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Regional normal-appearing white matter metabolic profiles reflect information processing speed and verbal learning in early RRMS: A multi-voxel ¹H-MRS cross-sectional study

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Abstract

Background: Cognitive impairment affects 40–70% of multiple sclerosis (MS) patients. Regional domain-selective associations between normal-appearing white matter (NAWM) metabolites and cognition remain underexplored.

Methods: In this cross-sectional study, 52 early RRMS patients (EDSS 0–3; all on interferon-beta) and 44 healthy controls underwent multi-voxel 3D ^{1}H-MRS (3T, PRESS) across six supracallosal NAWM regions. Between-group metabolite differences were FDR-corrected. Spearman correlations and age-adjusted partial correlations assessed MRS–cognition associations. ROC analysis with DeLong testing evaluated discriminative ability.

Results: Significant NAA/Cr differences were found in four of six regions. Left frontal NAA/Cr (R1) correlated with SDMT ($\rho = 0.350$, $p = 0.012$) and discriminated below-average processing speed (AUC = 0.724; 95% CI: 0.553–0.880; DeLong vs. age: $\Delta AUC = 0.208$, $p = 0.036$). Left parietal NAA/Cr (R5) showed a trend association with RAVLT z-score ($\rho = 0.261$, $p = 0.064$). Age-controlled partial correlations were directionally consistent but non-significant (both $p > 0.10$). Neither fatigue nor mood correlated with MRS parameters.

Conclusion: Multi-voxel ^{1}H-MRS reveals topographically heterogeneous NAWM damage with domain-selective cognitive associations in early RRMS. NAA/Cr R1 shows preliminary discriminative ability formally superior to age alone, but requires confirmation in larger cohorts.

1. Introduction

Cognitive impairment occurs in 40–70% of patients with multiple sclerosis (MS) across disease stages and directly affects vocational status and quality of life [1]. The most commonly affected cognitive domains are information processing speed — measured by the Symbol Digit Modalities Test (SDMT) — and verbal learning, measured by the Rey Auditory Verbal Learning Test (RAVLT) [2]. Of particular clinical importance is the fact that cognitive decline may precede measurable neurological disability on the Expanded Disability Status Scale (EDSS) by years, highlighting the need for sensitive neuroradiological biomarkers in early disease [3].

Conventional MRI parameters only moderately correlate with cognitive deficits (clinico-radiological paradox) [4], partly because diffuse microstructural NAWM damage remains invisible on standard sequences. ^{1}H-MRS quantifies neurochemical processes in vivo: NAA/Cr reflects axonal integrity, Cho/Cr marks demyelination, and mI/Cr indicates glial activation [5, 6].

Previous MRS studies have consistently shown NAA/Cr reduction in NAWM of MS patients [5, 6], but most studies have analyzed global or single-region changes. The disconnection syndrome hypothesis posits that damage to specific white matter pathways underlies domain-selective cognitive deficits: frontal damage affects processing speed, while parietal damage affects verbal learning [7, 8].

Accordingly, we designed a cross-sectional study of 52 patients with early RRMS (EDSS 0–3; all on interferon-beta) in which we determined metabolite ratios across six supracallosal NAWM regions and examined their domain-selective associations with SDMT, RAVLT z-score, and MSFC (Multiple Sclerosis Functional Composite). Additionally, we tested specificity by examining whether MRS parameters correlate with fatigue and depressive symptoms, common non-cognitive MS symptoms that could act as confounders.

2. Subjects and Methods

2.1 Subjects

This prospective cross-sectional study enrolled 52 adult patients with RRMS diagnosed according to the 2017 McDonald criteria [9] and 44 healthy controls (HC) matched for age and sex. Patient inclusion criteria were: EDSS 0–3.0, no clinical relapse or corticosteroid therapy within the preceding 30 days, and treatment with interferon-beta (IFN-β-1a or IFN-β-1b). One enrolled patient could not complete the MRI examination and was excluded from neuroimaging analysis (final MRS sample: n = 51). HC underwent MRI/MRS but not cognitive testing, serving exclusively as a metabolite reference group. All participants provided written informed consent. The study was approved by the institutional Ethics Committee and conducted in accordance with the Declaration of Helsinki.

