Published in final edited form as:

Neuropathology. 2013 April ; 33(2): 122–133. doi:10.1111/j.1440-1789.2012.01332.x.

Frontotemporal Lobar Degeneration with TDP-43 Proteinopathy and Chromosome 9p Repeat Expansion in *C9ORF72*: Clinicopathologic Correlation

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Abstract

Mutations in *C9ORF72* resulting in expanded hexanucleotide repeats were recently reported to be the underlying genetic abnormality in chromosome 9p-linked frontotemporal lobar degeneration with TAR DNA-binding protein of 43 kD (TDP-43) proteinopathy (FTLD-TDP), amyotrophic lateral sclerosis (ALS), and frontotemporal lobar degeneration with motor neuron disease (FTLD-MND). Several subsequent publications described the neuropathology as being similar to that seen in cases of FTLD-TDP and ALS without *C9ORF72* mutations, except that cases with mutations have p62 and ubiquitin positive, TDP-43 negative inclusions in cerebellum, hippocampus, neocortex, and basal ganglia. The identity of this protein is as yet unknown, and its significance is unclear. With the goal of potentially uncovering the significance of these TDP-43 negative inclusions, we compared the clinical, pathologic, and genetic characteristics in 5 cases of FTLD-TDP and FTLD-MND with *C9ORF72* mutations to 20 cases without mutations. We confirmed the apparent specificity of p62 positive, TDP-43 negative inclusions in cerebellum, hippocampus, cortex, and basal ganglia to FTLD with *C9ORF72* mutations. p62 positive, TDP-43 negative inclusions in hippocampus correlated with hippocampal atrophy, but no additional correlations were uncovered. However, although ambiguity of TDP sub-typing has previously been reported in cases with *C9ORF72* mutations, this is the first report to show that although most FTLD cases with *C9ORF72* mutations were TDP type B, some of the pathologic characteristics in these cases were more similar to TDP types A and C than to TDP type B FTLD cases without mutations. These features include greater cortical and hippocampal atrophy, greater ventricular dilatation, more neuronal loss and gliosis in temporal lobe and striatum, and TDP-43 positive fine neuritic profiles in the hippocampus in FTLD cases with *C9ORF72* mutations compared to FTLD-TDP type B cases without mutations, implying that the *C9ORF72* mutation modifies the pathologic phenotype of FTLD-TDP type B.

Keywords

C9ORF72; repeat expansion; p62; ubiquitin; TDP-43; FTLN; ALS

Introduction

Non-coding region mutations in *C9ORF72* consisting of expanded GGGGCC hexanucleotide repeats were recently reported to be responsible for cases of chromosome 9p-linked FTLN-TDP, ALS, and FTLN-MND¹⁻³. The function of the protein remains unknown, but it is highly conserved across species¹. In addition, an RNA gain-of-function disease mechanism, independent of *C9ORF72*, has also been suggested¹. Although the general neuropathology of cases with *C9ORF72* mutations is TDP-43 proteinopathy, some of the neuropathology appears to be unique. Specifically, inclusions in the cerebellar granular layer are positive for ubiquitin and p62 but negative for TDP-43⁴⁻⁹. However, one group also found these inclusions in three cases negative for *C9ORF72* mutations⁹. Additionally, some have reported unusual “star-shaped” inclusions in the hippocampus, neocortex, and basal ganglia that are also ubiquitin and p62 positive and TDP-43 negative⁵⁻⁸. These inclusions had previously been reported in cases that were linked to chromosome 9p, prior to identification of the repeat expansions¹⁰⁻¹³, and now their specificity seems apparent. Dot-like TDP-43 positivity in the hippocampal CA2-4 region, described as “synaptic,” as well as TDP-43 positive fine dystrophic neurites (DNs) in the hippocampal CA1 region and subiculum in cases with repeat expansion has also been reported⁴.

