Multicenter validation of [18F]-FDG PET and support-vector machine discriminant analysis in automatically classifying patients with amyotrophic lateral sclerosis versus controls

Multicenter validation of [^{18}F]-FDG PET and support-vector machine discriminant analysis in automatically classifying patients with Amyotrophic Lateral Sclerosis versus controls

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ABSTRACT

Purpose: Recent studies have shown that the early diagnosis of Amyotrophic Lateral Sclerosis (ALS) can be aided using FDG PET. Single-center studies using a support vector machine (SVM) approach to differentiate ALS from controls have shown high overall accuracy on an individual patient basis using local a priori defined classifiers. The aim of this study is to validate the accuracy of the SVM in one center to a large dataset acquired in another center.

Methods: A previously defined Belgian (BE) single center group of 175 ALS patients (61.9 ± 12.2 years, 120 M / 55 F) and 20 screened healthy controls (62.4 ± 6.4 years, 12 M / 8 F) was used to classify another large ALS and control data set from one center in Italy (IT), consisting of 195 patients (63.2 ± 11.6 years, 115 M / 78 F) – of which 2 patients had to be exluded - and 40 control subjects (62 ± 14.4 years; 29 M / 11 F) who underwent whole-body FDG PET-CT for lung cancer but with visually normal brain scans and no indication of neurological or psychiatric disorders. All were evaluated by local routine ^{18}F-FDG PET-CT. Group comparisons based on SPM were performed and SVM classifiers based on the local training sets was applied to differentiate ALS cases from controls from the other center.

Results: SPM group analysis showed only minor differences between both ALS groups, indicating consistency in diagnosis and pattern. SVM using the BE data set as training, classified 183/193 IT ALS patients correctly (accuracy of 94.8%). However, from the IT control population, 35/40 were misclassified into the ALS group (accuracy 12.5%). Inversely, using the Italian data set as a training, BE ALS group could not be distinguished from the Belgian control population (Why “inversely”? It is the same: in both sets patients and controls could not be discriminated using the “other” data set). SPM group analysis confirmed prefrontal hypometabolism in the IT vs BE control group, indicating subclinical brain changes in patients undergoing oncological WB scanning.

Conclusion: The results of this multicenter study confirm the diagnostic value of ^{18}F-FDG PET for ALS and a stable pattern over centers that can be discerned with high accuracy using SVM.

Furthermore, it highlights the importance of carefully selected control group for this analysis tool, as confounding subclinical frontal changes are present in patients in an oncological setting.

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a rapidly progressing adult-onset neurodegenerative disease, with both upper (UMN) and lower (LMN) motor neuron degeneration, resulting in progressive paralysis and poor prognosis. Besides motor neuron degeneration, significant extramotor cerebral pathology has been observed. In 50% of cases, cognitive and/or behavioral impairment exists, including overt frontotemporal dementia in 15% of these [1-5]. The clinical diagnosis of ALS is based on revised El Escorial and Awaji-Shima criteria[6-8], which use a combination of clinical and electrophysiological examinations. ALS can be classified as bulbar or spinal-onset disease depending on the site of onset of the first symptom. Currently, neuroimaging is mainly used to rule out other diseases mimicking ALS [6, 8-11].

^{18}F-Fluorodeoxyglucose (^{18}F-FDG) positron emission tomography (PET) has contributed to our knowledge of metabolic changes in ALS patients, but advances in hardware and methodology have also enabled accurate and patient-specific identification of specific ALS related metabolic patterns[12-14]. Moreover, FDG PET was shown to be useful for individual prognosis prediction, as frontotemporal hypometabolism reflects a worse prognosis with risk of associated frontotemporal dementia[1, 15, 16]. Although the described metabolic changes in ALS, such as hypometabolism in the (pre)motor cortex, the frontal cortex and hypermetabolism in the temporal cortex, midbrain and cerebellum, are consistent between studies, some discrepancies exist such as activity in the amygdala and parietal cortex, as well as primary visual cortex, but the latter is likely dependent on differences in acquisition circumstances[11, 14].

Our group recently validated a support vector machine (SVM) approach to more accurately differentiate ALS from screened healthy controls on an individual patient basis, using a priori defined classifiers from a training set (n = 70). As within-center study, this resulted in high discrimination accuracy (> 95 %). The most discriminating variables were hypometabolism in the prefrontal cortex and premotor cortex and hypermetabolism in the cerebellum, upper brain stem and medial temporal cortex[14]. In order to use such advanced discriminant analysis methods more widely, validation is needed with other centers, as potential added variability might be introduced resulting in lower

accuracy, which may be caused by different camera type, standard acquisition schemes, reconstruction variables and clinical parameters or referral timepoints. Therefore, we performed a retrospective validation study using the largest published dataset in ALS described until now in Italy (n=195)[11, 17] (17 had a limited ALS population). The purpose of the present study was therefore threefold: firstly, to investigate whether in comparative ALS patient groups, the metabolic changes were consistent using the same data analysis pipeline. Secondly, to validate a previously generated local SVM classifier to another center's ALS patient sample and evaluate potential causes of reduced accuracy by clinical and voxel-based group comparison of the training data sets.

MATERIAL AND METHODS

Participants

This retrospective study was approved by the local Ethics Committee from the University Hospital Leuven. Patient demographic and disease characteristics are given in Table 1.

