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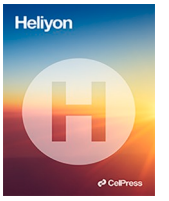

Research article

MRl and MRS hints for the differentiation of cerebellar multiple system atrophy from spinocerebellar ataxia type II

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ABSTRACT

Background and objectives: The differentiation of spinocerebellar ataxia type II (SCA 2) from idiopathic multiple systemic atrophy of the cerebellar type (MSA-C) is often difficult in patients with cerebellar ataxia when molecular testing is not available. Besides genetic testing, magnetic resonance imagining (MRI) and magnetic resonance spectroscopy (MRS) prove to be beneficial. Nevertheless, the characteristics observed through radiology change as the disease advances. Different radiological criteria may be needed across different stages of the disease. This study aimed to assess the radiological characteristics of MSA-C or SCA 2 patients across various stages of the disease and to identify potential distinguishing factors.

Methods: Between January 2000 and January 2020, a total of 390 patients, diagnosed with probable MSA-C according to the second consensus on MSA (317 cases) or with molecularly confirmed SCA 2 (73 cases), who had undergone at least one brain MRI and MRS targeting the cerebellar hemispheres, were enrolled in the study. The clinical parameters and neuroimaging features between these two diseases were compared and analyzed.

Results: A greater occurrence of a pontine hot cross bun sign (HCBS), higher scores on the scale for the assessment and rating of ataxia, and reduced levels of cerebellar N-acetyl aspartate (NAA)/creatine (Cr), and cerebellar choline (Cho)/Cr were found in MSA-C patients as compared with SCA 2 patients at similar disease durations. For the patients with an HCBS, a cerebellar Cho/Cr level of <0.53 was indicative of the potential presence of MSA-C, with significant level of specificity (85.96%).

Discussion: Discerning SCA2 from MSA-C using MRI and MRS appears to be plausible at various disease stages.

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1. Introduction

Idiopathic multiple systemic atrophy of the cerebellar type (MSA-C) is a neurodegenerative disorder that begins in adulthood, occurs sporadically, and has a dismal prognosis [1]. In clinical practice, the diagnosis relies on the presence of typical cerebellar ataxia, autonomic dysfunction, and/or a lack of positive response to levodopa, as outlined by the second consensus [2]. However, certain spinocerebellar ataxias (SCA), in particular, the rapidly progressive subtype SCA type II (SCA2) [3], could mimic MSA-C both clinically and radiologically [4]. Despite SCA is an autosomal dominant disorder, a considerable number of SCA2 patients might present with an uncertain family history. The unequivocal differentiation of SCA2 from MSA requires genetic testing [5].

When genetic testing is not readily available for a definitive diagnosis, magnetic resonance imaging (MRI) of brain and MR spectroscopy (MRS) prove to be valuable in everyday clinical practice to suggest either MSA-C or SCA2 [6]. MRI and MRS are widely utilized non-invasive techniques for assessing neurological abnormalities in the brain at both macroscopic and microscopic levels, respectively. In patients with SCA2 and MSA, cerebellar atrophy is frequently detected by MRI. A pontine hot cross bun sign (HCBS) [7], which manifests as a cruciform shape T2-weighted imaging hyperintensity at pons, is frequently seen in patients identified with MSA-C [8,9], however, it is important to note that pontine HCBSs can also be present in persons diagnosed with SCA2 [10], as was seen in 25.7% of cases [11,12]. Some in vivo brain metabolites changes are demonstrated by a clinical MRS protocol. A lower ratio of choline (Cho) to creatine (Cr) in the cerebellum is typically observed in MSA-C compared to SCA2. [12]; however, the imaging abnormalities would evolve with disease progression [12]. The changes at various disease stages have seldom been mentioned in the literature.

Based on our previous studies, we observed distinct variations in metabolite levels detected using MRS throughout varying stages of illness in patients identified with MSA-C and SCA2. Building upon these findings, our hypothesis is that the combination of MRI and MRS findings at various stages of the diseases may serve as a valuable tool for differentiating MSA-C from SCA2. This study aimed to identify changes in MRI and MRS data at different stages of the disease in patients with MSA-C or SCA 2.

2. Methods

2.1. Participants

The study received approval from the Institutional Review Board (IRB) of in a tertiary hospital (VGHTPE). Each participant provided their informed consent through a document ratified by the IRB (2018-01-017B), ensuring compliance with all pertinent guidelines and regulations.

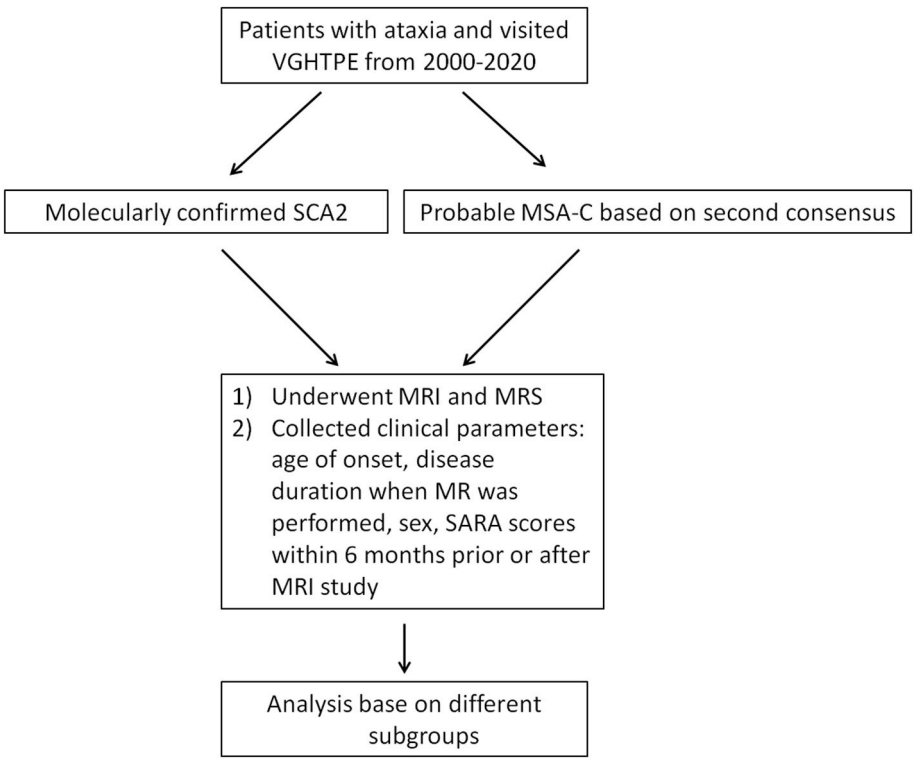

Fig. 1. The flowchart of patient inclusion details.

From January 2000 to January 2020, patients who met the criteria for either molecularly confirmed SCA2 or probable MSA-C, as outlined by the second consensus criteria for MSA-C, were recruited from the neurology department. This included those who had undergone at least one brain MRI and MRS targeting the cerebellar hemispheres and vermis. The study enrolled a total of 73 patients with SCA2 and 317 with MSA-C. Key clinical parameters such as age at onset of the disease, sex, duration of the disease at the time of the MRI, and scores from the scale for the assessment and rating of ataxia (SARA) within six months before or after the MRI (for patients diagnosed after 2006) were retrospectively reviewed and documented. The flowchart of patient inclusion details was presented as Fig. 1. The demographic characteristics of the patients are detailed in Table 1.

