Parkinsonism and Related Disorders 62 (2019) 226–230

Contents lists available at ScienceDirect

Parkinsonism and Related Disorders

journal homepage: www.elsevier.com/locate/parkreldis

Short communication

Stridor-related gray matter alterations in multiple system atrophy: A pilot study

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ARTICLE INFO

Keywords:
Stridor
Multiple system atrophy
Voxel based morphometry

ABSTRACT

Introduction: The neuroanatomical substrate of stridor associated with Multiple System Atrophy (MSA) remains unclear. We evaluated stridor-related gray matter (GM) changes in MSA.

Methods: 36 MSA patients underwent standardized nocturnal video-polysomnography and brain MRI. Differences in GM density between MSA patients with and without stridor and a sample of 22 matched healthy controls were evaluated with Voxel Based Morphometry protocol supplemented by a specific tool (SUIT) for analysing infratentorial structures.

Results: Stridor was confirmed in 14 patients (10 MSA-cerebellar variant; 10 M; mean ± SD age = 61.6 ± 8.9years; disease duration = 5.2 ± 2.9years) and absent in 22 (11 MSA-cerebellar variant; 18 M; age = 61.4 ± 9.9years; disease duration = 4.8 ± 3.4years). Compared to MSA without stridor, patients with stridor showed higher GM density in the cerebellum ($p < 0.05$, corrected for the MSA-cerebellar variant and uncorrected when considering both MSA-variants) and lower in the striatum ($p < 0.05$, uncorrected).

Conclusions: This preliminary study has demonstrated for the first time in MSA stridor-related GM changes in striatal and cerebellar regions. Abnormalities in these regions were previously reported in dystonic disorders affecting laryngeal muscles, suggesting the hypothesis that stridor pathophysiology is dystonia-related. These results need however to be confirmed in a larger sample of patients.

1. Introduction

Multiple System Atrophy (MSA) is a neurodegenerative disease characterized by dysautonomia associated with various combinations of parkinsonism and/or cerebellar dysfunction. Sleep disorders, in particular REM-sleep behavior disorder and sleep-related breathing disorders are common in MSA patients.

Stridor is a harsh, high-pitched inspiratory sound due to a laryngeal narrowing, which in MSA typically occurs during sleep, affecting up to 42% of MSA patients and being a possible cause of sudden death during sleep. Its early onset is considered a predictor of shorter survival [1,2].

Although the mechanisms underpinning the generation of stridor in MSA are not completely elucidated yet, two main hypotheses based on electrophysiological and neuropathological studies have been formulated: dystonic hyperactivation of laryngeal adductor muscles or hypoactivity of laryngeal abductor muscles [2,3].

Some neuroimaging studies focusing on spasmodic dysphonia (SD) have demonstrated an involvement of different regions of the sensorimotor pathway, in particular, the motor cortex, basal ganglia and cerebellum [4,5]. To our knowledge, no studies have evaluated stridor-related brain alterations in vivo in MSA patients.

The aim of this study was to investigate brain gray matter (GM) changes related to stridor in MSA patients, by applying a hypothesis-free method (Voxel Based Morphometry, VBM).

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https://doi.org/10.1016/j.parkreldis.2018.11.018
Received 21 May 2018; Received in revised form 9 November 2018; Accepted 14 November 2018

Table 1
Demographic and clinical features of the study sample.

