Structural MRI Signatures in Genetic Presentations of the Frontotemporal Dementia-Motor Neuron Disease Spectrum

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ABSTRACT

Objective. To assess cortical, subcortical and cerebellar grey matter (GM) atrophy using magnetic resonance imaging (MRI) in patients with disorders of the frontotemporal lobar degeneration (FTLD) spectrum with known genetic mutations.

Methods. Sixty-six patients carrying FTLD-related mutations were enrolled, including 44 with pure motor neuron disease (MND) and 22 with frontotemporal dementia (FTD). Sixty-one patients with sporadic FTLD (sFTLD) matched for age, sex and disease severity with genetic FTLD (gFTLD) were also included, as well as 52 healthy controls. A whole-brain voxel-based morphometry (VBM) analysis was performed. GM volumes of subcortical and cerebellar structures were obtained.

Results. Compared with controls, GM atrophy on VBM was greater and more diffuse in genetic FTD, followed by sporadic FTD and genetic MND cases, whereas sporadic MND (sMND) patients showed focal motor cortical atrophy. Patients carrying C9orf72 and GRN mutations showed the most widespread cortical volume loss, in contrast with GM sparing in SOD1 and TARDBP. Globally, gFTLD patients showed greater atrophy of parietal cortices and thalami compared with sFTLD. In volumetric analysis, gFTLD patients showed volume loss compared with sFTLD in the caudate nuclei and thalami, in particular comparing C9-MND with sMND cases. In the cerebellum, gFTLD patients showed greater atrophy of the right lobule VIIb than sFTLD. Thalamic volumes of gFTLD patients with a C9orf72 mutation showed an inverse correlation with Frontal Behavioral Inventory scores.

Conclusions. Measures of deep GM and cerebellar structural involvement may be useful markers of gFTLD, particularly C9orf72-related disorders, regardless the clinical presentation within the FTLD spectrum.
INTRODUCTION

The successful identification of new therapies for frontotemporal lobar degeneration (FTLD) critically depends on the ability to identify useful markers mirroring specific biological processes within this heterogeneous spectrum of clinical syndromes. Cases due to genetic mutations represent up to 30% of FTLD presentations, and provide an ideal model for studying these processes, as the underlying pathology can be inferred (even in the absence of post-mortem confirmation), facilitating the design of therapeutic trials targeting specific molecular mechanisms. Neuroimaging has demonstrated to provide in vivo, non-invasive measures of neurodegeneration in FTLD phenotypes. Distinctive patterns of atrophy on structural MRI contribute to establishing the correct diagnosis of behavioral variant of frontotemporal dementia (bvFTD) and primary progressive aphasia (PPA), help to distinguish FTLD patients from those with different pathologies and even provide hints about the underlying genetic and pathological substrate of each patient. Only recently, the role of pathological burden within grey matter (GM) structures other than the brain cortex has been investigated, suggesting a distinctive degeneration of deep GM and cerebellar structures in genetic FTLD (gFTLD) presentations, including motor neuron disease (MND). However, most previous studies were mainly focused on pure cognitive phenotypes, and an extensive characterization of subcortical and cerebellar damage across genetic forms of the FTD/MND spectrum (including C9orf72-related disorders) is still in progress.

The aim of this study was to explore the neuroanatomical structural correlates of genetic heterogeneity in a cohort of patients affected by the wide spectrum of FTLD disorders, including MND. More specifically, we assessed with a systematic approach the patterns of atrophy of cortical, subcortical and cerebellar structures using up-to-date MRI volumetric techniques, to identify neuroimaging measures associated with specific genetic alterations.

METHODS

Participants
A total of 658 patients with a suspected diagnosis of FTLD-related disorders were prospectively enrolled in four referral clinics in Lombardy, Italy and referred to San Raffaele Hospital in Milan between October 2007 and July 2019 to perform MRI on a 3T scanner, as part of their diagnostic work-up. Of these, 362 patients gave consent to be screened for known pathogenic mutations and evaluated for inclusion in the present multicenter, case-control study. Patients who underwent genetic screening had received a clinical diagnosis of FTD (n=110) according to either bvFTD\(^3\) or PPA\(^4\) clinical criteria, or MND variants (n=252), including ALS,\(^12\) progressive muscular atrophy (PMA)\(^13\) and primary lateral sclerosis (PLS).\(^14\) Sixty-six mutation carriers were identified, of which 22 presented with a clinical variant of FTD (i.e., bvFTD, n=12; bvFTD-ALS, n=5; nfvPPA, n=3; svPPA, n=1; and right-predominant variant of svPPA, n=1) and 44 with a pure MND phenotype (i.e., ALS, n=35; PMA, n= 6; and PLS, n=3). Patients fulfilling both bvFTD\(^3\) and ALS criteria\(^12\) were considered as FTD for the subsequent MRI analyses, considering that greater atrophy was expected compared with pure MND. Figure 1A outlines the screening process of gFTLD, whereas Table 1 and eTables 1 and 4 (available from Dryad https://doi.org/10.5061/dryad.1vhhmgqsz) summarize the demographic and clinical features of included subjects. Among patients who proved negative for known pathogenic mutations (i.e., sporadic FTLD [sFTLD]), we selected 61 subjects to be matched with gFTLD cases for age, sex, and MR scanner type, with comparable clinical diagnoses and disease severity assessed by FTLD-Clinical Dementia Rating (CDR-FTLD) scale\(^15\) for FTD and ALS Functional Rating Scale Revised (ALSFRS-r)\(^16\) for MND patients. Therefore, 16 sporadic FTD (sFTD, including bvFTD, n=12; nfvPPA, n=2; and svPPA, n=2) and 45 sporadic pure MND (sMND) patients (i.e., ALS, n=37; PMA, n=5; PLS, n=3) were included (Table 1). All patients underwent neurological examination, multi-domain cognitive testing and brain MRI at study entry. Fifteen sFTD patients (i.e., all but one with svPPA) and 10 gFTD patients also underwent lumbar puncture to exclude cerebrospinal fluid biomarker profile suggestive of Alzheimer’s disease pathology, as part of their diagnostic work-up (Table 1). No patient showed a p-tau/Aβ\(_{42}\) ratio >0.13, considered as pathological.\(^17\)
Fifty-two healthy controls comparable for age, sex, and MR scanner type with patient groups were recruited by word of mouth among subjects unrelated to the patient population. Controls were included if the following criteria were satisfied: normal neurological assessment; Mini-Mental Status Examination (MMSE) score $\geq 28$; no family history of neurodegenerative diseases.

