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ORIGINAL ARTICLE

Heterogeneous brain FDG-PET metabolic patterns in patients with C9orf72 mutation

Veronica Castelnovo[1,2] · Silvia Paola Caminiti[1,3] · Nilo Riva[4] · Giuseppe Magnani[4] · Vincenzo Silani[5,6] · Daniela Perani[1,3,7]

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Abstract

Objective The hexanucleotide repeat expansion in C9orf72 is an associated genetic cause in amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). In the "ALS/FTD" spectrum prevails clinical heterogeneity and an in vivo knowledge of the underling brain dysfunction in patients carrying C9orf72 mutation remain limited and only described at group level. The study aimed to assess the brain metabolic alterations characterizing patients with C9orf72 mutation using FDG-PET in single individuals.

Methods We applied a validated statistical parametric mapping (SPM) voxel-based procedure for FDG-PET data to obtain maps of brain relative hypometabolism and hypermetabolism at single-subject level in six FTD/ALS patients carrying the C9orf72 mutation.

Results Clinical diagnoses classified the patients as right semantic variant of frontotemporal dementia (one case, C9svFTD), behavioral variant of frontotemporal dementia (two cases, C9bvFTD), and bulbar amyotrophic lateral sclerosis (three cases, C9bALS). The FDG-PET SPM revealed a prevalent frontal hypometabolism in C9bvFTD cases, and right temporal polar and lateral involvement in C9svFTD, consistent with the clinical diagnosis. There was a quite comparable occipital and cerebellar hypermetabolism in these cases. The three C9bALS patients showed variable patterns of hypo- and hypermetabolism.

Conclusions The present work is the first in vivo FDG-PET study showing the heterogeneous patterns of brain regional hypo- and hypermetabolism in single patients sharing C9orf72 mutation. Brain hypometabolism was consistent with the clinical phenotypes, supporting the diagnostic importance of neuroimaging functional biomarkers to capture at single-subject level specific brain dysfunction.

Keywords ALS-FTD spectrum · C9orf72 · Heterogeneity · Positron emission tomography · Statistical parametric mapping

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✉ Daniela Perani
perani.daniela@hsr.it

1 Vita-Salute San Raffaele University, Milan, Italy

2 Neuroimaging Research Unit, Institute of Experimental Neurology, Division of Neuroscience, San Raffaele Scientific Institute, Milan, Italy

3 In vivo Structural and Molecular Neuroimaging Unit, Division of Neuroscience, San Raffaele Scientific Institute, Milan, Italy

4 Department of Neurology, San Raffaele Hospital, Milan, Italy

5 Department of Neurology and Laboratory of Neuroscience, IRCCS Istituto Auxologico Italiano, Milan, Italy

6 Department of Pathophysiology and Transplantation, "Dino Ferrari" Center, Università degli Studi di Milano, Milan, Italy

7 Nuclear Medicine Unit, San Raffaele Hospital, Milan, Italy

Introduction

In 2011, two international groups have identified in parallel the most common genetic cause of both amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD): the hexanucleotide repeat expansion C9orf72, a gene strongly implicated in neurodegeneration [1, 2]. The C9orf72 pathogenic expansion is characterized by a clinical heterogeneity, which reflects both the structural and pathological variance of ALS and FTD [3]. At present, according to the clinical, neuropathological, and genetic overlapping features, they are accounted to lay on a continuous spectrum, referred to as ALS/FTD [4–7].

18-Fluorodeoxyglucose positron emission tomography (FDG-PET) studies in patients carrying the C9orf72 mutation are limited and all conducted at a group level, reporting variable cortical and subcortical metabolic alterations [8–11].

These inconsistencies may be due to the clinical heterogeneity within the included cohorts that cannot be disentangled analyzing FDG-PET data at group level. In this study, we applied an optimized semi-quantitative method to assess brain metabolic alterations at single-subject level, in a clinically heterogeneous cohort of C9orf72 patients. This procedure possesses high statistical accuracy in detecting the specific typical metabolic patterns associated with several neurodegenerative conditions, including the FTLD spectrum [12–18].

The study aims at providing a detailed description of the brain hypo- and hypermetabolism patterns in six patients carrying the rare C9orf72 mutation and different clinical syndromes, namely ALS and FTD.

