

Supplementary Materials

Multivariate Analysis Identifies Distinct Latent Components and Highlights the Contribution of Motor Cortex Neurochemistry to Clinical Variance in Amyotrophic Lateral Sclerosis

Aakanksha Singh MSc,¹ Tobias R. Baumeister PhD,³⁻⁵ Nicholas Dupré MD, MSc,⁶⁻⁷ Angela Genge MD, FRCPC,³ Lawrence Korngut MSc, MD,⁸ Lorne Zinman MD, MSc, FRCPC,⁹⁻¹⁰ the Canadian ALS Neuroimaging Consortium (CALSNIC), Yasser Iturria-Medina PhD,³⁻⁵ Sanjay Kalra MD, FRCPC¹⁻²

1. Neuroscience and Mental Health Institute, Faculty of Medicine and Dentistry, University of Alberta, Edmonton, Alberta, Canada
2. Division of Neurology, Faculty of Medicine and Dentistry, University of Alberta, Edmonton, Alberta, Canada
3. Department of Neurology and Neurosurgery, Montreal Neurological Institute, McGill University, Montreal, Quebec, Canada
4. McConnell Brain Imaging Center, Montreal Neurological Institute, Montreal, Quebec, Canada
5. Ludmer Centre for Neuroinformatics & Mental Health, Montreal, Quebec, Canada
6. Axe Neurosciences, CHU de Québec, Université Laval, Québec City, Quebec, Canada
7. Department of Medicine, Faculty of Medicine, Université Laval, Québec City, Quebec, Canada
8. Hotchkiss Brain Institute, University of Calgary, Calgary, Alberta, Canada
9. Sunnybrook Health Sciences Centre, University of Toronto, Toronto, Ontario, Canada
10. Division of Neurology, Department of Medicine, University of Toronto, Toronto, Ontario, Canada

Supplementary Methods

For consistency, all citation numbers here correspond to those also used in the main manuscript.

References for citations used here are included again at the end of this document.

Supplementary Methods S1: MRI Acquisition Parameters by Site

Imaging data were acquired on 3T scanners across CALSNIC-1 sites as follows:

Site	Scanner	Coil
Site 1: Calgary	GE Discovery MR750	12-channel receive head coil
Site 2: Edmonton	Siemens Prisma	20-channel receive head coil
Site 3: Montreal	Siemens Tim Trio	32-channel receive head coil
Site 4: Toronto	GE Discovery MR750	8-channel receive head coil
Site 5: Vancouver	Philips Achieva	8-channel receive head coil

A multicentre harmonized scanning protocol, adjusted for scanner-specific differences, was used to acquire: 1) motor and mesial prefrontal cortex magnetic resonance spectroscopy (MRS), 2) diffusion-weighted imaging (DWI), and 3) T1-weighted (T1w) anatomical MRI data.

T1-weighted anatomical acquisition (MPRAGE):

- For anatomical localization and normalization, a high-resolution 3D T1w scan of the whole brain was acquired.
- Repetition time (TR): 2300 ms, Echo time (TE): 3.43 ms, Inversion time (TI): 900 ms, Flip angle: 9°, Field of view (FOV): 256 mm × 256 mm, Isotropic resolution: 1 mm³.

Diffusion-weighted acquisition (2D spin-echo, single-shot EPI, axial):

- A 2D spin-echo, single-shot, EPI pulse sequence was used for the acquisition of diffusion-weighted images axially.

- TR: 10,000 ms, TE: 90 ms, Flip angle: 90°, Slices: 70, b0 images: 5, Diffusion gradient directions: 30, b-value: 1000 s/mm², Isotropic resolution: 2 mm³.

Parameters above reflect the Siemens acquisition protocol. Data acquired on General Electric and Philips systems were harmonized to the Siemens protocol, with minor vendor-specific adjustments as required by hardware constraints.

Supplementary Methods S2: Magnetic Resonance Spectroscopy (MRS) Acquisition, Quantification, and Quality Control

MRS data acquisition and voxel placement procedures have been described in full detail previously [21].

Single-voxel MRS localization, dimensions, and acquisition (STEAM):

- Three-dimensional T1-weighted images (1 mm³) described above were used for MRS voxel localization in two regions of interest: midline motor cortex (20 × 50 × 20 mm) and midline mesial prefrontal cortex (25 × 32 × 25 mm).
- Single-voxel proton MRS was performed with a stimulated echo acquisition mode (STEAM) sequence.
- Motor cortex voxel: 20 × 50 × 20 mm, placed using anatomical landmarks in the left/right foot region of the primary motor cortex, centered symmetrically along the midline.
- Mesial prefrontal cortex (mPFC) voxel: 25 × 32 × 25 mm. mPFC voxel was centered symmetrically across the midline. The bottom edge of the voxel was parallel to the AC-PC line at the level of genu of the corpus callosum and the posterior edge touched the anterior edge of the genu of corpus callosum.

