophy and a Gaussian smoothing kernel of 4 mm for the analysis of BF atrophy. Following the matched-filter theorem, the optimal smoothing kernel in voxel-based analyses should be matched in size to the expected effects. The selection of the different smoothing kernels was based on the findings of our previous study where changes in the BF were better detectable in a univariate statistics using 4 mm smoothing than using 8 mm smoothing (Grothe, Heinsen, & Teipel, 2013).

2.5. BF mask

The delineation and localization of the cholinergic BF nuclei according to Mesulam's nomenclature including the localization of the NSP in MRI reference space was based on the histological serial coronal sections and post-mortem MRI scan of a brain from a 56 year old man, as previously described (Kilimann et al., 2014). In short, subregions of the cholinergic nuclei were identified from digitalized stained sections of the BF and manually transferred into the corresponding slices of the post-mortem MRI of the dehydrated brain in alcohol space. Employing SPM8 (Wellcome Trust Centre for Neuroimaging, London, UK; available at www.fil.ion.ucl.ac.uk/spm) the MRI brain volume in alcohol space was first transferred into the post-mortem in cranio MRI space applying a 12-parameter affine transformation followed by high-dimensional normalization (Ashburner, Andersson, & Friston, 1999). In a further step, the post-mortem in cranio MRI was transferred into MNI stereotaxic coordinates standard space with DARTEL registration method (Ashburner, 2007). The linear and non-linear transformations from alcohol through in cranio to MNI space were combined to spatially transform the BF mask into the MNI standard space. We used this map to relate the group effects in local deformations to the anatomical position of the BF nuclei in MNI space.

2.6. Statistical analysis

We used three complementary analysis approaches: (i) univariate voxel-based analysis, (ii) region of interest (ROI) analysis, and (iii) multivariate PLSs. Of note, all BF volumes have been adjusted for overall brain size by using only the non-linear component of the spatial normalization for modulation of GM voxel intensities as described above (Section 2.4).

(i) For the univariate voxel-based analysis we employed the general linear model within the SPM framework. We determined the between-group differences in GM volume between the PPA patients and the control subjects for the GM maps smoothed with an 8 mm kernel, and for the GM maps smoothed with a 4 mm kernel and masked for the BF regions, controlling for age, sex and scanner. For the cortical atrophy analysis, results were thresholded at $p < .05$, False discovery rate (FDR) corrected, with a minimum cluster extension threshold of 50 contiguous voxels. The BF results were assessed at a statistical threshold of $p < .05$, FDR corrected, with a minimum cluster extension threshold of five contiguous voxels, due to the smaller size of the search region.

(ii) For the ROI analysis, we averaged GM volumes within each of the subnuclei from the cholinergic BF mask and compared the average GM volumes between groups using a multivariate linear model, controlling for age, sex, and scanner, followed by pairwise post-hoc Scheffé test between PPA variants and controls. We used partial correlation for the analysis of the association between BF volume and cognitive function, controlling for diagnosis, age, sex, and scanner.

(iii) To determine the structural covariance of the BF nuclei we used PLS) analysis (McIntosh & Lobaugh, 2004). This approach operates on the covariance between brain voxels and allows assessment of an integrated network of brain regions that together covary with some external measure (Krishnan, Williams, McIntosh, & Abdi, 2011). Here, we used the GM volumes of the BF nuclei to derive the principal components from the GM maps. This approach has also been termed seed voxel analysis (McIntosh et al., 1999) because GM volume of a specific location of the brain is regressed on the covariance structure of the rest of the brain to uncover correlated networks of structural connectivity. PLS yields a new set of variables, so called latent variables (LVs) where each LV identifies a pattern of differences in brain GM between groups or a pattern of brain regions structurally connected with BF atrophy. Each voxel of the brain has a weight on each LV, called the salience of this voxel on the LV. The significance of each LV has been assessed

using permutation tests with 100 permutations at a $p < .05$ threshold. In addition, we used bootstrap estimation (with 100 bootstrap iterations) to determine the reliability of the saliences for the brain voxels determining each LV. The bootstrap ratios of salience follow a standard z-score distribution, where a ratio of >1.96 corresponds to a p value of <.05, a ratio of >2.58 to a p value of <.01. In contrast to univariate analysis, the permutation tests and the saliences are determined in one single analytical step so that there is no need for multiple comparison correction.