Methods

Study design

From a large clinical cohort referred to the Molecular Medicine Unit at San Raffaele Hospital (Milan, Italy) from 2013 to 2016, we selected patients who underwent FDG-PET acquisition, with a diagnosis of ALS or FTD and who carried the rare C9orf72 hexanucleotide expansion. Three out of the six patients were clinically evaluated at the Department of Neurology at San Raffaele Hospital (Milan, Italy), and three at the IRCCS Istituto Auxologico Italiano (Milan, Italy).

The FDG-PET scans were performed for each patient, to support differential diagnosis at entry as a standard of care in use in our center. Before FDG-PET, all patients provided written informed consent, following detailed explanation of the FDG-PET standard procedure. The retrospective study was approved by the San Raffaele Hospital Ethical Committee.

Genetic analyses

After informed consent, all patients underwent blood sampling, and DNA samples were screened for mutations in genes more commonly implicated in amyotrophic lateral sclerosis and frontotemporal dementia. The GGGGCC hexanucleotide repeat expansion was detected using repeat primed polymerase chain reaction (PCR) method. All six cases carried the C9orf72 repeat expansion [19].

Clinical and neuropsychological data

All cases were evaluated by experienced neurologists and neuropsychologists with a structured clinical interview, a full neurological examination, and an extended neuropsychological battery (see S3 for a description of the neuropsychological assessment, and Table 1 for demographics).

[18F]Fluoro-deoxy-glucose positron emission tomography image acquisition

The FDG-PET scans were acquired with a Discovery STE (GE Medical Systems, Milwaukee, WI, USA) multi-ring PET tomography system. Static emission images were acquired 45 min after injecting a dose of FDG radioactivity (125–50 MBq) and acquisition lasted 15 min. Image reconstruction followed ordered subset expectation maximization algorithm. FDG-PET scans were performed in compliance with the European Association of Nuclear Medicine procedure guidelines [20].

[18F]Fluoro-deoxy-glucose positron emission tomography statistical parametric mapping analysis

Image pre-processing and analysis were performed according to a statistical procedure previously developed and validated in our laboratory [12, 21] with SPM5 software (http://www.fil.ion.ucl.ac.uk/spm/software/spm5/) [22], running in Matlab 7.6 (MathWorks Inc., Sherborn, MA, USA). FDG-PET single-subject images were normalized using an FDG-PET specific template [21]. The normalized and smoothed images were then tested for relative brain hypometabolism and hypermetabolism by means of a two-sample t test implemented in SPM5, in which the single image was compared with a large normal control FDG-PET dataset, entering age as a nuisance covariate [12] (See Appendix S1).

To obtain the hypermetabolism SPM t-maps, we employed the same approach used by Cistaro et al. (2012), consisting in the use of a relative threshold > 1 [23].

The resulting SPM t-maps, thresholded at $p < 0.05$ family-wise error-corrected for multiple comparisons, represented the abovementioned basis for the ALS and FTD disease-specific pattern ratings.

Results

FDG-PET imaging findings In C9orf72 bulbar ALS patients, the FDG-PET single-subject optimized procedure provided heterogeneous patterns of brain hypo-and hypermetabolism (Fig. 1a).

In details, the SPM t-map of C9bALS patient no. 1 showed marked bilateral hypometabolism involving the dorsolateral frontal cortex, the orbitofrontal cortex, inferior frontal gyrus, postcentral gyrus, superior and inferior temporal gyrus, the left and right insula, caudate, nucleus accumbens, and thalamus. Regions of hypermetabolism were found in the vermis and cerebellar cortex. The SPM t-map of C9bALS patient no. 2 showed significant hypometabolism in the occipital cortex (left and right calcarine cortex, inferior occipital and lingual gyri), plus in the inferior frontal gyrus, and precuneus

Table 1 Clinical and demographic features

Patient	Age	Sex	Age at onset (years)	Disease duration (years)	Clinical diagnosis
1	59	F	55	4	C9bALS
2	71	F	70	1	C9bALS
3	62	M	60	2	C9bALS
4	71	M	66	5	C9bvFTD + parkinsonism
5	60	M	59	2	C9bvFTD
6	60	F	59	1	right C9svFTD

C9bALS, bulbar amyotrophic lateral sclerosis; *C9bvFTD*, behavioral variant of frontotemporal dementia; *right C9svFTD*, right semantic variant of frontotemporal dementia

bilaterally, cerebellum, and a diffuse cortical and subcortical regional hypermetabolism. C9bALS patient no. 3 was characterized by severe hypometabolism in the premotor and supplementary motor cortex, pre- and postcentral gyrus and thalamus, all bilaterally, and a diffuse cortical and subcortical hypermetabolism.