2.2 Cognitive and clinical assessment

Neurological disability was assessed using the EDSS. Cognitive testing was performed exclusively in RRMS patients. Information processing speed was assessed using the oral version of the SDMT (90-second administration). Below-average processing speed performance was defined as a raw score ≤ 56, corresponding to the mean of Serbian healthy controls in the BICAMS validation study (HC mean 56.3; Drulović et al. [10]) and conceptually aligned with the functional benchmark of occupational difficulties according to Benedict et al. [11]. This threshold is lower than the standard BICAMS impairment criterion ($z \leq -1.5$, raw score ≤ 40) and detects a broader spectrum of below-average cognitive performance. Verbal learning and memory were assessed using the RAVLT (five successive learning trials; total score A1–A5, range 0–75). RAVLT z-scores were calculated using Serbian normative data by Janićijević et al. [12], stratified by sex and age group (18–29, 30–39, 40–49, 50–59, ≥60 years); impairment was defined as $z \leq -1.5$. Global cognition was screened using the MoCA. Fatigue was quantified using the Fatigue Impact Scale (FIS, range 0–160). Mood was assessed using the Depression Anxiety Stress Scales-21 (DASS-21). Functional disability was quantified using the MSFC composite z-score (Timed 25-Foot Walk [T25FW], 9-Hole Peg Test [9HPT], and PASAT).

2.3 MRI and ^{1}H-MRS protocol

All examinations were performed on a 3T MRI scanner (Trio Tim, Siemens, Erlangen, Germany) at a single center. Conventional imaging (T1W, T2W, FLAIR, 3D T1W MPR, diffusion) confirmed the absence of focal lesions within spectroscopic voxels. Proton 3D multi-voxel MRS of supracallosal white matter

was performed using PRESS (TR/TE: 1700/135 ms long echo, 1700/30 ms short echo; FOV 160 × 160 × 160 mm; VOI 80 × 80 × 80 mm; resulting voxel size 10 × 10 × 10 mm [1.0 cm^3]; acquisition time 8 min 17 s). Six outer-volume saturation regions and automatic volume-selective shimming were applied, yielding a 64-voxel (8 × 8) grid. Six anatomical regions were defined: R1 = left frontal subcortical, R2 = right frontal subcortical, R3 = left deep white matter (corona radiata), R4 = right deep white matter, R5 = left parietal subcortical, R6 = right parietal subcortical. NAA (2.0 ppm), Cho (3.2 ppm), and tCr (3.0 ppm) were quantified on long echo time (TE = 135 ms); mI (3.5 ppm) on short echo time sequence (TE = 30 ms). All ratios were expressed relative to creatine, the internal reference standard. Metabolite ratios were determined using the Siemens Syngo software. Spectra with CRLB > 20% for NAA or Cr were excluded; mean CRLB for NAA was 6.2% (range 2.1–18.4%). Low signal-to-noise spectra were repositioned until obtaining spectra of satisfactory quality. Correction for cerebrospinal fluid partial volume within voxels was not applied, representing a potential source of systematic noise, particularly in patients with more pronounced atrophy (see Limitations). All the spectra were positioned and analyzed by an experienced neuroradiologist (over 15 years of experience). One extreme mI/Cr value for R4 (40.43; remaining range 0.34–0.93) was identified as a data entry error and excluded from analysis for that region (n = 50 for mI/Cr R4; noted in Table 2).

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2.4 Statistical analysis

Distribution normality was tested using the Shapiro–Wilk test. Group comparisons were performed using Student's t-test for normally distributed variables or the Mann–Whitney U test for non-normal distributions and ordinal data. Between-group MRS differences (18 comparisons: 3 metabolites × 6 regions) were corrected using the FDR procedure according to Benjamini–Hochberg [13]; effect sizes were expressed as Cohen's d. Associations between MRS ratios and cognitive/clinical outcomes were assessed using Spearman rank correlation. For primary hypothesized pairs (NAA/Cr R1 vs. SDMT; NAA/Cr R5 vs. RAVLT z-score), partial correlations controlling for age were calculated. The discriminative ability of NAA/Cr R1 for below-average SDMT performance was assessed using ROC curve analysis; the optimal cut-off was determined by the Youden index, and 95% CIs for AUC were obtained by bootstrap resampling (2,000 iterations, seed = 2024). The incremental value of NAA/Cr R1 beyond age was tested by comparing logistic regression models (NAA/Cr R1 alone, age alone, NAA/Cr R1 + age) via AUC comparison; the statistical significance of AUC differences between correlated models was assessed using the DeLong test [14]. Differences between cognitive subgroups were assessed using the Kruskal–Wallis test with subsequent Bonferroni-corrected pairwise tests. Statistical significance was defined at a two-sided $\alpha = 0.05$. All analyses were performed in Python 3.12 (SciPy 1.11).

3. Results

3.1 Subject characteristics

Fifty-two RRMS patients and 44 healthy controls were enrolled. Groups were matched for age (RRMS 39.7 ± 9.8 vs. HC 40.3 ± 9.5 years; p = 0.736) and sex (both groups ~81% female; p = 1.000). RRMS patients had significantly more years of formal education (median 16 [IQR 12–17] vs. 12 [12–16] years; p = 0.006). All patients were in early RRMS: EDSS 0.0–3.0 (median 2.0), disease duration 5.0 [IQR 3.8–10.2] years, all on IFN-β therapy. Detailed characteristics are presented in Online Resource Table S1.