Exclusion criteria for all subjects were: medical illnesses or substance abuse that could interfere with cognitive functioning; any (other) major systemic, psychiatric, or neurological illnesses; and other causes of focal or diffuse brain damage, including lacunae and extensive cerebrovascular disorders at routine MRI.

**Standard Protocol Approvals, Registrations, and Patient Consents**

Local ethical standards committee on human experimentation approved the study protocol and all participants provided written informed consent.

**Genetic analysis**

Blood samples were collected from all patients and genomic DNA was obtained and processed in each of the recruiting centers. The presence of GGGGCC hexanucleotide expansion in the first intron of the \textit{C9orf72} gene was assessed using fluorescent amplicon-length analysis and a repeat-primed polymerase chain reaction (PCR) assay. A cut-off of $\geq 30$ repeats combined with a typical saw-tooth pattern was considered pathological. In addition, \textit{GRN}, \textit{MAPT}, \textit{TARDBP}, \textit{SOD1}, \textit{FUS}, \textit{TBK1}, \textit{TREM2}, \textit{OPTN} and \textit{VCP} genes were analyzed by Next Generation Sequencing (NGS) and their mutations were confirmed by standard Sanger sequencing. All MND patients were systematically tested for \textit{C9orf72}, \textit{TARDBP}, and \textit{SOD1} mutations, and additional testing of \textit{FUS} and \textit{TBK1} was performed in the presence of positive family history of MND/dementia. Similarly, all FTD patients were tested for \textit{C9orf72}, \textit{TARDBP}, \textit{MAPT}, and \textit{GRN} mutations, with additional testing of \textit{FUS}, \textit{TBK1}, \textit{TREM2}, \textit{OPTN} and \textit{VCP} in the presence of positive family history.
**Clinical and neuropsychological evaluations**

Clinical evaluation was performed by experienced neurologists blinded to MRI results. For patients presenting with FTD variants, disease severity was assessed using the CDR-FTLD. For MND patients, site of disease onset, disease severity using the ALSFRS-r and manual muscle testing of strength based on the Medical Research Council (MRC) scale were recorded. The rate of disease progression was defined according to the formula: \([48–ALSFRS-r \text{ score}] / \text{time from symptom onset}\).

Neuropsychological assessment was performed by experienced neuropsychologists unaware of MRI results. A comprehensive multi-domain cognitive and behavioral battery was administered, as previously described. Based on available cognitive measures, a diagnosis according to the revised Strong criteria for cognitive/behavioral impairment in MND could be made for 30 sMND and 29 gMND patients.

**MRI acquisition**

All patients and healthy controls underwent brain MRI on a 3T scanner (Philips Medical Systems, Best, the Netherlands) at San Raffaele Hospital between 2007 and 2019. The original scanner (for brevity, Scanner 1) was substituted with an upgraded model from the same manufacturer in 2016 (Scanner 2).

Using Scanner 1, a 3D T1-weighted fast field echo (FFE) sequence was acquired (TR=25 ms, TE=4.6 ms, flip angle=30°, 220 axial slices with voxel size=0.89x0.89x0.8 mm, matrix size=256x256, FOV=230x182 mm²). Using Scanner 2, a 3D T1-weighted turbo field echo (TFE) with comparable resolution was acquired (TR=7 ms, TE=3.2 ms, TI=1000 ms, 204 sagittal slices with voxel size=1x1x1 mm, matrix=256x256, FOV=256x256 mm²).

**MRI analysis**

*Voxel-based morphometry*
Voxel-based morphometry (VBM) was performed using SPM12 (http://www.fil.ion.ucl.ac.uk/spm/) and Diffeomorphic Anatomical Registration Exponentiated Lie Algebra (DARTEL) registration method,\textsuperscript{20} to investigate GM volume alterations at a whole-brain level. Details of the VBM pipeline have been described previously.\textsuperscript{21}

**Volumetric analysis**

Volumes of the deep GM structures (i.e., bilateral caudate, globus pallidus, putamen, and thalamus), hippocampus and amygdala were obtained using the FMRIB's Integrated Registration and Segmentation Tool (FIRST) in FSL (http://www.fmrib.ox.ac.uk/fsl/first/index.html). Local GM volumes of the cerebellar lobules and vermis were calculated automatically using an atlas propagation and label fusion strategy based on the SUIT atlas.\textsuperscript{22,23} GM volumes were multiplied by the normalization factor derived from SIENAx (http://www.fmrib.ox.ac.uk/fsl/sienax/index.html) to correct for subject head size.