	MSA			HC	p-value
	All	*MSAwS*	*MSAwoS*		
N (%)	36 (100.0)	14 (38.9)	22 (61.1)	22	-
AAE (y) [mean ± SD (range)]	61.5 ± 9.4 (40.6–85.4)	61.6 ± 8.9 (47.6–79.0)	61.4 ± 9.9 (40.6–85.4)	56.7 ± 10.1 (40.0–83.0)	0.170 *(one-way ANOVA)*
SEX (M/F)	28/8	10/4	18/4	12/10	0.144 *(χ^2 Pearson's test)*
DD (y) [mean ± SD (range)]	4.9 ± 3.2 (1.0–14.0)	5.2 ± 2.9 (1.0–13.0)	4.8 ± 3.4 (1.0–14.0)	–	0.413 *(Mann-Whitney test)*
H − Y [mean ± SD (range)]	3.3 ± 1.0 (1.5–5.0)	3.5 ± 1.1 (2.0–5.0)	3.1 ± 1.0 (1.5–5.0)	–	0.311 *(Mann-Whitney test)*
MSA-C (%)	21 (58.3)	10 (71.4)	11 (50.0)	–	0.400 *(χ2 Pearson's test)*
RBD (%)	31 (86.1)	14 (100.0)	17 (77.3)	–	0.83 *(χ2 Pearson's test)*
Snoring (%)	26 (72.2)	10 (71.4)	16 (72.7)	–	0.932 *(χ2 Pearson's test)*
OSAS (%)	13 (36.1)	5 (35.7)	8 (36.4)	–	0.968 *(χ2 Pearson's test)*
Stridor age at onset (y) [mean ± SD (range)]	–	60.4 ± 9.2 (47–77)	–	–	–
Stridor onset latency (y) ^ [mean ± SD (range)]	–	3.9 ± 3.4 (0–13.0)	–	–	–
DD (y) after stridor onset [mean ± SD (range)]	–	2.8 ± 1.7 (1.0–7.0)	–	–	–
Stridor duration since MR scanning [mean ± SD (range)]	–	1.2 ± 2.0 (−1.5–6.7)	–	–	–

Legend. MSA: multiple system atrophy; HC: healthy controls; MSA-wS: MSA with stridor; MSA-woS: MSA without stridor; AAE: age at evaluation; SD: standard deviation; DD: disease duration; H − Y: Hoehn-Yahr modified scale; MSA-C: cerebellar variant of MSA; RBD: REM-sleep behavior disorder; OSAS: obstructive sleep apnoea syndrome. ^ stridor onset latency calculated from the onset of motor symptoms.

2. Materials and methods

2.1. Study sample

We retrospectively analyzed 36 patients with probable MSA who underwent brain MR scanning at the Functional MR Unit from January 2008 to January 2016 within their diagnostic work-up.

All patients were evaluated at least once a year during the disease course at the Movement Disorders Centre of the IRCSS Istituto delle Scienze Neurologiche di Bologna. The diagnosis of probable MSA was performed at the last follow-up evaluation according to international criteria [6]. The study protocol, approved by the local Ethical Committee, includes a retrospective and an ongoing prospective phase (Cod.: 17093, 13.07.2017). All patients retrospectively included gave written consent to process their data in the study.

Twenty-two age- and sex-comparable healthy controls (HC) were also included in the study, selected from the Functional MR Unit database of healthy volunteers (Ethical Committee approval Cod.: 120/2014, 7.10.2014). Demographic and clinical data of patients and controls are reported in Table 1.

2.2. Video-polysomnographic recording

In order to confirm the presence of stridor, all patients underwent whole night video-polysomnography (VPSG). Sleep habits and presence of sleep disorders were also evaluated by structured interview and checked with interviews of close relatives.

The whole night (from 11 p.m. to 7 a.m.) digitally recorded VPSG included electroencephalogram, surface electro-oculogram, surface electromyogram of the submental, wrist extensor, tibialis anterior and intercostalis muscles, electrocardiogram, microphone, oro-nasal, thoracic and abdominal respirograms, oxygen saturation, and continuous audio-visual acquisition. Stridor was defined as a strained, high-pitched, harsh respiratory sound on audio–video monitoring. Obstructive Sleep Apnoea Syndrome was diagnosed when more than ten apnoea-hypopnoea episodes per hour of sleep were observed.

2.3. Brain MRI protocol and analysis

MR acquisitions were performed on a 1.5T GE Medical Systems scanner with a volume head coil. The standardized protocol included coronal T2-weighted FLAIR (TR = 8000 ms, TI = 2000 ms, TE = 93.5 ms, 3 mm slices) and volumetric T1-weighted FSPGR images (TR = 12.5 ms, TE = 5.1 ms, TI = 600 ms, 1 × 1 × 1 mm^3 voxels).

