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Neuroimaging Correlates of Frontotemporal Dementia Associated with SQSTM1 Mutations

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Abstract.
Background: Frontotemporal lobar degeneration (FTLD) is a progressive dementia characterized by focal atrophy of frontal and/or temporal lobes caused by mutations in the gene coding for *sequestosome 1* (*SQSTM1*), among other genes. Rare *SQSTM1* gene mutations have been associated with Paget's disease of bone, amyotrophic lateral sclerosis, and, more recently, frontotemporal lobar degeneration (FTLD).
Objective: The aim of the study was to determine whether a characteristic pattern of grey and white matter loss is associated with *SQSTM1* dysfunction.
Methods: We performed a voxel-based morphometry (VBM) study in FTD subjects carrying *SQSTM1* pathogenic variants (*FTD/SQSTM1* mutation carriers; $n = 10$), compared with *FTD* subjects not carrying *SQSTM1* mutations (*Sporadic FTD*; $n = 20$) and healthy controls with no *SQSTM1* mutations *(HC/SQSTM1* noncarriers*;* $n = 20$). The groups were matched according to current age, disease duration, and gender.
Results: After comparing *FTD/SQSTM1* carriers with *Sporadic FTD,* a predominantly right cortical atrophy pattern was localized in the inferior frontal, medial orbitofrontal, precentral gyri, and the anterior insula. White matter atrophy was found in both medial and inferior frontal gyri, pallidum, and putamen. *FTD/SQSTM1* carriers compared with *HC/SQSTM1* noncarriers showed atrophy at frontal, temporal, and parietal lobes of both hemispheres whereas the MRI pattern found in S*poradic FTD* compared with controls was frontal and left temporal lobe atrophy, extending toward parietal and occipital lobes of both hemispheres.
Conclusions: These results suggest that fronto-orbito-insular regions including corticospinal projections as described in ALS are probably more susceptible to the damaging effect of *SQSTM1* mutations delineating a specific gene-linked atrophy pattern.

Keywords: Dementia, frontotemporal dementia, SQSTM1 protein, voxel-based morphometry

INTRODUCTION

Frontotemporal lobar degeneration (FTLD) is a progressive dementia characterized by atrophy of frontal and/or temporal lobes resulting from different pathological processes, frequently linked to genetic mutations. FTLD is associated (15%) with motor neuron disease (MND) or amyotrophic lateral sclerosis (ALS). A frequent cause of FTLD+/-MND is a *G4C2 repeat expansion in the chromosome 9 open reading frame 72 gene* (*C9ORF72*; OMIM#: 614260). Less frequently, FTLD is caused by mutations in other genes such as the *microtubule-associated protein tau gene* (*MAPT*; OMIM#: 157140) [1], *progranulin* (*GRN*; OMIM#: 138945) [2], *valosin containing protein* (VCP; OMIM#: 601023), *fused in sarcoma* (*FUS*; OMIM#: 137070), *TAr DNA-binding protein* (*TARDBP*; OMIM#: 605078) gene, and, very recently, in the gene coding for *sequestosome 1* (*SQSTM1*; OMIM#: 601530), which causes Paget disease of bone [3, 4, 5, 6, 7].

The *SQSTM1* gene is located in chromosome 5 and encodes for p62, a ubiquitin binding protein involved in protein phosphorylation and degradation, autophagosome formation, mediation of the NFkappaB activation pathway, and osteoclast growth factor regulation [8]. There are seven mutational studies reporting rare *SQSTM1* variants associated with FTLD or ALS, suggesting that *SQSTM1* has a significant role in both diseases [9, 10]. Since there is no information on the regional brain effects of *SQSTM1* variants in the literature, we hypothesized that VBM-studies in *SQSTM1*-carriers patients with FTLD can be useful for two reasons: first, in order to

determine whether *SQSTM1*mutations are associated with specific clinical FTLD phenotypes and secondly to identify whether the expression of *SQSTM1* mutations in the brain have any significant regional differences when compared with non-carriers, thus reflecting an increased regional susceptibility.

