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In vivo evidence for decreased scyllo-inositol levels in the supplementary motor area of patients with Progressive Supranuclear Palsy: A proton MR spectroscopy study

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ABSTRACT

Introduction: Several structural and functional neuroimaging studies have shown that the Supplementary Motor Area (SMA) is affected by tau pathology in patients with Progressive Supranuclear Palsy (PSP). The aim of the study was to investigate the biochemical profile of SMA in PSP patients, using proton magnetic resonance spectroscopy (^{1}H-MRS).

Methods: Sixteen PSP patients and 18 healthy controls participated in this study. ^{1}H-MRS was performed by using a Point RESolving Spectroscopy (PRESS) single-voxel sequence implemented on a 3-T scanner. A voxel of 25 × 25 × 15 mm involving the right and left SMA was acquired in all subjects. Peak areas of N-acetyl-aspartate + N-acetyl-aspartyl-glutamate (NAA), creatine with phosphocreatine (Cr), glycerophosphocholine + phosphocholine (Cho), glutamate + glutamine (Glx), glutathione (GSH), myo-Inositol (mI) and Scyllo-Inositol (Scyllo) were calculated using a version 6.3-1K of the fitting program LCModel. Comparative analysis was performed on both absolute concentrations and ratio values relative to Cr.

Results: PSP patients showed a significant decrease in Scyllo concentration and Scyllo/Cr ratio values in SMA, compared to controls, whereas no difference between groups was found for the other ratio values. Of note, the attention and working memory functions were positively related to Scyllo and Scyllo/Cr values in PSP patients.

Conclusions: Our study demonstrates that Scyllo and Scyllo/Cr were significantly reduced in the SMA of PSP patients. Because Scyllo seems to be able to protect against formation of toxic fibrils of amyloid-beta fragments and tau oligomers deposition, these preliminary findings may open new perspectives to investigate Scyllo as a new potential disease-modifying therapy for PSP.

1. Introduction

Progressive supranuclear palsy (PSP) is clinically defined by a progressive neurodegenerative disease associated with axial rigidity, bradykinesia, postural instability, vertical supranuclear gaze palsy, speech and swallowing dysfunctions, as well as fronto-executive and behavioral manifestations [1]. Neuropathologically, PSP is characterized by the deposition in the brain of misfolded 4-repeat tau in globose neurofibrillary tangles, tufted astrocytes, and neuropil threads. These features support the role of tau dysfunction in the pathogenesis of the disorder and the classification of PSP as a primary tauopathy [2]. Tau is a major target for future treatments of PSP, and a number of clinical treatment trials of therapies targeting tau are already underway.