2.2. Parameters of neuroimaging and spectroscopy

Brain MRIs and MRS data were acquired using a 1.5-T system (Signa EXCITE, GE Medical Systems, Milwaukee, WI, USA). The imaging protocol included an axial T1-weighted three-dimensional fast spoiled gradient-recalled acquisition in steady state with a repetition time (TR) of 8.58 ms, echo time (TE) of 3.62 ms, inversion time (TI) of 400 ms, and a voxel size of $0.75 \times 0.75 \times 1.5\ mm^3$). Additionally, an axial T2 fast spin-echo sequence with a TR of 4000 ms, TE of 256.5 ms, and a voxel resolution of 348×512, alongside an axial fluid-attenuated inversion recovery (FLAIR) sequence with a TE of 350 ms, TR of 5000 ms, field of view (FOV) of 256 mm, inversion time of 1800 ms, matrix size of 256×224, and a flip angle of 120°, were utilized. To assess the presence of the hot cross bun sign (HCBS), a T2 fast spin-echo or FLAIR image was employed. Horizontal and vertical high signal lines at the pons, indicative of grades 3 to 5 as per Horimoto's criteria, confirmed the presence of HCBS [13].

Following the MRIs, proton MRS data were collected using a single-voxel stimulated echo acquisition mode (STEAM) sequence, tailored with specific parameters to optimize the analysis. The sequence settings included a TR of 3000 ms, an TE of 15 ms, a mixing time of 13.7 s, and 96 excitations. The spectral width was set to 2500 Hz, with data points numbering 2048, and the voxel resolution was determined to be 2 cm $\times$ 2 cm x 2 cm. The precise placement of the voxel of interest (VOI) within the cerebellar hemispheres and cerebellar vermis for each participant was meticulously carried out by an experienced neuroradiologist (JFL). The VOI was carefully positioned at the center of the right (Fig. 2A) and left (Fig. 2B) cerebellar hemispheres, ensuring avoidance of any cerebrospinal fluid spaces at the medulla level. Similarly, the vermis VOI was placed at the center of the vermis (Fig. 2C). It is important to note that no voxel tilt was applied during the acquisition process. The analysis focused on the peak area for NAA at 2.02 parts per million (ppm), Cr at 3.03 ppm, and also Cho at 3.22 ppm, utilizing vendor provided Functool software (GE XVi, Milwaukee, WI). Ratios of metabolites intensity, such as NAA/Cr and Cho/Cr, were computed autonomously following each voxel acquisition. To guarantee the integrity of the MRS data, results displaying a full width at half maximum (FWHM) of 6 Hz for the water peak were excluded from the analysis. If poor quality MRS was obtained, we repeated the MRS by placing the VOI at previous or next axial slice instead to try to get good quality MRS.

2.3. Statistical analyses

The Mann–Whitney *U* test, a nonparametric method, was employed for analyzing continuous variables that did not follow a normal distribution, while the chi-squared test was utilized to examine the nominal variables across various disease stages in patients with MSA-C compared to patients with SCA2. The factors contributing to HCBS in the patients were analyzed using logistic regression with a forward method and quantified as odd ratios (ORs) with accompanying 95% confidence intervals (CIs). To distinguish MSA-C from SCA2, cutoff values were determined using a receiver operating characteristic (ROC) curve analysis. The results are presented as means $\pm$ standard deviations (SDs). A p-value of less than 0.05 was considered statistically significant.

3. Results

3.1. Patient characteristics (Table 1)

Patients with SCA2 exhibited an earlier age of onset compared to those diagnosed with MSA-C. The distribution of gender among the two groups of patients did not display any significant difference.

Table 1
The demographics of the patients with MSA-C or SCA 2.

	Total (n = 390)		SCA2 (n = 73, 18.72%)		MSA-C (n = 317, 81.28%)		*p*-value
Age of disease onset[a]	52.18 ± 12.35		38.28 ± 14.08		55.31 ± 9.43		0.000
Sex[b]							
Female	203	52.05%	31	42.47%	172	54.26%	0.20
Male	187	47.95%	42	57.53%	145	45.74%	

The numeric data are presented as means ± standard deviations.
[a] Mann–Whitney test.
[b] chi-squared test; MSA-C, multiple system atrophy, cerebellar type; SCA2, spinocerebellar ataxia type 2.

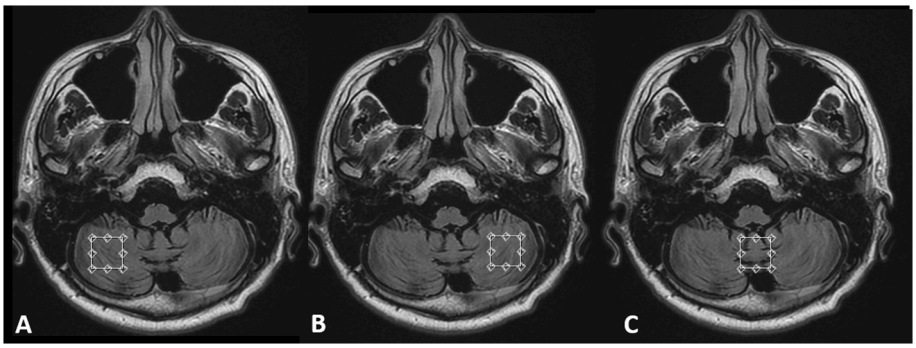

Fig. 2. Positioning of Magnetic Resonance Spectroscopy (MRS) Voxel of Interest (VOI). The VOIs for the cerebellar regions were precisely located at the center of the right (A) and left (B) cerebellar hemispheres, while the VOI for the vermis was centered within the vermis (C).

Table 2
The differences in the parameters between the patients with MSA-C or SCA2, with variable disease durations.

The differences in the parameters between patients with MSA-C or SCA 2 for all disease durations.					
		Total (n = 390)	SCA2 (n = 73)	MSA-C (n = 317)	*p*-value
HCBS[b]	Present	318	25	293	0.000
	Absent	72	48	24	
Disease duration[a]		4.45 ± 3.39	6.79 ± 4.66	3.92 ± 2.76	0.000
Cerebellum[a]	NAA/Cr	0.62 ± 0.15	0.66 ± 0.21	0.61 ± 0.13	0.000
	Cho/Cr	0.54 ± 0.16	0.59 ± 0.16	0.53 ± 0.16	0.000
Vermis[a]	NAA/Cr	0.67 ± 0.14	0.70 ± 0.19	0.66 ± 0.13	0.000
	Cho/Cr	0.57 ± 0.13	0.63 ± 0.12	0.56 ± 0.12	0.000
SARA score[a]		Total (n = 371)	SCA2 (n = 64)	MSA-C (n = 307)	0.000
		16.31 ± 7.99	12.69 ± 6.33	17.10 ± 8.11	
The differences in the parameters between the patients with MSA-C or SCA2 for those with a disease duration of less than 4 years.					
		Total (n = 239)	SCA2 (n = 25)	MSA (n = 214)	*p*-value
HCBS[b]	Present		7	194	0.000
	Absent		18	20	
Disease duration[a]		2.40 ± 1.18	2.60 ± 0.99	2.37 ± 1.2	0.148
Cerebellum[a]	NAA/Cr	0.64 ± 0.13	0.72 ± 0.18	0.63 ± 0.13	0.000
	Cho/Cr	0.58 ± 0.13	0.63 ± 0.11	0.57 ± 0.14	0.001
Vermis[a]	NAA/Cr	0.69 ± 0.13	0.72 ± 0.19	0.69 ± 0.12	0.047
	Cho/Cr	0.60 ± 0.11	0.68 ± 0.10	0.59 ± 0.10	0.001
SARA score[a]		Total (n = 224)	SCA2 (n = 20)	MSA-C (n = 204)	0.000
		14.27 ± 6.73	10.13 ± 5.21	14.69 ± 6.73	
The differences in the parameters between the patients with MSA-C or SCA2 with a disease duration of longer than 4 years.					
		Total (n = 151)	SCA2 (n = 48)	MSA (n = 103)	*p*-value
HCBS[b]	Present	117	18	99	0.000
	Absent	34	30	4	
Disease duration[a]		7.71 ± 3.18	8.97 ± 4.32	7.13 ± 2.27	0.000
Cerebellum[a]	NAA/Cr	0.58 ± 0.17	0.62 ± 0.22	0.56 ± 0.14	0.000
	Cho/Cr	0.49 ± 0.18	0.57 ± 0.18	0.46 ± 0.18	0.000
Vermis[a]	NAA/Cr	0.63 ± 0.16	0.69 ± 0.19	0.60 ± 0.14	0.000
	Cho/Cr	0.54 ± 0.14	0.60 ± 0.12	0.51 ± 0.14	0.000
SARA score[a]		Total (n = 147)	SCA2 (n = 44)	MSA-C (n = 103)	0.000
		19.54 ± 8.75	13.85 ± 6.48	22.23 ± 8.41	