1/ Belgian subjects

A total of 175 ALS patients (ALS-BE), as previously described[13, 14], were divided in 2 groups (a training set of 70 subjects, and a second set of 105 ALS patients for within-center validation). The training set (n = 70; 62.1 ± 12.5 years; 44 M / 26 F) was recruited at the tertiary neuromuscular clinic at the University Hospital Leuven (Belgium) between January 2011 and January 2013, whereas the second set patients (n = 105; 61.7 ± 12.0 years; 74 M / 31 F) was recruited between October 2012 and January 2015. All patients had a negative history of other neurologic disorders, underwent neurological investigation and were electrophysiologically tested by an experienced specialist in neuromuscular disorders. Sixty patients had definite ALS diagnosis, 81 patients probable and 34 patients possible ALS diagnosis, based on both the revised El Escorial and Awaji-Shima criteria. (Table 1). The majority (> 90%) of subjects underwent PET imaging within 4 months after diagnosis (median = 1.2 months, range = 0 to 17.0 months). Time from first symptom to PET scan was (15.0± 12.3 months).

The control subjects (CON-BE; n = 20; 62.4 ± 6.4 years; 12 M / 8 F) were also identical as in Van Weehaeghe et al. (2016) [14]. All subjects were part of a reference database set of carefully screened healthy volunteers, selected for various clinical trial purposes, and underwent a thorough general and psychiatric history, laboratory tests, clinical and neurological examination, as described previously [14].

2/ Italian subjects

All Italian ALS patients (ALS-IT; n = 195; 63.2 ± 11.6 years; 115 M / 78 F) were recruited at the Turin ALS Center between June 2011 and February 2013. They all had probable lab-supported,

probable, or definite ALS diagnosis according to the revised El Escorial criteria. The majority of subjects underwent PET imaging within 5 months after diagnosis (median = 4.1 months, range ?)[11]

The control subjects (CON-IT; n = 40; 62 ± 14.4 years; 29 M / 11 F) were oncological patients referred to the Turin PET center for suspected diagnosis of lung cancer, and this set consisted of patients where no oncological disease was observed in the whole-body ^{18}F-FDG PET-CT. Moreover, they all had a normal clinical neurological assessment. Main exclusion criteria were: major systemic illness, major vision disturbances, psychiatric illnesses and diseases which could affect brain metabolism aside from cancer[11].

^{18}F-FDG PET acquisition and reconstruction

1/ Belgian groups

Details of the methods used in the Belgian subjects were given in Van Weehaeghe et al. (2016)[14]. In short, ^{18}F-FDG PET was acquired using a Siemens ECAT HR+ camera (n = 169) or Siemens HiRez PET-CT camera (n = 6), operated in 3-dimensional mode. All subjects fasted for at least 6 hours, and glycemia was measured before scanning (< 130 mg/dL). Subjects were injected intravenously with 151 (± 8) MBq of ^{18}F-FDG under standard conditions (lying supine in a dimly lit, quiet room, with ears and eyes open). Thirty minutes after ^{18}F-FDG injection, a dynamic scan of 30 min (6 frames of 5 min each) was started. During the acquisition, the subject's head was immobilized by means of a vacuum pillow. On the HR+ camera, attenuation- and scatter-corrected images were reconstructed using 3-dimensional filtered back projection with a Hanning postfilter, resulting in FWHM of 7 mm. On the HiRez, ^{18}F-FDG images were reconstructed using iterative ordered-subset expectation maximization (4 iterations, 4 subsets) resulting in FWHM of 8 mm.

2/ Italian groups

As previously mentioned in Pagani et al.[11], all Italian subjects fasted for at least 6h before PET acquisition. Before injection, blood glucose was measured (< 130 mg/dl) in all patients. After a 10-min

rest in a silent and darkened room, with eyes closed and ears unplugged, the subjects were injected with ± 185 MBq of ^{18}F-FDG through an intravenous cannula. PET-CT scanning was started approximately 60 min after injection and lasted for 10 min. A polycarbonate head holder was applied to reduce head movements during the scan. All brain PET-CT scans were acquired using a Discovery STE system (GE Healthcare). CT data were used for attenuation correction of the PET data. Data were collected and ^{18}F-FDG images were reconstructed using iterative ordered-subset expectation maximization (2 iterations, 28 subsets).resulting in FWHM of 6 mm[17]

Image analysis

All further analysis of the reconstructed data was performed at one site (Leuven, Belgium) using the same analysis pipeline. First, images were individually checked for complete acquisition. Two ALS-IT patients were excluded because part of the brain was not in the field of view. Then a voxel-based analysis was executed by using Statistical Parametric Mapping (SPM version 8; Wellcome Trust Centre for Neuroimaging, London, UK), implemented in Matlab (R2016a; The MathWorks Inc., Massachusetts, USA).

All the scans were spatially normalized to the MNI (Montreal Neurological Institute) space using the SPM FDG PET-template in both procedures, followed by a non-rigid registration with 16 iterations and isotropic Gaussian smoothing kernel with a full width at half maximum of 8 mm. In SPM, all data were normalized to the average gray matter activity of each image.

As there were no significant age differences between the four groups (Table 1), the analysis was executed without age as nuisance variable. As for the Belgian group, patients and healthy volunteers were scanned on two different cameras, and the whole Italian group was scanned on the same camera, resulting in a total of three different cameras, camera type was included as nuisance variable. Groups were compared by two-sample unpaired t-tests.