**Statistical analysis**

Normal distribution assumption was checked by means of Q-Q plot and Shapiro-Wilks and Kolmogorov-Smirnov tests. Clinical, neuropsychological and MRI volumetric measures were compared between groups using age-, sex- and MR scanner-adjusted ANOVA models, followed by post-hoc pairwise comparisons, Bonferroni-corrected for multiple comparisons. The threshold of significance was set at \( p<0.05 \). The SPSS Statistics 22.0 software was used.

VBM group comparisons were tested using ANOVA models in SPM12, adjusting for total intracranial volume, age, sex and MR scanner type. Results were assessed at \( p<0.05 \), Family-wise error (FWE)-corrected for multiple comparisons.

Correlations between clinical, cognitive and MRI volumetric features of FTLD patients (i.e., gFTLD and sFTLD, separately) were tested by means of partial correlation analyses adjusted for age, sex and education. Subsequently, the same analysis was performed subdividing the gFTLD
group according to the \textit{C9orf72} status. The threshold of statistical significance was set at p<0.05, Bonferroni-corrected for multiple comparisons. The SPSS Statistics 22.0 software was used.

**Data Availability Statement**

The dataset used and analyzed during the current study is available from the corresponding author on reasonable request.

**RESULTS**

**Genetic findings**

The 66 FTLD mutation carriers (Figure 1A) showed pathogenic alterations of the following genes: \textit{C9orf72} (n=33); \textit{TARDBP} (n=10); \textit{GRN} (n=8); \textit{SOD1} (n=7); \textit{FUS} (n=2); \textit{TBK1} (n=2); \textit{TREM2} (n=1); one patient showed both a \textit{C9orf72} expansion and a mutation of the \textit{GRN} gene; and one had both a \textit{C9orf72} and a \textit{TARDBP} mutation.

**Clinical diagnosis according to genotype**

eTables 1 and 4 (available from Dryad [https://doi.org/10.5061/dryad.1vhhmgqsz]) report the clinical diagnoses of gFTLD patients, according to the identified mutation(s). We found that each mutation was specifically associated with either an FTD (as for \textit{GRN}, \textit{MAPT}, and \textit{TREM2}) or an MND presentation (as for \textit{TARDBP}, \textit{SOD1}, \textit{TBK1}, and \textit{FUS}), with the notable exception of patients with a \textit{C9orf72} mutation, 22 of which presented with MND (C9-MND) and 11 with FTD (C9-FTD). Patients carrying a double mutation (i.e., the C9orf72+GRN and C9orf72+TARDBP cases) presented, respectively, with FTD and MND.

**Clinical and sociodemographic features**
Sociodemographic and clinical characteristics of groups defined by clinical diagnosis and genetic status are reported in Table 1. Groups were comparable in terms of sex, education, age at MRI and disease duration at MRI, and specific measures of disease severity for FTD or MND.

When assessing the most sizeable gFTLD subgroups, according to underlying mutation (i.e. C9-MND, C9-FTD, SOD1, TARDBP and GRN), a faster disease progression rate was found in TARDBP patients as compared to sMND, C9-MND and SOD1 (eTable 1 available from Dryad https://doi.org/10.5061/dryad.1vhhmgqsz).

Neuropsychological features

eTable 2 available from Dryad (https://doi.org/10.5061/dryad.1vhhmgqsz) reports the neuropsychological test scores of groups defined by clinical diagnosis and genetic status. Both sFTD and gFTD patients showed significant impairment of memory, executive, and linguistic functions. Notably, only gFTD patients had significant visuospatial impairment, compared with healthy controls. Overall, neuropsychological features of MND groups were comparable with healthy controls, although 9 sMND and 10 gMND met criteria for mild cognitive and/or behavioral impairment. When comparing gMND and gFTD with the relative sporadic groups, no significant differences were detected.

eTables 3 and 4 available from Dryad (https://doi.org/10.5061/dryad.1vhhmgqsz) report detailed neuropsychological features according to the underlying mutation. Of note, GRN mutation carriers showed the greatest visuospatial impairment, although this was not statistically significant compared with other groups.

GM atrophy

For both VBM and volumetric analyses, characteristic patterns of GM atrophy in gFTLD were investigated using three different levels of comparison, following a systematic scheme that would allow an unbiased reading and interpretation of results (Figure 1B). First, three broad groups were
compared: gFTLD, sFTLD and healthy controls (Level 1). As a second step, FTLD patients were subdivided according to genetic status and phenotypic manifestation, so that sMND, sFTD, gMND and gFTD patients were considered separately (Level 2). Finally, for sufficiently sized genotypic groups, a further subdivision according to specific mutations was considered (Level 3). Considering the clinical rationale of the study, aiming at describing variability of atrophy according to the genetic background, and the expected greater atrophy in FTD compared with MND, comparisons between sporadic/genetic patients and healthy controls were performed separately for MND and FTD presentations for Levels 2 and 3.

**Voxel-based morphometry (VBM)**

**Level 1 (gFTLD vs sFTLD vs controls)**

Compared with healthy controls, both sFTLD and gFTLD patients showed significant GM volume loss of the prefrontal, anterior cingulate, insular and anterior temporal cortical regions, hippocampi, caudate nuclei and cerebellar crus II, bilaterally (Figure 2A-B, eTable 5 available on Dryad [https://doi.org/10.5061/dryad.1vhhmgqqs](https://doi.org/10.5061/dryad.1vhhmgqqs)); in addition to this, gFTLD showed a more widespread pattern of atrophy, also including the angular gyri, posterior temporal and posterior cingulate cortices, and the thalami (Figure 2B). When compared directly with sFTLD, gFTLD patients showed greater GM atrophy of the left angular gyrus (Figure 2C). No regions showing significant greater atrophy in sFTLD compared with gFTLD patients were found.