The two C9bvFTD patients were all characterized by an extended brain hypometabolism in the frontal regions (Fig. 1b). In details, C9bvFTD patient no. 4 showed marked bilateral hypometabolism in the dorsolateral prefrontal, orbitofrontal, medial frontal cortex, anterior and posterior cingulate gyrus, insula, inferior parietal lobule, and caudate; the C9bvFTD patient no. 5 showed bilateral hypometabolism in dorsolateral prefrontal and orbitofrontal cortex, nucleus accumbens, and in the right thalamus. The right C9svFTD patient no. 6 showed hypometabolism in the right hemisphere including the orbitofrontal cortex, the temporal pole, and the lateral and medial temporal cortex. All two C9FTD patients showed quite consistent hypermetabolic patterns mainly involving the occipital cortex, also extending to the associative temporal and parietal cortex, pre- and postcentral gyri and subcortical structures (putamen in patient nos. 5 and 6, see Appendix S2 for clinical features).

Discussion

In the present study, we assessed FDG-PET metabolic patterns at the individual level applying an optimized SPM voxel-based procedure in six patients carrying the C9orf72 hexanucleotide repeat expansion. FDG-PET studies available in literature on ALS/FTD cohorts positive for C9orf72 mutations provided hypometabolism pattern at group-level [8–11, 24]; the use of analysis at group-level may have prevented to describe more thoroughly the heterogeneous metabolic profiles in the clinical spectrum caused by this mutation.

Our sample was composed by three patients clinically diagnosed as FTD and three as ALS. In the C9bALS cases, the SPM *t*-maps revealed heterogeneous patterns of hypo- and hyper metabolism. Noteworthy, despite all the C9ALS patients presented a comparable bulbar onset, they showed different topographical distribution of brain metabolism alterations. It can be hypothesized that these differences are the effect of distinct neuropathological expressions within the same C9bALS group, as previously reported [25]. A previous study, comparing ALS with C9ALS brain hypometabolism at group-level, showed that C9ALS had a more widespread central nervous system involvement than ALS patients without genetic mutations [9]. However, in our series, only one patient showed a more widespread cortical and subcortical hypometabolism at individual level, namely patient no. 1. Also, our cohort of patients was heterogeneous from clinical standpoint; C9bALS patients differed as cognitive profiles: patient nos. 1 and 2 presented executive and language deficits at the Edinburgh Cognitive and Behavioral ALS Screen (ECAS) test), respectively. Their cognitive deficits were coherent with the hypometabolism in prefrontal regions [26], and in line with the growing evidence of cognitive changes in bulbar pathology [26, 27].

The C9FTD cases, conversely, revealed more consistent patterns of regional metabolism. Two C9bvFTD patients (cases 4 and 5) were characterized by an extensive dorsolateral frontal hypometabolism, consistent with the clinical core features, namely behavioral alterations and executive and language deficits. This pattern of brain hypometabolism was consistently reported by Origone et al. (2014) in a bvFTD patient carrying mutations in both TARDBP and C9orf72 genes. The C9bvFTD patient showed a moderately symmetrical hypometabolism pattern involving inferolateral frontal cortex, orbital frontal cortex, anterior and middle cingulated gyri, and caudate [28]. A previous case-report study reported instead brain frontal hypermetabolism without any significant regional brain hypometabolism in a C9bvFTD patient. This unexpected result was attributed to patient's concomitant manic symptoms [29].