3. Results

We found significant atrophy of **cortical GM** in the entire PPA group compared to controls in left predominant lateral temporal lobe and prefrontal lobe areas, extending into the medial temporal lobe and frontobasal areas (Supplemental Fig. 1). The pattern of atrophy in the individuals with PPA not further classified was focused in prefrontal lobe areas and almost entirely overlapped with atrophy of the whole PPA group (Supplemental Fig. 1). Atrophy in the semantic variant was focused on anterior and lateral temporal lobe of both hemispheres with slight left hemispheric predominance. Atrophy in the logopenic variant was focused in the prefrontal and lateral temporal lobe extending into the inferior parietal lobe, with a strong left hemispheric predominance. Atrophy effects in the nonfluent/agrammatic variant were focused in the lateral and anterior temporal lobe and the prefrontal lobe, with a strong left hemispheric predominance (Supplemental Fig. 2).

We found a significant atrophy of the brain size adjusted **total BF** in all PPA patients compared to healthy controls, controlling for age, sex, and scanner in the analysis of covariance (ANCOVA) model, $F(1, 82) = 20.2$, $p < .001$, as well as in each PPA subtype {nonfluent/agrammatic variant [$F(1, 45) = 7.5$, $p < .009$], logopenic variant [$F(1, 29) = 15.5$, $p < .001$], semantic variant [$F(1, 38) = 20.4$, $p < .001$] of PPA, and non-classified PPA cases [$F(1, 31) = 6.3$, $p < .02$]}.

Cohen's d effect size estimates (Cohen, 1988) for total BF atrophy were −.76 in nonfluent/agrammatic variant, −.27 in logopenic variant, −1.21 in semantic variant, and −.05 in non-classified PPA cases.

Using post-hoc Scheffé tests, we found significant atrophy of BF subnuclei Ch4p and NSP in the nonfluent/agrammatic and the semantic variant of PPA, respectively (see Fig. 1 for details). There were no significant differences in total and regional BF volumes between the PPA variants in a post-hoc Scheffé test. The voxel-wise distribution of atrophy within the BF is shown in Fig. 2, comparing the nonfluent/agrammatic, the logopenic and the semantic variants of PPA with healthy controls. At a p value of .05, FDR corrected, the PPA cases not further classified showed no significant decline of voxel-based GM volume within the BF compared to controls.

When we assessed associations between BF atrophy and **cognitive function**, we found no significant effects after controlling for age, sex, scanner and diagnosis (PPA *vs* controls).

We analyzed the **structural covariance of BF volumes** using PLS, controlling for age, sex, scanner, and (in the PPA cases)