**Level 2 (gMND vs sMND vs controls; gFTD vs sFTD vs controls)**

Compared with healthy controls, sMND patients showed selective atrophy of the left precentral cortex (Figure 3A, eTable 6 available on Dryad [https://doi.org/10.5061/dryad.1vhhmgqqs](https://doi.org/10.5061/dryad.1vhhmgqqs)); gMND patients showed atrophy of the left hippocampus, angular gyrus, occipital cortex, and Rolandic operculum, left crus II, cerebellar vermis VIIIa, and lobule VIIb, bilaterally (Figure 3B). Compared
with sMND, gMND patients showed greater atrophy of the left superior frontal and angular gyri (Figure 3E).

Compared with controls, sFTD patients showed widespread atrophy of the prefrontal, insular, anterior temporal and anterior cingulate cortical regions, caudate nuclei, and cerebellar crus I, bilaterally (Figure 3C); gFTD patients showed atrophy of similar regions, with the additional involvement of the thalami, posterior cingulate, fusiform and angular gyri bilaterally, left middle temporal gyrus, and right primary sensory cortex (Figure 3D). Compared with sFTD, gFTD patients showed greater GM atrophy of the thalami and superior parietal lobules, bilaterally, left angular gyrus and right posterior cingulate cortex (Figure 3F).

No regions showing significant greater atrophy in sporadic MND/FTD compared with genetic MND/FTD patients were found.

**Level 3 (C9-MND vs SOD1 vs TARDBP vs sMND vs controls; C9-FTD vs GRN vs sFTD vs controls)**

Compared with healthy controls, C9-MND patients showed atrophy of the left precentral and postcentral gyri, left supplementary motor area, right angular gyrus, and right occipital cortex, as well as the left thalamus and cerebellar vermis lobule V and lobule VIIb, bilaterally (Figure 4A, eTable 7 available on Dryad [https://doi.org/10.5061/dryad.1vhhmgqqs2]; MND patients with other mutations (i.e., SOD1 and TARDBP mutation carriers) did not show significant GM atrophy. Compared with sMND patients, C9-MND patients showed greater atrophy of the inferior frontal gyri, bilaterally, left postcentral gyrus, and right posterior thalamus (Figure 4D). No other statistically significant differences between MND groups were found.

C9-FTD patients showed widespread bilateral atrophy of the prefrontal, insular and cingulate cortices, angular gyri, caudate nuclei, thalami and cerebellar left crus II and vermis lobule IX (Figure 4B); GRN patients showed atrophy of the prefrontal, insular, and anterior cingulate cortices, hippocampi, bilaterally, left angular and fusiform gyri, caudate nuclei, thalami, and
cerebellar crus II, bilaterally (Figure 4C). Although no statistically significant differences between FTD groups were found, it is worth noting that thalamic involvement in C9-FTD was more widespread, involving both anterior and posterior regions, if visually compared with the selective damage of anterior thalamic regions observed in GRN cases.

**Volumetric GM analysis**

*Level 1 (gFTLD vs sFTLD vs controls)*

Compared with healthy controls, sFTLD patients showed decreased volume of the right thalamus, left putamen, left amygdala, and left hippocampus; gFTLD patients showed volumetric reduction of the caudate nuclei and thalami, bilaterally, left putamen, left hippocampus, right cerebellar crus II and right cerebellar lobule VIIb (eFigure 1 and eTable 8 available from Dryad https://doi.org/10.5061/dryad.1vhhmgqsz). Compared with sFTLD, gFTLD patients showed volumetric reduction of the thalami, bilaterally, right caudate, and right cerebellar lobule VIIb.

*Level 2 (gMND vs sMND vs controls; gFTD vs sFTD vs controls)*

Compared with healthy controls, sMND did not show any significant volumetric reduction of the considered GM structures, whereas gMND patients showed decreased thalamic volumes, bilaterally, and greater atrophy of the right cerebellar crus II and right lobule VIIb (Figure 5, eTable 9 available from Dryad https://doi.org/10.5061/dryad.1vhhmgqsz). Compared with sMND, gMND showed volumetric reduction of the left caudate and left thalamus, and a trend toward greater atrophy of the right crus II (p=0.07).

Compared with controls, sFTD patients showed decreased volume of the caudate nuclei, thalami, hippocampi, putamina and pallidi, bilaterally, and left amygdala; gFTD showed similar widespread volume loss of the caudate nuclei, thalami, hippocampi, putamina and pallidi, bilaterally, as well as a trend toward greater atrophy of the right cerebellar lobule VIIb (p=0.067).
No significant differences between sFTD and gFTD were found.

**Level 3 (C9-MND vs SOD1 vs TARDBP vs sMND vs controls; C9-FTD vs GRN vs sFTD vs controls)**

Compared with healthy controls, C9-MND patients showed significant volumetric reduction of the caudate nuclei, thalami, cerebellar lobules VII b, bilaterally, and right crus II (eFigure 2, eTables 10-11 available from Dryad https://doi.org/10.5061/dryad.1vhhmgqsza). Compared with sMND, C9-MND patients showed significant volumetric reduction of the thalami, bilaterally, and left caudate, and a trend toward greater atrophy of the right cerebellar lobule VII b (p=0.07).