General statistics

Unless specified otherwise, SPM comparisons were conducted using a p_{height} of less than 0.001 and a cluster extent threshold (k_E) set at 50 voxels. Secondly, a classical SVM with a linear kernel was used to classify each subject using the default soft margin option in the Spider software (version 1.71, http://people.kyb.tuebingen.mpg.de/spider/; running on Matlab version R2016a). The same brain mask was used in all analyses and was defined by those voxels exceeding 50% of the mean of the ^{18}F-FDG PET images.

Initially, a leave-one-out SVM approach was conducted to compare the within-center diagnostic accuracy (based on what, VOIs? How were them segmented?). The LOO SVM was trained to classify an image into one of two classes using all images less one. The remaining image served as test set. By means of permutations the entire dataset was used at least once as the test set. Besides the simple binary classification, the distances of each scan to the separating hyperplane will be reported.

Thereafter, a classical SVM approach was conducted. First, the a priori defined classifiers obtained in Van Laere *et al.* (2016)[13] were used to investigate the discriminative power to classify the Italian dataset using the classical SVM approach. Secondly, a subgroup of the Italian dataset (a random selection of ALS patients with bulbar and spinal onset (n = 80) and all CON-IT (n=40), based on time feasibility to run the software) was used as training set for classical SVM analysis to investigate the inverse classification accuracy for the Belgian dataset.

RESULTS

1/ Voxel based group comparison between the ALS groups versus same-center controls

To identify possible metabolic differences between both patient groups, group analyses were performed to detect differences in relative hypo- and hypermetabolism in both patient group versus the same-center controls and between ALS groups directly. In **ALS-BE** compared to **CON-BE**,(abbreviations not previously defined) hypometabolism was found in the frontal and parietal cortex and hypermetabolism in the temporal cortex, cerebellum and brainstem (Fig. 1A). The Italian ALS group compared to the controls showed hypometabolism in the frontal, motor and occipital cortex and hypermetabolism in the temporal cortex, cerebellum and brainstem (Fig. 1C). The Belgian population (ALS-BE vs CON-BE) had a relative hypermetabolism compared to the Italian population (ALS-IT vs CON-IT) in the hippocampus, cerebellum and occipital lobe (Fig. 1B); relative hypometabolism was observed in the lateral temporal lobe, frontal lobe, precuneus and posterior cingulum (Fig. 1C).

In direct group comparison to the Belgian ALS patients (ALS-BE), the group of ALS-IT showed slight hypermetabolism in the middle andinferior frontal cortex, the inferolateral temporal cortex and the occipital cortex, and hypometabolism in the premotor cortex, the prefrontal and orbitofrontal cortex, the medial temporal cortex, the hippocampus, the cingulate cortex and the cerebellum (Table 2).

HERE I DO NOT UNDERSTAND. IF THE TEXT IN YELLOW IS RIGHT THE ITALIAN POPULATION, I.E. SHOULD BE **HYPERMETABOLIC** AS COMPARED TO THE BELGIAN ONE IN HIPPOCAMPUS CEREBELLUM AND OCCIPITAL LOBE (SINCE IN THE OPPOSITE COMPARISON IS HYPOMETABOLIC). AND THE SAME FOR HYPO/HYPERMETABOLISM.

Regarding voxel difference intensities, the peak heights of the latter clusters was very minor and thus second-order compared to the differences in patient vs controls. For example, the peak voxel difference in the frontal cortex between ALS-BE and CON-BE was 20%, between ALS-IT and CON-IT 12% and between both ALS groups less than 2%.

2/ SVM using the Belgian dataset (training set, n = 70) as LOO and as training set (to be consistent with the text: LOO comes first)

The LOO SVM approach, based on the original Belgian training set (70 ALS-BE patients and 20 CON-BE), had a sensitivity of 95.7%, specificity of 80% and accuracy of 93.3% as was already described in Van Laere et al[13]. LOO should be performed using all 175 subjects, why limiting the analysis to the 70? Because of the numerosity differences with the controls? How were these 70 chosen among the 175? Only because they were the "original" 70 patients? Were they all examined with the same camera? And what about the remaining 105?If you describe them in the methods you have to include them into the analyses, isn't it?

Using the classical SVM approach based on the Belgian training dataset, only 10 of 193 (5.2%) ALS-IT patients were misclassified. However, in contrast to the high within-center specificity value of 80%[13], as much as 35 of the 40 CON-IT were misclassified into the ALS group, resulting in an overall sensitivity of 94.8%, specificity of 12.5% and **accuracy of 80.7%.** (It is a repetition of the text below and should be deleted)

The overall classification accuracy using the classical SVM approach for both CON-IT and ALS-IT together was 80.7% (Table 3). The distribution of scan distances from the hyperplane is illustrated in Figure 2.

I think here you should also mention the results of the JNM 2016 study in order to compare the different results between BEL and ITA dataset using classical SVM.