The identity of the protein in the p62 and ubiquitin positive, TDP-43 negative inclusions also remains unknown. Reportedly, they are negative for neurofilament, alpha-internexin, FUS, optineurin, and *C9ORF72*^{5, 13}. While the exact disease mechanism associated with this mutation currently remains unknown, c9FTD/ALS (the recommended terminology for cases with mutations in *C9ORF72*) is a new member of the class of non-coding repeat expansion disorders.

Materials and Methods

Cases

Twenty-five cases of FTLN-TDP or FTLN-MND were chosen based on their pathologic diagnosis from the files of the IRB-approved Northwestern University Cognitive Neurology and Alzheimer Disease Center Brain Bank and analyzed for *C9ORF72* expanded hexanucleotide repeats. Brain autopsies were performed under proper informed consent procedures. Although *C9ORF72* mutations are found also in patients with ALS only, because the cases in the current study are derived from an Alzheimer Center, none of them has ALS without FTD. Analysis for *C9ORF72* repeat expansion was performed on de-identified cases at Mayo Jacksonville (see below).

Clinical Data

Clinical information regarding gender, onset age, duration of illness, family history of any neurologic disorder, family history of dementia or ALS in a 1st or 2nd degree relative, and initial and final clinical diagnoses were recorded. In addition, presence or absence of motor neuron disease, extrapyramidal signs, memory disorder, language disorder, and behavioral/executive disorder were recorded.

Pathologic Data

All pathologic information except the cerebellar p62 immunostaining was obtained prior to analysis for the *C9ORF72* mutation. The p62 immunostaining of all 25 cases was interpreted with the neuropathologist blind to the results of the hexanucleotide repeat status of each individual case. Pathologic information included brain weight, gross frontal, temporal, parietal, motor, caudate, and hippocampal atrophy, ventricular dilatation, microscopic neuronal loss and gliosis in frontal, temporal, parietal, and motor cortex, striatum, hippocampal CA1 region (none had significant neuronal loss or gliosis in CA2-4), subiculum, substantia nigra, and hypoglossal nucleus, rarefaction of corticospinal tracts in the medullary pyramids, and presence of Bunina bodies in the hypoglossal nucleus. These gross and microscopic findings were semiquantitated as absent, mild, moderate, or severe. All gross and general microscopic evaluations were performed according to routine protocol, prior to obtaining immunohistochemical diagnostic information, i.e., “blind” to the pathologic diagnosis. While these evaluations are subjective, criteria are straightforward. Gross atrophy is defined as either no, mild, moderate, or severe gyral thinning and sulcal widening (Fig. 1). Ventricular dilatation is defined by the degree of rounding of the corners of the lateral ventricles, particularly near the striatum, and enlargement of the lateral ventricles, again as either no, mild, moderate, or severe (Fig. 2). Similarly, microscopic neuronal loss and gliosis is determined by the degree of decrease in neuronal density and increase in glial density (none, mild, moderate, or severe) (Fig. 3). Hippocampal sclerosis, for the purpose of this study, was defined as moderate or severe neuronal loss and gliosis in both the hippocampal CA1 region and subiculum.

The FTLTD-TDP pathologic sub-type was classified on the basis of morphologic analysis of TDP-43 immunohistochemistry results alone (i.e., not on Western blotting), as per the recently published “harmonized” classification scheme¹⁴. The presence or absence of TDP positive neuronal intranuclear inclusions (NIIs) was recorded. Cytoplasmic TDP positive cytoplasmic inclusions (CIs) in the hippocampal dentate gyrus, hippocampal CA1-4 region, and subiculum were scored as follows: absent = 0, rare (1 CI in several 200x fields) = 0.5, sparse (maximum of 1 CI per 200x field) = 1, moderate (maximum of 2–3 CI per 200x field) = 2, and frequent (>3 in most 200x fields) = 3 (Figs. 4 and 5). The sum of CIs in these three regions was given a “TDP CI score.” TDP positive fine dystrophic neurites (DNs) and dot-like “synaptic” labeling in the hippocampal subiculum and CA1 and CA2-CA4 region, as described in a recent publication⁴, were scored as follows: TDP-43 positive DN and synaptic positivity was semi-quantitated on a scale from 0 to 3, as for CIs, in each hippocampal CA1 region and subiculum as well as the CA2-4 region and the sum in each region was given a “TDP neurites score” (Figs. 6 and 7). p62 positive, TDP-43 negative “star-shaped” inclusions in the hippocampal CA1-CA4 region, and small p62 positive, TDP-43 negative cytoplasmic inclusions in the cerebellar granular layer were semi-quantitated on the same scale as above from 0–3 and recorded for each case. Lastly, CERAD plaque scores and Braak & Braak tangle stages were recorded for each case^{15, 16}.