Compared with healthy controls, C9-FTD patients showed volumetric reduction of the caudate nuclei and thalami, bilaterally; GRN patients showed volumetric reduction of the caudate nuclei, thalami and hippocampi, bilaterally, and left putamen (eFigure 2, eTables 10-11 available from Dryad https://doi.org/10.5061/dryad.1vhhmgqsza). Compared with sFTD, no statistically significant differences of MRI volumetric measures of C9-FTD and GRN patients were found.

**Correlations between clinical, cognitive and MRI volumes of FTLD patients**

Thalamic volumes of gFTLD patients showed an inverse correlation with Frontal Behavioral Inventory total scores (left: r=-0.473, p=0.031; right: r=-0.638, p=0.002). After subdividing gFTLD according to C9orf72 status (i.e., C9-FTLD and non-C9 gFTLD), this correlation remained significant only for C9-FTLD (left: r=-0.579, p=0.024; right: r=-0.613, p=0.025) (Figure 6).

No other statistically significant correlations between clinical/cognitive and MRI volumetric features of FTLD patients were found.

**DISCUSSION**
The present study provides a comprehensive report of clinical and GM structural MRI findings in a cohort of patients affected by disorders of the FTLD spectrum with known genetic mutations. Genetically determined FTLD patients (i.e., both FTD and MND presentations) consistently showed greater GM disruption, compared with sporadic cases who were matched for clinical presentation and degree of functional and cognitive impairment. In particular, the involvement of parietal cortices, thalami and cerebellar regions was observed consistently in gFTLD cases, in contrast with sFTD showing atrophy mostly affecting fronto-temporo-insular regions and basal ganglia, and sMND displaying focal damage of motor cortical regions. We have described distinctive patterns of atrophy that associate with each specific mutation, identifying the reduction of thalamic volumes as mostly indicative of C9orf72-mutated cases, in particular for patients presenting with MND. The results provide interesting insights into the pathophysiology of gFTLD and suggest possible neuroimaging markers of underlying pathology that may help to disentangle the heterogeneity of disorders of the FTLD spectrum.

First of all, some important observations can be drawn regarding the relationship between clinical phenotype and the underlying genetic background in the present sample. Our cohort of gFTLD included a prevalent proportion of MND cases, compared with previous studies which were mostly focused on FTD. Only studies assessing C9orf72 mutation carriers had a larger representation of MND, consistent with the known association of this mutation with both cognitive and motor FTLD presentations. In fact, in our cohort, C9orf72 was the only mutation that was observed both in FTD and MND cases, and all patients presenting with a mixed FTD-ALS phenotype carried this genetic alteration. The composition of our cohort also allowed the identification of relatively sizeable groups of less common MND-related mutation carriers, whose neuroanatomical damage has been rarely described, such as SOD1 or TARDBP. In addition, we highlighted that not only ALS, but also other MND phenotypes (i.e., PLS and PMA) can be associated with C9orf72, TARDBP or SOD1 mutations. PLS/PMA cases with a C9orf72 expansion have been rarely described, whereas an association with SOD1 or TARDBP mutations is
practically anecdotic.\textsuperscript{31} Therefore, our findings suggest that the common notion of PLS and PMA as sporadic MND presentations should be at least reconsidered.\textsuperscript{31}

The sporadic groups of our sample were selected to be matched with gFTLD cases for clinical presentation and disease severity. Consistently, gMND and gFTD were comparable with the respective sporadic groups in terms of the main clinical and cognitive measures. A notable exception was provided by \textit{TARDBP} mutation carriers, whose disease progression rate was faster than other MND groups, although with a high interindividual variability consistent with previous reports.\textsuperscript{32} Although we did not detect significant differences in neuropsychological measures between genetic FTD/MND patients and the respective sporadic groups, gFTD (in particular, \textit{GRN} mutation carriers) had the most severe impairment of visuospatial skills, suggesting a more rapid evolution to multidomain cognitive impairment, similar to previous reports in \textit{GRN} mutation carriers.\textsuperscript{33}

The most consistent result provided by VBM was a more severe and widespread GM atrophy in gFTLD patients, compared with sFTLD. We revealed a characteristic involvement of the inferior parietal, posterior cingulate, thalamic and posterior cerebellar regions in gFTD and gMND patients, that the respective sporadic groups lacked. GM atrophy was generally greater and more diffuse in gFTD cases, followed by sFTD and gMND cases, whereas sMND showed very focal, subtle atrophy of the motor cortex (which was shared by gMND). The presence of diffuse neuroanatomical damage in gFTLD compared with sFTLD, extending to posterior cortical and subcortical regions despite a comparable disease severity and duration, supports the notion that an “unfavorable” genetic background might accelerate neurodegeneration in neuronal populations that are relatively distant from those classically involved in FTLD.\textsuperscript{34,35}

