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## BACKGROUND AND AIM

Evidence suggests a strict relationship between frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS) that has not been fully elucidated yet. FTD and ALS have been described as a continuum since they share clinical, genetic, and pathologic features. However, comparisons of biomarkers between patients with behavioral variant FTD (bvFTD) with and without motor involvement are rare. **This multi-biomarker study aims to assess the variability of clinical, genetic, and biological markers in the ALS-FTD spectrum at the time of diagnosis, while integrating a machine learning approach to enhance data interpretation.**

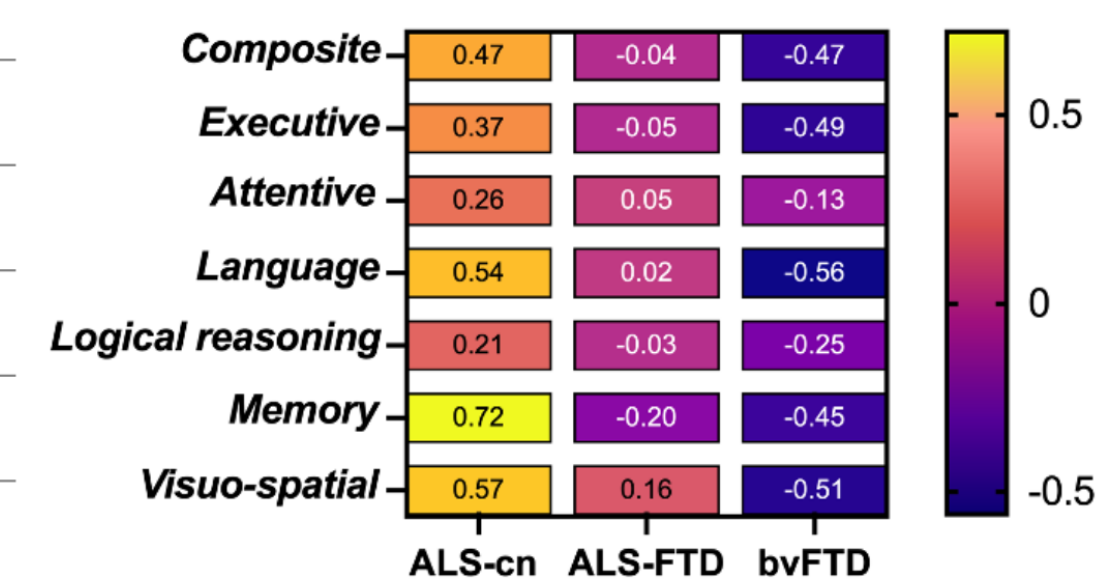
## METHODS

Participants, genetically screened for known ALS- and FTD-associated mutations, underwent neuropsychological assessments, CSF analysis, and brain imaging through 18-fluorodeoxyglucose positron emission tomography ([<sup>18</sup>F]FDG-PET). Neuropsychological data were analyzed calculating, for each cognitive domain, a composite score by averaging the rank-transformed z-scores of all tests measuring the same domain. [<sup>18</sup>F]FDG-PET analysis was performed using a validated voxel-based SPM method at single-subject and group-level. To evaluate the ability of the identified markers to differentiate ALS-cn, ALS-FTD, and bvFTD, machine-learning models — including support vector machine (SVM) and random forest (RF) — were applied, offering a streamlined, data-driven approach to improve diagnostic precision across this spectrum of disorders.