On cognitive testing, 38/52 (73.1%) had SDMT ≤ 56, and 14/52 (26.9%) had RAVLT impairment (z ≤ −1.5); 12 (23.1%) had dual impairment, 12 (23.1%) had no impairment, and 26 (50.0%) had isolated below-average SDMT performance. MoCA (26.9 ± 2.3) did not correlate with MRS parameters (all p > 0.05).

3.2 Regional NAWM metabolite changes

Multi-regional MRS revealed topographically inhomogeneous metabolic changes (Table 2; Fig. 1). After FDR correction, significant NAA/Cr differences were found in four of six regions (R1, R3, R5: decreased in RRMS; R4: paradoxically elevated — likely a methodological artifact, see Discussion). Cho/Cr was elevated in R1, R3, R5, and R6 (all p_FDR < 0.01); mI/Cr showed a regionally heterogeneous pattern.

[Table 2 about here]

3.3 Correlations between NAWM metabolites and cognitive outcomes

Spearman analysis revealed a domain-selective pattern (Table 3; Fig. 2): NAA/Cr R1 significantly correlated with SDMT (ρ = 0.350, p = 0.012; ρ² ≈ 12.2%), while NAA/Cr R5 showed a trend association with RAVLT z-score (ρ = 0.261, p = 0.064; ρ² ≈ 6.8%). Both associations were regionally specific.

Partial correlations controlling for age showed attenuated, directionally consistent, but statistically non-significant values: SDMT–NAA/Cr R1 (partial r = 0.205, p = 0.148) and RAVLT z–NAA/Cr R5 (partial r = 0.151, p = 0.290). These findings suggest that age partly mediates both associations and that the correlations are preliminary in nature; definitive confirmation requires larger samples and multivariate models.

Neither FIS nor DASS-21 correlated with NAA/Cr (all p > 0.50). EDSS correlated with SDMT (ρ = −0.343, p = 0.013), and depressive symptoms with RAVLT z-score (ρ = −0.396, p = 0.004).

[Table 3 about here]

3.4 MSFC and frontal NAWM integrity

MSFC composite scores significantly correlated with NAA/Cr in three regions: R1 left frontal (ρ = 0.325, p = 0.020), R2 right frontal (ρ = 0.375, p = 0.007), and R6 right parietal (ρ = 0.325, p = 0.020). Bilateral frontal associations were the strongest MRS–clinical correlations in the dataset. We note that these

correlations were not corrected for multiple testing beyond the primary hypotheses and should be considered exploratory findings requiring confirmation.

3.5 Discriminative ability and cognitive subgroup analysis

NAA/Cr R1 discriminated below-average from intact patients: AUC = 0.724 (95% CI: 0.553–0.880; Fig. 2c), optimal threshold NAA/Cr ≤ 1.890 (sensitivity 64.9%, specificity 78.6%). Logistic regression with age as a covariate showed that age alone has negligible discriminative ability (AUC = 0.515); the DeLong test confirmed that NAA/Cr R1 had significantly greater discriminative ability than age alone (ΔAUC = 0.208; z = 2.094; p = 0.036). Adding age to the NAA/Cr R1 model did not improve discrimination (AUC = 0.730 vs. 0.724; DeLong p = 0.706). Supplementary analysis with the BICAMS threshold (SDMT ≤ 40): AUC = 0.635 (95% CI: 0.445–0.820; 11 impaired vs. 40 intact).

The Kruskal–Wallis test confirmed a NAA/Cr R1 gradient across cognitive profiles (H = 7.79, p = 0.020; n = 49): no impairment 1.989 ± 0.127; SDMT-only 1.818 ± 0.242; dual impairment 1.777 ± 0.172 (Fig. 2d; post-hoc: no impairment vs. dual p_adj = 0.013).

4. Discussion

4.1 Summary of key findings

This study demonstrates three findings: (1) regionally heterogeneous NAWM metabolic changes (FDR-significant in 4/6 regions); (2) domain-selective associations — frontal NAA/Cr correlates with SDMT, parietal with RAVLT z-score (trend); (3) absence of correlation with fatigue and depression. Partial correlations lose significance when controlling for age, and explained variance remains modest ($\rho^2 \approx 12\%$ for SDMT), positioning the findings as preliminary.