Structural magnetic resonance imaging (MRI) [3,4], functional MRI

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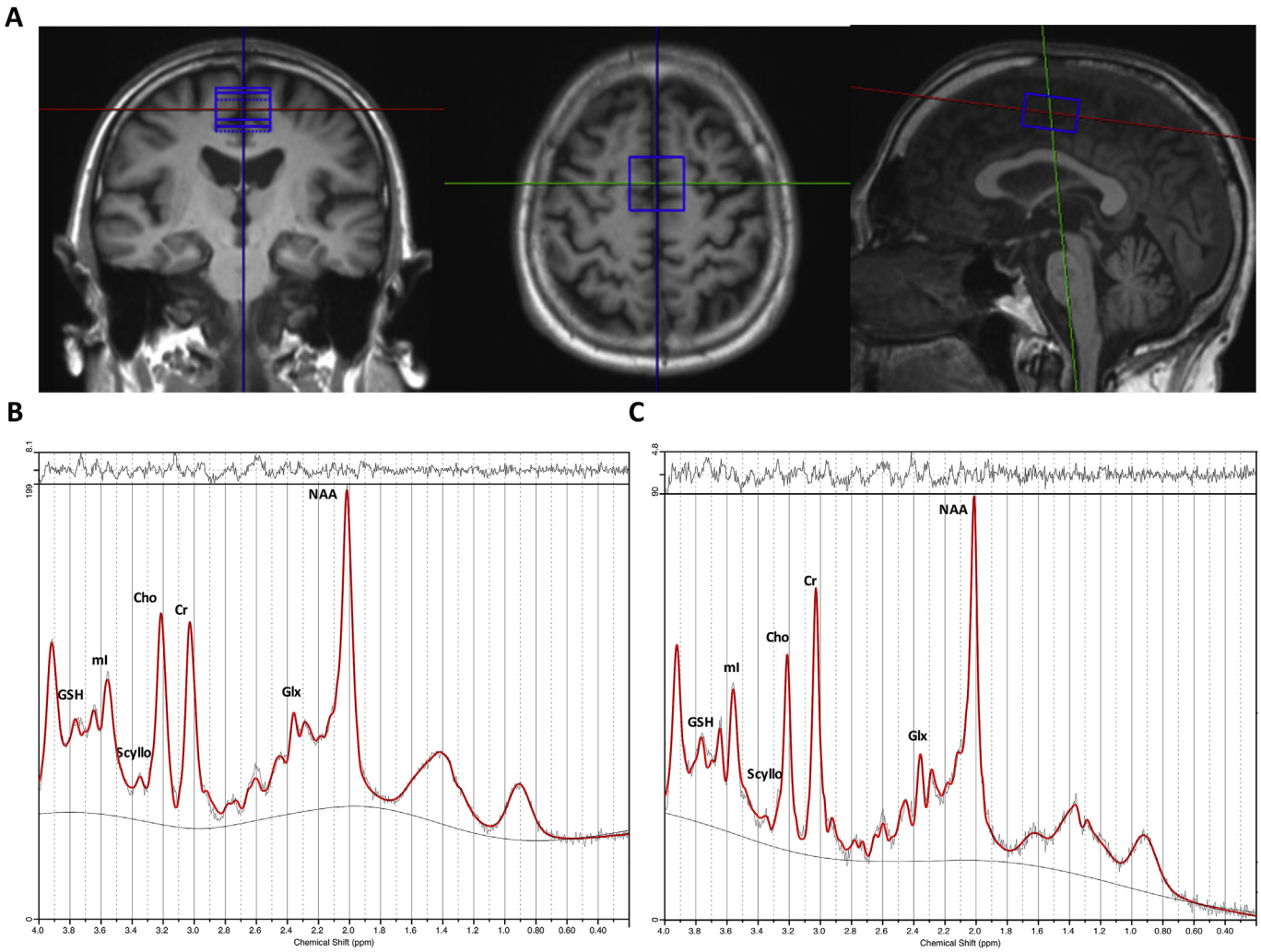

Fig. 1. VOI localization and examples of ^{1}H-MRS spectra obtained from study subjects.
(**A**) ^{1}H-MRS localization of the volume of interest involving both right and left SMA on coronal, axial, and sagittal T1 images from a PSP patient. (**B-C**) Proton spectra obtained from the SMA of a healthy control (**B**) and a PSP patient (**C**), whose PVE-corrected Scyllo concentrations and Scyllo/Cr ratios were respectively 0.42 and 0.08 for the healthy control, 0.12 and 0.04 for the PSP patient. The gray line is the actual spectra; the red line represents the LCModel fit; at the top are plotted the residuals between LCModel fit and actual spectra. Scyllo peak appear with a "little singlet" shape at 3.35 ppm, between mI and Cho peaks.

(fMRI) [5] and [^{18F}]-fluoro-deoxyglucose positron-emission tomography (FDG-PET) studies [6] have respectively demonstrated that the supplementary motor area (SMA) is affected in PSP patients, in terms of atrophy and microstructural damage, functional dysconnectivity, and decreased metabolic activity. Recent PET studies using ligands specifically targeted to tau proteins in the brain (i.e. [^{18F}]AV-1451) have found increased tracer uptake in the SMA of PSP patients [7], suggesting the involvement of this brain area in this disease.

Proton MR spectroscopy (^{1}H-MRS) is a non-invasive technique that is able to quantify *in vivo* the neurometabolic pattern in brain tissue. The main metabolites that can be detected by ^{1}H-MRS at 3T are N-acetyl-aspartate + N-acetyl-aspartyl-glutamate (NAA), creatine + phosphocreatine (Cr), glycerophosphocholine + phosphocholine (Cho), glutamate + glutamine (Glx), as well as myo-inositol (mI) [8], glutathione (GSH), and scyllo-inositol (Scyllo) [9]. This latter has been shown to prevent and reverse Alzheimer's Disease in a transgenic mouse model [10,11], inhibiting and blocking the aggregation of amyloid-beta peptide, with a consequent improvement of cognitive functions [12–14]. Moreover, in vitro studies found that Scyllo is able to protect against formation, not only of toxic fibrils of amyloid-beta fragments, but also tau oligomers deposition [14], that is a hallmark of PSP pathology. Hence, the therapeutic application of Scyllo open new perspectives for the treatment of disorders of protein aggregation, including other tauopathies such as PSP.