To exclude possible bias related to chronic vascular white matter lesions, we performed semi-automated lesion segmentation on T2-weighted FLAIR images (Jim7.0, Xinapse Systems) and a lesion filling of volumetric images.

For VBM analyses (SPM12.0), GM maps were segmented from structural images, non-linearly registered to create a study specific template, then smoothed and intensity-modulated to account for deformations.

To preserve anatomical details of cerebellar sub-regions and get a more accurate inter-subject alignment at the infratentorial level, SUIT (Spatially Unbiased Infratentorial Toolbox, http://www.diedrichsenlab.org/imaging/suit.htm) was used [7].

2.4. Statistical analysis

Demographic and clinical variables were compared, according to their type, using ANOVA, Pearson's χ [2] or a Mann-Whitney test (IBM® SPSS® v.21).

Voxel-wise non parametric testing was conducted on GM maps using the FSL tool *randomize* (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/Randomise), for both comparisons between groups and correlations with clinical variables, considering $p < 0.05$ corrected for multiple

comparisons (family-wise error rate) as significant. Given the preliminary nature of the study, when stridor-related differences did not reach a statistically significant level, clusters where the spatial extent was greater than 100 mm^3 were considered without multiple comparisons correction to explore potential gray matter density alterations to be successively confirmed in a larger sample. After an ANCOVA with age, sex, total intracranial volume and MSA variant as covariates, post-hoc tests between groups were performed with analogous covariates.

The same analyses were also conducted considering only the MSA-C patients subgroup in order to exclude that possible GM differences could be due to different proportion of MSA-C variant in the two groups (with and without stridor).

3. Results

Stridor was confirmed in 14 MSA patients and absent in 22; no significant differences were present in age and sex among the three groups, nor in clinical variables between MSA patients with (MSA-wS) and without stridor (MSA-woS) (Table 1).

Whole brain analysis showed that, compared to HC, both MSA-wS and MSA-woS had lower GM density within putamen and cerebellum ($p < 0.05$, corrected), with reductions extending further for MSA-woS in the cerebellum (Fig. 1A). MSA-wS showed lower GM density than MSA-woS in the putamen and higher within the cerebellum ($p < 0.05$, uncorrected) (Fig. 1A).

The cerebellar analyses showed that GM alterations in MSA-woS and MSA-wS compared to HC were mainly located in bilateral crus I, vermis VI, VIIIa and left V, bilateral VI, right I-IV and VIIb lobules ($p < 0.05$, corrected) (Fig. 1B).

Higher GM density in MSA-wS compared to MSA-woS was found in right crus I, right VIIb-VIIIa lobule, bilateral VI lobule and vermis VI ($p < 0.05$, uncorrected) (Fig. 1B). No infratentorial regions had a significant lower GM density in MSA-wS compared to MSA-woS.

When only MSA-C variant was considered, whole brain VBM showed that compared to HC, both MSA-wS and MSA-woS had lower GM density in the cerebellum, MSA-wS had lower GM density also in the putamen ($p < 0.05$, corrected). MSA-wS showed lower GM density than MSA-woS in the putamen ($p < 0.05$, uncorrected) and higher density within the cerebellum ($p < 0.05$, corrected) (Fig. 1A).

In MSA-C, SUIT cerebellar analyses showed that GM alterations in MSA-woS and MSA-wS compared to HC were mainly located in bilateral crus I, II, bilateral I-IV, V, VI, VIIb, IX lobules, right VIIIa lobule, and vermis VI, VIIb, VIIIa, VIIIb, IX ($p < 0.05$, corrected) (Fig. 1B).

MSA-wS compared to MSA-woS in MSA-C variant showed a significantly higher GM density in bilateral crus I ($p < 0.05$, corrected) (Fig. 1B). No infratentorial regions had a significant lower GM density in MSA-wS compared to MSA-woS.

4. Discussion

We have shown for the first time preliminary evidence of stridor-related GM abnormalities in a group of MSA patients, located in the putamen and cerebellum. In particular, patients with stridor compared to patients without, showed higher GM density in the cerebellum and lower in the striatum, suggesting a possible involvement of these regions in stridor pathophysiology.