MATERIALS AND METHODS

Subject selection and comparison groups

This is a multicenter retrospective and exploratory collaborative study in which all the members of the EU EOD consortium [10] and other authors of published studies analyzing the *SQSTM1* gene [10, 11] were asked to provide available brain MRI scans from the subjects screened in previous studies whether they were *FTD/SQSTM1 carriers* or not. A subject was considered to be a *FTD/SQSTM1 carrier* when she/he fulfilled research criteria for FTLD [12] and carried a disease-segregating SQSTM1 genetic variant or, if DNA samples from relatives to demonstrate familial segregation were unavailable, their *SQSTM1* variant, was only found in FTLD cases or the variants were more frequent among the cases [9, 11].

The T1-weighted images were obtained from five European research centers: the Center for Aging Brain and Neurodegenerative Disorders. Neurology Unit, University of Brescia, Brescia, Italy; University of Turin, Department of Neuroscience "Rita Levi Montalcini" Studi di Torino, Turin, Italy; Center de Recherche de I' Institut du Cerveau et de la Moelle Epinière, Hôpital de la Pitié-Salpêtrière - France; Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal and the Clínica Universidad de Navarra, Pamplona, Spain (Table 1). Seven MRI scans from the S*poradic FTD subjects* were discarded because of poor image quality or movement artifacts. On the other hand, two *FTD/SQSTM1* carrier patients were discarded, one because the images were not isotropic and another one because the patient carried two heterozygous FTLD-causing mutations: p.P392L *SQSTM1* and the C9orf72 expansion.

Once we obtained the MRI scans, the group of *FTD/SQSTM1 carrier* subjects was double-matched with a group of *Sporadic FTD/SQSTM1* subjects and a group of cognitively intact *SQSTM1 noncarriers*. The matching process was performed in order to have similar gender, age at onset, disease duration, and age at evaluation across groups (Table 2). Finally, except for a patient with semantic dementia, the FTD patients were matched across groups by their clinical FTD presentation. *Sporadic FTD* subjects did not carry mutations in the *GRN*, *MAPT*, or *C9ORF72* genes.

Demographic and clinical data and analysis

Normality and variance homogeneity for the variables age at evaluation, Mini-Mental State Examination (MMSE), disease duration, and age at onset were included in the study and tested using the Shapiro-Wilk and Levene tests, respectively. Analysis of variance was applied to test the differences between the means of age at evaluation and MMSE variables among the three study groups. When only the two groups (*FTD/SQSTM1* carriers and *Sporadic FTD*) were compared, a t-test was applied to evaluate age at onset and disease duration. FTD subtype and family history variables were compared using the exact χ^2-test. The analysis was carried out using the program SPSS 15.0 software for Windows (SPSS Inc., Chicago, Illinois, USA). Bonferroni's correction was applied for multiple comparisons in all tests performed (Table 2).

Table 1
Number of participants in each group for center

Centers	FTD/SQSTM1 carriers	Sporadic FTD	HC/SQSTM1 noncarriers
	Number of participants		
Centre for Aging Brain and Neurodegenerative Disorders. Neurology Unit, University of Brescia, Brescia, Italy	2	8	0
University of Turin, Department of Neuroscience "Rita Levi Montalcini" degli Studi di Torino, Turin, Italy	2	5	0
Centre de Recherche de I' Institut du Cerveau et de la Moelle Epinière, Hôpital de la Pitié-Salpêtrière - France	0	5	0
Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal	3	0	0
Clínica Universidad de Navarra, School of Medicine in the Memory Disorders Unit, Department of Neurology, Pamplona, Spain	1	14	20