To date, only a few studies have focused on ^{1}H-MRS changes in PSP patients, showing neurometabolic alterations in the lentiform nucleus (i.e. putamen and globus pallidus), brainstem, centrum semiovale, frontal and precentral cortex [15–18], but no studies have assessed the neurometabolic pattern of the SMA in PSP patients, and no studies have quantified Scyllo in PSP, by using an *in vivo* spectroscopic approach.

The current study aimed to investigate the biochemical underpinnings of PSP in the SMA using a short echo-time single-voxel ^{1}H-MRS approach in order to test the hypothesis of Scyllo depletion.

2. Methods

2.1. Subjects

2.1.1. Clinical assessment

From April 2017 to June 2018, we enrolled 16 patients affected by PSP-Richardson Syndrome (PSP-RS) and 18 age-matched healthy controls with no history of neurological or severe general medical disease. All patients were referred to our Movement Disorders Centre. For PSP patients, inclusion criteria were: 1) diagnosis of probable PSP-RS

according to new criteria for clinical diagnosis of PSP [19] (a trained neurologist [M.M.] with more than 10 years of experience in movement disorders made the diagnosis according to established criteria, and a senior neurologist [A.Q.], with over 40 years of clinical experience, reviewed and confirmed the diagnosis of all PSP-RS patients); 2) negative history of neurological or psychiatric diseases other than PSP; 3) no presence of vascular risk factors; 4) no evidence of movement artifacts, brain tumor, and/or marked cortical atrophy, and/or diffuse white matter hyperintensities on MRI scan (an expert neuroradiologist [U.S.] with 20 years' experience examined all MRIs to exclude potential brain abnormalities, as apparent on conventional FLAIR, T2-weighted, and T1-weighted images). For each patient, a complete medical history with neurological examination were performed, including the Unified Parkinson's Disease Rating Scale-Motor Examination (UPDRS-ME). All study procedures and ethics aspects were approved by the institutional review board, in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants who were examined as part of the study.

2.1.2. Neuropsychological assessment

A trained neuropsychologist (C.C.) evaluated, in all participants, the following cognitive functions: 1) global cognitive functions (Mini Mental State Examination [MMSE]); 2) executive control (Frontal Assessment Battery [FAB]); 3) short- and long-term verbal memory (Rey Auditory-Verbal Learning Test [RAVLT]); 4) attention and working memory (Digit Span Forward and Backward); 5) visuospatial skills (Judgement of Line Orientation [JLO]); 6) verbal fluency (Controlled Oral Word Association Test [COWAT]); and 7) anxiety and depression (Hamilton Rating Scale Anxiety [HAM-A]), Beck Depression Inventory [BDI]). The neuropsychological session lasted 1 h.

2.2. ^{1}H-MRS

2.2.1. ^{1}H-MRS acquisition

All participants underwent the same MRI scanning protocol using a 3-T scanner (Siemens). The MRI protocol included conventional, three-dimensional, T1-weighted, T2-weighted, FLAIR scanning and single-voxel ^{1}H-MRS. 3D T1-weighted images (TR = 2300 ms; TE = 2.34 ms; slice thickness = 1 mm with no inter-slice gap; matrix size = 256 × 256) were acquired for spectral localization.

A volume of interest (VOI) of 25 × 25 × 15 mm involving right and left SMA was acquired in all subjects (Fig. 1A). The VOI was placed by visual inspection as to contain both right and left SMA: on the axial image in which the central and precentral sulci appeared, the posterior side of the voxel was medial to the precentral sulcus and the centre of the voxel involved the interhemispheric fissure.