These results need however to be evaluated with caution as statistical differences were observed with proper correction only when considering the MSA-C variant alone and important co-variates such as disease progression and stridor severity were not included in the analysis.

Increasing evidence from neuroimaging studies indicates that an altered interconnection among basal ganglia, motor cortex, cerebellar cortex and dentate nuclei may be involved in the pathophysiology of dystonia [8]. In particular, cerebellar dysfunction may contribute to the development of dystonia mainly by inducing a disruption of the connections with the thalamus and the brain cortex [4,8].

Accordingly, in our MSA cohort with stridor, we found higher GM

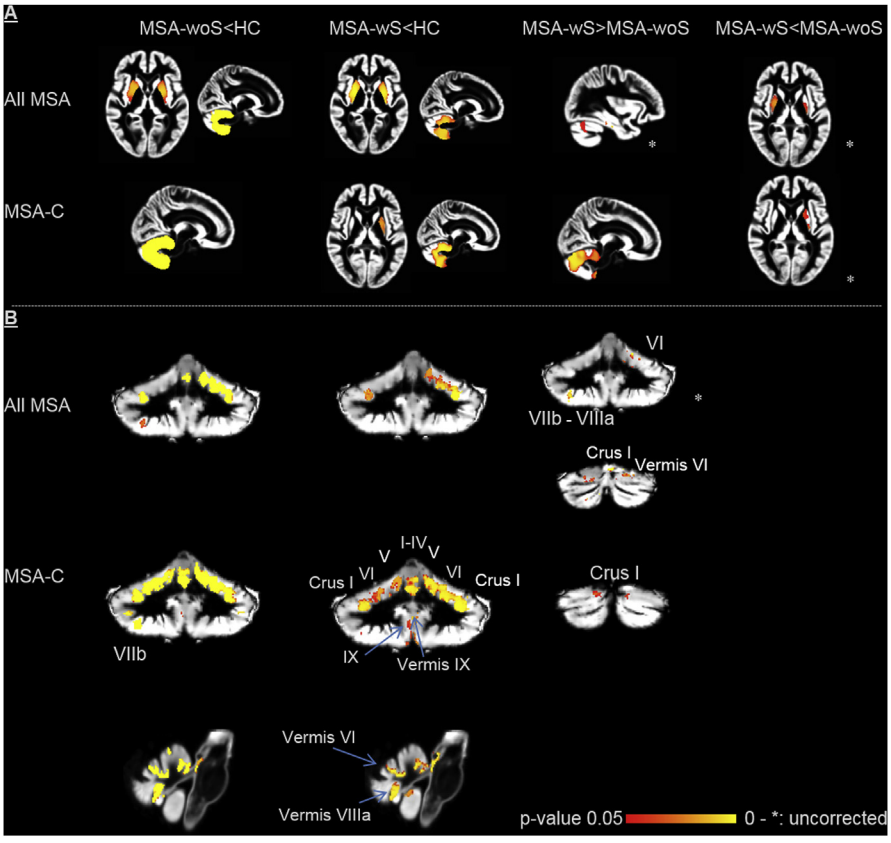

Fig. 1. VBM results A: Whole-brain VBM results, considering all MSA patients (top) and only MSA-C patients (bottom). B: VBM results for the infratentorial compartment (SUIT), considering all MSA patients, (top) and only MSA-C patients (bottom). Images are shown in radiological convention. P-values maps are shown on the study specific templates, $p < 0.05$ corrected (a part from the cases marked with *, $p < 0.05$ uncorrected). The color bar represents the significance of voxel-wise non parametric tests for comparisons between groups expressed as p-value, ranging from 0.05 (red) to 0 (yellow). MSA: Multiple System Atrophy, MSA-woS: MSA without stridor, MSA-wS: MSA with stridor, HC: healthy controls. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

density of bilateral crus I, right VIIb-VIIIa lobules, left VI lobule and vermis VI, although these results need to be evaluated with an appropriate degree of caution.