The numeric data are presented as means ± standard deviations.
[a] Mann–Whitney test.
[b] chi-squared test; HCBS, hot cross bun sign; MSA-C, multiple system atrophy, cerebellar type; SCA2, spinocerebellar ataxia type 2; SARA, scale for the assessment and rating of ataxia; NAA, N-acetyl aspartate; Cr, creatinine; Cho, choline.

3.2. Comparison of parameters in patients with MSA-C and SCA2 based on the duration of the disease (Table 2)

Among our study participants, 34.25% of SCA2 patients demonstrated HCBS, while 92.43% of the 317 MSA-C patients showed pontine HCBS on T2 weighted images (T2WI) or FLAIR MRI scans. Patients diagnosed with MSA-C exhibited a notably higher occurrence of the HCBS than those with SCA2, regardless of the disease duration. At all stages of the disease (disease duration of 0–4 years and longer than 4 years), individuals with MSA-C demonstrated reduced levels of cerebellar NAA/Cr (as shown in Fig. 3A and B) and Cho/Cr (as indicated in Fig. 3C and D), alongside lower NAA/Cr and Cho/Cr in the vermis, and elevated scores on the SARA. Fig. 3

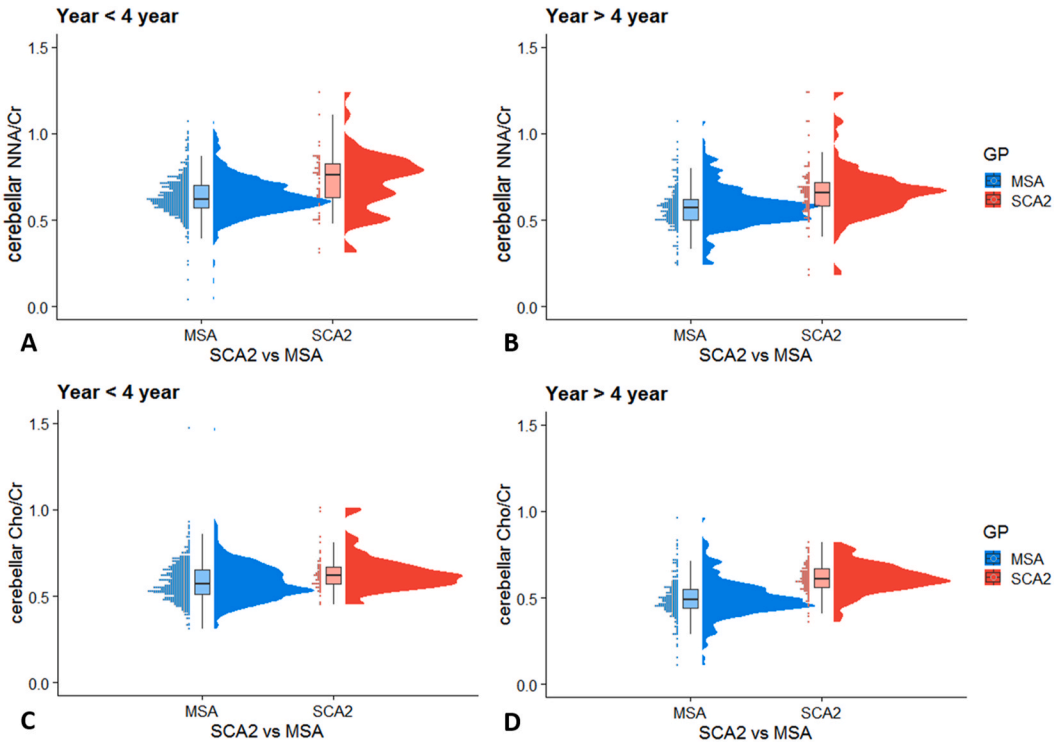

Fig. 3. The distribution plots of cerebellar NAA/Cr and cerebellar Cho/Cr with MSA or SCA2 across different disease durations. Specifically, panels A and B illustrate the distribution of NAA/Cr ratios, while panels C and D depict Cho/Cr ratios. For disease durations of 0–4 years (A and C) and durations exceeding 4 years (B and D), MSA-C patients exhibit significantly lower NAA/Cr and Cho/Cr ratios compared to those with SCA2.

illustrates the distribution plots of NAA/Cr ratios in the cerebellum and Cho/Cr ratios in the cerebellum.

Through ROC curve analysis, it was determined that both NAA/Cr and Cho/Cr ratios in cerebellum have comparable areas under the curve (AUCs), effectively differentiating between SCA2 and MSA-C in patients at the early stages of the disease, specifically within the first 0–4 years of diagnosis. The cut off value for cerebellar NAA/Cr was established at 0.75, indicating a sensitivity of about 89.61% and specificity of about 55.17%, resulting in an AUC around 0.868. For cerebellar Cho/Cr, the cutoff was set at 0.56, achieving a sensitivity of around 49.63% and specificity of about 77.59%, yielding an AUC of 0.656.

Table 3
The differences in the parameters between the patients in early or late stages of disease.

MSA-C		Total (n = 317)	0–4 years (n = 214)	>4 years (n = 103)	*p*-value
HCBS[b]	Positive	293	194	99	0.015
	Negative	24	20	4	
Cerebellum[a]	NAA/Cr	0.61 ± 0.13	0.63 ± 0.13	0.56 ± 0.14	0.000
	Cho/Cr	0.53 ± 0.16	0.57 ± 0.14	0.46 ± 0.18	0.000
Vermis[a]	NAA/Cr	0.66 ± 0.13	0.69 ± 0.12	0.60 ± 0.14	0.000
	Cho/Cr	0.56 ± 0.12	0.59 ± 0.10	0.51 ± 0.14	0.000
SARA score[a]		Total (n = 307)	MSA-C (n = 204)	MSA-C (n = 103)	0.000
		17.10 ± 8.11	14.69 ± 6.73	22.23 ± 8.41	
SCA2		Total (n = 73)	0–4 years (n = 25)	>4 years (n = 48)	*p*-value
HCBS[b]	Positive	25	7	18	0.251
	Negative	48	18	30	
Cerebellum[a]	NAA/Cr	0.66 ± 0.21	0.72 ± 0.18	0.62 ± 0.22	0.002
	Cho/Cr	0.59 ± 0.16	0.63 ± 0.11	0.57 ± 0.18	0.196
Vermis[a]	NAA/Cr	0.70 ± 0.19	0.72 ± 0.19	0.69 ± 0.19	0.362
	Cho/Cr	0.63 ± 0.12	0.68 ± 0.10	0.60 ± 0.12	0.060
SARA score[a]		Total (n = 64)	SCA2 (n = 20)	SCA2 (n = 44)	0.001
		12.69 ± 6.33	10.13 ± 5.21	13.85 ± 6.48	

The numeric data are presented as means ± standard deviations.