- TR: 3000 ms, TE: 160 ms, mixing time (TM): 40 ms, two acquisitions of 32 signal averages for each voxel.

Metabolite quantification: LCModel (version 6.1) was used for automated metabolite quantification of the proton MR spectra. Peak area estimates of choline (Cho), myo-inositol (mIn), creatine (Cr), N-acetylaspartate (NAA), and N-acetylaspartylglutamate (NAAG) were of interest. NAA and NAAG peak areas were combined to quantify total N-acetyl aspartyl moieties (referred to as NAA throughout the manuscript). Total NAA metabolite ratios (NAA/Cho, NAA/Cr, NAA/mIn) were derived for statistical analysis.

Quality control: Manual inspection was conducted for each spectrum with respect to linewidth, lineshape, signal-to-noise ratio, and the presence of spurious signal from eddy current effects. Spectra not meeting these criteria, or with an LCModel fit standard deviation greater than 15%, were excluded from further analysis.

Supplementary Methods S3: DWI Preprocessing and Diffusion Tensor Imaging (DTI)

DTI data were processed using ExploreDTI (v4.8.6; <https://www.exploredti.com>).

Quality control: Visual inspection was performed to assess scan quality, head motion, and signal artefacts prior to preprocessing.

Preprocessing steps (performed in ExploreDTI):

- Correction for signal drift, correction for Gibbs ringing artefacts, head motion correction, eddy current correction.

Diffusion metrics computation: Following preprocessing, voxel-wise maps were calculated for four diffusion metrics: fractional anisotropy (FA), mean diffusivity (MD), radial diffusivity (RD), and axial diffusivity (AD).

Regional extraction: Voxel-wise FA, MD, RD, and AD maps were masked using the motor and frontal MRS voxel locations (see Supplementary Methods S2) to obtain regional values. The motor and mPFC voxels were used as regions of interest (ROIs), and average diffusion metric values were computed within each ROI using the ‘fslmeans’ function in FSL (<https://fsl.fmrib.ox.ac.uk/fsl>).

Supplementary Methods S4: Texture Analysis Preprocessing

T1-weighted 3D images were processed using the CAT12 toolbox [22]. For each participant:

1. Deformation fields mapping native-space anatomy to MNI template space were estimated using the DARTEL method [23] but not yet applied to the images.
2. Segmentation of gray matter, white matter, and cerebrospinal fluid (CSF) was performed on the native-space images, with CSF excluded from further analysis.
3. Corrections were applied for non-uniformity variation and global intensity standardization, yielding bias-corrected images in native space.

Texture computation: Bias-corrected, native-space images were subjected to texture analysis using the three-dimensional Orthogonal Texture Maps method within SPM8 (<http://www.fil.ion.ucl.ac.uk/spm/software/spm8>). A Gray Level Co-occurrence Matrix (GLCM) was computed for each voxel based on its neighboring voxels, using the VGLCM-TOP-3D method [24], with the autocorrelation (AutoC) feature calculated across three orthogonal planes and reported as the average of all three.

GLCM parameters:

- Standard parameters were employed for GLCM computation. Neighborhood radius = 1, Distance = 1, Quantization level = 8, Smoothing kernel = 0.

The autocorrelation (AutoC) texture feature was selected based on promising results from previous studies [15,16,25]. Texture maps were subsequently normalized to MNI standard space using the deformation fields estimated in step (1) above. Averaged gray matter and white matter autocorrelation values were computed within the same regions used for MRS (motor and frontal voxels).

Supplementary Tables

Supplementary Table S1: Site-wise Sample Sizes

Site	<i>N</i>
Site 1: Calgary	10
Site 2: Edmonton	21
Site 3: Montreal	12
Site 4: Toronto	23
Site 5: Vancouver	9
Total	75

Supplementary Table S2: Missing Data by Feature

Feature	% Missing
Symptom duration	0%
Frontal FA, MD, RD, AD	0%
ALSFRS-R	1.3%
Disease progression rate (DPR)	1.3%
Motor NAA/Cr	1.3%
Motor FA, MD, RD, AD	1.3%
Frontal NAA/Cho	5.3%
Motor AutoC	5.3%
Frontal AutoC	5.3%
Motor NAA/Cho	8.0%
Frontal NAA/Cr	8.0%
UMN burden	9.3%
Bilateral finger- and foot-tapping	12.0%
Frontal NAA/mIn	14.7%
ECAS total and ALS-specific	14.7%
LMN burden	16.0%
Motor NAA/mIn	18.7%
FVC	24.0%
Cortical thickness	24.0%

Missing values were imputed using a trimmed scores regression (TSR) algorithm, which estimates missing values iteratively using the correlational structure among all available variables [44-47]. At the participant level, 33.3% of subjects had no missing data. For the remaining subjects, 86.7% had missing data below 13.8% with no subject having more than 24.1% missing data.