Table 2
Demographic and clinical data of the groups analyzed

Group	MMSE	Gender (male)	Age at evaluation	Disease duration	Age at onset	FTD Subtype* (n)		Family history (n)	
			(Years) Mean ± SD (range)						
FTD/SQSTM1 carriers *(n = 10)*	20.5 ± 6.2 (9–28)	50%	70.1 ± 9.3 (52–81)	4 ± 2.4 (1–8)	66 ± 10.3 (44–79)	bvFTD SD PNFA	7 0 3	FTD FTD+PAGET None No data	8 1 1 0
Sporadic *FTD/SQSTM1* *(n = 20)*	22.6 ± 4.9 (10–29)	50%	70.6 ± 7.9 (54–82)	3.4 ± 2.8 (1–12)	67 ± 7.9 (51–80)	bvFTD SD PNFA	12 1 7	FTD FTD+PAGET None No data	4 0 10 6
HC/SQSTM1 noncarriers *(n = 20)*	29.0 ± 0.7 (28–30)	50%	70.0 ± 8.4 (54–80)	–	–	–		–	
		ANOVA		*t*-Test		χ^2			
Statistics	$F = 18.1$ $p < 0.0001$	–	$F = 0.028$ $p = 0.9$	$t = 0.52$ $p = 0.6$	$t = -0.3$ $p = 0.7$	*FTD/SQSTM1* carriers versus Sporadic *FTD* $\chi^2 = 0.65$ $p = 0.72$		*FTD/SQSTM1* carriers versus Sporadic *FTD* $\chi^2 = 1414$ $p = 0.003$	

Numerical data were presented as mean ± standard deviation (range). Categorical data are presented as percentage. *1st degree family history of FTD. All *p*-values were two sided. The level of significance considered was 0.05 after Bonferroni correction. FTD, frontotemporal dementia; PAGET, Paget's disease; FTD subtypes: SD, semantic dementia; bvFTD, behavioral; and PNFA, progressive nonfluent aphasia. * number of cases.

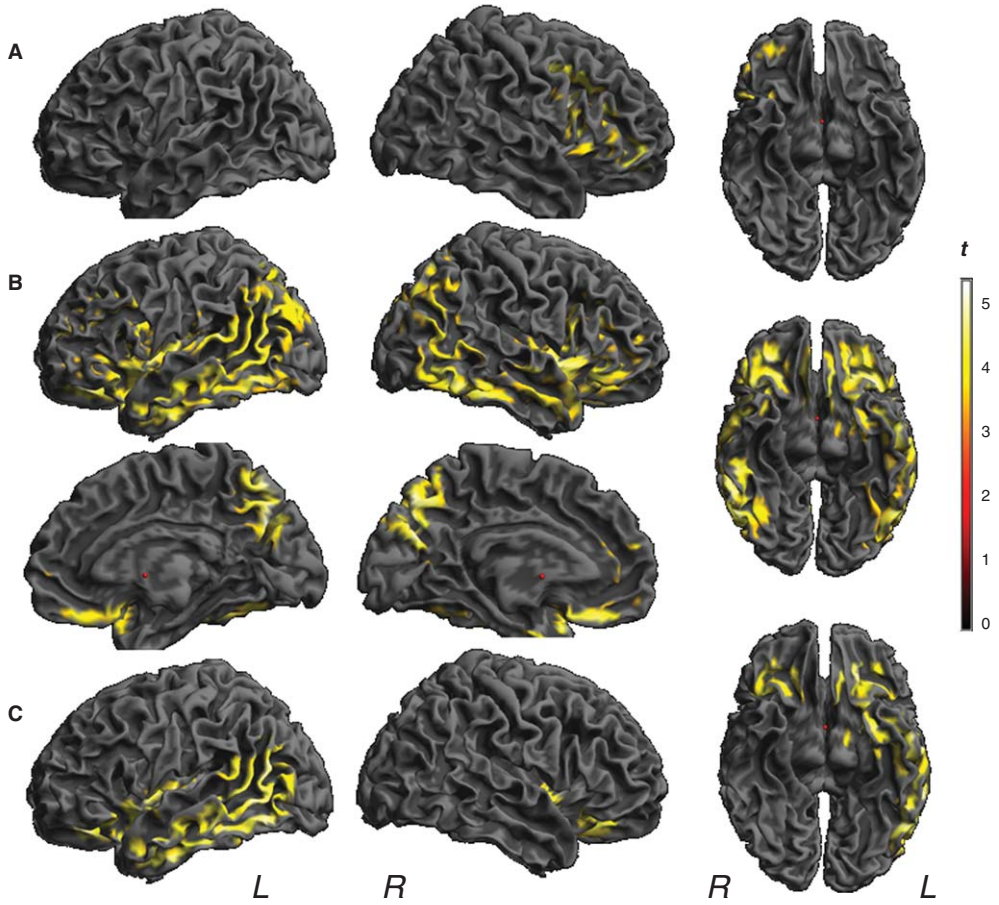

Fig. 1. Analysis displaying grey matter loss on the rendered surface of the brain in the following contrasts: A) Contrast *FTD/SQSTM1 carriers* versus *Sporadic FTD* (Threshold $p < 0.05$. FWE cluster-wise $p < 0.01$); B) *FTD/SQSTM1 carriers* versus *HC/SQSTM1 noncarriers*; and C) *Sporadic FTD* versus *HC/SQSTM1 noncarriers* (Threshold $p < 0.05$. FWE cluster-wise $p < 0.001$).