Vermis VI has been associated to sensorimotor processes involving the head/face [9]. Although cerebellar crus I and lobules VI-VII have been mainly associated with higher-level processes, their functional connection with sensorimotor cortex has been demonstrated in healthy volunteers [10].

The finding of reduction of putaminal volume in relation to stridor is in agreement with striatal lesions triggering dystonia and previous findings of striatal GM changes [8].

Our preliminary results, despite requiring confirmation in a larger cohort, hint that stridor may be considered as a form of laryngeal dystonia being, overall, in line with previous MR studies in spasmodic dysphonia and, more generally, in craniofacial and primary focal dystonia in which structural and functional alterations in infratentorial structures were demonstrated [4], in particular an increase of GM volume in cerebellar crus II.

The dystonic nature of stridor has also been suggested by neurophysiological studies on small cohorts of MSA patients demonstrating a tonic hyperactivation of vocal cords muscles during sleep with a phasic activation during inspiration [3]. Moreover, the injection of botulin toxin, used to threat dystonia, has been reported to ameliorate stridor, by reducing laryngeal muscle hyperactivation [11].

The comparison of GM density between MSA patients and controls showed a prominent atrophy in striatum and cerebellum. Our results for the MSA-C variant are in line with those of the study of Minnerop et al. [12], even though we did not find cortical alterations.

The atrophy of brainstem nuclei such as nucleus ambiguus and pre-Bötzinger complex might be expected given their role in the control of striated musculature of pharynx and larynx and also of breathing. Nevertheless, detection of gray matter changes in brainstem remains an intrinsic limitation of VBM analysis, since its internal architecture is difficult to resolve by tissue segmentation algorithms of T1-weighted images. A combination of different MR contrast images could resolve these structures [examples in Refs. [13,14]].

The main limitation of this study is the relatively small study sample that however reflects the prevalence of MSA, collected at a single centre. Further studies are needed to replicate these results. Taking into account the exploratory nature of this investigation, we applied a voxel-wise method and also considered results uncorrected for multiple comparisons, although this procedure makes the results provisional. Nevertheless, when the analysis was restricted to the MSA-C variant, stridor-related GM differences in bilateral crus I are significant after multiple comparison correction, giving a stronger value to the cerebellar pattern of alterations. A larger sample of MSA-P patients would permit a better balance between the two variants in the analyses of the stridor effect.

In conclusion, we have shown preliminary evidence of stridor-related GM changes, previously reported in dystonic disorders affecting laryngeal muscles, in MSA for the first time, localized in striatal and cerebellar regions. This exploratory study paves the way for further studies on larger samples and including functional/structural analyses to corroborate the hypothesis presented. If the hypotheses were to be confirmed, this would motivate possible specific therapeutic approaches.

Funding sources for study

None.

Authors' roles

Dr. Testa: drafting and revising the manuscript content, study concept and design, analysis and interpretation of data, statistical analyses, final approval of the version.

Dr. Calandra-Buonaura: revising the manuscript content, study concept and design, analysis and interpretation of data, final approval of the version.

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Dr. Talozzi: drafting and revising the manuscript content, interpretation of data, final approval of the version.

Dr. Manners: drafting and revising the manuscript content, analysis and interpretation of data, statistical analyses, final approval of the version.

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Prof. Cortelli: revising the manuscript content, study concept and design, interpretation of data, final approval of the version.

Financial disclosures of all authors

Dr. Testa reports no disclosures.
Dr. Calandra-Buonaura reports no disclosures.
Dr. Evangelisti reports no disclosures.
Dr. Giannini reports no disclosures.
Dr. Provini reports no disclosures.
Dr. Ratti reports no disclosures.
Dr. Cecere reports no disclosures.
Dr. Talozzi reports no disclosures.
Dr. Manners reports no disclosures.
Prof. Lodi reports no disclosures.
Prof. Tonon reports no disclosures.
Prof. Cortelli received honoraria for speaking engagements or consulting activities from Allergan, AbbVie, Chiesi Farmaceutici, Eli Lilly, Novartis, Teva, UCB, Zambon.