Single-voxel ^{1}H-MRS was performed by using a Point RESolved Spectroscopy (PRESS) single voxel localization sequence with TR = 2000 ms, TE = 30 ms, and total number of scans = 276. This volume-localized sequence was used with chemical-shift-selective saturation water suppression and outer-volume suppression contiguous to the single VOI. Four outer-volume suppressions were placed far from the VOI at the brain-bone interface. Automatic shimming was conducted before the spectra were obtained. After shimming, a water suppression value at 95% and a spectral line width of less than 12.77 Hz on the water signal were considered as acceptable values [20,21].

2.2.2. ^{1}H-MRS post-processing

Metabolite quantification was performed with the LCModel software (http://www.s-provencher.com) for the automatic quantitation of *in vivo* ^{1}H-MRS spectra. The LCModel method automatically performs phase adjustments, frequency alignment, baseline subtraction, and eddy current correction. Relative metabolite concentrations (and their uncertainties) are estimated by fitting the spectrum to a linear combination of 'basis spectra' of each individual metabolite, obtained from solutions acting as concentration references for the *in vivo* acquisitions. For this study a basis set provided by the LCModel software for a 3T PRESS acquisition with TE = 30 ms was used. The unsuppressed water spectrum is then used to normalize the initial fit to generate a first estimate of metabolite concentration in the tissue. LCModel defines the concentrations of the pertinent metabolites by scaling the relative areas and chemical shifts across the two sets of spectra. The fitting of the spectral peaks was thus achieved with a priori knowledge of their actual characteristics.

The main brain metabolites of interest were the NAA peak at 2.0 ppm, the Cr peak at 3.0 ppm, the Cho peak at 3.2 ppm, the Glx peak at 2.2–2.4 ppm, the GSH peak at 3.78 ppm, the mI peak at 3.56 ppm, and Scyllo peak at 3.35 ppm [8,9] (Fig. 1B–C, Figure e − 1 and e − 2). To define a criterion for rejection of poor quality spectra, the Cramér-Rao Lower Bounds (given as % Standard Deviation (SD)-value by the LCModel) were used. Only those spectra with a %SD < 20 were included in the study. Signals of NAA, Cr, Cho, Glx, GSH, mI, and Scyllo were quantified. Absolute concentrations of the metabolites were expressed as arbitrary units because they were not corrected for the significant T2 relaxation of the metabolites. Moreover, the ratios of metabolites to Cr were also determined [20,21].

2.2.3. Imaging-based partial volume correction of metabolite concentrations

Since the spectroscopic voxels involved dissimilar areas and included different proportions of cerebrospinal fluid (CSF), gray matter (GM) and white matter (WM), atrophy of the SMA could affect these proportions and, consequently, the spectroscopic values. Assuming zero metabolite concentration in CSF, partial volume effect (PVE) arises due to the inclusion of CSF in the voxel could lead to underestimation of metabolite concentrations in quantitative MRS.

Then, the size and location of each area from the spectra file headers were extracted to obtain the SMA masks for each subject. For segmenting the MRI scans, the FMRIB Software Library v5.0 tools were used. In particular, first, the structural images were skull-stripped with the brain extraction tool. FAST was applied for obtaining segmented tissues and the partial volume maps for GM, WM, and CSF. From these partial volume estimation maps, the percentage of CSF, GM, and WM content within the spectroscopic voxel were calculated, allowing for correction of the CSF fraction of the spectroscopic values [22]. The metabolite concentrations (C_0) were corrected for the PVE due to CSF by finding the average fractional content of CSF in each voxel (F_{CSF}), and applying the following equation: $C = C_0 * [1/(1\text{-}F_{CSF})]$. Subsequently, these PVE-corrected metabolite concentrations were controlled for the GM fraction $[F_{GM}/(F_{GM} + F_{WM})]$ of each subject [23], in order to minimize the atrophy-related PVE.

2.3. Statistical analysis

Statistical analyses were performed with SPSS (v12.0, Chicago, IL, USA). To compare sex distributions among groups, we used the χ^2 test. To investigate the differences in clinical data between PSP and control groups, we used the unpaired *t*-test and the Mann–Whitney *U* test for normally and non-normally distributed variables, respectively, as revealed by the Shapiro-Wilk test.

PVE-corrected metabolite concentrations and ratio values between groups were compared using a general linear model (GLM). The GM fraction and age were included as covariates in the GLM [23].

Pearson correlation coefficient was calculated to explore the relationships of clinical variables with the metabolite that survived to the normalization with the Cr.