When we assessed patients defined on the specific underlying mutation, a greater damage of the inferior parietal cortices (namely, the angular gyrus) and the thalami was shared by C9-MND, C9-FTD and \textit{GRN} mutation carriers, compared with sporadic cases. The greater involvement of the inferior parietal regions is consistent with previous reports in \textit{C9orf72}\textsuperscript{24,36–38} and \textit{GRN} mutation carriers.
carriers, even from the presymptomatic stages. GRN patients showed a left-sided prevalence of parietal cortical damage, consistent with the relatively large proportion (3/8) of PPA presentations in our cohort and the known asymmetrical atrophy of this group, although the overall average pattern of GM atrophy was relatively symmetrical. Of note, characteristic posterior thalamic and cerebellar atrophy was found in C9orf72 mutation carriers with either FTD or MND presentations, in contrast with the involvement of anterior thalamic regions in GRN mutation carriers. Our findings are in line with previous studies highlighting the specific involvement of the pulvinar and posterior cerebellar regions in FTD cases with a C9orf72 expansion, and expanding the validity of these findings to pure MND cases. By contrast, we were not able to detect significant atrophy in the cortical regions of TARDBP and SOD1 patients, although these groups were similarly sized when compared with C9-MND. To our knowledge, there are no published reports of MRI volumetric findings in a cohort of TARDBP patients, due to the rarity of this mutation. Although our findings need to be confirmed in larger samples, these suggest a substantial absence of GM involvement in MND patients carrying a TARDBP mutation, in contrast with the few case reports demonstrating frontotemporal atrophy in TARDBP-related FTD cases. The absence of brain GM atrophy in our SOD1 group is in line with studies suggesting a different distribution of the non-TDP-43 pathology associated with this mutation, that prevalently involves the lower motor neurons in the spinal cord.

Intrigued by the results that we obtained at the whole-brain level, we then focused on the involvement of deep GM and cerebellar structures, in order to identify quantitative volumetric markers that could provide relevant group-specific measures of neurodegeneration in gFTLD. In this case, the difference in severity of atrophy between MND and FTD patients was even more relevant compared with the VBM analysis, since both sFTD and gFTD showed a similarly widespread severe involvement of basal ganglia, thalami and hippocampi, in contrast with a general preservation of these structures in sMND and gMND. The only notable exception was the significant bilateral thalamic atrophy identified in gMND, that emerged particularly when only C9-
MND were considered, as a distinctive feature compared with sMND cases. In fact, our findings strengthen previous evidence that thalamic atrophy in FTLD is highly indicative of a genetic underlying cause, mostly pointing toward a \textit{C9orf72} expansion.\textsuperscript{7,24,40,41} We have also shown distinctive atrophy of the caudate nucleus in C9-MND cases, consistent with previous MRI studies demonstrating typical basal ganglia involvement in \textit{C9orf72}-related MND\textsuperscript{10} and subtle functional rearrangements in the thalami and basal ganglia of \textit{C9orf72} mutation carriers, even in presymptomatic phases.\textsuperscript{42,43} We have also found an inverse correlation between thalamic volumes and behavioral impairment in gFTLD patients, which was mostly driven by \textit{C9orf72} mutation carriers. This suggests a significant influence of such characteristic neuroanatomical damage over the progression of neurobehavioral impairment in \textit{C9orf72}-related FTLD, consistent with the involvement of the thalami in cognition and complex behavior.\textsuperscript{44} Therefore, our results strongly point towards the use of measures of deep GM involvement as useful markers of \textit{C9orf72}-related disorders, regardless of the clinical presentation within the FTLD spectrum.

The analysis of cerebellar volumes showed a substantial preservation of these regions in sFTLD, in contrast with atrophy of the lobule VIIb and crus II detected in gFTLD. Particularly, the single cerebellar structure showing greater damage in gFTLD compared with sFTLD was the right lobule VIIb. When looking at single genetic alterations, we identified gMND and, particularly, C9-MND as the subjects driving these results, which are consistent with recent studies indicating the involvement of the cognitive/affective regions of the posterior cerebellum (particularly, lobule VII and crus regions, involved in the modulation of emotions and social behaviors) as indicative of the presence of a \textit{C9orf72} mutation,\textsuperscript{7} possibly as a consequence of the close structural and functional connections with the thalami through cerebello-thalamo-cortical networks.\textsuperscript{42,45} Similar alterations have been demonstrated in presymptomatic \textit{C9orf72} mutation carriers,\textsuperscript{37,38,46} correlating with cognitive inhibition deficits.\textsuperscript{46} Although the role in cognition and the topological organization of the cerebellar cortex has started being elucidated only in recent years,\textsuperscript{45} this is an exciting area of
developing research for biomarkers of disease pathology in the FTLD spectrum (including MND),
that might be combined with other more established measures of cortical damage.

This study is not without limitations. First, in order to include the largest possible number of
gFTLD cases, we included subjects acquired using two different MRI scanners. For this reason, we
corrected all analyses for scanner type. Moreover, we lacked neuropathological post-mortem
diagnosis of the sporadic cases. This might have partially influenced the results when comparing
sFTD patients with gFTD who, in our cohort, were almost exclusively due to mutations leading to
FTLD-TDP pathology. Finally, we did not involve presymptomatic mutation carriers, that would be
needed to understand how early the volumetric changes that we detected can be observed in the
course of the disease. Future longitudinal studies involving these subjects will be fundamental to
understand the potential clinical relevance of these measures for early identification of patients
close to symptom onset and as outcome measures in clinical trials targeting specific FTLD-related
molecular mechanisms.

In conclusion, this study encompassed the entire FTLD spectrum, providing an accurate
overview of clinical, neuropsychological and MRI volumetric findings in patients with a genetically
determined FTD/MND clinical phenotype. We have described also mutations that have been rarely
reported previously in the neuroimaging literature and identified specific imaging measures of
FTLD genotypes, which proved especially useful when it comes to C9orf72-associated
presentations. Our results strongly suggest that neuroimaging can provide useful volumetric
measures applicable to future clinical trials targeting these genetic mutations.
Acknowledgments: We thank the patients and their families for the time and effort they dedicated to the research; and Laura Pozzi (MSc, Experimental Neuropathology Unit, IRCCS San Raffaele Scientific Institute, Milan, Italy), for providing technical assistance with genetic data.
## Appendix 1: Authors

<table>
<thead>
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<th>Name</th>
<th>Location</th>
<th>Contribution</th>
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<tbody>
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References


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**Table 1.** Main socio-demographic and clinical characteristics of healthy controls and FTLD patients classified by clinical presentation and genetic status.