4.2 Methodological advantages of multi-regional MRS over single-voxel studies

Most previous MRS studies of cognition in MS used single-voxel spectroscopy (SVS), which assumes NAWM damage uniformity — our data refute this (Cohen's d ranged from 0.209 to 2.195), consistent with Sun et al. [15]. Additionally, large SVS voxels (8–25 cm^3) risk lesional contamination, whereas our 1 cm^3 voxels allow individual T2/FLAIR screening; and SVS cannot test spatial hypotheses [8, 16]. Our 3D PRESS protocol at 3T [17] overcomes these limitations with clinically acceptable acquisition time.

4.3 Regional specificity and the disconnection syndrome hypothesis

Our multi-regional design reveals a directionally dissociative pattern: frontal NAA/Cr (R1) correlates with SDMT ($\rho = 0.350$) but not with RAVLT z-score; parietal NAA/Cr (R5) shows a trend association with RAVLT z-score ($\rho = 0.261$, $p = 0.064$) but not with SDMT. This dissociation is neuroanatomically coherent — SDMT depends on fronto-striatal circuits [18], while RAVLT involves parietal-hippocampal networks [19]. The stepwise NAA/Cr R1 gradient across cognitive profiles (H = 7.79, p = 0.020) suggests a dose-

dependent relationship between axonal integrity and cognitive status, consistent with the disconnection syndrome hypothesis [8].

4.4 Inferential limitations: partial correlations and effect size

Both primary correlations lose significance when controlling for age: SDMT–NAA/Cr R1 (partial r = 0.205, p = 0.148) and RAVLT z–NAA/Cr R5 (r = 0.151, p = 0.290). NAA/Cr may reflect general aging rather than MS-specific pathology; alternatively, aging and MS neurodegeneration share common mechanisms (oxidative stress, mitochondrial dysfunction), and partial correlation may overcorrect by removing genuinely MS-relevant variance. Without longitudinal data, these interpretations cannot be distinguished.

Explained variance is modest ($\rho^2 \approx 12\%$ for SDMT, ~6.8% for RAVLT z), positioning NAWM metabolic changes as one of several pathophysiological mechanisms of cognitive impairment in MS; comprehensive prediction requires multivariate models combining MRS, DTI, lesion burden, and volumetry.

4.5 Asymmetric distribution and the R4 artifact

Relative preservation of right frontal (R2) and right parietal (R6) NAWM may reflect asymmetric demyelination distribution known in MS; the R2–MSFC correlation (uncorrected for multiple testing) requires confirmation. The finding of higher NAA/Cr in RRMS at R4 (d = 1.602) likely reflects a methodological artifact due to corona radiata heterogeneity: hemispheric white matter asymmetry, differing shimming quality near the paranasal sinuses, or corpus callosum signal contamination. Without DTI co-registration, precise identification of the cause remains speculative.

4.6 Fatigue, depression, and neurobiological specificity

The absence of correlation between NAA/Cr and FIS or DASS-21 (all $p > 0.50$) provides evidence of specificity of MRS markers for cognitive outcomes, consistent with the literature on different mechanisms of fatigue in MS [20, 21]. Depressive symptoms correlate with RAVLT z-score ($\rho = -0.396$, $p = 0.004$), but the partial correlation of NAA/Cr R5–RAVLT controlling for age and depression ($r = 0.162$, $p = 0.257$) is numerically higher than without controlling for depression ($r = 0.151$), suggesting that depression does not act as a confounder of this association [22].

4.7 Clinical implications and perspectives

The discriminative ability of NAA/Cr R1 (AUC = 0.724) is formally superior to age alone (AUC = 0.515; DeLong $\Delta AUC = 0.208$, $p = 0.036$), while adding age to the NAA/Cr R1 model does not improve discrimination (DeLong $p = 0.706$). This indicates that discriminative power derives from the metabolic marker rather than from an age effect. Replication in larger cohorts ($n \geq 150$) with multivariate correction remains necessary. The fact that ~73% of patients on IFN-β still show below-average SDMT performance is consistent with the smouldering MS concept [23]; the difference from the prevalence in Drulović et al.

[10] (52.7%) reflects the difference in applied thresholds (functional ≤ 56 vs. BICAMS z ≤ −1.5). Cognitive reserve may mitigate the rate of decline even in the presence of existing metabolic changes [24].

4.8 Study limitations

Limitations include: (1) cross-sectional design; (2) small sample size (power ≈ 48% for ρ ≈ 0.27; ≈ 72% for ρ = 0.35); (3) healthy controls without cognitive testing; (4) residual confounding by education; (5) RAVLT norms [12] based on a preprint study (n = 200); (6) MSFC correlations without multiple testing correction; (7) exclusive use of IFN-β limits generalizability; (8) no CSF partial volume correction — the creatine ratio partially compensates but incompletely, as CSF differentially affects metabolites; future studies should include absolute quantification.