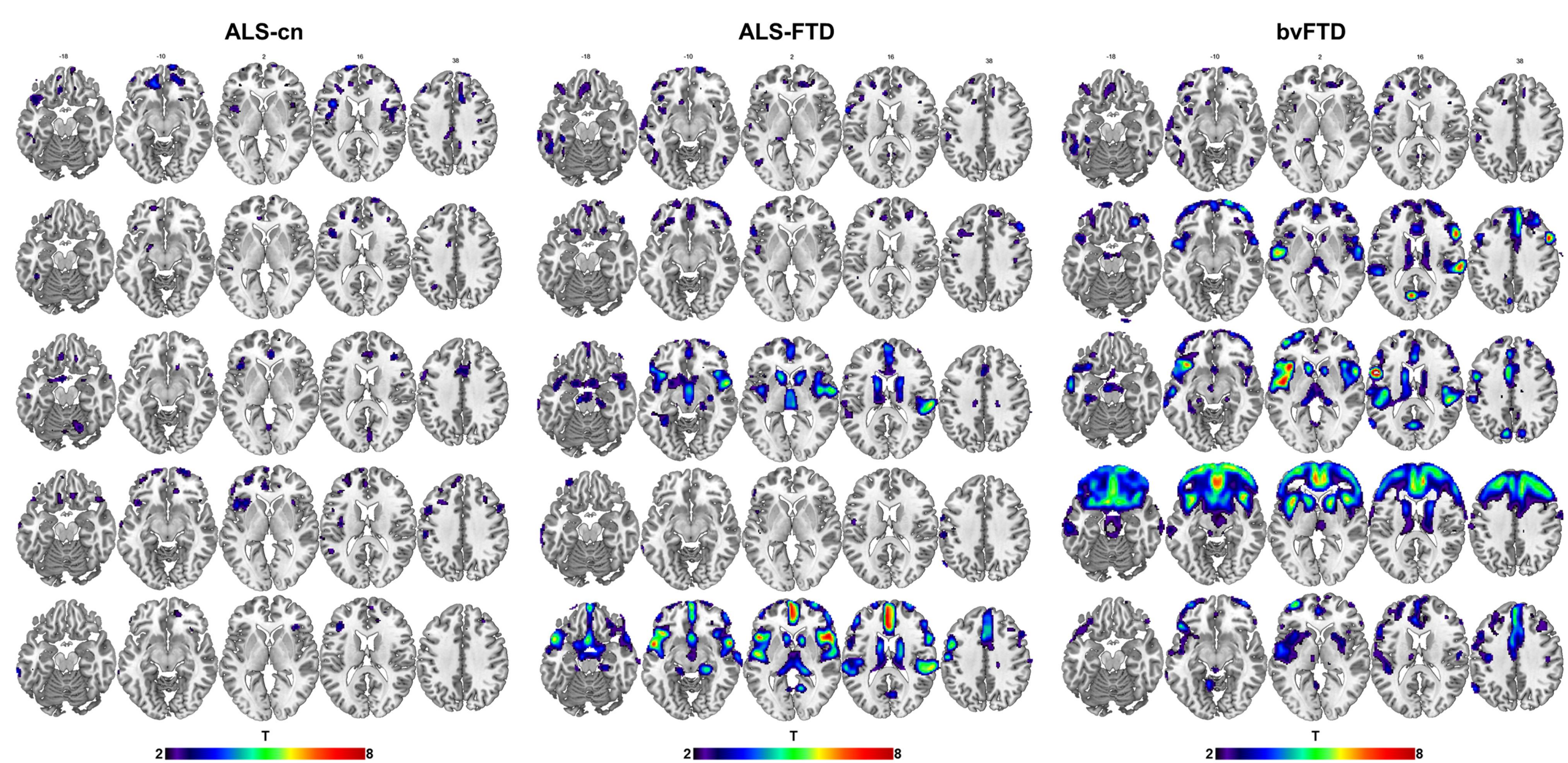
## RESULTS

20 ALS-cn, 19 ALS-FTD, and 21 bvFTD patients were included. Neuropsychological composite z-scores revealed significant differences across groups, underlining worse performance in bvFTD regarding memory, visuospatial and executive functions (**Figure 1**). Brain [<sup>18</sup>F]FDG-PET showed a pattern of hypometabolism increasing from ALS-cn to ALS-FTD and reaching its greater extent in bvFTD. Specifically, brain hypometabolism was mainly confined to the sensorimotor cortices and the frontobasal regions in the ALS-cn group, whereas in the ALS-FTD group, it was extended to the supplementary motor area and the dorsolateral frontal cortex, and in the bvFTD group, a widespread hypometabolism further affected the frontomesial and orbitofrontal cortices (**Figure 2**). No significant differences in CSF biomarkers were observed. SVM correctly classified 83% of patients, indicating a good level of classification performance, while RF showed perfect accuracy (100%). The two models shared eight to ten most relevant features in the classification system, namely age, disease duration from symptoms onset to diagnosis, total composite z-score, superior frontal gyrus (left), middle frontal gyrus (left), middle frontal gyrus - pars orbitalis (left and right), and anterior cingulate cortex (left).

	ALS-cn	ALS-FTD	bvFTD	F, P	P (ALS-cn vs ALS-FTD)	P (ALS-cn vs bvFTD)	P (ALS-FTD vs bvFTD)
Global composite z-score (m ± SD)	0.47 ± 0.35	-0.04 ± 0.61	-0.47 ± 0.41	21.07; < 0.001	0.007	< 0.001	0.030
Executive z-score (m ± SD)	0.37 ± 0.48	-0.05 ± 0.63	-0.49 ± 0.78	8.16; < 0.001	0.027	< 0.001	0.083
Attentive z-score (m ± SD)	0.26 ± 0.79	0.05 ± 1.06	-0.13 ± 0.65	0.50; 0.610	0.599	0.242	0.050
Language z-score (m ± SD)	0.54 ± 0.82	0.02 ± 0.94	-0.56 ± 0.74	5.52; 0.007	0.003	0.001	0.538
Logical reasoning z-score (m ± SD)	0.21 ± 1.17	-0.03 ± 0.93	-0.25 ± 0.98	0.38; 0.686	0.539	0.439	0.913
Memory z-score (m ± SD)	0.72 ± 0.69	-0.20 ± 0.62	-0.45 ± 0.54	14.75; < 0.001	< 0.001	< 0.001	0.218
Visuo-spatial z-score (m ± SD)	0.57 ± 0.47	0.16 ± 0.71	-0.51 ± 0.80	10.40; < 0.001	0.068	< 0.001	0.015



**Figure 1.** Comparison of cognitive performance across ALS-cn, ALS-FTD, and bvFTD groups in different cognitive domains.



**Figure 2.** Group-level [<sup>18</sup>F]FDG-PET analysis in the three groups (ALS-cn, ALS-FTD and bvFTD) vs. healthy controls.

## DISCUSSION

Our study identified significant differences in the biomarkers according to the neurodegenerative clinical groups within the same disease spectrum. These differences were evident in neuropsychological profiles and brain hypometabolism patterns, successfully addressing the study's aim and providing valuable insights for differential diagnosis into ALS-FTD continuum heterogeneity.