Significance level was set at 0.05 after false discovery rate (FDR) correction for multiple comparisons.

Table 1
Demographic, clinical, and neuropsychological features of the study subjects.

Characteristic	PSP-RS (n = 16)	HC (n = 18)	*p value*
Demographic measures			
Sex, M/F	10/7	8/10	*0.5* [a]
Age, y (SD)	69.6 (5.0)	66.2 (8.8)	*0.2* [b]
Education, y (SD)	9.1 (4.2)	9.3 (5.0)	*0.9* [b]
Clinical measures			
Age at onset, y (SD)	66.7 (4.9)	–	–
Disease duration, y (SD)	3.0 (1.9)	–	–
UPDRS-ME, score (SD)	36.2 (7.3)	–	–
Neuropsychological data			
Global cognitive functions			
MMSE, score (SD)	24.7 (2.5)	26.0 (2.6)	*0.2* [c]
Executive control			
FAB, score (SD)	9.5 (2.8)	14.8 (2.8)	***< 0.001*** [b]
Short-term verbal memory			
RAVLT (IR), score (SD)	28.3 (10.8)	33.5 (10.3)	*0.2* [b]
Long-term verbal memory			
RAVLT (DR), score (SD)	4.5 (3.7)	5.1 (2.6)	*0.7* [b]
Attention and working memory			
Digit Span Forward, score (SD)	4.4 (1.2)	4.8 (0.8)	*0.3* [b]
Digit Span Backward, score (SD)	2.8 (0.9)	3.0 (0.7)	*0.5* [c]
Visuospatial skills			
JLO, score (SD)	11.5 (9.5)	19.3 (8.8)	*0.1* [b]
Verbal fluency			
COWAT, score (SD)	14.1 (6.5)	24.9 (11.4)	***0.02*** [b]
Ansiety			
HAM-A, score (SD)	12.0 (7.2)	14.6 (3.6)	*0.3* [b]
Depression			
BDI, score (SD)	12.4 (3.9)	15.1 (5.2)	*0.4* [c]

Abbreviations – PSP-RS = Progressive Supranuclear Palsy- Richardson Syndrome. HC = healthy controls. M = male; F = female. UPDRS-ME = Motor Examination of the Unified Parkinson's Disease Rating Scale. MMSE = Mini-Mental State Examination. FAB = Frontal Assessment Battery. RAVLT (IR) = Rey Auditory Verbal Learning Test (Immediate Recall). RAVLT (DR) = Rey Auditory Verbal Learning Test (Delayed Recall). JLO = Judgement of Lines Orientation. COWAT = Controlled Oral Word Association Test. HAM-A = Hamilton Anxiety Rating Scale. BDI = Beck Depression Inventory.
Significant p-values are indicated in bold.
[a] χ^2 test.
[b] Unpaired *t*-test.
[c] Mann-Whitney *U* test.

3. Results

3.1. Inter-group comparisons

Table 1 summarizes the main demographic, clinical, and neuropsychological features of study subjects. Groups were matched for sex, age and education. PSP-RS patients did not differ from controls on neuropsychological tests investigating global cognitive functions, short-term and long-term verbal memory, attention and working memory, and visuo-spatial skills. However, as also reported in previous studies [24,25], PSP-RS patients displayed statistically significant lower mean scores in executive control (FAB) and verbal fluency (COWAT). Finally, no differences between groups were found in anxiety and depression scales.

Obtained spectra showed high quality and an optimal signal to noise ratio (SNR = 28.3 ± 7.4). Fig. 2A and B shows the results of the comparative analysis of ^{1}H-MRS data. No significant differences in PVE-corrected concentrations of Cho, Glx, GSH, and mI were found between groups, whereas NAA, Cr, and Scyllo were significantly decreased in PSP-RS patients (FDR-corrected p-values were 0.02, 0.02, and 0.01, respectively). Scyllo/Cr was the only ratio significantly reduced in PSP-RS patients (FDR-corrected p-value = 0.02), whereas no inter-group differences were found in other ratios.