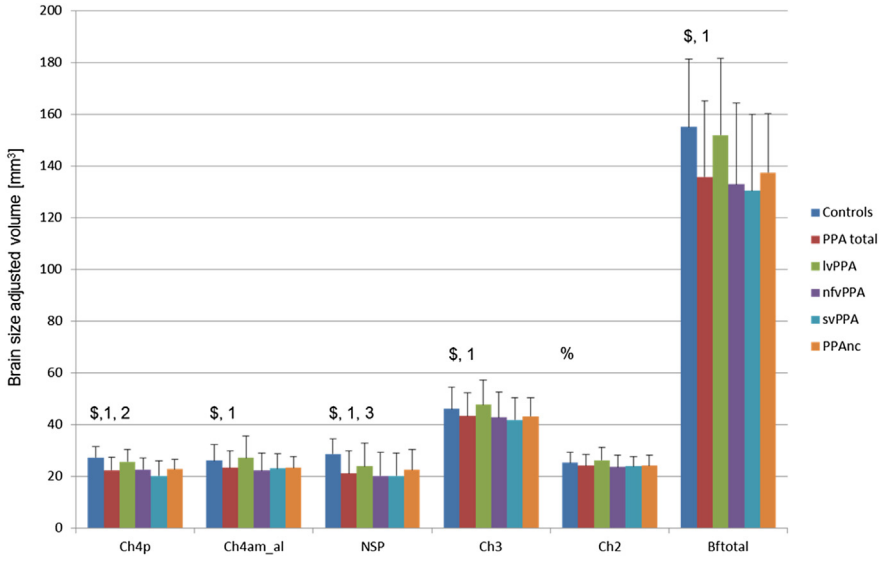

Fig. 1 – ROI based analysis of BF volumes in PPA variants and controls. Bar diagram of mean values and standard deviations of brain size adjusted BF subregions and total BF in controls and the whole PPA group (PPA total), the logopenic variant (lvPPA), the nonfluent/agrammatic variant (nfvPPA), and the semantic variant of PPA (svPPA), and the cases with PPA not further classified (PPAnc). The x-axis shows the BF regions according to Mesulam's nomenclature (Mesulam & Geula, 1988), including the entire BF volume (BFtotal). $ – Significantly different across all groups, controlling for age, sex, and scanner in an ANCOVA model, $p < .002$. % – Not significantly different between groups, controlling for age, sex, and scanner in an ANCOVA model, $p = .08$. 1 – Significantly different between controls and all PPA patients, controlling for age, sex, and scanner in an ANCOVA model, $p < .001$. 2 – Significantly different between controls and nfvPPA and svPPA, post-hoc Scheffé test, $p < .012$. 3 – Significantly different between controls and nfvPPA and svPPA, post-hoc Scheffé test, $p < .015$.