3/ SVM approach using the Italian training set and LOO

The LOO SVM approach, based on the Italian **training set**, (sorry but I continue to not understand why the LOO should be performed on a subset of patients and not on all of them. LOO and classical approach are different ways to analyze the data, why limiting the use of the former to the training set?) resulted in a sensitivity of 85.0%, a specificity of 52.5% and an accuracy of **82.5%.** (if 31 subjects

were misclassified the overall accuracy should be around 75%). Nineteen CON-IT were misclassified as ALS and 12 ALS-IT patients were misclassified as controls. In Neurology 2014 we had much better results with all 195 subjects

The classical SVM approach based on the subset of the ALS-IT and CON-IT as a training set, could not reliably distinguish the ALS-BE from the CON-BE as all cases were classified as ALS (Fig. 2)

DISCUSSION

In this study, a classical SVM approach with previously defined classifiers was used to discriminate ALS patients from controls in a multicentric setting.

First, metabolic differences between ALS patients described in previously published studies were processed by the same pipeline. Using a voxel based analysis we observed second-order differences in glucose metabolism between both ALS groups that were below 2 % in difference. Several sources of bias may explain these intricate small differences. Firstly, patient preparation (eyes open in ALS-BE versus eyes closed in ALS-IT) likely explains the relative occipital hypermetabolism seen in ALS-IT compared to ALS-BE. Secondly, there was a time difference between FDG injection and PET scan in both groups (start acquisition 30 minutes p.i. for BE groups, vs. 60 minutes for IT groups). Later timing may produces relatively higher relative FDG-uptake (+ 2%) in bilateral posterior cingulate gyrus, parietal and frontal association cortices, and subcallosal cortices[18, 19], and a relatively lower uptake (- 2%) in cerebellum and orbitofrontal areas at 60 minutes p.i. [18, 19],thereby providing an explanation for the relative hypometabolism bias seen in the cerebellum in ALS-IT vs ALS-BE.

Furthermore, demographic comparison between the two ALS groups, revealed that all Italian ALS patients had a probable or definite ALS diagnosis according to the revised El Escorial criteria, whereas 2/3th of the Belgian patients had a possible of probable diagnosis of ALS, based on both the revised El Escorial and Awaji-Shima criteria. Additionally, 10.9% of the Belgian ALS population had a C9ORF72 mutation compared to only 7.7% of the Italian ALS population. Possibly, the described demographic differences could account for the second-order differences seen in the frontal, temporal and cingulate cortex.

Despite these small differences, the classical SVM analysis with the Belgian population as a training set, resulted in a sensitivity of 94.8% . This sensitivity is much higher than the value of 80%, which is the range of the overall sensitivity of the combination of the Awaji-Shima and El Escorial criteria[18].

This finding illustrates the robustness and clinical surplus value of the SVM approach to classify ALS patients on a multicentric level and with different demographic characteristics.

To address the discrepancy induced by including the CON-IT group, several hypotheses were investigated. The main clinical difference between CON-BE and CON-IT groups was that the CON-BE were carefully screened healthy volunteers, whereas CON-IT groups were oncological patients referred in the context of malignancy screening but presumed oncological disease-free (normal FDG findings). To analyze the effect of patient controls versus carefully screened controls, additional tests were performed using the classical SVM based on the BE ALS/CON training set with various BE control sets: a/ using another set of 15 screened healthy controls (n = 15; mean age 31.5 ± 8.1 years; 6 M / 9 F;) from a later control study population gathered on the same camera in 2016 (CON-BE2), b/ 20 patients (mean age 59.5 ± 13.9 years; 12 M / 8 F) referred in 2016 in the context of cancer with positive abnormality findings described in the final medical protocol (ONC-BE), and c/ another set of 24 patients (mean age 48.7 ± 11.6; 9 M / 15 F) referred for fever of unknown origin, without abnormalities in the definitive protocol (FUO-BE).

From this analysis, all 15 screened healthy controls were all classified as controls, whereas the large majority of the other 2 BE patient groups, respectively 16/20 and 18/24, were classified into ALS (Supplemental Fig. 1), with relatively smaller to comparable distances to the hyperplane as most subjects in the CON-IT group. The consistency in these evaluations of the patient-control groups, leads us to hypothesize that patient group characteristics rather than camera differences has given rise to the classification discrepancies.

To interpret and evaluate the regional metabolic information represented in these data, an SPM group comparison was also carried out (Supplemental Fig. 2). Compared to the CON-BE, ONC-BE showed relative hypometabolism in the prefrontal cortex, and hypermetabolism in the occipital cortex and cerebellum. The same was true for FUO-BE and ONC-IT patients. In panel D, it can be seen that the CON-IT patients were very similar to the FUO-BE group regarding cerebral metabolism. All groups showed some prefrontal hypometabolism compared to CON-BE as well as relative cerebellar hypermetabolism.

Since the discriminating regions of this particular SVM set in ALS vs CON is mainly based on the relative prefrontal hypometabolism and cerebellar hypermetabolism (Supplemental Fig. 3), this can explain the lower discrimination accuracy in non-ALS pathologies that show particularly alterations in these regions. In oncological patients alteration of cerebral metabolism have been described, in particular frontotemporal hypometabolism even before chemotherapy administration [20, 21].These results and extra hypothesis testing, shows that certainly in this context of ALS vs CON, it is crucial to carefully select the used control population for brain studies as several pathophysiological features may have a significant impact on brain metabolism in the discriminant regions.

Nevertheless, despite the small metabolic differences observed in CON-IT patients, the within-center Italian LOO SVM approach demonstrated a high accuracy of 82.5% in discriminating between ALS and CON. This can be explained by the use of different classifiers and weight of the classifiers as illustrated in Supplemental Fig. 3.