Immunohistochemistry

TDP-43 immunostains were performed using the phosphorylated monoclonal TDP-43 antibody pS409/410-2 at a 1:5000 dilution, AEC chromogen (Cosmo-Bio USA, Carlsbad CA). p62 immunostains were performed using the monoclonal anti-p62 Ick ligand antibody at a 1:100 dilution, DAB chromogen (BD Transduction Laboratories, San Jose CA). Ubiquitin immunostains were performed using the DAKO polyclonal antibody at a 1:1000 dilution (DAKO, Carpinteria, CA).

Genetic Data

Genomic DNA was extracted from samples using the standard procedures. Twenty cases had Apolipoprotein E (*APOE*) genotype data. Eighteen were analyzed for progranulin (*GRN*) mutations, 8 for *TARDBP* mutations, 5 for *MAPT* mutations, and one for mutations in *FUS/TLS* as previously described^{17–20}.

For each case, the presence of an expanded GGGGCC hexanucleotide repeat in *C9ORF72* was detected using a two-step protocol. First, in all samples, the hexanucleotide repeat was PCR amplified using one fluorescently labeled primer followed by fragment length analysis on an automated ABI3730 DNA-analyzer as described¹. All patients that appeared homozygous in this assay were further analyzed using the repeat primed PCR method as described¹. A characteristic stutter amplification pattern on the electropherogram was considered evidence of a pathogenic repeat expansion.

Statistical Analysis

The *C9ORF72* status was related to categorical variables using Fisher's exact test, and to continuous variables using the Wilcoxon rank sum test. Results with $p < 0.05$ were considered statistically significant.

Results

Five of the 25 cases (20%) had *C9ORF72* repeat expansion. There was a family history of at least some type of neurologic disorder in four of the five cases, and a family history of dementia or ALS in a 1st or 2nd degree relative in three of these four. Family history was unknown in one case. In the 20 cases without mutations, there was a family history or ALS in a 1st or 2nd degree relative in seven cases and a family history of some type of neurologic disorder in 10. Family history was unknown in one case. Clinical ALS was present in one case with *C9ORF72* mutations, and that individual had FTLT-MND pathology. FTLT-MND pathology was present in two additional cases without clinical ALS. All but two of the FTLT-MND cases without *C9ORF72* mutations had clinical ALS and none of the FTLT-TDP cases without mutations had clinical ALS. There was no significant difference between those with and those without mutations with respect to ApoE status, gender distribution, onset age, duration of illness, family history of any neurologic disorder, family history of ALS or dementia in a 1st or 2nd degree relative, presence of clinical ALS, extrapyramidal signs, psychiatric signs, memory, language, or behavioral/executive disorder, initial or final clinical diagnosis, pathologic diagnosis, or degree of AD pathology. None of the patients had psychosis.

Four of the cases with hexanucleotide repeat expansion had TDP type B, as is most often seen in cases with FTLT-MND. Two of the type B cases had pathologic FTLT-MND but only one of these had clinical ALS. Two had no clinical or pathologic ALS and had the pathology of FTLT-TDP¹⁴. The fifth case had pathologic FTLT-MND, without clinical ALS, but interestingly had TDP type A pathology, most often seen in cases without ALS and present in all cases with *GRN* mutations¹⁴.