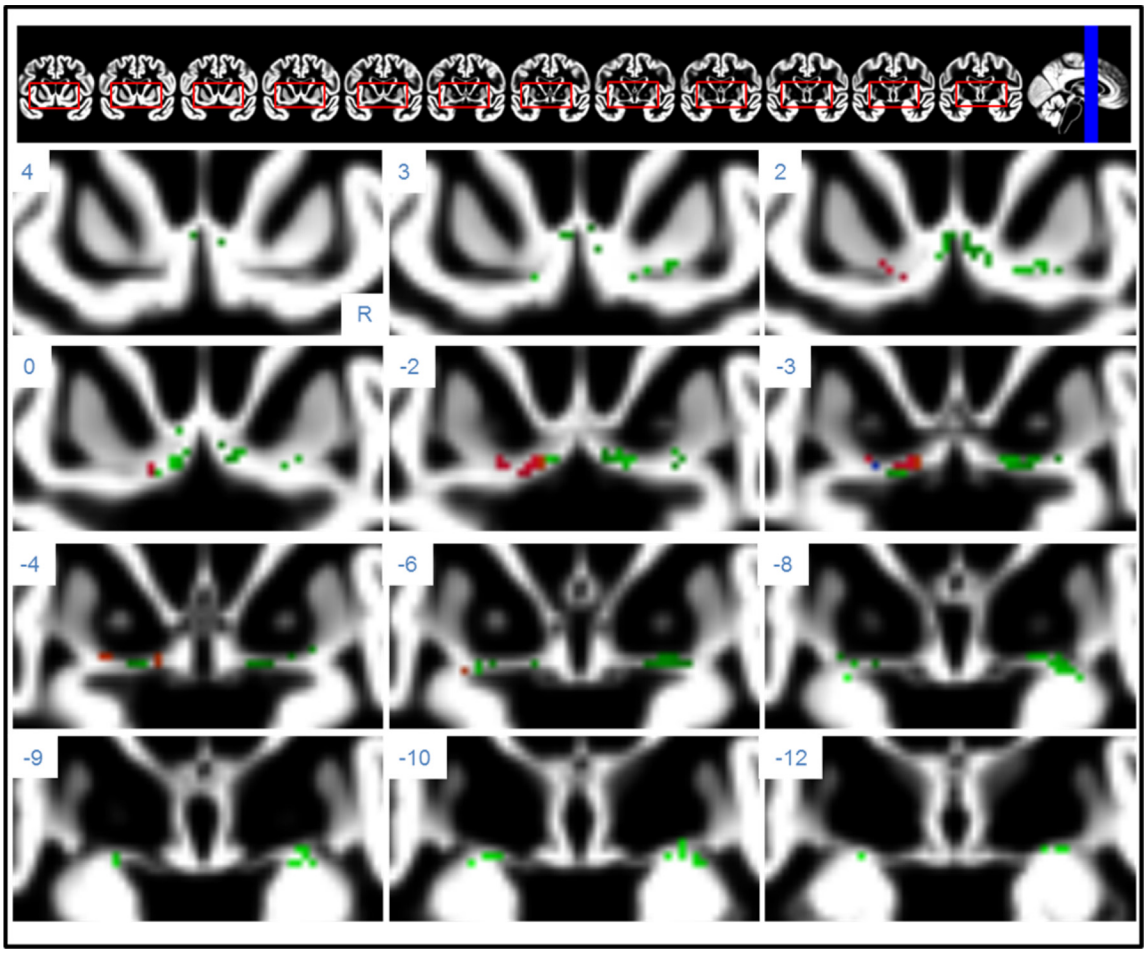

Fig. 2 – Voxel-based BF atrophy in PPA variants. Coronal sections from MNI coordinate y = 4 to y = −12, sections are 1.5 mm apart. Right of image (R) is right of brain (view from posterior). Clusters of at least five voxels surpassing a significance threshold of .05, FDR corrected, are shown for the nonfluent/agrammatic variant of PPA (red), the logopenic variant of PPA (blue), and the semantic variant of PPA (green) compared with controls. Upper left numbers indicate the level of the coronal section according to the MNI space y-coordinate.

the PPA variants. In the *healthy controls*, total BF was associated with left hemispheric predominant lateral temporal, superior parietal, and prefrontal lobe GM, hippocampus and insular cortex (Fig. 3). The GM volume in the anterior medial and anterior lateral region of the BF showed a significant association with the cortical GM in bilateral antero-lateral and antero-medial temporal lobes, left predominant prefrontal lobe, including Broca's area, and inferior parietal cortex (Fig. 3). The NSP volume was associated with left hemispheric predominant lateral temporal cortex, prefrontal lobe, including Broca's area, superior lateral parietal cortex, hippocampus and insular cortex (Fig. 3). The GM volume in the posterior region of the BF showed a significant association with bilateral GM volume of hippocampus and entorhinal cortex, cingulate gyrus, prefrontal lobe, lateral temporal cortex, and superior parietal lobe (Fig. 3). All effects were significant at a permuted p value < .001.

In the *PPA patients* the volume of the total BF showed a significant association with right hemispheric predominant GM in the anterior medial frontal lobe, the hippocampus, the insula cortex and the anterior temporal pole (Fig. 4). The GM volume in the anterior medial and anterior lateral region of the BF showed a significant association with the cortical GM in right hemispheric predominant areas of the frontal and parietal operculum, the anterior temporal pole, anterior medial frontal, and medial occipital regions and the hippocampus (Fig. 4). The NSP GM volume was significantly associated with the right hemispheric cortical GM volume in the insular cortex, the anterior temporal lobe, the hippocampus and the head of the caudate nucleus (Fig. 4). The posterior BF GM volume showed a significant association with left hemispheric predominance in the cortical GM of the insular cortex, the anterior temporal lobe, and the hippocampus (Fig. 4). All effects were significant at a permuted p value < .001.

In a PLS regression analysis of the interaction effect of BF volume and diagnosis (PPA *vs* controls), controlling for age, sex, and scanner, we found a significantly higher covariance of the total BF volume with left hemispheric GM volume in the healthy control cases compared to the PPA cases at $p < .001$, including lateral temporal cortex, temporal pole, insular cortex, hippocampus, putamen and inferior frontal gyrus (Fig. 5).