Overall however, our results do show that an SVM analysis using the a priori defined classifiers shows very promising results with an accuracy of significant clinical surplus value compared to the clinical criteral as accuracies of more than 90% in ALS vs CON is obtained, irrespective of slight diagnostic differences of ALS patients and PET scanning instrumentation, implying the robustness of the method as long as an appropriate control group is used.

There are some limitations of this study. First, MRI imaging was not available so we could not perform partial volume correction to correct for atrophy[22]. However, as glucose uptake is not only a measure of cellular function but also of more macroscopic atrophy effects, consequently the validity of these results remains unchanged[9, 23].

Secondly, in this study, we did not consider ALS mimics (such as cervical spondylotic myeloradiculopathy (CSM) and multifocal motor neuropathy (MMN)) that may be more difficult to separate from ALS patients that CON. Due to the robustness and good results of this SVM approach, the next step would be to include a large set of single- and multicenter acquired ALS mimics to calculate the discrimination accuracy for these versus ALS patients, which is a highly important

remaining clinical question, both in the setting of early diagnosis as well as for prognosis and inclusion in experimental trials[24].

CONCLUSION

This multicentric study highlights the robustness of ^{18}F-FDG PET brain imaging with voxelbased SVM analysis to discriminate ALS patients from healthy controls. Secondly, this study also stresses the importance of a carefully selected healthy control population in this setting for multicenter use of the SVM technique, as the major discriminatory regions such as frontotemporal areas can be effected and reduce classification accuracy.

DISCLOSURES

No conflict of interest relevant to this article was reported. The authors acknowledge the skilled help of the radiopharmacy, technologist and medical physics team at UZ Leuven (Marva Bex, Tjibbe de Groot, Kim Serdons, Kwinten Porters, Jef Van Loock, Kristof Baete, Michel Koole, Johan Nuyts and Jenny Ceccarini).

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FIGURE LEGENDS

Figure 1: Group comparison of relative glucose metabolism in patients with Amyotrophic Lateral Sclerosis (ALS) and controls using a voxel-based discriminant analysis.

A. Surface projections of areas with relative hypometabolism (red, $p_{FWEcor} < 0.05$; orange, $p_{uncor} < 0.001$) and hypermetabolism (blue, $p_{uncor} < 0.001$) for Belgian (BE) ALS patients vs. BE healthy controls.
B. Surface projections of areas with relative hypermetabolism (red, $p_{FWEcor} < 0.05$; orange, $p_{uncor} < 0.001$) for the Belgian (ALS-BE vs CON-BE) versus the Italian population (ALS-IT vs CON-IT)
C. Surface projections of areas with relative hypometabolism (red, $p_{FWEcor} < 0.05$; orange, $p_{uncor} < 0.001$) and hypermetabolism (blue, $p_{uncor} < 0.001$) for Italian (IT) ALS patients vs. IT controls.
D. Surface projections of areas with relative hypometabolism (red, $p_{FWEcor} < 0.05$; orange, $p_{uncor} < 0.001$) for the Belgian (ALS-BE vs CON-BE) versus the Italian population (ALS-IT vs CON-IT)