Our C9svFTD case was clinically characterized by language deficits, such as anomies and loss of word-meaning, apathy, exacerbation of the premorbid obsessive traits, and prosopagnosia. The FDG-PET revealed a right hypometabolism in the temporal lobe (i.e., the pole and lateral temporal cortex) and in the orbitofrontal cortex. Taken together, these cases showed dysfunctional patterns consistent with

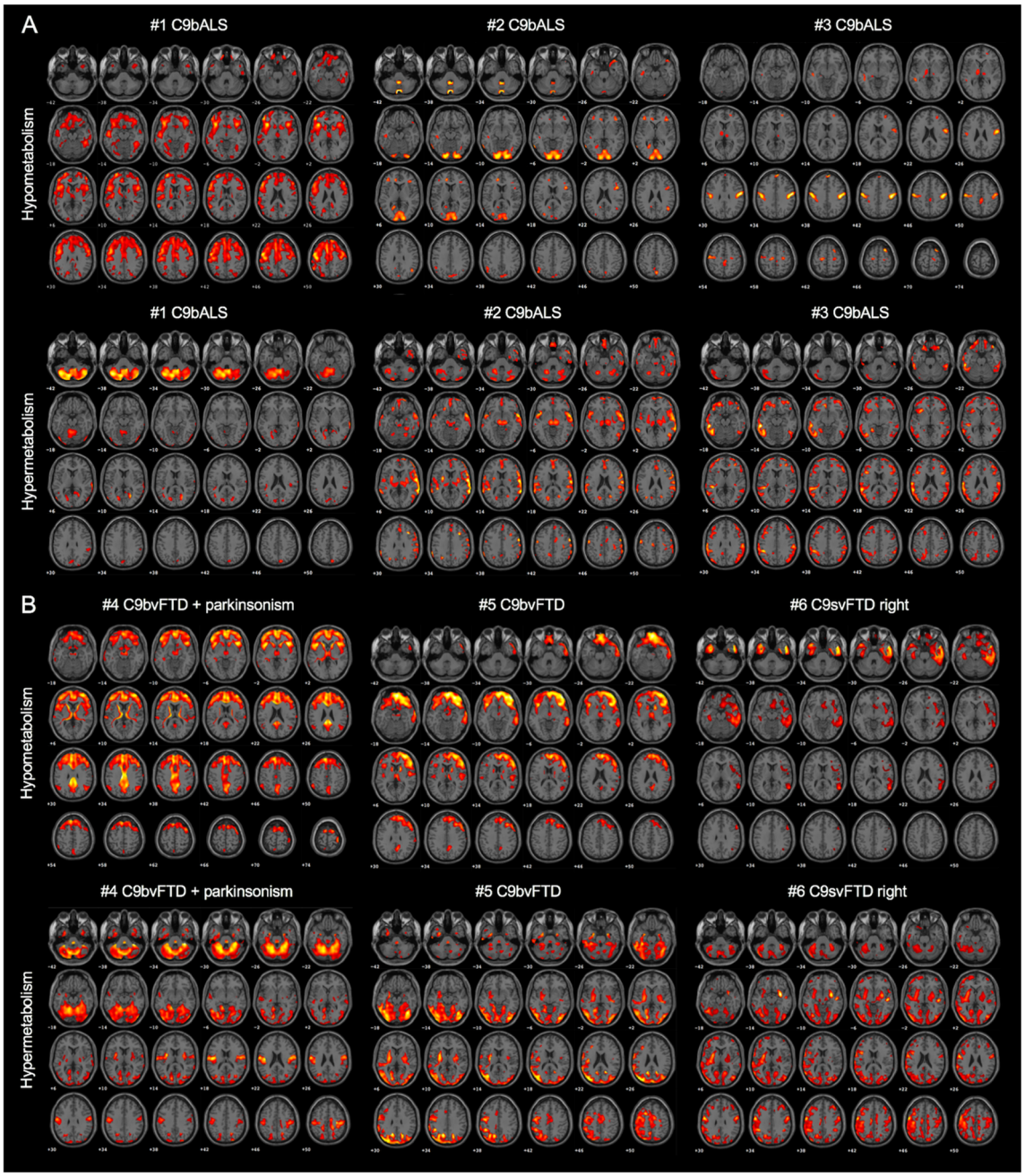

Fig. 1 FDG-PET SPM *t*-map of relative hypo- and hypermetabolism on axial view of an MRI standardized in **a** C9bALS patients and **b** C9FTD patients. *T*-maps were obtained from the comparison with 112 normal scans and applying a family wise error (FWE) correction, with minimum cluster size = 100 voxels and statistical threshold $p = 0.05$. C9bvFTD, behavioral variant of frontotemporal dementia; right C9svFTD, right semantic variant of frontotemporal dementia; C9bALS, bulbar amyotrophic lateral sclerosis

the typical hypometabolism reported in the literature, for the semantic [30], and the behavioral variant FTD [31], respectively. Our findings add further evidence in cases carrying the C9orf72 gene mutation.