Neuroimaging assessment

Data acquisition

The T1-weighted images obtained from five centers used five different 1.5 T scanners. Three *FTD/SQSTM1* carriers underwent their MRI scan in 1.5 T GE Medical Systems scanner at two different centers, 5 *FTD/SQSTM1* carriers in 1.5-T Siemens Symphony scanner at two different centers, and 2 in 1.5-T Philips Medical Systems at only one center. The MRI scans from *Sporadic FTD* and 20 *HC/SQSTM1* subjects were acquired in 1.5-T Siemens Symphony scanners at two different centers. All T1-weighted images had a voxel size of $1 \times 1 \times 1$ mm.

MRI pre-processing

Determination of image quality followed the local internal protocol consisting of examination of the voxel size, the isotropic characteristic of the images and the movement artifacts. Secondly, after the preprocessing, we performed a covariance matrix that showed the covariance between volumes in each subject. These covariance values (Supplementary Figure 1) demonstrated the homogeneity of the sample.

We used the Statistical Parametric Mapping 8 software (SPM8; Wellcome Trust Centre for Neuroimaging; University College London, UK) implemented in Matlab 7.1 environment (MathWorks, Inc.) for image preprocessing. All images were transformed from the standard DICOM format (Digital Imaging and Communication in Medicine) to the NIfTI format (Neuroimaging Informatics Technology Initiative); the MNI localization of each image was manually rectified using the anterior and posterior commissures and anatomical references. First, we generated a custom template using the Diffeomorphic Anatomical Registration with the Exponentiated

Lie Algebra algorithm tool (DARTEL) [13]. Subsequently, the images were processed using the New Segment tool for defining tissue probability maps through non-linear deformation and registration, in order to obtain grey matter (GM), white matter (WM) and cerebrospinal fluid images [13]. These individual GM and WM images were spatially normalized with the DARTEL- custom template made with the images of the population tested in this study. In this image processing method, we multiplied each GM and WM image spatially normalized by its total intracranial volume before and after normalization (modulation step). Finally, they were smoothed with a full-width-half-max (FWHM) 8-mm Gaussian kernel to increase the variation signal in the anatomical structure.

Statistical group analysis

Segmented images from *FTD/SQSTM1* carriers, *Sporadic FTD and HC/SQSTM1* noncarriers were compared in a voxel-wise manner by performing two ANOVA analyses (one for GM and another for WM): these ANOVAs included a 'group' factor with three levels (*FTD/SQSTM1* carriers, *Sporadic FTD and HC/SQSTM1* noncarriers). Since the subjects were age- and gender-matched, the scanner type was included as covariate with the goal of eliminating its possible effect on the results. After determining a significant mean effect of the group factor, the following pair-wise SPM-t comparisons were carried out: *FTD/SQSTM1* carriers versus *Sporadic FTD* noncarriers, *FTD/SQSTM1* carriers versus *HC/SQSTM1* noncarriers, and *Sporadic FTD* versus *HC/SQSTM1* noncarriers.