[a] Mann–Whitney test.

[b] chi-squared test; HCBS, hot cross bun sign; MSA-C, multiple system atrophy, cerebellar type; SCA2, spinocerebellar ataxia type 2; SARA, scale for the assessment and rating of ataxia; NAA, N-acetyl aspartate; Cr, creatinine; Cho, choline.

For patients who have had the disease for more than 4 years, the AUCs of cerebellar NAA/Cr and cerebellar Cho/Cr were comparable for distinguishing SCA2 from MSA-C. The cut value of cerebellar NAA/Cr was set at 0.62, with a sensitivity of about 75.96% and specificity of around 70.67%, resulting with AUC about 0.775. The cutoff value of cerebellar Cho/Cr was determined to be 0.54, demonstrating a sensitivity of around 73.37% and a specificity of about 89.19%, which resulted in an AUC of 0.844.

3.3. Comparison of parameters in patients at different disease stages (Table 3)

In patients diagnosed with MSA-C, a disease duration exceeding 4 years was associated with more frequent HCBS, notably reduced levels of cerebellar NAA/Cr and Cho/Cr, along with lower vermis NAA/Cr and Cho/Cr, and elevated SARA scores when compared to patients whose disease duration was 4 years or shorter. Among SCA2 patients, a longer disease duration (over 4 years) led to significantly decreased cerebellar NAA/Cr and increased SARA scores. However, the occurrence of HCBS, along with the levels of cerebellar Cho/Cr, vermis NAA/Cr, and vermis Cho/Cr, showed no significant variation as compared to patients with a disease duration of under 4 years.

3.4. Comparison of parameters in patient with and without HCBS (Table 4)

MSA-C patients exhibiting HCBS often have extended durations of the disease, higher SARA scores, and marked reduced in cerebellar NAA/Cr, Cho/Cr, and vermis NAA/Cr compared to those without HCBS. Multivariate analysis revealed that MSA-C patients with HCBSs had longer disease durations (OR of 1.235; 95% CI, 1.049–1.454) and lower cerebellar NAA/Cr (OR of 0.35; 95% CI, 0.003–0.422) than those without HCBS. Among SCA2 patients, those with HCBS tended to have significantly higher SARA scores and lower r NAA/Cr, Cho/Cr ratios in cerebellum, and Cho/Cr ratio in vermis compared to those without HCBS. Multivariate analysis showed that those with HCBSs tended to have higher SARA scores (OR of 1.249; 95% CI, 1.132–1.378).

3.5. Distinguishing MSA-C from SCA2 in patients exhibiting HCBS (Table 5)

Patients with MSA-C showed a greater occurrence of pontine HCBS compared to those with SCA2, regardless of the disease duration. Nonetheless, individuals with SCA2 also displayed HCBS quite frequently. Distinguishing SCA2 from MSA-C in patients with

Table 4
The differences in the parameters between the patients with and without HCBSs.

MSA-C		Total (n = 317)	HCBS positive (n = 293)	HCBS negative (n = 24)	*p*-value	Univariate *p*-value	Multivariate *p*-value	OR	95% CI
Duration[a]		3.92 ± 2.76	4.03 ± 2.77	2.50 ± 2.24	0.000	0.000	0.011	1.235	1.049–1.454
Cerebellum[a]	NAA/Cr	0.61 ± 0.13	0.60 ± 0.13	0.68 ± 0.12	0.000	0.000	0.008	0.35	0.003–0.422
	Cho/Cr	0.53 ± 0.16	0.53 ± 0.15	0.60 ± 0.11	0.001	0.000			
Vermis[a]	NAA/Cr	0.66 ± 0.13	0.66 ± 0.12	0.72 ± 0.10	0.006				
	Cho/Cr	0.56 ± 0.12	0.55 ± 0.13	0.61 ± 0.07	0.050				
SARA score[a]		Total n = 307	n = 283	n = 24	0.009	0.001			
		17.10 ± 8.11	17.34 ± 8.15	14.36 ± 7.18					
SCA2		Total (n = 73)	HCBS positive (n = 25)	HCBS negative (n = 48)	*p*-value	Univariate *p*-value	Multivariate *p*-value	OR	95% CI
Duration[a]		6.79 ± 4.66	7.64 ± 5.37	6.34 ± 4.21	0.068	0.183			
Cerebellum[a]	NAA/Cr	0.66 ± 0.21	0.62 ± 0.21	0.68 ± 0.21	0.003	0.063			
	Cho/Cr	0.59 ± 0.16	0.56 ± 0.16	0.61 ± 0.16	0.009	0.038			
Vermis[a]	NAA/Cr	0.70 ± 0.19	0.62 ± 0.25	0.74 ± 0.11	0.107				
	Cho/Cr	0.63 ± 0.12	0.58 ± 0.15	0.65 ± 0.09	0.016				
SARA score[a]		Total n = 64	n = 25	n = 39	0.000	0.000	0.000	1.249	1.132–1.378
		12.69 ± 6.33	16.76 ± 7.22	10.55 ± 4.57					

The numeric data are presented as means ± standard deviations.
[b] chi-squared test; HCBS, hot cross bun sign; MSA-C, multiple system atrophy, cerebellar type; SCA2, spinocerebellar ataxia type 2; SARA, scale for the assessment and rating of ataxia; NAA, N-acetyl aspartate; Cr, creatinine; Cho, choline.
[a] Mann–Whitney test.

Table 5
HCBS-positive MSA patients vs. HCBS-positive SCA2 patients for all disease durations.

		Total (n = 318)	SCA2 (n = 25)	MSA (n = 293)	*p*-value
Disease duration[a]		4.31 ± 3.20	7.64 ± 5.37	4.03 ± 2.77	0.000
Cerebellum[a]	NAA/Cr	0.60 ± 0.14	0.62 ± 0.21	0.60 ± 0.13	0.841
	Cho/Cr	0.53 ± 0.16	0.56 ± 0.16	0.53 ± 0.16	0.006
Vermis[a]	NAA/Cr	0.65 ± 0.15	0.62 ± 0.25	0.66 ± 0.13	0.173
	Cho/Cr	0.56 ± 0.13	0.58 ± 0.15	0.56 ± 0.13	0.061
SARA score[a]		Total (n = 308)	SCA2 (n = 25)	MSA (n = 283)	0.931
		17.30 ± 8.07	16.76 ± 7.22	17.34 ± 8.14	

The numeric data are presented as means ± standard deviations.
[b] chi-squared test; HCBS, hot cross bun sign; MSA-C, multiple system atrophy, cerebellar type; SCA2, spinocerebellar ataxia type 2; SARA, scale for the assessment and rating of ataxia; NAA, N-acetyl aspartate; Cr, creatinine; Cho, choline.
[a] Mann–Whitney test.

pontine HCBS in a clinical setting can be challenging when genetic testing is not readily available. In this study, 293 MSA-C patients and 25 SCA2 patients had pontine HCBSs. Patients diagnosed with MSA-C exhibited notably reduced cerebellar Cho/Cr levels compared to those diagnosed with SCA2. Through ROC analysis, a cerebellar Cho/Cr cutoff of 0.53 was identified to suggest MSA-C presence, featuring a sensitivity of roughly 48.12%, a specificity of about 85.11%, and an AUC of 0.64. Fig. 4 illustrates the distribution plots of cerebellar NAA/Cr (Fig. 4A) and cerebellar Cho/Cr (Fig. 4B) in patients with HCBS.