5. Conclusion

Multi-voxel ^{1}H-MRS reveals topographically selective NAWM metabolic changes in early RRMS with domain-consistent associations: frontal NAA/Cr correlates with processing speed, while parietal NAA/Cr shows a trend association with verbal learning. Specificity for cognitive — rather than affective — outcomes supports the biological basis of the findings. Partial correlations lose significance when controlling for age, indicating synergistic aging and neurodegenerative processes whose contributions cannot be disentangled in the present sample. NAA/Cr R1 shows preliminary discriminative ability (AUC 0.724) formally superior to age alone (DeLong p = 0.036), but definitive confirmation requires validation in larger cohorts with multivariate control for age, lesion burden, and atrophy.

Statements and Declarations

Competing Interests: The authors declare that they have no competing interests.

Ethics approval: This study was approved by the institutional Ethics Committee. Informed consent was obtained from all individual participants included in the study.

Data availability: De-identified data are available from the corresponding author upon reasonable request.

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References

1. Chiaravalloti ND, DeLuca J (2008) Cognitive impairment in multiple sclerosis. Lancet Neurol 7:1139–1151. https://doi.org/10.1016/S1474-4422(08)70259-X
2. Sumowski JF, Benedict R, Enzinger C et al (2018) Cognition in multiple sclerosis: state of the field and priorities for the future. Neurology 90:278–288.

https://doi.org/10.1212/WNL.0000000000004977

3. Kappos L, Wolinsky JS, Giovannoni G et al (2020) Contribution of relapse-independent progression vs relapse-associated worsening to overall confirmed disability accumulation in typical relapsing multiple sclerosis. JAMA Neurol 77:1132–1140. https://doi.org/10.1001/jamaneurol.2020.1568
4. Barkhof F (2002) The clinico-radiological paradox in multiple sclerosis revisited. Curr Opin Neurol 15:239–245. https://doi.org/10.1097/00019052-200206000-00003
5. De Stefano N, Matthews PM, Fu L et al (1998) Axonal damage correlates with disability in patients with relapsing-remitting multiple sclerosis. Brain 121:1469–1477. https://doi.org/10.1093/brain/121.8.1469
6. De Stefano N, Narayanan S, Francis GS et al (2001) Evidence of axonal damage in the early stages of multiple sclerosis and its relevance to disability. Arch Neurol 58:65–70. https://doi.org/10.1001/archneur.58.1.65
7. Schoonheim MM, Meijer KA, Geurts JJG (2015) Network collapse and cognitive impairment in multiple sclerosis. Front Neurol 6:82. https://doi.org/10.3389/fneur.2015.00082
8. Dineen RA, Vilisaar J, Hlinka J et al (2009) Disconnection as a mechanism for cognitive dysfunction in multiple sclerosis. Brain 132:239–249. https://doi.org/10.1093/brain/awn275
9. Thompson AJ, Banwell BL, Barkhof F et al (2018) Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. Lancet Neurol 17:162–173. https://doi.org/10.1016/S1474-4422(17)30470-2
10. Drulović J, Tončev G, Nadj Č et al (2022) Validation of the Brief International Cognitive Assessment for Multiple Sclerosis (BICAMS) in a large cohort of relapsing-remitting MS patients. Acta Clin Croat 61:62–68. https://doi.org/10.20471/acc.2022.61.01.08
11. Benedict RH, Drake AS, Irwin LN et al (2016) Benchmarks of meaningful impairment on the MSFC and BICAMS. Mult Scler 22:1874–1882. https://doi.org/10.1177/1352458516633517
12. Janićijević N, Purić D (2025) Reliability and convergent validity of the Rey-Auditory Verbal Learning Test (RAVLT) against working memory tasks in a Serbian community sample. Preprint. https://osf.io/4ywpr/
13. Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc Ser B 57:289–300. https://doi.org/10.1111/j.2517-6161.1995.tb02031.x
14. DeLong ER, DeLong DM, Clarke-Pearson DL (1988) Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. Biometrics 44:837–845. https://doi.org/10.2307/2531595
15. Sun J, Song H, Yang Y et al (2017) Metabolic changes in normal appearing white matter in multiple sclerosis patients using multivoxel magnetic resonance spectroscopy imaging. Medicine 96:e6534. https://doi.org/10.1097/MD.0000000000006534
16. Heckova E, Dal-Bianco A, Strasser B et al (2022) Extensive brain pathologic alterations detected with 7.0-T MR spectroscopic imaging associated with disability in multiple sclerosis. Radiology 303:199–209. https://doi.org/10.1148/radiol.210614