Acknowledgements

We thank Dr. Stefano Zanigni for his advice in the study design and Dr. Claudio Bianchini for technical support.

References

[1] G. Giannini, G. Calandra-Buonaura, F. Mastrolilli, M. Righini, M.L. Bacchi-Reggiani, A. Cerere, G. Barletta, P. Guaraldi, F. Provini, P. Cortelli, Early stridor onset and stridor treatment predict survival in 136 patients with MSA, Neurology 87 (13) (2016) 1375–1383.
[2] T. Ozawa, K. Sekiya, N. Aizawa, K. Terajima, M. Nishizawa, Laryngeal stridor in multiple system atrophy: clinicopathological features and causal hypotheses, J. Neurol. Sci. 361 (2016) 243–249.
[3] R. Vetrugno, R. Liguori, P. Cortelli, G. Plazzi, C. Vicini, A. Campanini, R. D'Angelo, F. Provini, P. Montagna, Sleep-related stridor due to dystonic vocal cord motion and neurogenic tachypnea/tachycardia in multiple system atrophy, Mov. Disord. 22 (5) (2007) 673–678.
[4] K. Simonyan, C.L. Ludlow, Abnormal structure-function relationship in spasmodic dysphonia, Cerebr. Cortex 22 (2) (2012) 417–425.
[5] V.S. Kostic, F. Agosta, L. Sarro, A. Tomić, N. Kresojević, S. Galantucci, M. Svetel, P. Valsasina, M. Filippi, Brain structural changes in spasmodic dysphonia: a multimodal magnetic resonance imaging study, Park. Relat. Disord. 25 (2016) 78-74.
[6] S. Gilman, G.K. Wenning, P.A. Low, D.J. Brooks, C.J. Mathias, J.Q. Trojanowski, N.W. Wood, C. Colosimo, A. Dürr, C.J. Fowler, H. Kaufmann, T. Klockgether, A. Lees, W. Poewe, N. Quinn, T. Revesz, D. Robertson, P. Sandroni, K. Seppi,

M. Vidailhet, Second consensus statement on the diagnosis of multiple system atrophy, Neurology 71 (2008) 670–676.
[7] J. Diedrichsen, A spatially unbiased atlas template of the human cerebellum, Neuroimage 33 (1) (2006) 127–138.
[8] S. Lehericy, M.A.J. Tijssen, M. Vidailhet, R. Kaji, S. Meunier, The anatomical basis of dystonia: current view using neuroimaging, Mov. Disord. 28 (7) (2013) 944–957.
[9] C. Mottolese, N. Richard, S. Harquel, A. Szathmari, A. Sirigu, M. Desmurget, Mapping motor representations in the human cerebellum, Brain 136 (2013) 330–342.
[10] L. Sang, W. Qin, Y. Liu, W. Han, Y. Zhang, T. Jiang, C. Yu, Resting-state functional connectivity of the vermal and hemispheric subregions of the cerebellum with both the cerebral cortical networks and subcortical structures, Neuroimage 61 (4) (2012) 1213–1225.
[11] L.M. Merlo, A. Occhini, C. Pacchetti, E. Alfonsi, Not paralysis but dystonia causes stridor in Multiple System Atrophy, Neurology 58 (4) (2002) 649–652.
[12] M. Minnerop, K. Specht, J. Ruhlmann, N. Schimke, M. Abele, A. Weyer, U. Wüllner, T. Klockgether, Voxel-based morphometry and voxel-based relaxometry in multiple system atrophy-a comparison between clinical subtypes and correlations with clinical parameters, Neuroimage 36 (4) (2007) 1086–1095.
[13] C. Lambert, A. Lutti, G. Helms, R. Frackowiak, J. Ashburner, Multiparametric brainstem segmentation using a modified multivariate mixture of Gaussians, Neuroimage Clin 2 (2013) 684–694.
[14] M. Ezra, O.K. Faull, S. Jbabdi, K.T. Pattinson, Connectivity-based segmentation of the periaqueductal gray matter in human with brainstem optimized diffusion MRI, Hum. Brain Mapp. 36 (9) (2015) 3459–3471.