All five cases with *C9ORF72* mutations had small p62 positive cytoplasmic inclusions and rare intranuclear inclusions in cerebellar granular neurons while none without mutations had these inclusions (Fig. 8A & B). Fewer were positive with ubiquitin than with p62 (Fig. 8C). In some cases there were also cytoplasmic inclusions in molecular layer basket cells and rarely in Purkinje cells (Fig. 8D). The cases with mutations also all had p62+ "star-shaped" cytoplasmic inclusions in the hippocampal CA1-CA4 region, and occasional "dot-like" intranuclear inclusions (Fig. 9). Fewer star-shaped inclusions were labeled with ubiquitin than with p62. Neither cerebellar nor hippocampal star-shaped inclusions were labeled with

TDP-43. More hippocampal dentate gyrus inclusions were labeled with p62 and ubiquitin than with TDP-43 (Fig. 5). The cases with repeat expansion had more cortical inclusions labeled with p62 and ubiquitin than with TDP-43, and occasional star-shaped inclusions were noted with p62 and ubiquitin but not seen with TDP-43 (Fig. 10). Rare star-shaped inclusions were seen in the basal ganglia.

Three cases with mutations had fine TDP-43 positive DNPs in the CA1 region of the hippocampus and subiculum (Fig. 7) and four had dot-like synaptic TDP-43 labeling in the CA2-4 region of the hippocampus (Fig. 6), variably labeled with p62. Nine of the 20 cases without mutations had fine TDP-43 positive DNPs in the CA1-subiculum region and only six of 20 had TDP-43 positive synaptic labeling in the CA2-4 region. Combined TDP-43 positive fine DNPs and synaptic labeling were seen in all cases with mutations, and all TDP types A and C cases (12/12), but they were present in only four of the 13 type B cases without mutations ($p=0.04$). In none of the 25 cases did either TDP-43 positive thin DNPs or synaptic labeling or their combined TDP scores correlate with either hippocampal sclerosis or with the presence of a memory, behavioral or language disorder.

Cerebellar p62 positive, TDP-43 negative inclusions did not correlate with clinical ALS, extrapyramidal signs, initial or final clinical diagnosis, pathologic diagnosis, or TDP type. Hippocampal star-shaped p62 positive, TDP-43 negative inclusions, interestingly, did correlate with hippocampal atrophy ($p=0.04$), but did not correlate with hippocampal neuronal loss and gliosis, TDP type, NIIs, hippocampal TDP CI score, hippocampal TDP DNPs or synaptic pathology, the presence of a memory disorder, the initial or final clinical diagnosis, or the pathologic diagnosis.

Cases with repeat expansion had greater temporal atrophy ($p=0.008$), motor atrophy ($p=0.006$), hippocampal atrophy ($p=0.0247$), and motor cortex neuronal loss and gliosis ($p=0.004$). There was no difference between groups in frontal, parietal, or caudate atrophy, ventricular dilatation, neuronal loss and gliosis in frontal, temporal, or parietal lobes, striatum, hippocampus, substantia nigra, or hypoglossal nucleus, corticospinal tract rarefaction in medullary pyramids, hypoglossal Bunina bodies, TDP type, presence of NIIs, hippocampal/dentate gyrus TDP-43 positive CIs, or hippocampal TDP-43 positive DNPs or synaptic pathology.