17. Inglese M, Li BS, Rusinek H et al (2003) Diffusely elevated cerebral choline and creatine in relapsing-remitting multiple sclerosis. Magn Reson Med 50:190–195. https://doi.org/10.1002/mrm.10481
18. Riccitelli GC, Pagani E, Rodegher M et al (2019) Imaging patterns of gray and white matter abnormalities associated with PASAT and SDMT performance in relapsing-remitting multiple sclerosis. Mult Scler 25:204–216. https://doi.org/10.1177/1352458517743091
19. Wagner AD, Shannon BJ, Kahn I, Buckner RL (2005) Parietal lobe contributions to episodic memory retrieval. Trends Cogn Sci 9:445–453. https://doi.org/10.1016/j.tics.2005.07.001
20. Induruwa I, Constantinescu CS, Gran B (2012) Fatigue in multiple sclerosis — a brief review. J Neurol Sci 323:9–15. https://doi.org/10.1016/j.jns.2012.08.007
21. Tartaglia MC, Narayanan S, Francis SJ et al (2004) The relationship between diffuse axonal damage and fatigue in multiple sclerosis. Arch Neurol 61:201–207. https://doi.org/10.1001/archneur.61.2.201
22. Arnett PA, Barwick FH, Beeney JE (2008) Depression in multiple sclerosis: review and theoretical proposal. J Int Neuropsychol Soc 14:691–724. https://doi.org/10.1017/S1355617708081174
23. Giovannoni G, Popescu V, Wuerfel J et al (2022) Smouldering multiple sclerosis: the real MS. Ther Adv Neurol Disord 15:17562864211066751. https://doi.org/10.1177/17562864211066751
24. Sumowski JF, Rocca MA, Leavitt VM et al (2014) Brain reserve and cognitive reserve protect against cognitive decline over 4.5 years in multiple sclerosis. Neurology 82:1776–1783. https://doi.org/10.1212/WNL.0000000000000433

Tables

Table 2 NAWM MRS ratios in RRMS vs. healthy controls (FDR-corrected)

NAWM region	RRMS (n=51) Mean ± SD	HC (n=44) Mean ± SD	p (FDR)	Cohen's d	Direction
NAA/Cr (N-acetylaspartate/creatine)					
R1: Frontal subcortical (L)	1.843 ± 0.247	2.016 ± 0.258	**0.009**	0.709	↓ MS
R2: Frontal subcortical (R)	1.832 ± 0.239	1.770 ± 0.250	0.106	0.251	n.s.
R3: Deep white matter (L)	1.969 ± 0.278	2.686 ± 0.372	**<0.001**	2.195	↓ MS
R4: Deep white matter (R)	2.004 ± 0.275	1.597 ± 0.226	**<0.001**	1.602	↑ MS*
R5: Parietal subcortical (L)	2.077 ± 0.404	2.230 ± 0.315	**0.002**	0.417	↓ MS
R6: Parietal subcortical (R)	2.085 ± 0.443	2.004 ± 0.313	0.581	0.209	n.s.
Cho/Cr (choline/creatine)					
R1: Frontal subcortical (L)	1.187 ± 0.184	0.939 ± 0.173	**<0.001**	1.381	↑ MS
R2: Frontal subcortical (R)	1.174 ± 0.180	1.153 ± 0.142	0.562	0.129	n.s.
R3: Deep white matter (L)	1.189 ± 0.173	0.792 ± 0.179	**<0.001**	2.245	↑ MS
R4: Deep white matter (R)	1.180 ± 0.186	1.113 ± 0.124	0.063	0.417	n.s.
R5: Parietal subcortical (L)	1.127 ± 0.203	0.984 ± 0.169	**0.001**	0.758	↑ MS
R6: Parietal subcortical (R)	1.154 ± 0.209	0.892 ± 0.093	**<0.001**	1.583	↑ MS
mI/Cr (myo-inositol/creatine)					
R1: Frontal subcortical (L)	0.527 ± 0.130	0.436 ± 0.135	**0.002**	0.672	↑ MS
R2: Frontal subcortical (R)	0.548 ± 0.136	0.575 ± 0.139	0.157	0.198	n.s.
R3: Deep white matter (L)	0.515 ± 0.148	0.405 ± 0.150	**<0.001**	0.731	↑ MS
R4: Deep white matter (R)‡	0.537 ± 0.131	0.542 ± 0.146	0.462	0.035	n.s.