Noteworthy, we found brain regions with hypermetabolism, in particular the occipital cortex, the pre- and postcentral gyri, and subcortical structures. The cerebellum showed also consistent hypermetabolism in both C9ALS and C9FTD patients. Comparable findings were consistently reported by Cistaro et al. [9] in a group of C9ALS patients. The cerebellum is involved in C9ALS and ngsC9FTD both histologically and macroanatomically, and it may be associated with the genesis of symptoms in both disorders [32]. In addition, different histopathological studies reported the presence of Ub+,

p62+, and TDP-43, mainly in cerebellum, thalamus, and hippocampus [33–36]. The reason for an upregulation of metabolism needs to be investigated. There are however, hypotheses that advocate local increases in microglia and astrocyte activation [23, 37, 38]. In a study using 11C-(R)-PK11195 in ALS patients, Turner et al. (2003), found a widespread microglial activation in regions of neurodegeneration, such as the motor cortex, pons, and thalamus [39]. Recently, Grabert et al. (2016) also suggested the role of metabolic demand of microglia [40].

From a clinical standpoint, the C9bALS and C9bvFTD cases presented the key symptoms of the sporadic diseases [35], plus features typical of the C9orf72 mutation, such as psychosis and hallucinations [41, 42]. Consistent with the neuropsychiatric manifestations and the personality changes, C9bvFTD patients showed, at a single-subject level, an extensive hypometabolism affecting the limbic structures (anterior cingulate cortex, orbitofrontal cortex, bilateral insula, and ventral striatum). The same hypometabolism pattern was observed in C9bALS patient no. 1, who presented dysexecutive and behavioral features.

A peculiar clinical phenotype was found in C9bvFTD patient no. 4. Together with the typical features of bvFTD, the patient showed also parkinsonism, the most observed motor symptom in C9orf72 patients initially presenting with FTD [35]; in addition, the patient presented hypophonia, speech monotony, occasional dysphagia for liquids, and drooling. Even if this patient was clinically diagnosed with the behavioral variant of FTD, these latter symptoms were suggestive of a concomitant ALS. Consistently with these motor clinical features, the brain FDG-PET revealed hypometabolism in motor-related structures. This imaging finding suggests that this case may lie in the middle of the "ALS/FTD" clinical spectrum. In a previous single-case report, a patient presenting with amnestic mild cognitive impairment, which evolved into Alzheimer's disease (AD)-type dementia and later ALS, showed an asymmetric cerebral hypometabolism suggestive of an alternative diagnosis within the FTLD spectrum disorders rather than AD [43]. The patient, resulting positive for C9orf72 mutation was then diagnosed as C9ALS/FTD. This study supports the critical role of FDG-PET measure at individual level when clinical picture is not clear enough. Differential diagnosis between AD and C9ALS/FTD may be difficult, because of overlapping symptoms and sometime presence of mixed pathology. Accordingly, Saint-Aubert et al. reported a case diagnosed with the logopenic variant of PPA, and carrier of C9ORF72 expansion, despite cerebrospinal fluid, FDG-PET and 11C-PiB PET imaging were suggestive of AD pathology [44]. C9orf72 repeat expansion was reported in 6 out of 771 subjects with a clinical diagnosis of probable AD [45]. In a report on two siblings, both presenting C9orf72 mutation, the differential diagnosis between AD and C9ALS/FTD was further supported by the combined use of FDG-PET and 11C-PiB PET imaging [46].

Conclusions

Our results provide evidence for heterogeneous brain metabolic profiles in patients with C9orf72 mutation but consistent with the different clinical phenotypes, supporting the importance of neuroimaging biomarkers such as FDG-PET brain metabolism, as measured with optimized voxel-wise approaches, to capture at single-subject level specific brain dysfunctions. Additional research studies in larger cohorts will be necessary to evaluate the role of FDG-PET imaging as diagnostic biomarker.

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