The statistical significance of the contrasts of *FTD/SQSTM1* carriers versus *HC/SQSTM1* noncarriers and *Sporadic FTD* versus *HC/SQSTM1* noncarriers was set up with a primary threshold of voxel-wise $p = 0.001$, which yielded a cluster-extent based threshold of $k = 41976$ and 2018 in GM ($t = 3.2$) and k 1022 and 825 in WM ($t = 3.2$), respectively. For the contrast of *FTD/SQSTM1* carriers versus *Sporadic FTD* noncarriers, a primary threshold of voxel-wise $p < 0.01$ was set up due to low statistical power, which yielded a cluster-extent based thresholding of $k = 6124$ in GM ($t = 2.4$) and 5590 in WM ($t = 2.4$). Finally, the threshold for the resulting clusters in each contrast was limited $p < 0.05$ family-wise error (FWE) correction. The cluster-extent based thresholding was calculated with the Gaussian random field (GRF) in Statistical Parametric Mapping (SPM8) using the estimated intrinsic smoothness based on residual images. Tables were created using the SPM8 Anatomy toolbox version 1.5 [14], and anatomical areas reported were based on its V20 maps for GM. For WM evaluation we used a tractography-based atlas of human brain connections from the Natbrainlab website (http://www.natbrainlab.com/).

RESULTS

Subjects available for the VBM analysis

In this study, ten *FTD/SQSTM1* carriers (median age = 70.1; range = 52-81; 50% male) were compared with twenty *Sporadic FTD* (median age = 70.6; range = 54-82; 50% male) and twenty *HC/SQSTM1* noncarriers (median age = 70.0; range = 54-80; 50% male) (Table 2). All groups were matched by gender, age at evaluation, and disease duration among patients. The *FTD/SQSTM1* carrier group included two subjects with the *SQSTM1* p.L238del variant and the other subjects carried one of the following *SQSTM1* variants: p.P392L, p.P387L, pA33, E319K, P348L, P439L, T430P, and p.D329G (Table 1). With regard to the clinical subtypes of the *FTD/SQSTM1* carriers, seven patients were diagnosed with behavioral *FTD* subtype (bvFTD) and three with progressive non-fluent aphasia (PNFA). In addition, one patient also had ALS, one Paget's disease, two presented parkinsonian signs and one psychotic symptoms. Among the *Sporadic FTD*, twelve patients had the bvFTD subtype, seven PNFA, and one semantic dementia (SD).

Demographic and clinical analyses

There were no significant differences in age at evaluation across the groups ($F = 0.02$, $p = 0.9$). There were no differences between *FTD/SQSTM1* carriers and *Sporadic FTD* in the other demographic variables such as the age at onset ($t = –0.3$, $p = 0.7$), disease duration ($t = 0.5$, $p = 0.6$), and FTD clinical subtype ($\chi^2 = 0.65$, $p = 0.72$). We found significant differences in MMSE (group factor $F = 18.1$, $p < 0.0001$) in *FTD/SQSTM1* carriers *versus HC/SQSTM1* noncarriers ($p < 0.0001$) and *Sporadic FTD versus HC/SQSTM1* noncarriers comparisons ($p < 0.0001$). There were no MMSE score differences between *FTD/SQSTM1* carriers and *Sporadic FTD* ($p = 0.5$). Finally, differences were also found in the frequency of family history of FTD and/or ALS, which was higher in the *FTD/SQSTM1* carriers ($\chi^2 = 1414$, $p = 0.003$) (Table 2).

Table 3
Volumetric grey matter loss contrast between *FTD/SQSTM1* carriers versus *Sporadic FTD*

Side	Area	BA	k	*p-value*	*t*	x	y	Z
R	Inferior Frontal Gyrus p. Triangularis	45	6124	0.01	3.78	42	11	24
R	Inferior Frontal Gyrus p. Opercularis	44			3.74	41	11	22
		44			3.17	53	11	12
R	Inferior Frontal Gyrus p. Orbitalis	44			3.27	45	41	–3
R	Precentral Gyrus	6			3.20	45	–2	45
					3.00	33	–1	48
R	Middle Frontal Gyrus	8			3.46	38	14	42
					3.37	35	23	37
R	Middle Orbital Gyrus	10, 11, 46			3.32	35	53	–3
R	Insula Lobe	44			3.37	41	12	3

Statistically significant white matter loss; Side: laterality; BA: Brodmann Areas; k: number of voxels per cluster. $p < 0.05$ FWE cluster-wise corrected $p = 0.01$; *t*: T-test score; x.y.z : MNI coordinates.