R5: Parietal subcortical (L)	0.497 ± 0.119	0.612 ± 0.171	**0.001**	0.782	↓ MS
R6: Parietal subcortical (R)	0.520 ± 0.189	0.402 ± 0.110	**<0.001**	0.744	↑ MS

Data: mean ± SD. *Value higher in MS; likely methodological artifact (see text). ‡One extreme value excluded (n = 50 for mI/Cr R4; see Methods). Bold p-values: FDR-corrected p < 0.05. n.s. not significant

Table 3 Spearman correlation between NAWM NAA/Cr and clinico-cognitive outcomes

Outcome	MRS ratio	NAWM region	Spearman ρ	p-value	Part. r (adj. age)
SDMT (processing speed)	NAA/Cr	R1: Front. subcort. (L)	+0.350	0.012*	0.205 (p=0.148, n.s.)
		R2–R6 all regions	n.s.	—	—
RAVLT z-score (verbal learning)	NAA/Cr	R5: Pariet. subcort. (L)	+0.261	0.064	0.151 (p=0.290, n.s.)
		R1: Front. subcort. (L)	+0.187	0.190	—
MSFC composite	NAA/Cr	R1: Front. subcort. (L)	+0.325	0.020*	—
		R2: Front. subcort. (R)	+0.375	0.007**	—
		R6: Pariet. subcort. (R)	+0.325	0.020*	—
FIS (fatigue)	All	All 6 regions	n.s.	—	—
DASS-21 (mood)	All	All 6 regions	n.s.	—	—

ρ Spearman coefficient, ρ² explained variance. Partial r adjusted for age (Pearson, residual method). *p < 0.05; **p < 0.01. n.s. not significant. RAVLT z-scores calculated using Serbian norms (Janićijević et al. [12]), stratified by sex and age group. FIS Fatigue Impact Scale, DASS-21 Depression Anxiety Stress Scales-21, MSFC Multiple Sclerosis Functional Composite

Figures

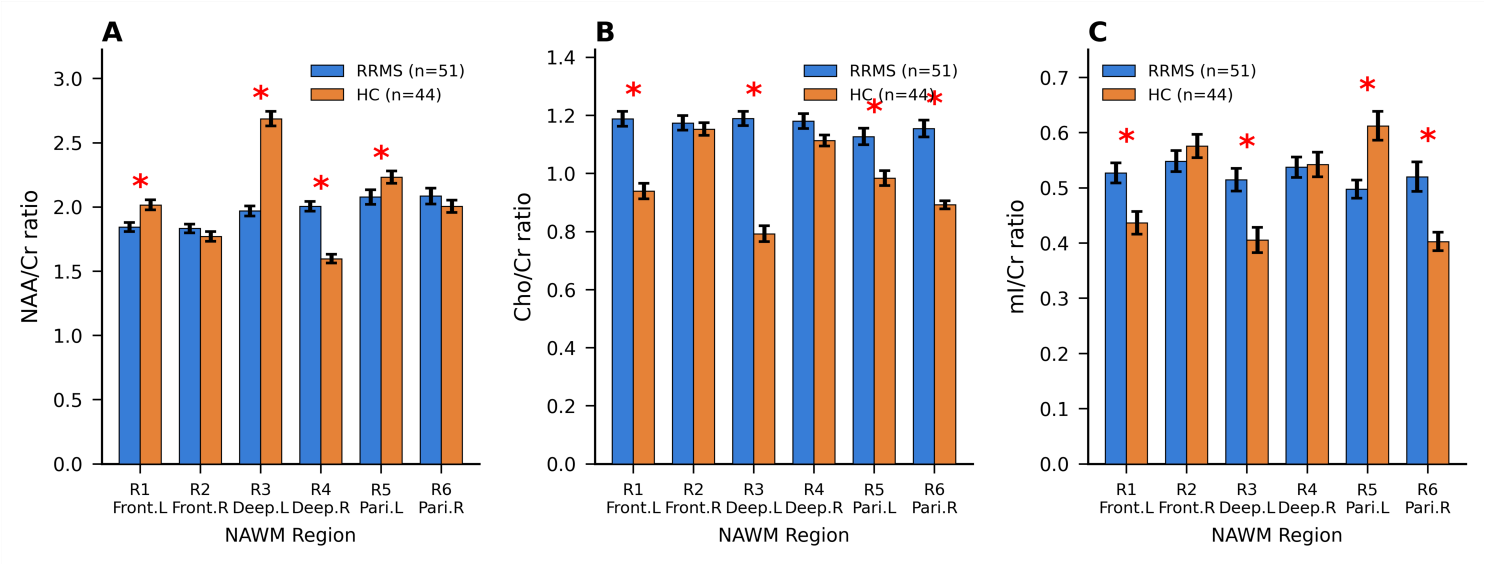


Figure 1

Regional NAWM metabolite differences between RRMS and healthy controls. Bar plots (mean ± SEM) of NAA/Cr, Cho/Cr, and mI/Cr across six NAWM regions. Asterisks indicate FDR-corrected significance ($p < 0.05$). HC healthy controls, R1–R6 as defined in Methods