3.6. Comparison of parameters based on SARA scores (Table 6 and Fig. 5)

Patients were categorized into three groups based on their clinical manifestations using SARA scores: 0–11, 12–20, or 21–40. Patients with SARA scores between 0 and 11 were generally able to walk with variable difficulties, while those with scores higher than 12 were largely wheelchair-bound.

For patients with SARA scores between 0 and 11, patients with MSA-C displayed HCBS more frequently and had shorter disease durations relative to those with SCA2. MSA-C patients had significantly reduced cerebellar NAA/Cr levels compared to those with SCA2, as illustrated in Fig. 5A. ROC curve analysis identified a NAA/Cr cutoff of 0.64 for distinguishing SCA2 from MSA-C, featuring a sensitivity of around 46.9%, a specificity of approximately 78.0%, and an AUC of 0.620.

In patients exhibiting SARA scores between 12 and 20, MSA-C patients still exhibited higher frequencies of HCBS and shorter disease durations. MSA-C patients exhibited significantly lower levels of cerebellar NAA/Cr, as shown in Fig. 5B, and Cho/Cr, highlighted in Fig. 5E. ROC curve analysis indicated that cerebellar Cho/Cr demonstrated significantly greater AUCs than cerebellar NAA/Cr for predicting MSA-C. The Cho/Cr cutoff was established at 0.58, with a sensitivity of around 59.6% and a specificity of about 76.7%, leading to an AUC of 0.681. Meanwhile, the NAA/Cr cutoff was set at 0.67, achieving a sensitivity of about 71.1% and a specificity of around 46.7%, resulting in an AUC of 0.594.

For patients with a SARA score between 21 and 40, MSA-C patients had shorter disease durations and lower cerebellar Cho/Cr (Fig. 5F). Using ROC curve analysis, the cutoff for Cho/Cr was 0.53 for distinguishing SCA.

Fig. 6 presents the cerebellar MRS data for patients diagnosed with MSA-C (labeled as A-F) or SCA2 (labeled as G-L), encompassing a range of disease durations and SARA scores, and indicating the presence or absence of HCBS. A 69-year-old female diagnosed with MSA with her disease duration of 1 year and a SARA score of 7 had an MRI of her brain that showed the presence of an HCBS (Fig. 6A), and the MRS (Fig. 6B) readouts for her cerebellum were an NAA/Cr of 0.6 and a Cho/Cr of 0.49. A 74-year-old female MSA patient, who has been living with the disease for 5 years and has a SARA score of 18, had an MRI that revealed an HCBS (Fig. 6C), and the MRS

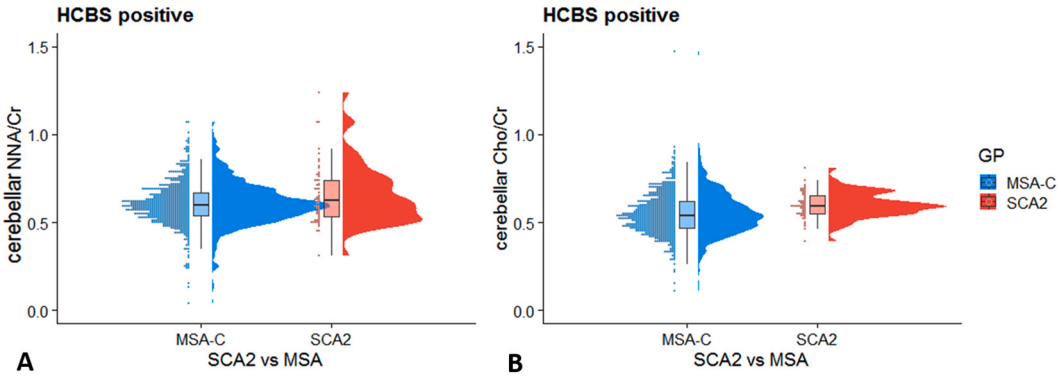

Fig. 4. Distribution plots depicting the ratios of NAA/Cr in the cerebellum (A) and Cho/Cr in the cerebellum (B) for patients with HCBS-positive MSA-C or SCA2. It is observed that patients diagnosed with MSA-C exhibit significantly lower cerebellar Cho/Cr ratios when compared with those diagnosed with SCA2.

Table 6
The differences in the parameters between the patients with variable SARA scores.

The differences in the parameters between the patients with MSA-C and those with SCA2 with SARA scores of between 0 and 11.					
		Total (n = 100)	SCA2 (n = 25)	MSA (n = 75)	*p*-value
HCBS[b]	Positive	69	4	65	0.000
	Negative	31	21	10	
Disease duration[a]		2.72 ± 2.24	4.92 ± 2.39	1.99 ± 1.62	0.000
Cerebellum[a]	NAA/Cr	0.68 ± 0.13	0.71 ± 0.13	0.67 ± 0.13	0.011
	Cho/Cr	0.61 ± 0.12	0.62 ± 0.08	0.61 ± 0.13	0.563
Vermis[a]	NAA/Cr	0.74 ± 0.08	0.75 ± 0.09	0.73 ± 0.08	0.520
	Cho/Cr	0.63 ± 0.08	0.66 ± 0.09	0.62 ± 0.07	0.218
SARA score[a]		8.34 ± 2.55	7.82 ± 2.75	8.51 ± 2.46	0.138
The differences in the parameters between the patients with MSA-C and those SCA2 with SARA scores of between 12 and 20.					
		Total (n = 171)	SCA2 (n = 32)	MSA (n = 139)	*p*-value
HCBS[b]	Positive	143	14	129	0.000
	Negative	28	18	10	
Disease duration[a]		4.24 ± 3.08	7.80 ± 4.06	3.42 ± 2.08	0.000
Cerebellum[a]	NAA/Cr	0.63 ± 0.13	0.67 ± 0.19	0.62 ± 0.11	0.023
	Cho/Cr	0.57 ± 0.11	0.63 ± 0.10	0.56 ± 0.11	0.000
Vermis[a]	NAA/Cr	0.70 ± 0.09	0.71 ± 0.12	0.70 ± 0.08	0.426
	Cho/Cr	0.60 ± 0.08	0.64 ± 0.08	0.59 ± 0.08	0.009
SARA score[a]		15.22 ± 2.56	14.53 ± 2.26	15.38 ± 2.61	0.021
The differences in the parameters between the patients with MSA-C and those with SCA2 with SARA scores of between 21 and 40.					
		Total (n = 88)	SCA2 (n = 7)	MSA (n = 81)	*p*-value
HCBS[b]	Positive	86	7	79	0.516
	Negative	4	0	4	
Disease duration[a]		6.25 ± 3.46	10.43 ± 8.28	5.89 ± 2.42	0.011
Cerebellum[a]	NAA/Cr	0.56 ± 0.13	0.64 ± 0.23	0.56 ± 0.12	0.454
	Cho/Cr	0.50 ± 0.10	0.58 ± 0.05	0.49 ± 0.10	0.000
Vermis[a]	NAA/Cr	0.61 ± 0.13	0.56 ± 0.32	0.61 ± 0.11	0.672
	Cho/Cr	0.55 ± 0.10	0.53 ± 0.14	0.55 ± 0.10	0.528
SARA score[a]		27.36 ± 5.33	25.00 ± 3.10	27.56 ± 5.43	0.134