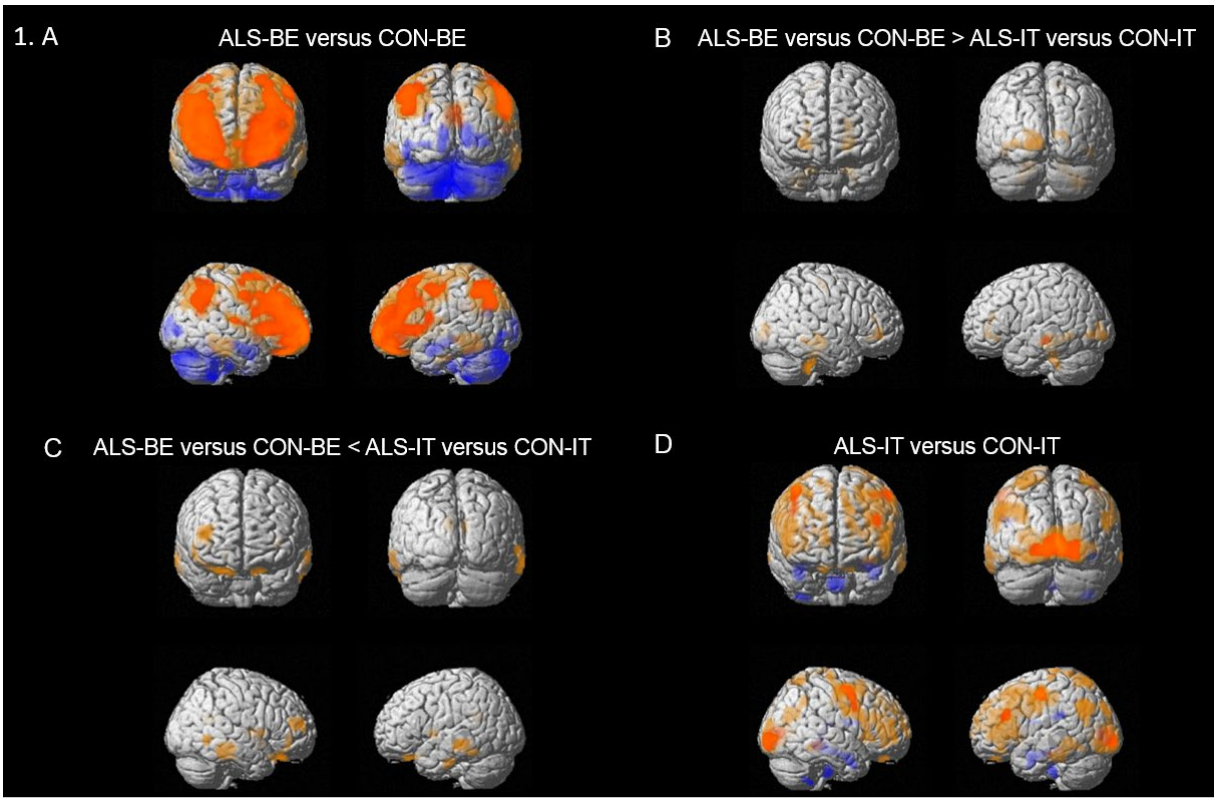

1. A
ALS-BE versus CON-BE
B
ALS-BE versus CON-BE > ALS-IT versus CON-IT
C
ALS-BE versus CON-BE < ALS-IT versus CON-IT
D
ALS-IT versus CON-IT

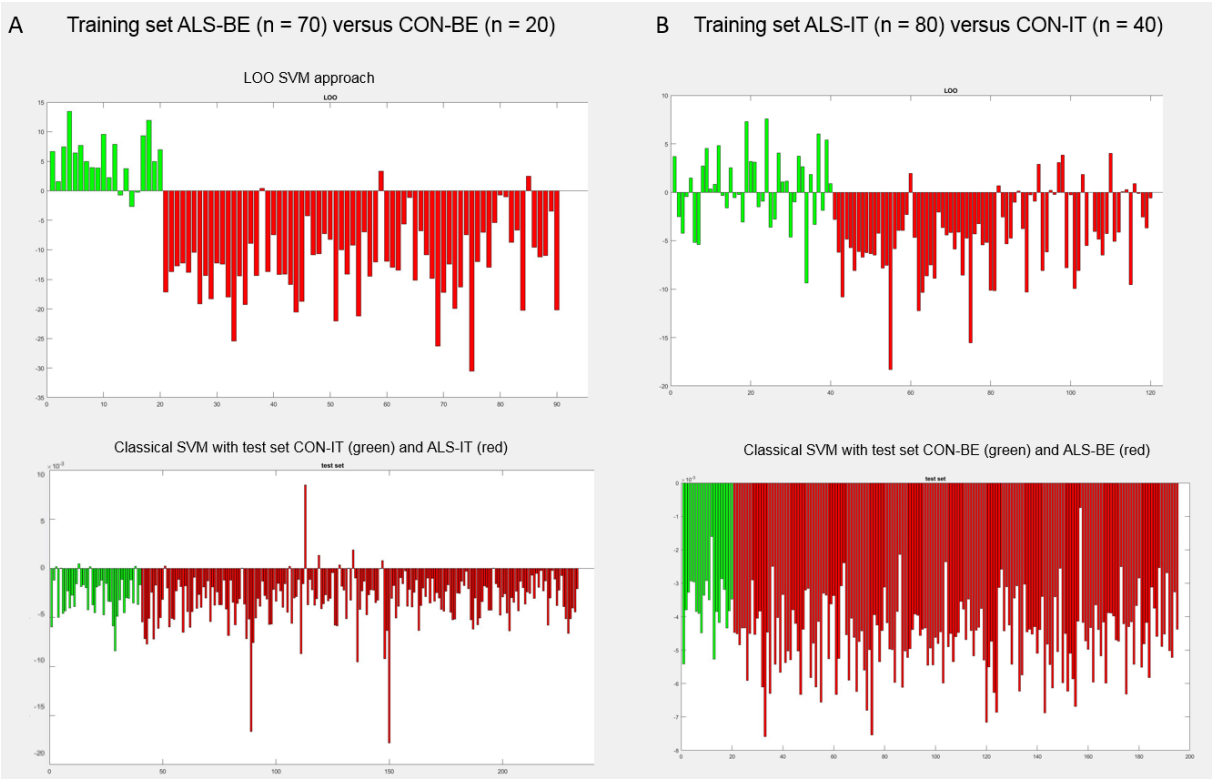
Figure 2: Support Vector Machine (SVM) analysis and LOO approach of Amyotrophic Lateral Sclerosis (ALS) vs. control cases.

A. Upper part: discriminative analysis of CON-BE vs. ALS-BE using the leave-one-out approach.

   Lower part: Plots of distance to the classifier for controls from Italy (CON-IT) and ALS patients from Italy (ALS-IT) using classical SVM approach with Belgian training set.

B. Upper part: discriminative analysis of CON-IT vs. ALS-IT using the leave-one-out approach.

   Lower part: Plots of distance to the classifier for healthy controls from Belgium (CON-BE) and ALS patients from Belgium (ALS-BE) using classical SVM approach with Italian training set.



TABLES

Table 1: Demographic and clinical patient and control characteristics.