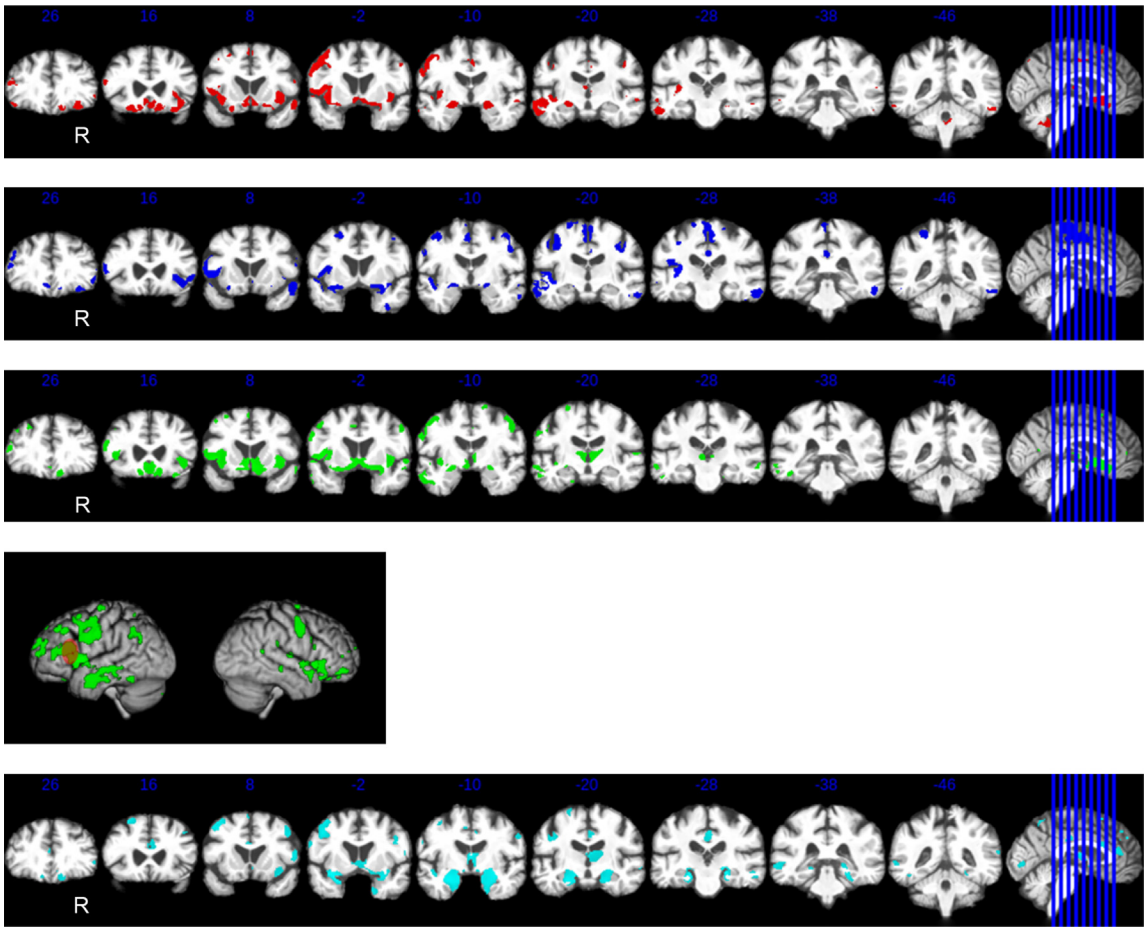

Fig. 3 – Association of BF and cortical GM in controls as determined using PLS analysis. LVs representing brain regions where cortical GM volume was associated with GM volume of the total BF (upper row, red), the medial and lateral anterior BF (second row, blue), the NSP (third row, green), and the posterior BF (lower row, cyane), projected on an MRI scan in MNI standard space. Row four shows the LV for the NSP volume projected onto the rendered surface of an MRI in standard space, with a red oval in the left hemisphere highlighting the location of Broca's area. Coronal sections go from MNI coordinate $y = 26$ to $y = -46$, sections are 9 mm apart. Upper numbers indicate the level of the coronal section according to the MNI space y-coordinate. Colored voxels represent a significant bootstrap ratio of $p < .05$. Right of image (R) is right of brain, view from posterior.

In contrast, the PPA cases showed no areas of increased covariance compared with controls.

4. Discussion

We studied atrophy and structural covariance of BF volumes in a relatively large sample of PPA cases retrieved from a prospective multicenter cohort study. We found significant atrophy of total BF volume in PPA cases compared with healthy control subjects with effects most pronounced in the posterior BF and the area of the NSP, and most severe in the nonfluent/agrammatic and the semantic variants of PPA. We used PLS analysis as a robust and powerful approach to the assessment of structural covariance and connectivity (McIntosh & Lobaugh, 2004). The structural covariance of the total BF and most of its subregions was predominant in the left hemisphere, including prefrontal, lateral temporal and superior parietal cortical areas in healthy controls, whereas the structural covariance of the BF regions in the PPA patients was predominantly located in the right hemisphere, involving hippocampus, insular cortex, lateral temporal cortex and anterior temporal pole, and extending into medial frontal and to some extent into inferior and superior parietal lobule areas.

The pattern of cortical atrophy in PPA and its variants in our study replicated previous findings in independent studies, with bilateral involvement of anterior temporal lobes in semantic variant PPA, extension of atrophy into left hemispheric inferior parietal lobe areas in logopenic variant of PPA and strong involvement of left prefrontal lobe areas in the nonfluent/agrammatic variant of PPA (Gorno-Tempini et al., 2004; Rogalski et al., 2011). Different to previous studies, the parietal lobe areas were not involved in our logopenic variant cases, but due to the small sample size in this subgroup the statistical power to detect group differences was limited.