<table>
<thead>
<tr>
<th></th>
<th>HC</th>
<th>sMND</th>
<th>gMND</th>
<th>sFTD</th>
<th>gFTD</th>
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<td>Number</td>
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<td>45</td>
<td>44</td>
<td>16</td>
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<td>Diagnosis</td>
<td>-</td>
<td>37 ALS, 5 PMA, 3 PLS</td>
<td>35 ALS, 6 PMA, 3 PLS</td>
<td>12 bvFTD, 2 nfvPPA, 2 svPPA</td>
<td>12 bvFTD, 5 bvFTD/ALS, 1 svPPA, 3 nfvPPA, 1 R-SD</td>
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<td>Sex (M/F)</td>
<td>26/26</td>
<td>23/22</td>
<td></td>
<td>22/22</td>
<td>9/7</td>
<td>11/11 0.99</td>
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<tr>
<td>Genetic mutation</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>22 C9orf72, 10 TARDBP, 7 SOD1, 2 TBK1, 2 FUS, 1 C9orf72 + TARDBP</td>
<td></td>
<td>11 C9orf72, 8 GRN, 1 MAPT, 1 TREM2, 1 C9orf72 + GRN</td>
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<td>Family history (+/-)</td>
<td>0/52</td>
<td>3/42</td>
<td>18/26</td>
<td></td>
<td></td>
<td>13/9 0.001</td>
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<tr>
<td>Education (years)</td>
<td>12.75 ± 3.70 (5 – 20)</td>
<td>12.07 ± 4.10 (5 – 24)</td>
<td>10.84 ± 3.23 (5-20)</td>
<td>10.36 ± 3.81 (5 – 17)</td>
<td>11.11 ± 4.19 (5 – 21)</td>
<td>0.13</td>
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<tr>
<td>Age at MRI (years)</td>
<td>59.2 ± 6.6 (44.7 – 72.7)</td>
<td>58.03 ± 9.7 (36 – 71)</td>
<td>57.25 ± 10.01 (31 – 75)</td>
<td>61.4 ± 5.7 (46 – 71)</td>
<td>60.26 ± 4.78 (49 – 67)</td>
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<td>Disease duration (months)</td>
<td>-</td>
<td>29.6 ± 42.9 (4 – 277)</td>
<td>25.70 ± 26.57 (4 – 112)</td>
<td>30 ± 10.4 (22 – 48)</td>
<td>29.35 ± 30.35 (9 – 119)</td>
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<tr>
<td>CDR</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td>1.23 ± 0.97 (0 - 3) 1.18 ± 0.93 (0-3)</td>
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<tr>
<td>CDR-FTLD</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
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<td>10.20 ± 6.70 (1 – 23) 7.25 ± 6.96 (0.5 – 20)</td>
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<tr>
<td>CDR-sb</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td>7.53 ± 5.3 (1 – 17) 6.14 ± 4.55 (1 – 15) 6.81 ± 5.45 (1 – 15)</td>
</tr>
<tr>
<td>MMSE (%)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.98 ± 0.03 (0.90-1)</td>
<td>0.97 ± 0.03 (0.90-1)</td>
<td>0.96 ± 0.05 (0.71-1)</td>
<td>0.77 ± 0.2 (0.2-0.93)&lt;sup&gt;b,d&lt;/sup&gt;</td>
<td>0.78 ± 0.2 (0.33-0.97)&lt;sup&gt;b,d&lt;/sup&gt;</td>
<td>0.003</td>
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<tr>
<td>FBI total (0-72)</td>
<td>-</td>
<td>1.95 ± 1.83 (0-6)</td>
<td>2.82 ± 3.13 (0-13)</td>
<td>28.00 ± 11.6 (15-45)&lt;sup&gt;b,d&lt;/sup&gt;</td>
<td>17.33 ± 13.09 (0-35)&lt;sup&gt;b,d&lt;/sup&gt;</td>
<td>&lt;0.001</td>
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<tr>
<td>ALSFRS-r (0-48)</td>
<td>-</td>
<td>37.36 ± 6.2 (23 – 47)</td>
<td>36.71 ± 7.22 (20 – 46)</td>
<td>-</td>
<td>35.2 ± 5.6 (26 – 41)*</td>
<td>0.76</td>
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<tr>
<td>MRC sum score (0-120)</td>
<td>-</td>
<td>97.08 ± 20.63 (34 – 120)</td>
<td>95.25 ± 20.96 (41 – 120)</td>
<td>-</td>
<td>90.4 ± 12.58 (78 – 110)*</td>
<td>0.77</td>
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<tr>
<td>Disease progression rate</td>
<td>-</td>
<td>0.64 ± 0.6 (0.07 – 2.4)</td>
<td>0.91 ± 0.84 (0.22 – 4)</td>
<td>-</td>
<td>1.18 ± 0.6 (0.75 – 2.2)*</td>
<td>0.11</td>
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<tr>
<td>Onset bulb/limb/bulb+limb</td>
<td>-</td>
<td>8/36/1</td>
<td>4/40/0</td>
<td>-</td>
<td>2/3/0*</td>
<td>-</td>
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<tr>
<td>CSF β-amyloid 42 (pg/mL)</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td>732.18 ± 241.23 (447-1101) 785 ± 325.9 (452-1470)</td>
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<tr>
<td>CSF total tau (pg/mL)</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td>193.73 ± 67.08 (121-350) 286.3 ± 177.24 (72-751)</td>
</tr>
<tr>
<td>CSF phosphorylated tau (pg/mL)</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td>31.49 ± 12.51 (15-57) 38.7 ± 16.62 (20-64)</td>
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</table>
Values are reported as means ± standard deviations (range). P values refer to ANOVA models, Bonferroni-corrected for multiple comparisons. # = ratio between correct and administered items, considering patient’s motor disability; * = bvFTD/ALS cases; - = not applicable; a= statistically significant difference with HC; b= statistically significant difference with sMND; c= statistically significant difference with sFTD; d= statistically significant difference with gMND. Abbreviations: ALSFRS-r= Amyotrophic Lateral Sclerosis Functional Rating Scale, revised version; bvFTD: behavioral variant frontotemporal dementia; CDR= Clinical Dementia Rating scale; CDR-sb= Clinical Dementia Rating scale, sum of boxes; CSF= cerebrospinal fluid; FBI= frontal behavioral assessment; gMND= genetic motor neuron disease; gFTD= genetic frontotemporal dementia; HC= healthy controls; MND= motor neuron disease; nfvPPA= non-fluent variant primary progressive aphasia; sFTD= sporadic frontotemporal dementia; sMND= sporadic motor neuron disease; R-SD= right-sided semantic dementia; svPPA= semantic variant primary progressive aphasia.
Figure legends