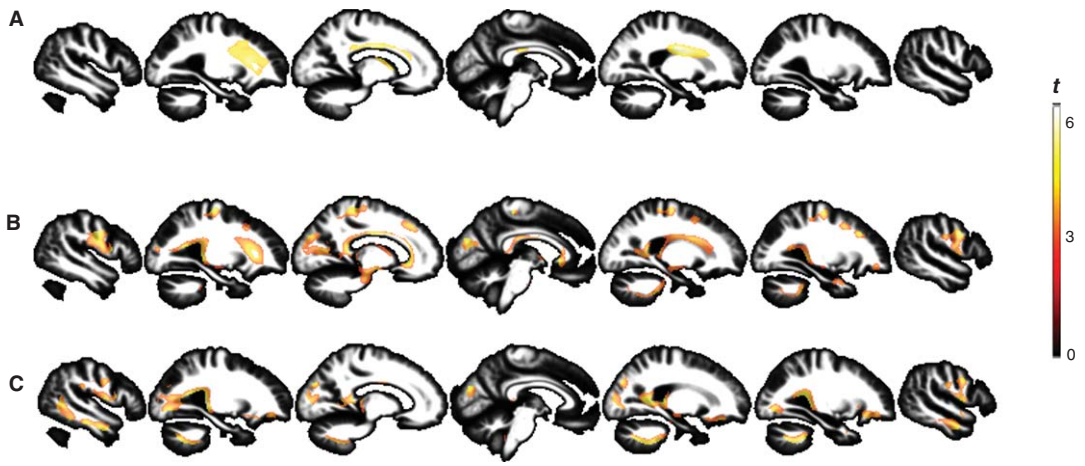

Fig. 2. Analysis displaying white matter loss on the rendered surface of the brain in the following contrasts: A) Contrast *FTD/SQSTM1 carriers* versus *Sporadic FTD* (Threshold $p < 0.05$. FWE cluster-wise $p < 0.01$); B) *FTD/SQSTM1 carriers* versus *HC/SQSTM1 noncarriers*; and C) *Sporadic FTD* versus *HC/SQSTM1 noncarriers* (Threshold $p < 0.05$. FWE cluster-wise $p < 0.001$).

Grey matter VBM analysis

ANOVA analyses of GM data showed a significant main effect of the Group factor ($F = 8.057$, $p < 0.0001$).

Comparison between FTD/SQSTM1 carriers versus Sporadic FTD groups

On a direct comparison, the *FTD/SQSTM1* carriers had smaller GM volumes than the *Sporadic FTD/SQSTM1* predominantly in the right hemisphere, including inferior and middle frontal gyrus, middle orbital gyrus, insula, and precentral gyrus (Fig. 1A, Table 3, and Supplementary Table 1). These results reflected a grey matter loss pattern among the *FTD/SQSTM1* carriers with impairment of right prefrontal-orbital-insular regions with relative preservation of the temporal lobes. In short, *FTD/SQSTM1* carriers' atrophy pattern was specific to the prefrontal cortex, involving premotor and primary motor regions (Fig. 1 and Table 3). The prefrontal right atrophic clusters were extended bilaterally when this same contrast was thresholded to $t = 2.4$, $p < 0.01$ uncorrected (Supplementary Figure 2).

Comparison between FTD/SQSTM1 carriers versus HC/SQSTM1 noncarriers

The 10 *FTD/SQSTM1* carriers were compared with 20 *HC/SQSTM1* noncarriers and showed severe GM volume loss ($p < 0.05$ FWE cluster-wise corrected) in the following regions: insula, inferior temporal gyrus, precuneus, and inferior frontal gyrus of both hemispheres, plus the right temporal lobe and cuneus and middle orbital gyrus of the left hemisphere (Fig. 1B, Supplementary Table 1).

Comparison between Sporadic FTD versus HC/SQSTM1 noncarriers

We observed widespread GM loss in the 20 *Sporadic FTD* compared with the 20 *HC/SQSTM1* noncarriers ($p < 0.05$ FWE cluster-wise corrected) with a predominant distribution in temporal and frontal lobes. This pattern was consistent with the results of previous VBM studies performed in FTLD-control series where the underlying genetic causes were not known [15]. The temporal atrophic clusters were located in the left middle temporal gyrus, temporal pole and superior temporal gyrus and in frontal regions. The brain atrophy was specifically intense at the insula and inferior and middle orbital gyrus (Fig. 1C and Supplementary Table 2).