3.2. Intra-group correlations

In PSP-RS patients, attention and working memory functions, as measured by Digit Span Forward test, were positively correlated with PVE-corrected Scyllo concentrations (Fig. 2C, $r = 0.7$, FDR-corrected *p-value* = 0.02). The relationship between Digit Span Forward test scores and Scyllo/Cr ratio values was significant before correction, but did not survive FDR correction (Fig. 2D, $r = 0.6$, uncorrected *p-value* = 0.02). No further significant correlations were found in PSP-RS group.

4. Discussion

In the current study, we have demonstrated *in vivo* the presence of neurometabolic changes in SMA of PSP-RS patients. By using ^{1}H-MRS, we found that PSP-RS patients had lower Scyllo concentration and Scyllo/Cr ratio values in the SMA in comparison with control subjects. It is noteworthy that the decreased Scyllo values correlated significantly with attention and working memory functions, as measured by digit span forward test.

Few previous ^{1}H-MRS studies [15–18] explored the presence of neurometabolic alterations in PSP-RS patients by using a 1.5T MR scanner. The authors restricted their analysis only to few metabolites (i.e. NAA, Cho and Cr) and to structures other than SMA. As reported in previous studies, lentiform nucleus [15–18], frontal cortex [17,18], frontoparietal cortex [16], brainstem, and centrum semiovale [18] can display a neural density marker reduction in PSP patients.

To our knowledge, this is the first study reporting an *in vivo* decrease of Scyllo in the SMA of the patients with PSP-RS, by using a 3T MR scanner. Indeed, no studies have investigated the neurometabolic pattern of SMA of PSP-RS patients. Moreover, 3T MR scanner allowed us to obtain high quality spectra, with an optimal signal to noise ratio, and to detect Scyllo, which showed a rather small peak compared to other metabolites. Scyllo is one of five naturally occurring stereo-isomers of inositol and it is found in relatively high concentrations in human brain, although it has a concentration about 12 times lower than mI [13]. Abnormally high levels of cerebral Scyllo have been identified by ^{1}H-MRS in mitochondrial enzyme deficiency, some brain tumors, and human immunodeficiency virus infection [26]. Conversely, Scyllo depletion was detected in hepatic encephalopathy [27].

We have demonstrated for the first time that SMA concentration of Scyllo is decreased significantly in patients with PSP, but the pathological significance of Scyllo depletion in PSP patients is unclear. It may be due to reduced blood-brain transport or, alternatively, to decreased biosynthesis in the brain [27], and both mechanisms could depend on abnormal protein-related brain damage. McLaurin and colleagues were the first to investigate the interactions between Alzheimer amyloid-beta peptides with phospholipid membranes, such as inositol stereo-isomers [10,11], demonstrating their inhibitory effect on amyloid-beta aggregation, and suggesting their potential therapeutic role. Scyllo, in particular, being an orally available natural compound that penetrates into the brain, has been suggested as a promising therapeutic agent [11,12] in Alzheimer's disease. In fact, oral administration of Scyllo to a mouse model of Alzheimer's disease inhibited amyloid-beta aggregation, attenuated amyloid-beta-induced impairments of spatial memory, reduced cerebral amyloid-beta pathology, and decreased the rate of mortality [11]. Hence, Scyllo has received a fast-track designation from the United States Food and Drug Administration for treatment of mild to moderate Alzheimer's disease, and Bacillus subtilis was improved in order to efficiently produce Scyllo [12].

Moreover, recent studies have demonstrated that Scyllo seems help protect against formation, not only of toxic fibrils of amyloid-beta fragments, but also tau oligomers deposition in mouse models [13,14], opening new therapeutic approaches in other tauopathies, such as PSP.