**Figure 1. Sample selection and study design.** (A) A total of 362 patients with disorders of the FTLD spectrum referred between 2007 and 2019 were screened for known pathogenic mutations. Sixty-six mutation carriers were identified. Detected mutations are reported in the last pie chart. (B) Diagram showing the hierarchical organization of the three-level statistical analysis. Abbreviations: FTD= frontotemporal dementia; gFTD= genetic frontotemporal dementia; gMND= genetic motor neuron disease; MND= motor neuron disease.
Figure 2. Patterns of GM atrophy in genetic and sporadic FTLD patients. Results of voxel-based morphometry analysis showing regions of significant GM atrophy in sFTLD and gFTLD patients when compared with HC (A-B) and between each other (C). Significant clusters are overlaid on the axial sections of the Montreal Neurological Institute standard brain. Analyses were corrected for age, sex, and total intracranial volume. Statistical threshold for significance was p<0.05, FWE-corrected for multiple comparisons. Abbreviations: FTLD = frontotemporal lobar degeneration; GM = grey matter; g = genetic; HC = healthy controls; s = sporadic.
Figure 3. Patterns of GM atrophy in FTLD patients according to genetic status and clinical presentation. Results of voxel-based morphometry analysis showing regions of significant GM atrophy in sporadic and genetic FTD/MND patients when compared with HC (A-D) and between each other (E-F). Significant clusters are overlaid on the axial sections of the Montreal Neurological Institute standard brain. Analyses were corrected for age, sex, and total intracranial volume. Statistical threshold for significance was p<0.05, FWE-corrected for multiple comparisons. Abbreviations: FTD= frontotemporal dementia; g= genetic; GM= grey matter; HC= healthy controls; MND= motor neuron disease; s= sporadic.
Figure 4. GM volumes of subcortical and cerebellar structures in FTLD patients according to genetic status and clinical presentation. Comparisons between groups were made using age-, sex- and MR scanner-adjusted ANOVA models, followed by post-hoc pairwise comparisons, Bonferroni-corrected for multiple comparisons. Symbols: * = p < 0.05 compared with HC; # = p < 0.05 compared with sMND . Abbreviations: FTD = frontotemporal dementia; g = genetic; GM = grey matter; HC = healthy controls; MND = motor neuron disease; s = sporadic.
Figure 5. Patterns of GM atrophy in FTLD patients according to genetic mutation. Results of voxel-based morphometry analysis showing regions of significant GM atrophy in FTLD genetic subgroups when compared with HC, sporadic patients and between each other. Significant clusters are overlaid on the axial sections of the Montreal Neurological Institute standard brain. Analyses were corrected for age, sex, and total intracranial volume, Statistical threshold for significance was p<0.05, FWE-corrected for multiple comparisons. 

Abbreviations: C9-FTD= frontotemporal dementia patients carrying a C9orf72 mutation; C9-MND= motor neuron disease patients carrying a C9orf72 mutation; FTD= frontotemporal dementia; GM= grey matter; HC= healthy controls; MND= motor neuron disease; s= sporadic.
Figure 6. Relationship between thalamic volume and FBI total scores in FTLD patients.

Plots showing significant inverse correlation only in the C9-FTLD group (left thalamus: r= -0.579, p=0.024; right thalamus: r= -0.613, p=0.025). Orange dots represent plotted values of sFTLD patients (n=26, based on availability of FBI scores), blue dots represent C9-FTLD patients (n=19), and green dots represent gFTLD patients with mutations other than C9orf72 (n=12). Partial correlation analyses were Bonferroni-corrected for multiple comparisons, adjusted for age, sex and education. Abbreviations: C9-FTLD= frontotemporal lobar degeneration patients carrying a C9orf72 mutation; FBI= frontal behavioral inventory; FTLD= frontotemporal lobar degeneration; g= genetic; s= sporadic.
Structural MRI Signatures in Genetic Presentations of the Frontotemporal Dementia-Motor Neuron Disease Spectrum

Edoardo Gioele Spinelli, Alma Ghirelli, Silvia Basaia, et al.

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