Replicating findings from a previous study in an independent small sample of 10 PPA cases and 18 controls (Teipel, Flatz, et al., 2014), we found significant atrophy of the total

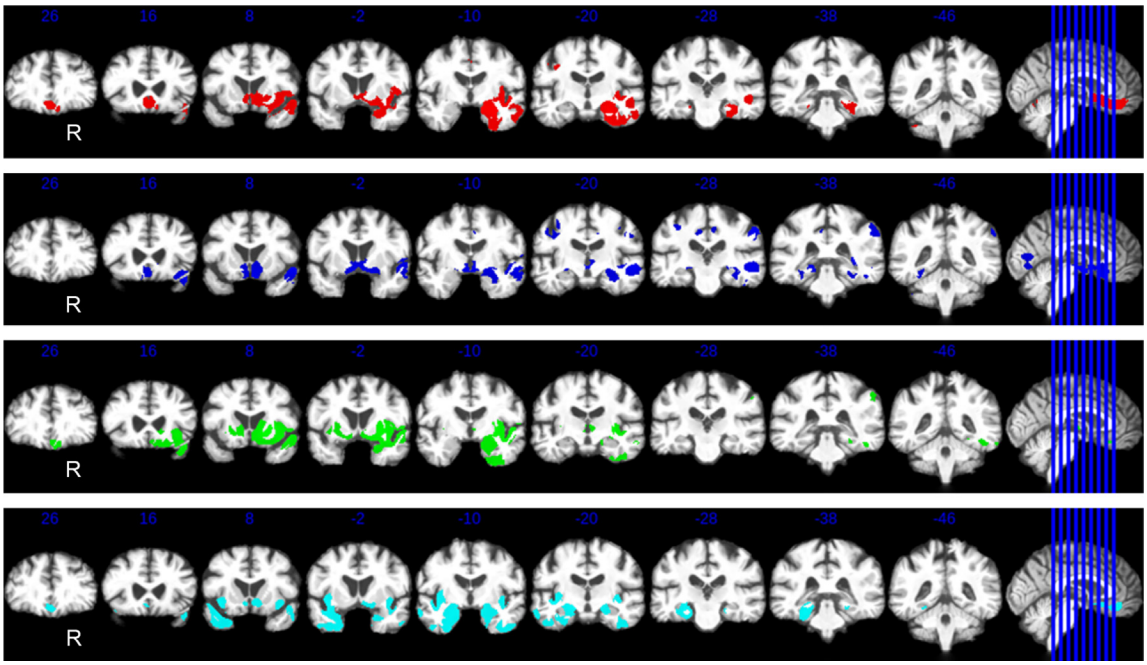

Fig. 4 – Association of BF and cortical GM in PPA as determined using PLS analysis. LVs representing brain regions where cortical GM volume was associated with GM volume of the total BF (upper row, red), the medial and lateral anterior BF (second row, blue), the NSP (third row, green), and the posterior BF (lower row, cyane), projected on an MRI scan in MNI standard space. Coronal sections go from MNI coordinate y = 26 to y = −46, sections are 9 mm apart. Upper numbers indicate the level of the coronal section according to the MNI space y-coordinate. Colored voxels represent a significant bootstrap ratio of $p < .05$. Right of image (R) is right of brain, view from posterior.

BF in PPA patients. Extending the previous findings, we found a differential pattern of atrophy between PPA variants. Effects were most pronounced in the semantic variant and nonfluent/agrammatic variant cases with smaller effect sizes in logopenic variant cases and non-classified PPA cases. Within the BF, the effects were most pronounced in the most posterior BF region (Ch4p) as well as the NSP. Ch4p has consistently been found to be the most early affected area in dementia due to Alzheimer's disease as well as in amnestic mild cognitive impairment (MCI) (Grothe et al., 2013; Teipel, Heinsen, et al., 2014), considered to represent an at risk stage of Alzheimer's disease dementia (Petersen et al., 2006). Therefore, one could speculate that the strong effects in the Ch4p region may partly arise from the underlying Alzheimer's disease pathology in a subset of the PPA cases. The involvement of the NSP would agree with its putative role in language development that has been pointed out by Simic et al. (1999): the NSP has only been described in humans and anthropoid monkeys, shows a strong left laterality, and is located in close proximity to the external capsule and thus the cortical language areas. Our findings of a prominent atrophy of this region support a potential involvement of the NSP in PPA. In addition, the pattern of structural covariance of the BF, particularly the NSP, in the healthy control subjects included left hemispheric areas, such as Broca's area, lateral temporal cortex and superior parietal cortex, including angular gyrus. This pattern of structural connectivity would agree with an involvement of BF regions, particularly the NSP, in language function. This association, however, was not specific for the NSP and requires confirmation in longitudinal design to study the onset of regional BF