White matter VBM analysis

ANOVA analyses of WM data showed a significant main effect of the Group factor ($F = 8.057$, $p < 0.0001$).

Comparison between FTD/SQSTM1 carriers versus Sporadic FTD/ SQSTM1

The contrast between WM brain volume *FTD/ SQSTM1* carriers versus *Sporadic FTD/ SQSTM1* resulted in significant differences in regions underlying both medial and left inferior frontal gyrus, triangular area, and in neighboring regions to left pallidum globe and putamen (Fig. 2A and Supplementary Table 3).

Comparison between FTD/SQSTM1 carriers versus HC/SQSTM1 noncarriers

This contrast showed WM volume loss ($p < 0.05$ FWE cluster-wise corrected) in underlying regions to the inferior frontal gyrus, primary motor cortex, precuneus, superior occipital gyrus and particularly along the borders of the insula, amygdala, and hippocampus (Fig. 2B and Supplementary Table 4).

Comparison between Sporadic FTD versus HC/SQSTM1 noncarriers

Finally, *Sporadic FTD* versus *HC/SQSTM1 noncarriers* contrast ($p < 0.05$ FWE cluster-wise corrected) showed an atrophy pattern in cortico-ponto-cerebellar regions such as the superior and inferior cerebellar peduncles. We also found WM volume loss in occipital and frontal regions, the middle temporal lobe and hippocampus (Fig. 2C and Supplementary Table 5).

DISCUSSION

The present study is the result of a multicenter collaborative effort provided by the EU EOD consortium [10] where members were invited along with researchers involved in the other studies that have screened the *SQSTM1* gene in FTLD subjects [10, 16] to collect available brain MRI scans from subjects with and without *SQSTM1* mutations. The aim of our study was to analyze whether *SQSTM1* pathogenic variants lead to a specific regional neurodegenerative pattern. For this purpose we used VBM MRI, which we have used successfully in the past to identify regional brain atrophy associated with other genetic variants, even in relatively small samples [17, 18]. In these studies, we reported that morphometric neuroimaging studies could identify brain regions sensitive to the damaging effects of specific genetic variants [15, 17, 18, 19].

In the present study, we found brain volume differences between *FTD/SQSTM1* carriers versus *Sporadic FTD*, where the GM loss in *FTD/SQSTM1* carrier group followed an asymmetric right pattern, with involvement of fronto-orbital regions, right inferior frontal gyrus (pars triangularis, pars opercularis and pars orbitalis), insula lobe, premotor and motor areas (Fig. 1 and Table 3). The right inferior frontal gyrus has been associated with cognitive emotion regulation and executive functions, especially for salience of external clues, regardless of whether that detection is followed by the inhibition of a motor response, the generation of a motor response, or no external response at all [20]. The right anterior insula belongs to the ventral attention system with connectivity with frontal lobe regions implicated in goal-directed behavior [21, 22]. On the other hand, the premotor cortex is involved in visuomotor transformation tasks, spatial orientation, learning, and cognitive functions [23, 24]. The pattern of atrophy found in *FTD/SQSTM1* carriers involved the impairment not only of the precentral cortex and corticospinal tracks but also premotor and prefrontal cortex, which are also impaired in human motorneuron disease [25].

Mutations in *SQSTM1* gene were first described to cause Paget's disease [7] and years later familial ALS [9]. In fact, the substantial loss of brain volume in pre-motor cortical areas and in the cortico-spinal

tract observed in our *FTD/SQSTM1* carriers group was not found in the *Sporadic FTD* when compared to the control group. We suggest that this pattern can be due to a subclinical impairment of the premotor cortical areas and the cortico-spinal tract in the *FTD/SQSTM1* carriers group, since *SQSTM1* is also a disease-causing ALS gene. In a VBM study of ALS with behavioral symptoms [26], the ALS patients with apathy, depression, and anxiety showed an atrophy profile very similar to that found in the present study after comparing the *FTD/SQSTM1* carriers with *Sporadic FTD,* comprising premotor and motor areas and corticospinal tracts. These results reinforce the idea that there could be an important association between mutations in the *SQSTM1* gene and the FTD-MND spectrum [9–11]. We want to highlight the point that our study collected brain MRI scans from FTD subjects with different mutations in diverse *SQSTM1* gene domains that can result in variable structural and functional abnormalities of the protein leading to different disease phenotypes [10] and, subsequently, to specific patterns of brain atrophy. However, since mutations in *SQSTM1* gene are so rare, it was not possible to assess the individual morphological brain effect for each of the *SQSTM1* variants.