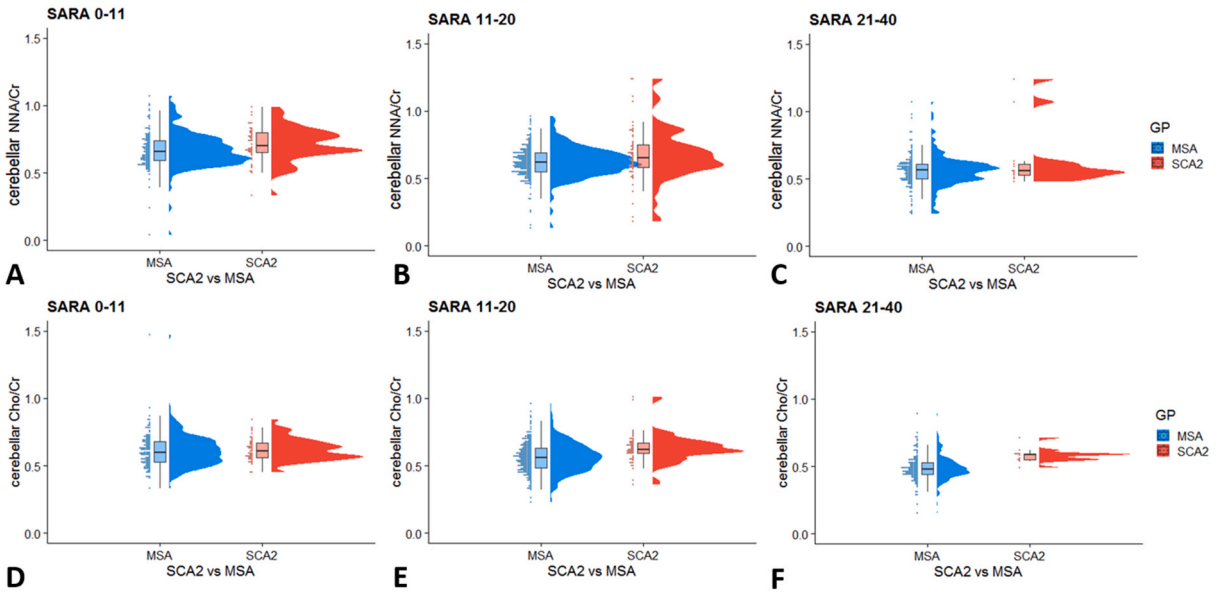

Fig. 5. Comparative distribution plots illustrating cerebellar NAA/Cr and Cho/Cr ratios in patients diagnosed with MSA-C or SCA2, stratified by various SARA scores. In individuals with SARA scores from 0 to 11, MSA-C patients exhibited significantly lower cerebellar NAA/Cr ratios compared to SCA2 patients (A), without significant changes in cerebellar Cho/Cr ratios (D). For those with SARA scores between 12 and 20, both cerebellar NAA/Cr (B) and Cho/Cr ratios (E) were significantly lower in MSA-C patients. In patients with severe disease, indicated by SARA scores of 21–40, MSA-C patients had lower cerebellar Cho/Cr ratios (F), but no significant difference in cerebellar NAA/Cr ratios (C) was observed.

(Fig. 6D) readouts for her cerebellum were an NAA/Cr of 0.55 and a Cho/Cr of 0.47. A 72-year-old female with MSA with a disease duration of 5 year and a SARA score of 25 had an MRI that presents an HCBS (Fig. 6E), and the MRS (Fig. 6F) ratios in cerebellum were an NAA/Cr of 0.59 and a Cho/Cr of 0.45. A 73-year-old male diagnosed with SCA2 with his disease duration of 2 year and a SARA score

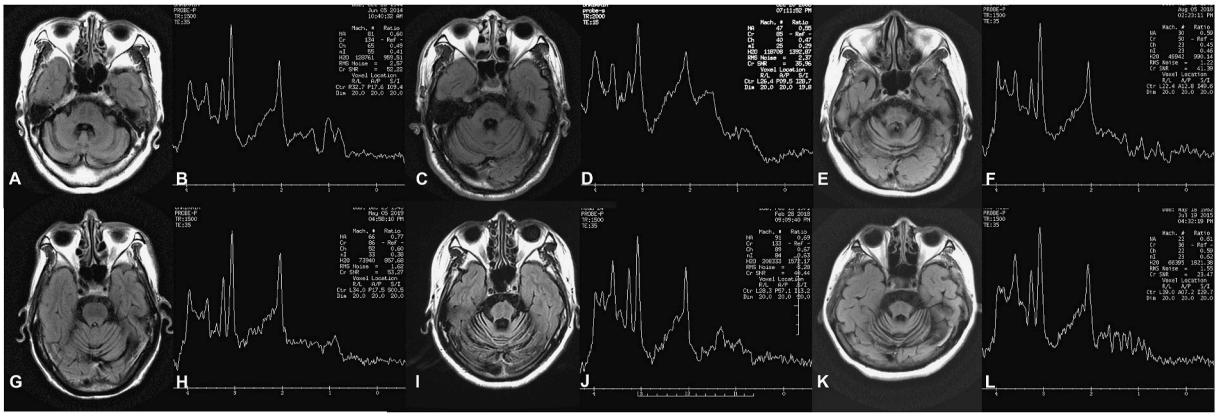

Fig. 6. This figure showcases axial FLAIR images of the pons and cerebellar MRS data for patients diagnosed with MSA-C (A–F) and SCA2 (G–L), categorized by disease duration and segmented according to SARA scores into three ranges: 0–11 (A, B, G, H), 12–20 (C, D, I, J), and over 21 (E, F, K, L). Through showcasing patient profiles from the early to advanced stages of MSA-C and SCA2, highlight the utility of MRS readouts in differentiating between these conditions at various stages of disease progression, demonstrating the potential of specific metabolite ratios as diagnostic markers.

of 3 underwent an MRI that disclosed no HCBS (Fig. 6G), and the metabolites ratios in his cerebellum (Fig. 6H) were an NAA/Cr of 0.77 and a Cho/Cr of 0.6. A 47-year-old female with SCA2 with a disease duration of 5 years and a SARA score of 12 had an MRI that showed the presence of an HCBS (Fig. 6I), and the MRS (Fig. 6J) readouts for her cerebellum were an NAA/Cr of 0.69 and a Cho/Cr of 0.67. A 53-year-old woman diagnosed with SCA2, having lived with the condition for 8 years and holding a SARA score of 24, underwent an MRI that identified a HCBS (Fig. 6K). Further, the MRS results for her cerebellum (Fig. 6L) demonstrated a NAA/Cr ratio of 0.61 and a Cho/Cr ratio of 0.59. The cutoff values provided by our study could reliably differentiate SCA2 from MSA at various disease stages.