	ALS patients		Control groups	
	ALS-BE	**ALS-IT**	**CON-BE**	**CON-IT**
N	175	193	20	40
Age (y)	61.9 ± 12.2	63.2 ± 11.6	62.4 ± 6.4	62 ± 14.4
Gender (M/F)	120/55	115/78	12/8	29/11
Onset type (S/B)	130/45	135/58	-	-
Onset (mo)	15.0 ± 12.3		-	-
C9ORF72 mutation	19	15	-	-

Values are mean ± SD. y = year; M = male; F = female; S = spinal; B = bulbar; mo = months

Table 2: Voxel-based brain mapping analysis results of ALS-BE vs. ALS-IT. Coordinates (MNI) and statistics of hypermetabolic regions in ALS-IT in comparison with ALS-BE (part A) and hypometabolic regions in ALS-IT in comparison with ALS-BE (part B).

(S = superior; I = inferior; L = lateral; M = medial)

A

Cluster-level		**Peak-level**		**Coordinates (mm)**			**Brodmann area (BA)**
P_{uncorr}	k_E	P_{uncorr}	T-value	x	y	z	
0.0001	1018	< 0.0001	6.4	-16	-57	-3	18
< 0.0001	2079	< 0.0001	5.6	70	-31	-13	20 (L), 21
0.001	1036	< 0.0001	5.5	-60	-57	-1	20 (L)
0.002	739	< 0.0001	4.6	54	35	-7	11 (I), 46
0.012	456	< 0.0001	4.2	18	-57	1	18, 19

B.

Cluster-level		**Peak-level**		**Coordinates (mm)**			**Brodmann area (BA)**
P_{uncorr}	k_E	P_{uncorr}	T-value	x	y	z	
< 0.0001	4027	< 0.0001	7.6	24	41	-3	6, 10, 11 (S), 23, 47, 48
< 0.0001	1309	< 0.0001	6.4	16	-27	-23	20 (M), 30, 36, 37
0.001	1023	< 0.0001	5.9	-14	-29	-21	20 (M), 30, 37
0.007	542	< 0.0001	5.2	-38	1	-33	20 (M), 36
0.0016	418	< 0.0001	4.5	0	-69	-13	Cerebellum
0.0013	444	< 0.0001	4.4	16	-5	57	6, 32

extent threshold: k = 50 voxels, $p_{uncorr} < 0{,}001$

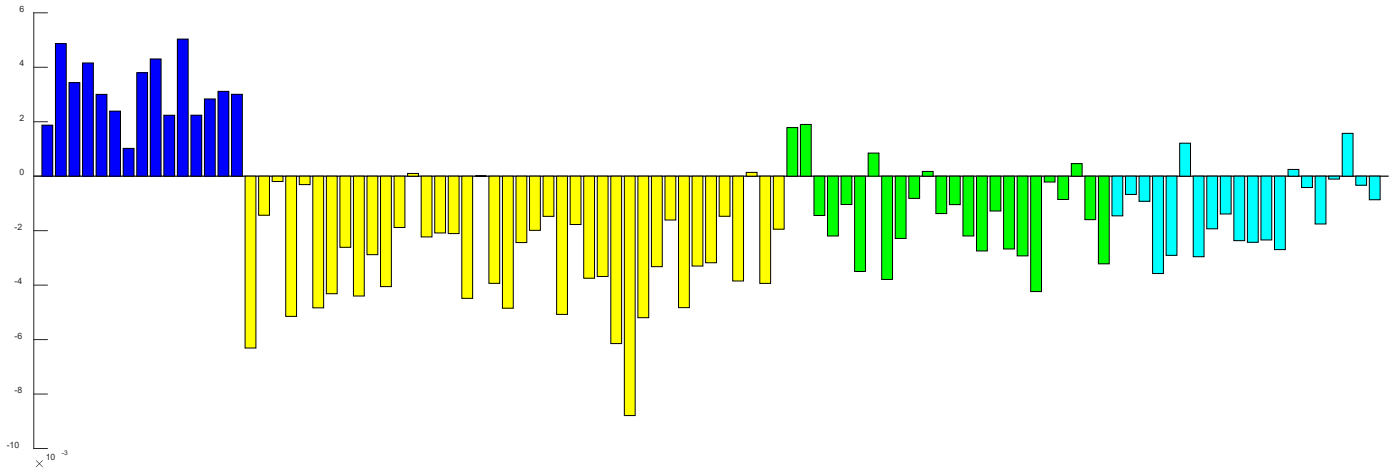
Table 3: SVM-based classification matrix of Italian ALS (ALS-IT) and controls (CON-IT) using the Belgian training set.

Group	CON	ALS	% correct
CON-IT	5	35	12.5
ALS-IT	10	183	94.8
All IT	15	218	80.7

SUPPLEMENTAL MATERIAL

Supplemental Figure 1:

Support Vector Machine (SVM) analysis of additional control cases (blue, n=15, CON-BE2), the Italian control group (CON-IT, yellow), patients with fever of unknown origin (n=15, FUO-BE) and new oncological cases (ONC-BE). Plots of distance to the classifier for the different groups using classical SVM approach with Belgian training set.



(CON-BE2 (n = 15; blue), CON-IT (n = 40; yellow), FUO-BE (n = 24; green), ONC-BE (n = 20; cyan.