The cases with *C9ORF72* mutations included three with FTL-D-MND, two with TDP type B and one with TDP type A, and two with FTL-D-TDP, both TDP type B. The cases without mutations included five with TDP type A, 13 with TDP type B, and two with TDP type C. All type A and type C cases without mutations had pathologic FTL-D-TDP and all type B cases without mutations had pathologic FTL-D-MND. When the group with mutations was compared to these three groups, some interesting correlations were found. Although three of the five had FTL-D-MND, and four of the five were TDP type B, many of the pathologic features of the cases with *C9ORF72* mutations were more similar to those of TDP types A and C, or type A alone, than type B. The cases with mutations were similar to TDP type A & C in atrophy of frontal ($p=0.016$), temporal ($p<0.0001$), and parietal cortex ($p=0.0005$), ventricular dilatation ($p=0.022$), and neuronal loss and gliosis in the temporal lobe ($p=0.0005$). The cases with mutations were similar to TDP type A in hippocampal atrophy ($p=0.011$) and neuronal loss and gliosis in the striatum ($p=0.004$), and had greater motor cortex atrophy ($p=0.013$) and motor neuronal loss and gliosis ($p=0.006$) than all TDP subtypes without mutations. The three cases with moderate or severe motor cortex atrophy include two FTL-D-MND and one FTL-D-TDP case, while the two with mild motor cortex atrophy include one FTL-D-MND and one FTL-D-TDP case. The four cases with moderate or severe motor cortex neuronal loss and gliosis include one FTL-D-TDP and three FTL-D-MND cases. Rarefaction of the medullary pyramids correlated with the presence of ALS

(FTLD-MND) ($p=0.0002$), but did not correlate with either motor cortex atrophy ($p=0.35$) or microscopic neuronal loss and gliosis in motor cortex ($p=0.35$).

Four of the five cases with *C9ORF72* mutations had minimal AD tangle pathology, with Braak stages of 1 or 2; one had no tangles and none had plaques. According to new AD neuropathologic diagnostic criteria, all five cases with mutations were “not AD”^{21, 22}. Of the 20 cases without mutations, 12 had tangles ranging from Braak stage 1 to 3, and five had sparse to frequent cortical plaques. Using the new criteria, 15 were “not AD,” two had low Alzheimer disease neuropathologic change (ADNC), and three had intermediate ADNC^{21, 22}. There was no difference between groups in the presence of AD pathology.

APOE genotypes were available for 20 of the 25 cases, four with *C9ORF72* mutations (two ϵ 33; one ϵ 34; one ϵ 23) and 16 without (seven ϵ 33; seven ϵ 34; two ϵ 23). There was no difference in *APOE* genotype in cases with and without mutations. In those cases where *MAPT*, *GRN*, *TARDBP* and *FUS* mutation screening was available, no pathogenic mutations were identified. One of the *C9ORF72* cases, case 4, did have a *TARDBP* 3'UTR variant as previously published²³.

Discussion

This study confirms that in FTLD, only cases with *C9ORF72* mutations had the previously reported p62 positive, TDP-43 negative inclusions in cerebellum, hippocampus, and neocortex, and to a lesser extent in basal ganglia^{5–13}. No specific clinical, pathologic, or genetic features, other than the presence of the *C9ORF72* repeat expansion, correlated with the cerebellar dentate or hippocampal p62 positive, TDP-43 negative inclusions. Hippocampal star-shaped inclusions correlated with hippocampal atrophy, which is intriguing, but because they did not correlate with hippocampal neuronal loss and gliosis, the presence of a memory disorder, the initial or final clinical diagnosis, or the pathologic diagnosis, their significance is difficult to understand. They also did not correlate with TDP type, the presence of TDP positive NIs, hippocampal TDP CI or TDP neurites score. Cerebellar inclusions did not correlate with clinical ALS, extrapyramidal signs, initial or final clinical diagnosis, pathologic diagnosis, or TDP type. Whether we are looking at the correct clinical and pathologic characteristics for these correlations and whether these inclusions have any involvement in the clinicopathologic process leading to FTLD-TDP, FTLD-MND, and ALS in cases with *C9ORF72* expanded repeats is unclear at the present time. Although no insight was gained into the significance or the identity of the major protein component of these inclusions, important observations were made with respect to some of the other results of this study.