4. Discussion

Patients with MSA-C often present with clinical symptoms similar to those observed in SCA2 [4]. The lack of genetic testing availability can make clinical diagnosis challenging. MRI and MRS serve as important non-invasive methods for evaluating neurological abnormalities [6]. Notably, MSA-C patients generally show a higher frequency and earlier appearance of HCBS compared to SCA2 patients. We established cutoff values for cerebellar Cho/Cr (0.56 and 0.54) and cerebellar NAA/Cr (0.75 and 0.62) for differentiating SCA2 from MSA-C, considering disease durations within 0–4 years or longer than 4 years. For patients presenting with pontine HCBS or SARA scores exceeding 21, cerebellar Cho/Cr with a cutoff of 0.53 may aid in distinguishing SCA2 from MSA-C.

MRS is a valuable tool for noninvasively detecting changes in microscopic chemical components. In the present study, we utilized a 1.5 T MRI system due to its widespread availability. Nonetheless, it is acknowledged that the magnetization strength impacts MRS outcomes. High-field MRI markedly improves MRS by enhancing the signal-to-noise ratio (SNR), thereby aiding in the more sensitive and precise detection and quantification of metabolites. It also provides improved chemical shift resolution, which allows for the more accurate differentiation and quantification of metabolites that have closely spaced or overlapping peaks. However, employing high magnetic fields introduces the potential for increased thermal effects, posing concerns for patient safety and comfort. Commonly used metabolite ratios in MRS includes NAA/Cr and Cho/Cr. N-acetylaspartate (NAA) is regarded as a marker of neuronal integrity, and a decrease in the NAA/Cr ratio in the brain suggests neuronal damage or loss [14]. MSA is characterized by oligodendroglial alpha-synucleinopathies, leading to marked neuronal loss, gliosis, fading of myelin coloration, and loss of axonal function [15]. In patients with SCA2, significantly loss of neuron and gliosis are commonly found in the pontine nucleus, inferior olivary nucleus, and cerebellar cortex [16]. Previous studies [12,17] and our current findings all show reduced cerebellar NAA/Cr ratio in patients diagnosed with SCA2 or MSA-C.

The choline peak signal primarily arises from phosphocholine and glycerol-phosphocholine and reflects membrane phospholipid metabolism [18]. An increase in Cho/Cr can indicate an intensified membrane turnover rate, while a decrease may signify reduced membrane turnover or cell loss. Decreased ratio of Cho/Cr has also been observed in various neurodegenerative disorders, including Parkinson's disease [19] and dyskinesia [20]. Similar to cerebellar NAA/Cr, previous studies [12,17] and our current research reveal lower cerebellar Cho/Cr in patients with SCA2 or MSA-C. These changes in NAA/Cr and Cho/Cr ratios provide insight into the neuronal integrity and membrane metabolism disturbances characteristic of MSA-C and SCA2, highlighting the extensive neurodegeneration that underpins these conditions.

Patients diagnosed with MSA-C, even with comparable disease durations, typically exhibit higher SARA scores, a greater incidence of HCBS [8], and reduced levels of cerebellar NAA/Cr and Cho/Cr, along with vermis NAA/Cr and Cho/Cr in comparison to individuals with SCA2. These findings suggest a faster clinical deterioration, rapid neuronal demise, and cell membrane damage in MSA-C.

For MSA-C patients, those with extended disease durations typically demonstrate reduced levels of cerebellar and vermis NAA/Cr and Cho/Cr ratios than those with shorter disease durations. This suggests ongoing neuronal and membrane damage as the disease

progresses. On the other hand, individuals with SCA2, especially those with extended disease durations or with HCBS, typically demonstrate reduced cerebellar NAA/Cr level but similar cerebellar Cho/Cr, vermis NAA/Cr, and vermis Cho/Cr levels. This divergence may be attributed to differential involvement of specific cells and structures at various disease stages. Neuronal demise appears to occur earlier in SCA2, while membrane breakdown reaches a plateau and slows down after 4 years. Consequently, cerebellar Cho/Cr does not further decrease with longer disease duration in SCA2 patients.

HCBS, identified by selective reduce of transverse myelinated fibers at the pontine base and pontine tegmentum myelinated fibers, along with neuronal loss and astrogliosis in the pontine raphe, is a hallmark of the disease [21]. The frequency of HCBS in patients diagnosed with MSA-C or SCA2 corresponds with previous reports [7,8,22]. Patients with HCBS tend to have higher SARA scores, with a mean score of 17 in both MSA-C and SCA2, indicating that the presence of HCBS is associated with more severe clinical manifestations [23]. Furthermore, patients with HCBS typically have longer disease durations. The mean disease duration for the appearance of HCBS is 4 years in MSA-C, significantly shorter than the 7.6 years observed in SCA2 patients, highlighting the rapidly deteriorating nature of MSA-C. In contrast, the primary association factor for HCBS in SCA2 patients is the SARA score, with patients in the later disease stages of SCA2 tending to exhibit higher frequencies of HCBS. The appearance of HCBS in SCA2 patients suggests that progressive cerebellar Cho/Cr reduction has reached a nadir, with no further decrease expected. The likely distinguishing point between SCA2 and MSA-C may be cerebellar Cho/Cr at 0.53.

When comparing patients with comparable SARA scores, individuals with MSA-C generally exhibit shorter disease durations than their SCA2 counterparts. In the early ataxia stage (SARA 0–11), MSA-C patients exhibit lower cerebellar NAA/Cr, indicating greater neuronal and myelinated fiber loss, despite similar clinical severity. In later stages, distinguishing between SCA2 and MSA-C may be feasible using cerebellar Cho/Cr, with cutoff values of 0.58, 0.53, and 0.53 for patients with SARA scores of 12–20, 21–40, and HCBS, respectively.

Several limitations exist in our study. Firstly, we did not include healthy controls in the current analysis. In a previous study, we compared MRS findings between MSA-C, SCA, and healthy controls [12]. For this specific study, our focus was solely on determining MRS parameter cutoff values to differentiate between SCA2 and MSA-C. To maintain the study's specific objective and avoid data duplication, we omitted healthy controls from our current analysis. Secondly, our MRS methodology may not represent the latest advancements in the field, and we did not employ quantification or post-processing techniques such as LC model analysis. Additionally, calculating tCr concentrations was not feasible with the available data. This retrospective study aimed to identify a readily accessible parameter for clinical differentiation between SCA2 and MSA-C. Thus, we conducted a retrospective analysis of NAA/Cr and Cho/Cr ratios spanning 20 years (2000–2020) through vendor-provided on-scanner analysis. While our sample sizes were relatively large compared to previous studies, limited case numbers in subgroup analyses based on various disease durations made it challenging to draw definitive conclusions. We could only separate disease durations using a unit of 4 years. Furthermore, our study had a limited number of patients in the early symptomatic stages, making confident differentiation between SCA2 and MSA-C more challenging. Finally, our study relied solely on SARA scores as an indicator of clinical severity. Future studies employing a prospective design, recording additional clinical parameters, recruiting healthy controls, larger sample sizes with more preclinical or early symptomatic subjects, and incorporating up-to-date MRS techniques, including three-dimensional acquisition of morphological images, and quantification methods such as LC model analysis and exploring tCr concentrations' stability across different disease populations, are warranted to provide valuable additional insights.

5. Conclusion

By using pontine HCBSs and cerebellar NAA/Cr and Cho/Cr levels, we identified a way to differentiate between SCA2 and MSA-C at variable disease stages, with differing levels of clinical severity, even in patients with HCBS.

Statement and Declarations

The authors declare no conflicts of interest.

Data availability

The datasets used and/or analyzed during the current study have not been deposited into a publicly available repository because the data will be made available on request.

