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The contribution of cerebellar proton magnetic resonance spectroscopy in the differential diagnosis among parkinsonian syndromes

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ABSTRACT

Introduction: The *in vivo* differential diagnosis between idiopathic Parkinson's disease (PD) and atypical parkinsonian syndromes (PS), such as multiple system atrophy [MSA with a cerebellar (C) and parkinsonian (P) subtype] and progressive supranuclear palsy – Richardson's Syndrome (PSP-RS) is often challenging. Previous brain MR proton spectroscopy (^{1}H-MRS) studies showed biochemical alterations in PS, despite results are conflicting. Cerebellum plays a central role in motor control and its alterations has been already demonstrated in atypical PS. The main aim of this study was to evaluate diagnostic accuracy of cerebellar ^{1}H-MRS in the differential diagnosis between PD and atypical PS.
Methods: We obtained ^{1}H-MRS spectra from the left cerebellar hemisphere of 57 PS (21 PD, and 36 atypical PS) and 14 unaffected controls by using a 1.5 T GE scanner. N-acetyl-aspartate (NAA)/Creatine (Cr), choline-containing compounds (Cho)/Cr, myoinositol (mI)/Cr, and NAA/mI ratios were calculated.
Results: NAA/Cr and NAA/mI ratios were significantly lower ($p < 0.01$) in atypical PS compared to PD and controls, and in MSA-C compared to PD, MSA-P, PSP-RS and controls. PSP-RS group showed reduced NAA/Cr ratios compared to PD ($p < 0.05$) and controls ($p < 0.05$), and reduced NAA/mI compared to controls ($p < 0.01$). NAA/Cr ratio values higher than 1.016 showed 100% sensitivity and negative predictive value, 62% positive predictive value and 64% specificity in discriminating PD.
Conclusion: Cerebellar biochemical alterations detected by using ^{1}H-MRS could represent an adjunctive diagnostic tool to improve the differential diagnosis of PS.

1. Introduction

Parkinsonian syndromes (PS) are an heterogeneous group of neuropathologically distinct degenerative disorders characterized by extrapidamidal signs associated with pyramidal, cerebellar, autonomic and cognitive dysfunction. The main forms are represented by idiopathic Parkinson's disease (PD), Progressive Supranuclear Palsy – Richardson's Syndrome (PSP-RS), and the cerebellar and parkinsonian variant of Multiple System Atrophy (MSA-C and MSA-P respectively). Despite differences in clinical presentation, disease progression and response to dopaminergic treatment, the *in vivo* differential diagnosis remains challenging, especially in the earlier stages of the disease. International criteria have been established in order to permit a clinical "possible" or "probable" diagnosis, although the definite diagnosis is only anatomopathological and can be reached *post-mortem* [1–3]. In the last decades, various biomarkers have been evaluated in order to increase diagnostic accuracy of clinical criteria and to permit a differential diagnosis in the early disease's stages [4]. In particular, the

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contribute of neuroimaging in the differential diagnosis of PS has been established. The role of conventional brain MRI is crucial to distinguish secondary forms from neurodegenerative PS and to highlight pathognomonic alterations suggestive of different neurodegenerative syndromes such as the "hummingbird sign" due to tegmental midbrain atrophy in PSP and the "hot-cross bun sign" in MSA, signifying selective degeneration of ponto-cerebellar tracts [5–7]. Advanced MR techniques, such as volumetric and morphometric analysis, diffusion tensor imaging (DTI), proton MR spectroscopy (^{1}H-MRS), and functional MRI, associated to conventional imaging, may contribute to define macro- and microstructural, biochemical and functional alterations in PS [5,8,9]. In particular, brain ^{1}H-MRS permits an *in vivo* quantitative evaluation of biochemical profile within specific brain volumes of interest (VOIs). In particular, it has been shown that a reduction of N-acetylaspartate (NAA) concentration is related to neuronal and/or axonal damage as in neurodegenerative disorders such as dementias and ataxias, and increased myo-inositol (mI) is considered as a marker of glial reaction [9,10].

^{1}H-MRS studies have been carried out in PS in order to better elucidate their pathophysiology and to contribute to differential diagnosis [10]. Different structures, such as substantia nigra, basal ganglia, hemispheric white and gray matter have been evaluated by this technique and the principal studies are summarized in Table 1. Because of the clinical variability of samples, different technical and methodological issues, the results of these studies are conflicting. Moreover, for the small size of the main brain structures involved in PD and atypical PS pathophysiology, such as substantia nigra and the basal ganglia, it is challenging to obtain an accurate metabolic profile by using ^{1}H-MRS avoiding the partial volume effect of the adjacent structures [44].