To the best of our knowledge, there are no VBM studies in patients with FTD and *SQSTM1* mutations, though one preliminary study measuring gray matter density in twelve patients with Paget's disease of bone and *SQSTM1* mutations without FTD described a predominantly left frontotemporal atrophy pattern among patients [27].

Among the previous literature on VMB MRI analysis in other FTLD mutated genes [28, 29], Rohrer et al. [29] performed a VMB MRI analysis of *MAPT* and *GRN* mutations in FTLD carriers compared with healthy controls and found among *GRN* mutation carriers a brain atrophy pattern with areas in common with that which we identified in our study, which included the orbitofrontal cortex and ventral insula, with marked extra atrophy of the anterior and medial temporal lobes and cingular cortex with a strong asymmetric pattern. However, we have to consider that they used a different methodology that enhances the GM loss. By contrary, the VBM pattern found among *MAPT* carriers was predominantly temporal and symmetrical. On the other hand, our VBM atrophy pattern (*FTD/SQSTM1* carriers versus *Sporadic FTD*) was quite different from the one found in another VBM study of FTLD *GRN* carriers compared to healthy controls, which showed greater loss in posterior temporal and parietal lobes in this group [27].

These authors performed an MRI analysis of three groups of subjects with *GRN and MAPT* mutations as well as *C9ORF72* expansion carriers [30] describing prominent atrophy of the anteromedial temporal lobes in the *MAPT* group, in the inferior temporal and parietal lobes in the *GRN* group, and in posterior cerebral regions including the cerebellum in the *C9ORF72* group. Interestingly, the brain atrophy pattern described for the *Sporadic FTD* group (35% of them diagnosed with FTD-ALS) was more consistent with the atrophy pattern that we found in the *FTD/SQSTM1* carriers versus *Sporadic FTD* comparison.

In another VBM MRI study comparing *C9ORF72* expansion carriers and noncarriers, a thalamic atrophy and cerebellar volume loss in *C9ORF72* expansion carriers was found [31]. In fact, the brain atrophy profile was completely different from the one found in our *FTLD SQSTM1* carriers' group which encompassed the inferior frontal-orbital regions, right IFG: pars triangularis, pars opercularis and pars orbitalis, insula lobe, premotor, and motor areas. These results can be influenced by a higher frequency of the PNFA subtype in our patients' group.

All the data derived from the VBM MRI studies in subjects with FTD-causing mutations highlight the fact that brain atrophy patterns can diverge according to the mutated gene, probably as a result of differences in mRNA expression and/or differential regional brain susceptibilities.

Although, we have previously identified patterns of regional brain atrophy associated with other genetic variants with similar study designs to the present one [15, 17, 18], our study still has several limitations: the number of subjects is low, but this was expected considering the low frequency of *SQSTM1* mutations among FTD patients. In addition, the distribution of the three populations studied (*FTD/SQSTM1* carriers, *Sporadic FTD* and *HC/SQSTM1* noncarriers) was not homogeneous among centers. This methodological imbalance, in spite of the common quality control, can introduce a bias, which was perhaps not totally corrected when introduced as a covariate in the analyses.

CONCLUSIONS

In summary, our results reflect that *FTD/SQSTM1* carriers show a right atrophy pattern in prefrontal-

orbital-insular regions with relative preservation of the temporal lobes different from that observed in *Sporadic FTD*. The WM atrophy was present in regions underlying the right medial frontal gyrus and left medial and inferior frontal gyrus and close to the left pallidum and putamen. However, our study is retrospective and exploratory and has a number of limitations (small sample size, multicenter study with different MRI scanners, and absence of DTI data). To better define the specificity of these patterns, larger cross-sectional and longitudinal imaging studies will be needed.

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SUPPLEMENTARY MATERIAL

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