As structural MRI, fMRI, FDG- and [^{18F}]AV-1451-PET studies have

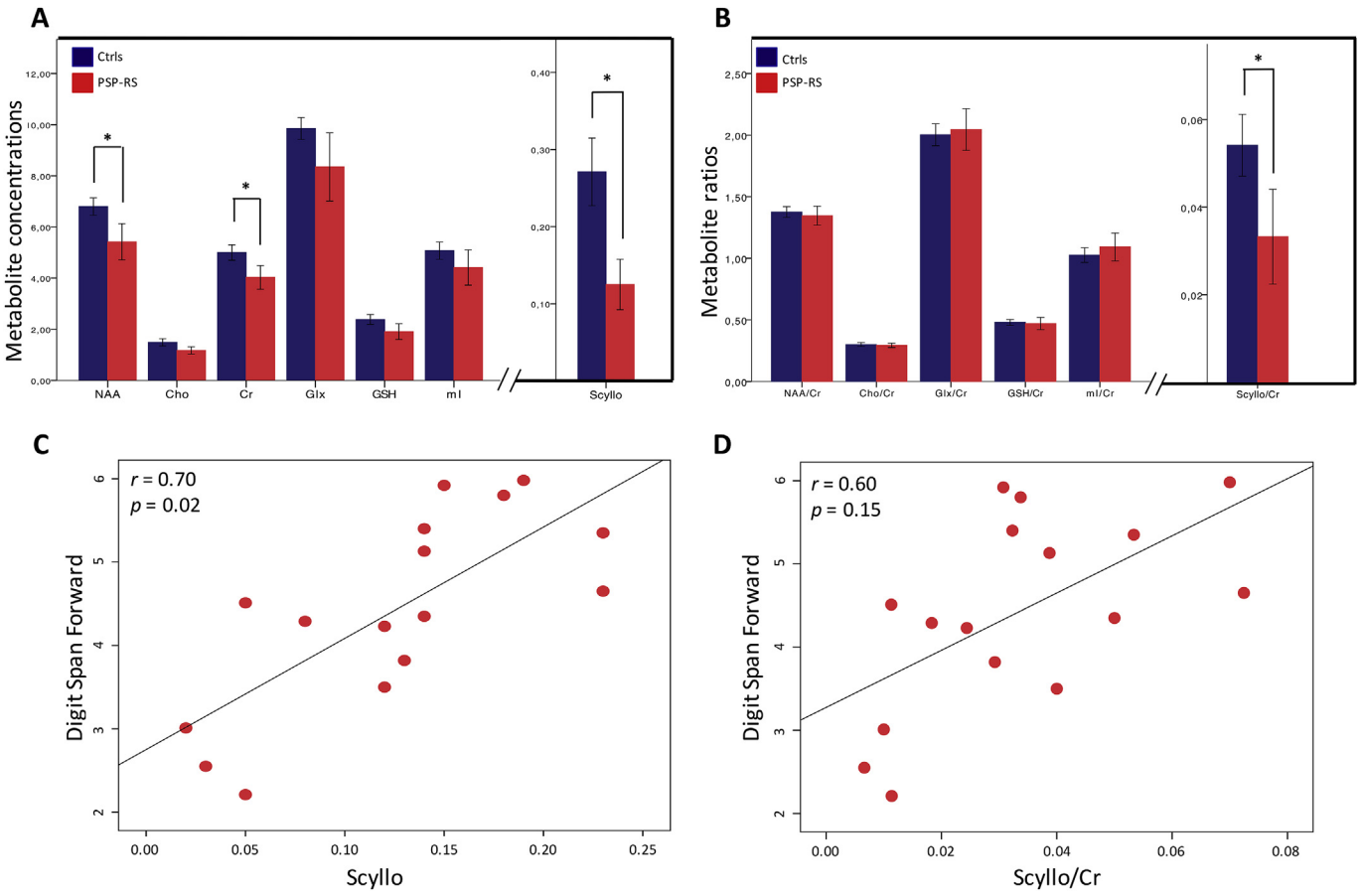

Fig. 2. Metabolic inter-group differences and correlations between spectroscopic data and neuropsychological scores.
(**A–B**) Differences in PVE-corrected metabolic concentrations (**A**) show that NAA, Cr, and Scyllo are lower in PSP patients than in controls, whereas differences in metabolic ratios (**B**) show that only Scyllo/Cr ratio is lower in PSP patients than in controls. * = FDR-corrected p-value < 0.05. (**C–D**) Correlation scatterplots of the Digit Span Forward scores with PVE-corrected absolute quantification of Scyllo concentrations (**C**) and with Scyllo/Cr ratios (**D**) in PSP patients. r = Pearson's correlation coefficient; p = FDR-corrected p-value.