The cerebellum is one major subcortical structure involved in motor control [45]. It is functionally and anatomically connected with the basal ganglia and its alterations have been demonstrated in PD [45]. Moreover, neuroimaging and neuropathological studies demonstrated macro- and microstructural changes in the cerebellum in MSA-C and PSP [47–50]. Previous studies evidenced the usefulness of cerebellar ^{1}H-MRS in evaluating neurochemical alterations in inherited and sporadic ataxias [51–56]. Although the cerebellar involvement in movement disorders' pathophysiology, spectroscopic studies on cerebellum for the differential diagnosis of PS are lacking to date. In the only ^{1}H-MRS study performed on cerebellar hemispheres Chaudhuri and colleagues reported normal NAA/Cr values in a small PD cohort compared to healthy controls [15].

The main aim of our study was to evaluate cerebellar biochemical profile in different forms of PS by using single voxel ^{1}H-MRS and to assess its accuracy in the differential diagnosis between PD and atypical PS. As secondary outcome, we also evaluated differences in cerebellar biochemical profile between parkinsonisms with cerebellar signs (cerebellar subgroup: PSP-RS and MSA-C) and without cerebellar signs (parkinsonian subgroup: PD and MSA-P).

2. Materials and methods

2.1. Subjects

We retrospectively included in this study all patients with PS referred between 2008 and 2013 to the Functional MR Unit, Policlinico S.Orsola – Malpighi, Bologna (IT), to perform brain MR as part of the diagnostic workup. We acquired and analyzed brain conventional MR images and ^{1}H-MRS spectra of 57 PS: 21 PD, 36 atypical PS (15 MSA, 8 MSA-C and 7 MSA-P, and 21 PSP) and 14 controls, without evidence of neurological disorders. Controls, matched to patients for age and sex, were selected among a sample of healthy volunteers, enrolled among University and Hospital workers and their relatives, that underwent brain MR in order to obtain normative values for quantitative MR parameters for clinical and research purposes. Demographic and clinical features of cases and controls are summarized in Table 2. Diagnosis were performed by neurologists with more than 10 years of experience in movement disorders (SZ, GC-B, MG, AG, PC) according to international criteria for PD [1], PSP [2], and MSA [3]. All PSP patients presented the Richardson's Syndrome variant (PSP-RS) [57]. Only 2 out of 21 PD patients were at Hoehn-Yahr stage 1 and presented parkinsonian signs only in one side (one patient right and the other left), while the other 19 presented bilateral symptoms with side prevalence, 9 with a right and 10 with a left prevalence. On the basis of clinical data obtained from the patients and from their clinical records at the day of MR scan, 12 PD patients fulfilled criteria for possible and 9 for probable PD, 1 PSP-RS patient was diagnosed as possible and 20 as probable PSP-RS, while all MSA patients fulfilled criteria for probable MSA. Because MR scans acquisitions were performed years before the analysis, in 7 PD patients the diagnosis evolved from possible to probable PD. The study protocol was approved by the local Ethical Committee and we obtained the written informed consent to personal data processing for research purposes from all participants.

2.2. Brain magnetic resonance imaging and spectroscopy acquisition

Brain MR studies were performed using a 1.5 T GE® Medical Systems Signa HDx 15, equipped with a quadrature birdcage head coil. Structural imaging included axial FLAIR T2-weighted images (repetition time, TR = 8000 ms, inversion time, TI = 2000 ms, echo time, TE = 93.5 ms, 3 mm slice thickness with no inter-slice gap), FSE coronal T2-weighted images (TR = 7000 ms, TE = 100 ms, 3 mm slice thickness), and 3D volumetric T1-weighted fast spoiled gradient-echo (FSPGR) images (TR = 12.5 ms, TE = 5.1 ms, TI = 600 ms, 25.6 cm^2 FOV; 1 mm isotropic voxels).

MR images obtained from each subject were visualized by expert neuroradiologists (RL, CaT) in order to exclude secondary causes of parkinsonism and other signal intensity or morphology changes.

A VOI of 2.0 × 2.0 × 1.5 cm^3 was selected in the left cerebellar hemisphere (Fig. 1) using the three planes of high resolution 3D FSPGR T1 sequence to optimise the localisation. The selected VOI included cerebellar nuclei and white matter. Suppressed-water proton MR spectra were acquired using the PRESS single-voxel localization sequence (PROBE) with TR = 4000 ms, TE = 35 ms, and averaging 64 FIDs for each acquisition [58]. The acquisition time for MR spectroscopy sequence was 5 min 52 s.

We decided to select the VOI in the left cerebellar hemisphere in order to standardize our acquisition and analysis protocol for all parkinsonian syndromes (with asymmetrical and symmetrical signs).

2.3. MR spectroscopic data analysis

Peak areas of NAA + NAA glutamate (NAAG), Cr + phospho-Cr (PCr), Glycero-phospho Cho (GPC)+ phospho Cho (PCh), and mI, were calculated using version 6.3 of the fitting program LCModel [59,60], a fully user-independent software, that analyzes spectra as a linear combination of complete model spectra of metabolite solutions in vitro. Metabolite content was expressed relative to Cr + PCr. The exclusion criterion for metabolite evaluation was an LCModel estimated fitting error >20%, this being a reliable indicator of poor quality spectra.