Supplementary Table S3: Benjamini-Hochberg False Discovery Rate (FDR) Correction**Across All 12 Latent Variables**

Latent Variable	Rank (i)	Uncorrected Permutation p-value	B-H Critical Value $((i/12) \times 0.05)$	Survived FDR Correction
LV-1	1	0.002	0.004	Yes
LV-2	2	0.002	0.008	Yes
LV-3	3	0.016	0.013	No
LV-4	4	0.034	0.017	No
LV-5	5	0.236	0.021	No
LV-6	6	0.283	0.025	No
LV-7	7	0.932	0.029	No
LV-8	8	0.966	0.033	No
LV-9	9	0.994	0.038	No
LV-10	10	1.000	0.042	No
LV-11	11	1.000	0.046	No
LV-12	12	1.000	0.050	No

Significance testing and False Discovery Rate (FDR) correction for the full Partial Least Squares (PLS) decomposition. Permutation testing (1,000 iterations) generated uncorrected p -values for all 12 latent variables (LVs). To control for multiple comparisons across the full set of components, a Benjamini-Hochberg FDR correction was applied at an alpha level of 0.05. The largest rank (i) where the uncorrected p -value was less than or equal to the critical value $((i/12) \times 0.05)$ was Rank 2. Consequently, only LV-1 and LV-2 survived FDR correction and are interpreted as statistically significant in the present sample.

Supplementary Table S4: Benjamini-Hochberg False Discovery Rate (FDR) Correction

Across All 11 Latent Variables for Sensitivity Analysis

Sensitivity Analysis Latent Variable	Rank (i)	Uncorrected Permutation <i>p</i>-value	B-H Critical Value $((i/11) \times 0.05)$	Survived FDR Correction
LV-1	1	0.002	0.005	Yes
LV-2	2	0.002	0.009	Yes
LV-3	3	0.044	0.014	No
LV-4	4	0.046	0.018	No
LV-5	5	0.144	0.023	No
LV-6	6	0.591	0.027	No
LV-7	7	0.930	0.032	No
LV-8	8	0.988	0.036	No
LV-9	9	0.998	0.041	No
LV-10	10	1.000	0.046	No
LV-11	11	1.000	0.050	No

Significance testing and False Discovery Rate (FDR) correction for the full PLS decomposition for sensitivity analysis after removing cortical thickness and forced vital capacity (FVC). Permutation testing (1,000 iterations) generated uncorrected *p*-values for all 11 latent variables (LVs). To control for multiple comparisons across the full set of components, a Benjamini-Hochberg FDR correction was applied at an alpha level of 0.05. The largest rank (i) where the uncorrected *p*-value was less than or equal to the critical value $((i/11) \times 0.05)$ was Rank 2. Consequently, only LV-1 and LV-2 survived FDR correction and are interpreted as statistically significant in the present sample.

Supplementary Table S5: Primary and Sensitivity Analysis Comparison Summary

	Primary Analysis ($n = 29$ features)	Sensitivity Analysis ($n = 27$ features, FVC & CT excluded)
Rank (max possible LVs)	12	11
Number of significant components (FDR-corrected)	2 (LV-1, LV-2)	2 (LV-1, LV-2)
LV-1 explained covariance	56.7%	57.6%
LV-1 r (uncorrected p)	0.54 ($p = 0.002$)	0.53 ($p = 0.002$)
LV-2 explained covariance	19.8%	20.6%
LV-2 r (uncorrected p)	0.51 ($p = 0.002$)	0.49 ($p = 0.002$)
Combined explained covariance (LV-1 + LV-2)	76.6%	78.3%
LV-1 imaging salience correlation vs. primary	—	$r = 0.997$
LV-1 clinical salience correlation vs. primary	—	$r = 0.999$
LV-2 imaging salience correlation vs. primary	—	$r = 0.995$
LV-2 clinical salience correlation vs. primary	—	$r = 0.997$

The primary analysis consisted of 29 total (17 imaging + 12 clinical) features (rank = 12) while the sensitivity analysis consisted of 27 total (16 imaging + 11 clinical) features (rank = 11) after the exclusion of cortical thickness and FVC.

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