shown, the SMA is involved in patients with PSP, displaying volume loss [3], microstructural damage [4], functional dysconnectivity [5], hypometabolism [6], and tau deposition [7]. However, in our PSP-RS group, despite NAA and Cr were significantly decreased, the lack of alterations of NAA/Cr, Cho/Cr, Glx/Cr, GSH/Cr, and mI/Cr ratios detected by ^{1}H-MRS suggest that in the SMA of PSP patients there are no changes in neuron density, damage of cellular membrane (including cell bodies, axons and dentrites), excitotoxicity, oxidative stress, or glial damage. A potential explanation for this apparent inconsistency may lie in the fact that, as previous studies have demonstrated [28], microstructural damages may occur before changes in neuron density or membrane markers. Indeed, we included patients with a short duration of disease, and it is possible that some spectroscopic alterations appear in more advanced stages of the disease. Therefore, studies including patients in more advanced PSP stages are needed to explore the presence of other spectroscopic alterations in the SMA. Conversely, Scyllo was the only metabolite that survived normalization to Cr, supporting the evidence that it may play a role in the dysfunction of the SMA in PSP patients. The positive relationship of attention and working memory functions with both Scyllo/Cr ratio values and Scyllo concentration in the SMA observed in our PSP patients further supports an involvement of this inositol stereo-isomer in the PSP-related frontal dysfunction, in which the SMA may play an important role. Previous fMRI data also point toward SMA involvement in time processing, which in turn is pivotal in the control of the attention and working memory [29]. Also, recent fMRI studies confirm that lesions involving the SMA can cause working memory deficit [30]. Our data demonstrate that, in PSP patients, Scyllo concentrations in the SMA are positively related to digit span forward scores. This result indicates that attention and working memory functions can be higher when there is a greater Scyllo concentration, and it suggests that Scyllo may help protect against tau oligomers deposition in the SMA [7]. In addition, the anterior cingulate below the SMA mediates attention and could have contributed to the ^{1}H-MRS signals and their correlations with neuropsychological functions.

The present study has some limitations to be acknowledged. First, no definite diagnosis of PSP-RS was confirmed on post-mortem analysis, and it cannot assume that all patients had a tauopathy. However, internationally validated criteria with good sensitivity and specificity [19] were used by two expert neurologists. Second, single-voxel approach did not allow us to explore neurometabolic pattern of other structures potentially affected in PSP, such as other cortical, subcortical or infratentorial structures. Third, our study was limited by the cross-sectional design. Furthermore, caution should be taken in attempting to relate these findings without further inquiry, because it cannot be excluded that a decrease of Scyllo in the SMA could not be related to the tau pathology [7]. For this reason, future investigations should combine multiple MRI parameters (such as structural MRI and ^{1}H-MRS) with nuclear medicine techniques (FDG-PET and/or AV-1451-PET) to further investigate the relationships between the structural, biochemical and metabolic abnormalities of patients with PSP. Moreover, another crucial investigation could be to longitudinally study the neurometabolic changes in the SMA, in order to clarify the role of Scyllo and other metabolites in the neuropsychological or motor dysfunctions in PSP.

In conclusion, our findings provide the first *in vivo* evidence of decreased Scyllo levels in the SMA of PSP patients by using ^{1}H-MRS, opening new perspectives to investigate the Scyllo as a new potential disease-modifying therapy for PSP.

Declaration of interest

None.

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Author's contribution

Barbagallo Gaetano: (1) the conception and design of the study, acquisition of data, analysis and interpretation of data, (2) drafting the article and revising it critically for important intellectual content, (3) final approval of the version to be submitted.

Morelli Maurizio: (1) acquisition of data, (2) revising the article critically for important intellectual content, (3) final approval of the version to be submitted.

Quattrone Andrea: (1) acquisition of data, (2) revising the article critically for important intellectual content, (3) final approval of the version to be submitted.

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Fabiana Novellino: (1) acquisition of data, (2) revising the article critically for important intellectual content, (3) final approval of the version to be submitted.

Alessia Sarica: (1) analysis and interpretation of data, (2) revising the article critically for important intellectual content, (3) final approval of the version to be submitted.

Gennarina Arabia: (1) acquisition of data, (2) revising the article critically for important intellectual content, (3) final approval of the version to be submitted.

Sabatini Umberto: (1) acquisition of data, (2) revising the article critically for important intellectual content, (3) final approval of the version to be submitted.

Quattrone Aldo: (1) the conception and design of the study, analysis and interpretation of data, (2) revising the article critically for important intellectual content, (3) final approval of the version to be submitted.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.parkreldis.2018.12.008.

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