Four of five *C9ORF72* cases had TDP-43 positive fine neuritic and dot-like synaptic labeling in the CA2-4 region of the hippocampus⁴. This is interesting, because four out of five of them have TDP type B pathology, and, while in cases without mutations, CA2-4 neuritic pathology was found in four of five cases with TDP type A pathology, it was present in only two of 13 cases with TDP type B pathology. This fine neuritic pathology has also been described as “synaptic,” because the linear dot-like positivity is reminiscent of dendritic labeling⁴. In that report, however, synaptic TDP-43 positive CA2-4 pathology was more common in cases with Mackenzie TDP-43 type 3 (harmonized type B)⁴. This discrepancy deserves further investigation. However, the fine neuritic pathology may suggest that normal TDP-43 plays a role in synaptic function and that the “synaptic” pathology seen with antibodies to phosphorylated TDP-43 signifies alterations in synaptic functioning in cases with the *C9ORF72* mutation. TDP-43 is present in RNA granules in dendrites, co-localizes with the post-synaptic protein PSD-95, and behaves as a translational repressor, suggesting that TDP-43 functions to regulate neuronal plasticity²⁴. Interestingly,

altered synapse biology has been implicated in FTLD-TDP with progranulin mutations, which almost always have TDP type A pathology²⁵. The fine neuritic pathology seen in the hippocampus in cases with *C9ORF72* mutations and FTLD with TDP type A may reflect dysfunction in this process. The absence of fine neuritic TDP-43 pathology in most cases of FTLD-TDP type B raises the possibility that these cases do not have hippocampal synaptic dysfunction, which also requires further investigation. Lastly, fine, thin, TDP-43 positive dystrophic neurites in hippocampal CA1-subiculum have previously been reported to correlate with the presence of hippocampal sclerosis⁴. In the current study, there was no correlation between either the CA1-subiculum DN, the CA2-4 synaptic labeling, or the combined subiculum-CA4 pathology and the presence of hippocampal sclerosis. In fact, it may be that the CA1-subiculum DN is composed of linear, more confluent, dot-like “synaptic” labeling like that in the CA2-4 region (Figs. 6 & 7), and may represent more extensive, but similar, pathology.

Cases with *C9ORF72* mutations, as noted in a recent paper⁴, have some interesting pathologic differences from the typical TDP subtypes described in the harmonized classification scheme¹⁴. In the current study, cases without mutations have the expected TDP sub-types; for example, all FTLD-TDP cases are either TDP type A or C, and all FTLD-MND cases are TDP type B. The cases with mutations, however, are not so neatly classified. Of the five cases with mutations, three have FTLD-MND and two have FTLD-TDP. Two of the FTLD-MND cases have the expected TDP type B, but one is TDP type A; and the two FTLD-TDP cases both have TDP type B. Another interesting finding in this correlation study is that the five cases have several pathologic features that are more similar to FTLD-TDP types A and C cases, or to type A alone, than to FTLD-MND type B cases. These pathologic features include greater frontal, temporal, parietal, and hippocampal atrophy, greater ventricular dilatation, greater neuronal loss and gliosis in temporal lobe and striatum, and the presence of TDP-43 positive fine neurites and dot-like labeling in the hippocampal pyramidal layer. Even more interesting is the fact that there is more motor cortex atrophy and neuronal loss and gliosis in the cases with mutations than in any of the other sub-types, and that this does not seem to correlate with the presence or absence of ALS, or with rarefaction of the corticospinal tracts in the medullary pyramids. It seems likely, then, that the atrophy of and neuronal loss and gliosis in motor cortex is unrelated to loss of upper motor neurons. Indeed, the motor atrophy and neuronal loss and gliosis are less severe in cases of FTLD-MND than FTLD-TDP and in most cases of ALS they are inapparent (personal observation). Although motor atrophy was not significantly correlated with frontal atrophy ($p=0.08$), it may be that it is related more to the overall atrophy in the frontal cortex in FTLD, the exact basis of which is not currently clearly defined. While neuronal loss is usually apparent in FTLD frontal cortex, it is unclear whether or not there is neuropil “shrinkage” related to neuropil loss. Data has been conflicting--some have described synaptic loss in FTLD as determined by decreased synaptophysin measured biochemically²⁶ while others have found increased immunohistochemical expression of synaptophysin and decreased expression of synaptosomal-associated protein of molecular weight 25 kDa (SNAP-25)²⁷. Therefore, the significance of greater motor atrophy, and also of greater temporal and hippocampal atrophy in cases with *C9ORF72* mutations, deserves further investigation. However, as has been previously suggested⁴, our results confirm the difficulty of classifying cases with *C9ORF72* mutations in the harmonized scheme, and additional studies are warranted.