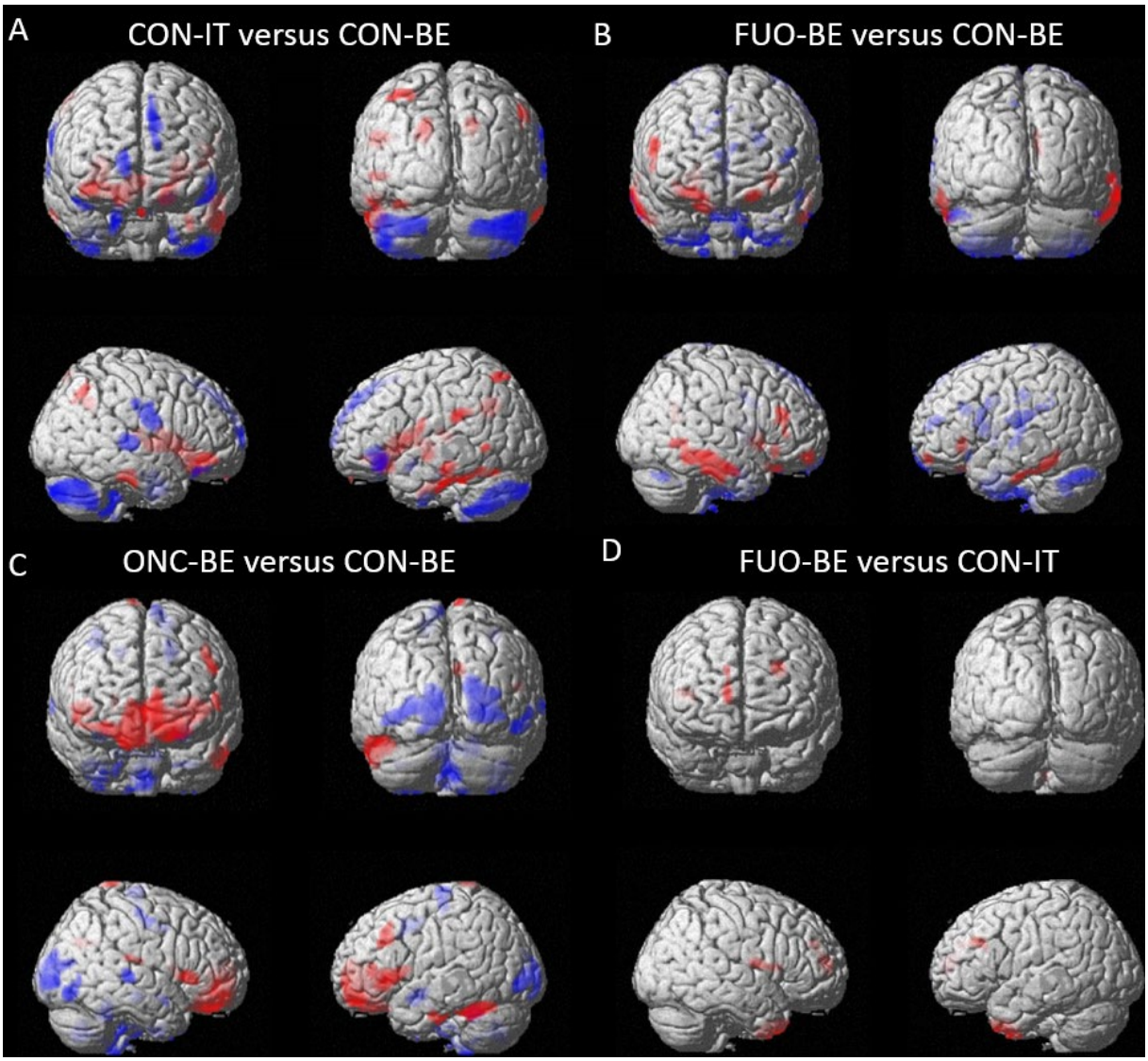
Supplemental Figure 2: Voxel-based comparison (at p_{uncorr} <0.001) between the screened healthy controls (CON-BE) and different patient control groups (ONC-BE, FUO-BE, CON-IT) and another screened healthy control sample (CON-BE2). Red = relative hypermetabolism, blue = relative hypermetabolism.

A. Surface projections of areas with relative hypo- and hypermetabolism for CON-IT versus CON-BE. The peak voxel difference in the frontal cortex was <5%.

B. Surface projections of areas with relative hypo- and hypermetabolism for FUO-BE versus CON-BE. The peak voxel difference in the prefrontal cortex cluster was <5%.

C. Surface projections of areas with relative hypo- and hypermetabolism for ONC-BE versus CON-BE. The peak voxel difference in the pre frontal cortex cluster was 11%.

D. Surface projections of areas with relative hypo- and hypermetabolism for FUO-BE versus CON-IT. The peak voxel difference in the frontal cortex was <5%.


A
CON-IT versus CON-BE
B
FUO-BE versus CON-BE
C
ONC-BE versus CON-BE
D
FUO-BE versus CON-IT

Supplemental Figure 3: Feature weights of classifier for ALS-BE training set (n = 70) vs. CON-BE (n = 20) and ALS-IT subgroup (n = 80) vs. CON-IT (n = 40) projected onto normalized structural MR image in Montreal Neurologic Institute space. Clusters indicate areas with high discriminative impact based on relative hypometabolism (yellow-red) and relative hypermetabolism (blue). Scale of feature weights represents how much a voxel contributes. Scale was normalized so that sum of all weights is 1. Only voxels with weight of more than 0.002 in absolute value are shown.