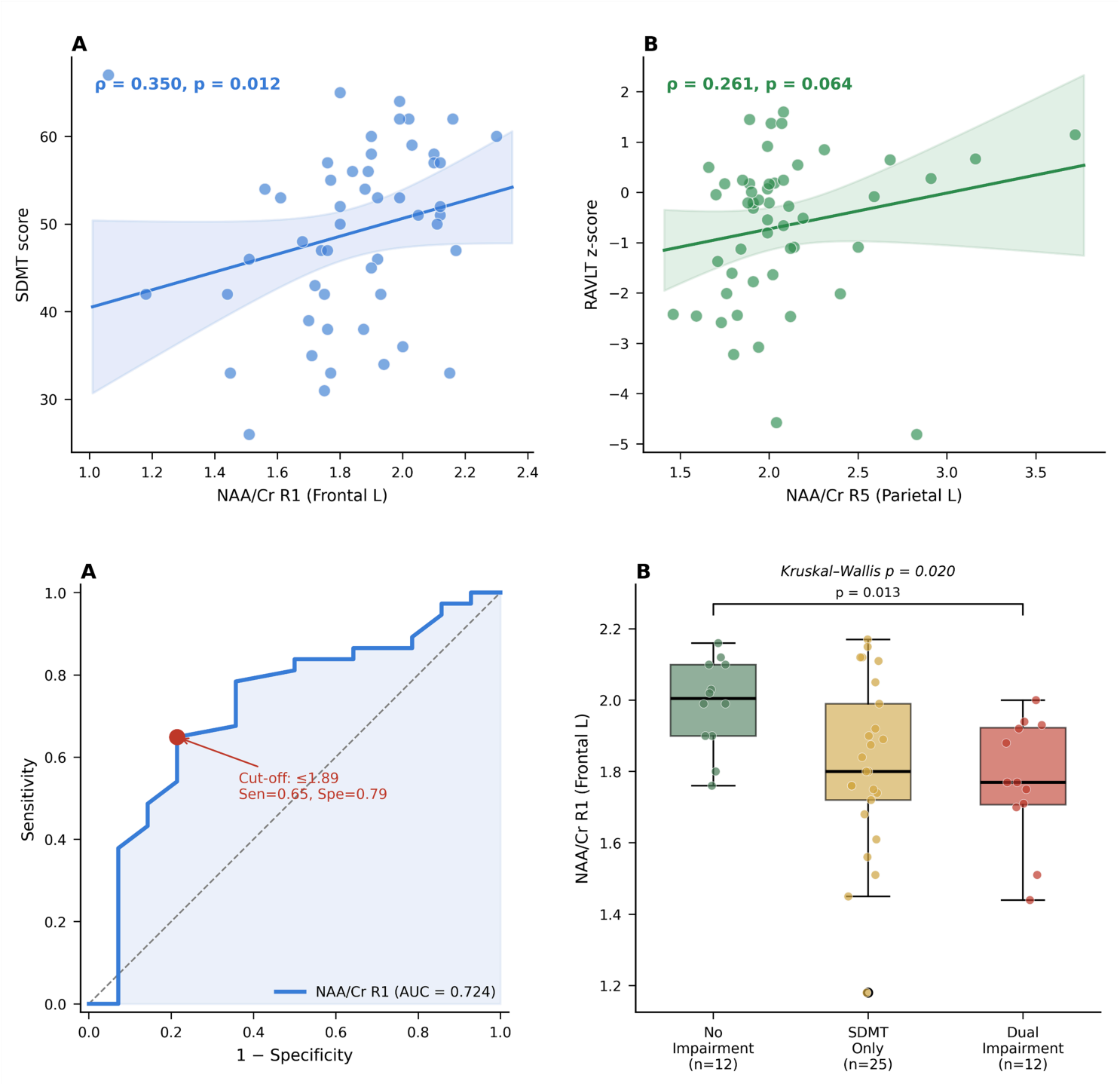


Figure 2

Domain-selective MRS–cognition correlations and discriminative ability. a SDMT vs. NAA/Cr R1 (ρ = 0.350, p = 0.012). b RAVLT z-score vs. NAA/Cr R5 (ρ = 0.261, p = 0.064). c ROC curve for NAA/Cr R1 as a discriminator of below-average SDMT (AUC = 0.724; 95% CI 0.553–0.880). d NAA/Cr R1 by cognitive profile (KW p = 0.020; post-hoc: no impairment vs. dual p_adj = 0.013)

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- ANBOnlineResourceTableS1.docx