CRediT authorship contribution statement

Hung-Chieh Chen: Writing – original draft, Formal analysis, Data curation. **Li-Hua Lee:** Data curation. **Jiing-Feng Lirng:** Writing – review & editing, Conceptualization. **Bing-wen Soong:** Writing – review & editing, Supervision, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Bing-wen Soong reports financial support was provided by Ministry of Science and Technology (MOST 107-2314-B-010-017 & MOST 107-2314-B-038-111), Taiwan, Republic of China.

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References

[1] D.J. Lin, K.L. Hermann, J.D. Schmahmann, Multiple system atrophy of the cerebellar type: clinical state of the art, Mov. Disord. 29 (3) (2014) 294–304.
[2] S. Gilman, G.K. Wenning, P.A. Low, D.J. Brooks, C.J. Mathias, J.Q. Trojanowski, N.W. Wood, C. Colosimo, A. Durr, 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 (9) (2008) 670–676.
[3] H.J. Kim, M. Stamelou, B. Jeon, Multiple system atrophy-mimicking conditions: diagnostic challenges, Parkinsonism Relat. Disorders 22 (Suppl 1) (2016) S12–S15.
[4] I. Lastres-Becker, U. Rub, G. Auburger, Spinocerebellar ataxia 2 (SCA2), Cerebellum 7 (2) (2008) 115–124.
[5] J.S. Kim, J.W. Cho, Hereditary cerebellar ataxias: a Korean perspective, J Mov Disord 8 (2) (2015) 67–75.
[6] S. Cocozza, G. Pontillo, G. De Michele, M. Di Stasi, E. Guerriero, T. Perillo, C. Pane, A. De Rosa, L. Ugga, A. Brunetti, Conventional MRI findings in hereditary degenerative ataxias: a pictorial review, Neuroradiology 63 (7) (2021) 983–999.
[7] Y.C. Lee, C.S. Liu, H.M. Wu, P.S. Wang, M.H. Chang, B.W. Soong, The 'hot cross bun' sign in the patients with spinocerebellar ataxia, Eur. J. Neurol. 16 (4) (2009) 513–516.
[8] G. Carre, J.L. Dietemann, O. Gebus, S. Montaut, O. Lagha-Boukbiza, T. Wirth, S. Kremer, I.J. Namer, M. Anheim, C. Tranchant, Brain MRI of multiple system atrophy of cerebellar type: a prospective study with implications for diagnosis criteria, J. Neurol. 267 (5) (2020) 1269–1277.
[9] S. Kasahara, Y. Miki, M. Kanagaki, T. Kondo, A. Yamamoto, E. Morimoto, T. Okada, H. Ito, R. Takahashi, K. Togashi, "Hot cross bun" sign in multiple system atrophy with predominant cerebellar ataxia: a comparison between proton density-weighted imaging and T2-weighted imaging, Eur. J. Radiol. 81 (10) (2012) 2848–2852.
[10] A.K. Naidoo, C.D. Wells, Y. Rugbeer, N. Naidoo, The "hot cross bun sign" in spinocerebellar ataxia types 2 and 7-case reports and review of literature, Mov Disord Clin Pract 9 (8) (2022) 1105–1113.
[11] I. Stankovic, N. Quinn, L. Vignatelli, A. Antonini, D. Berg, E. Coon, P. Cortelli, A. Fanciulli, J.J. Ferreira, R. Freeman, G. Halliday, G.U. Hoglinger, V. Iodice, H. Kaufmann, T. Klockgether, V. Kostic, F. Krismer, A. Lang, J. Levin, P. Low, C. Mathias, W.G. Meissner, L.N. Kaufmann, J.A. Palma, J.N. Panicker, M. T. Pellecchia, R. Sakakibara, J. Schmahmann, S.W. Scholz, W. Singer, M. Stamelou, E. Tolosa, S. Tsuji, K. Seppi, W. Poewe, G.K. Wenning, G. Movement, Disorder Society Multiple System Atrophy Study, A critique of the second consensus criteria for multiple system atrophy, Mov. Disord. 34 (7) (2019) 975–984.
[12] J.F. Lirng, P.S. Wang, H.C. Chen, B.W. Soong, W.Y. Guo, H.M. Wu, C.Y. Chang, Differences between spinocerebellar ataxias and multiple system atrophy-cerebellar type on proton magnetic resonance spectroscopy, PLoS One 7 (10) (2012) e47925.
[13] Y. Horimoto, I. Aiba, T. Yasuda, Y. Ohkawa, T. Katayama, Y. Yokokawa, A. Goto, Y. Ito, Longitudinal MRI study of multiple system atrophy - when do the findings appear, and what is the course? J. Neurol. 249 (7) (2002) 847–854.
[14] B. Ross, S. Bluml, Magnetic resonance spectroscopy of the human brain, Anat. Rec. 265 (2) (2001) 54–84.
[15] Z. Ahmed, Y.T. Asi, A. Sailer, A.J. Lees, H. Houlden, T. Revesz, J.L. Holton, The neuropathology, pathophysiology and genetics of multiple system atrophy, Neuropathol. Appl. Neurobiol. 38 (1) (2012) 4–24.
[16] C. Ishida, K. Komai, K. Yonezawa, K. Sakajiri, E. Nitta, A. Kawashima, M. Yamada, An autopsy case of an aged patient with spinocerebellar ataxia type 2, Neuropathology 31 (5) (2011) 510–518.
[17] S.M. Boesch, C. Wolf, K. Seppi, S. Felber, G.K. Wenning, M. Schocke, Differentiation of SCA2 from MSA-C using proton magnetic resonance spectroscopic imaging, J. Magn. Reson. Imag. 25 (3) (2007) 564–569.
[18] I.M. Burtscher, S. Holtas, Proton MR spectroscopy in clinical routine, J. Magn. Reson. Imag. 13 (4) (2001) 560–567.
[19] G. Barbagallo, G. Arabia, M. Morelli, R. Nistico, F. Novellino, M. Salsone, F. Rocca, A. Quattrone, M. Caracciolo, U. Sabatini, A. Cherubini, A. Quattrone, Thalamic neurometabolic alterations in tremulous Parkinson's disease: a preliminary proton MR spectroscopy study, Parkinsonism Relat. Disorders 43 (2017) 78–84.
[20] Y. Kuznetsov, A. Khiat, P.J. Blanchet, Y. Boulanger, Proton magnetic resonance spectroscopy study of dyskinesia patients, Mov. Disord. 22 (7) (2007) 957–962.
[21] T. Ando, F. Yokoi, Y. Riku, A. Akagi, H. Miyahara, M. Hasegawa, M. Katsuno, M. Yoshida, Y. Iwasaki, The hot cross bun sign in corticobasal degeneration, Neuropathology 41 (5) (2021) 376–380.
[22] M. Higashi, K. Ozaki, T. Hattori, T. Ishii, K. Soga, N. Sato, M. Tomita, H. Mizusawa, K. Ishikawa, T. Yokota, A diagnostic decision tree for adult cerebellar ataxia based on pontine magnetic resonance imaging, J. Neurol. Sci. 387 (2018) 187–195.
[23] S. Zhu, B. Deng, Z. Huang, Z. Chang, H. Li, H. Liu, Y. Huang, Y. Pan, Y. Wang, Y.X. Chao, L.L. Chan, Y.R. Wu, E.K. Tan, Q. Wang, "Hot cross bun" is a potential imaging marker for the severity of cerebellar ataxia in MSA-C, NPJ Parkinsons Dis 7 (1) (2021) 15.