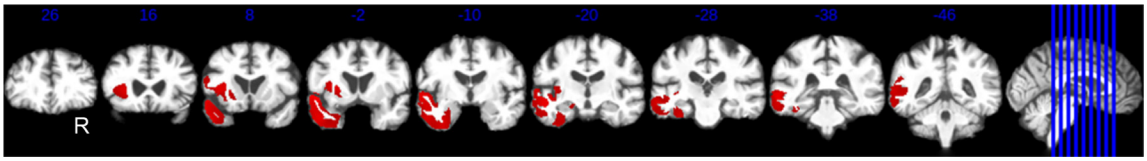

Fig. 5 – Interaction of diagnosis by total BF volume on cortical GM as determined using PLS analysis. LV representing brain regions where the association of cortical GM volume with GM volume of the total BF was significantly higher in healthy controls than in PPA cases, projected on an MRI scan in MNI standard space. Coronal sections go from MNI coordinate y = 26 to y = −46, sections are 9 mm apart. Upper numbers indicate the level of the coronal section according to the MNI space y-coordinate. Red voxels represent a significant bootstrap ratio of $p < .05$. Right of image (R) is right of brain, view from posterior.

atrophy in PPA. In contrast, the posterior BF regions, corresponding to Mesulam's Ch4p, showed more prominent associations with medial temporal regions and cingulate gyrus in the controls, areas that are early involved in Alzheimer's disease both at autopsy (Braak & Braak, 1991; Price, Davis, Morris, & White, 1991; Price et al., 2009) and in structural in vivo imaging (Baron et al., 2001; Chetelat et al., 2005; Schroeter, Stein, Maslowski, & Neumann, 2009; Teipel et al., 2006) studies so that this pattern of connectivity agrees with the early involvement of the posterior BF region in mild cognitive impairment and dementia stages of Alzheimer's disease (Grothe et al., 2013; Teipel, Heinsen, et al., 2014).

When we looked on the pattern of structural covariance of the NSP (and the other BF regions) in the PPA sample, we found a right hemispheric predominance of effects, involving hippocampus, insular cortex, frontobasal areas and lateral temporal cortex. This pattern does not represent the typical language areas. This change in pattern of structural covariance from left hemispheric areas involving cortical language areas in healthy older individuals to right hemispheric regions involving more posterior and more limbic brain areas in PPA patients suggests that cortical destruction in PPA destroys the pattern of structural covariance of BF areas, with relative preservation of covariance into less affected cortical areas. This is supported by the results of the interaction analysis. Here, structural covariance of cortical GM with total BF volume was significantly higher in healthy controls than in PPA cases in the left hemisphere. This would also imply that BF atrophy in PPA may be a secondary event, following primary left hemispheric cortical destruction in PPA as evidenced by the partially preserved pattern of covariance in the BF in the PPA patients with right hemispheric cortical areas. The interpretation of BF volume as a secondary event in PPA is further supported by the lack of an association of cognitive functions with BF volume in our PPA cases. In contrast, in a recent study on patients with mild cognitive impairment due to Alzheimer's disease, BF volume was found to correlate with both memory and executive function deficits (Grothe et al., 2015). This agrees with the important role of cholinergic function for executive function and attention as documented in trials on anticholinergic treatment effects (Dumas & Newhouse, 2011; Pomara, Nolan, & Halpern, 1995; Snyder et al., 2014) and the role of cholinergic degeneration as a primary event in Alzheimer's disease pathogenesis (M.M. Mesulam, 2013). It is important to note that our interpretation that the shift in covariance reflects a temporal sequence of primary cortical and secondary BF atrophy in PPA needs further replication, and confirmation in a longitudinal design. The German consortium for frontotemporal lobar degeneration is a still ongoing cohort study so that hopefully in the upcoming years we will have the data available to determine whether atrophy in left hemispheric cortical areas indeed precedes atrophy of the cholinergic BF in early clinical or even preclinical stages of PPA.

Autopsy data that would help to corroborate the notion of BF involvement into PPA are still scarce. While cholinergic markers have been studied post mortem in the behavioural variant of FTLD without evidence of major alterations (Procter et al., 1999), we are not aware of a post-mortem study on cholinergic markers in PPA so far. Another approach to understanding the role of cholinergic dysfunction in PPA is provided by treatment studies using acetylcholinesterase inhibitors. Over a short period of eight weeks, galantamine showed a trend towards stabilization of language function and global cognition in a small sample of PPA patients compared to placebo ($N = 10$ verum, $N = 10$ placebo) (Kertesz et al., 2008). An open label study showed improved behavioural symptoms in 20 subjects with the diagnosis of FTLD treated with rivastigmine compared to 20 untreated FTLD subjects over 12 months. Part of these effects may be due to underlying AD pathology in a subset of the patients (Mendez, 2009; Vossel & Miller, 2008). Injecting .4–.6 mg of scopolamine disrupted cholinergic activity in healthy young women, leading to impairments in reading, writing, verbal fluency and object naming (Aarsland, Larsen, Reinvang, & Aasland, 1994). This further supports the hypothesis that cholinergic depletion affects language.

There are several limitations associated with our study. First, this is a multicenter study, inducing additional variance in the volumetric measurements. In a previous study, we have shown that variability of BF measurements is slightly increased due to a multicenter design (Kilimann et al., 2014). Here, we used a prospectively harmonized acquisition protocol, but had variability in scanner platforms and field strengths. We accounted for scanner effects in all statistical models, including the structural covariance analyses. Secondly, the post-mortem mask of our study is based on only one single brain. We compared the distribution of our mask with the distribution of 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). There was a high general agreement between all three masks, with the only major difference being that Ch4p was located more ventrally in the first mask (Teipel et al., 2005) compared to the probabilistic mask (thresholded at 50%) (Zaborszky et al., 2008) and our mask. These findings suggest that the anatomy of the BF nuclei is relatively stable across subjects. Thirdly, although the overall number of PPA cases was relatively large, stratification into different variants yielded small subsamples limiting the statistical power to detect between variant effects in pattern of BF atrophy. Of particular notion, the effects in the logopenic variant were unexpectedly small. There were no significant effects in this variant in the ROI based analysis, which was, however, not only related to the small number of nine logopenic variant PPA cases, but also was reflected in the small effect size for volume differences between the logopenic variant cases and the healthy controls. Based on the assumption that the logopenic variant PPA in a considerable proportion of cases represents an atypical presentation of Alzheimer's disease one would have expected a particular involvement of the BF in these individuals. Still, this is the first study on BF atrophy in logopenic variant PPA so that we cannot be sure if the language variant of Alzheimer's disease may even present with an atypically low involvement of BF areas. Finally, the clinical stratification of PPA cases in different variants always is a matter of debate. Here, we used a two-step process of classification in the multicenter design of the German consortium for frontotemporal lobar degeneration. In the first step, all clinical raters of the consortium during the recruitment phase of the study underwent

regularly scheduled bi-annual rater training in the instruments and criteria for clinical classification of frontotemporal lobar degeneration, including PPA variants (Otto et al., 2011). In the second step, all cases that were rated as low confidence in the classification were rated by an independent neuropsychologist based on the neuropsychological data and the recorded sample of spoken language. In nine out of 62 cases, such a language sample was not available so that classification was based on the first step classification only. The pattern of cortical atrophy in our PPA variants replicates patterns of cortical atrophy in previous studies so that the overall classification of individuals seems appropriate although this does not guarantee correct classification in every individual case. Classification accuracy is, actually, a heuristic problem when there is no real gold standard to test the validity of any classification scheme. Therefore, we operationalized the process of classification as far as possible in such a multicenter design.

In summary, we found significant atrophy of the cholinergic BF in PPA cases compared with healthy older control individuals. Effects were most prominent in the posterior area of the BF and in the area of the NSP. The structural covariance pattern of the BF nuclei, particularly the NSP, in the healthy control individuals agree with an involvement of the BF in language function. The pattern of structural covariance of the BF in PPA cases compared with controls suggests a shift of covariance from left anterior to more right posterior brain areas from healthy aging to PPA, possibly as a consequence of the profound destruction of the cortical language areas in PPA. Our findings would agree with the assumption that BF atrophy is a secondary event, after earlier destruction of cortical language areas, in PPA, but this hypothesis needs further confirmation in longitudinal data.

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Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.cortex.2016.07.004.

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