Two possible disease mechanisms for cases with *C9ORF72* repeat expansion have thus far been proposed. On the one hand there is the possible contribution of *C9ORF72* haploinsufficiency^{1, 2}, while the accumulation of toxic RNA fragments composed of the repeated nucleotides, as has been shown in other non-coding expansion repeat disorders such as myotonic dystrophies, fragile-X associated tremor/ataxia syndrome, and several

spinocerebellar ataxias, is another likely mechanism¹. RNA misprocessing has been implicated in both ALS and FTD²⁸. RNA foci have been shown to sequester normal RNA and proteins involved in transcription regulation² and a family now known to carry the *C9ORF72* mutation had previously been shown to have aberrant RNA metabolism^{2, 29}. Are the small p62 positive intranuclear inclusions (Fig. 9) related to RNA foci? Although theoretically possible, the fact that we see just one in each nucleus, compared to several RNA foci in each nucleus, as has been previously shown¹, makes this unlikely. Interestingly, one paper reports that similar RNA foci are present not only in cases with *C9ORF72* mutations, but also in three cases with *MAPT* mutations and in a normal control, none of which had repeat expansion⁸. This may signify a role for altered RNA processing in other FTLDs, but it is difficult to understand the presence of GGGGCC RNA foci in the absence of repeat expansion. Because another group reports that p62 positive, TDP-43 negative cerebellar dentate inclusions can be found in cases without *C9ORF72* mutations, comprehending the significance of these inclusions becomes imperative⁹.

One limitation of the current study is the small sample size. On the other hand, these cases were chosen for analysis based on the pathologic rather than the clinical diagnosis, and because of that are drawn from a smaller cohort. In addition, all but four of the cases negative for *C9ORF72* mutation were followed longitudinally at the same center, using the same clinical measures, by two clinicians (SW and M-MM), and all pathology was interpreted by the same neuropathologist (EB) using the same standardized methodology, prior to identification of the specific molecular pathology of the autopsy brains. Further studies into the nature of the *C9ORF72* protein and the nature of the as-yet unidentified p62 positive, TDP-43 negative protein present in *C9ORF72* cases should open avenues of investigation for future clinicopathologic correlation studies, with the goal of identifying a potential therapeutic target.

Acknowledgments

Supported in part by an Alzheimer Disease Core Center grant to Northwestern University (P30 AG013854), an Alzheimer Disease Research Center grant to the Mayo Clinic (P50 AG16574) both from the National Institute on Aging, grants R01 NS065782 and R01 AG026251 from the National Institutes of Neurological Disorders and Stroke, and grants from the ALS Associations and the ALS Therapy Alliance. As always, we would like to express our gratitude to the patients and their families whose generosity makes research possible.

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Figure 1.

Examples of varying degrees of gross cortical atrophy. Severe atrophy of the posterior portions of the superior frontal gyrus (sfg) and middle frontal gyrus (mfg) and of the entire motor cortex (m), moderate atrophy of the frontal pole and the posterior portion of the superior temporal gyrus (stg), mild atrophy of the remainder of the frontal and temporal cortex, and no appreciable atrophy of the parietal cortex. Note: Moderate gross atrophy of the frontal pole of case 4 is shown in Figure 3 of reference #23.

Figure 2.
Examples of varying degrees of ventricular dilatation. Mild (a), moderate (b), and severe (c) ventricular dilatation.

Figure 3. Examples of cortical neuronal loss and gliosis. Cortex with no (a), mild (b), moderate (c), and severe (d) neuronal loss and gliosis.

Figure 4. Dentate gyrus cytoplasmic inclusions. Sparse (case 3) (a) and moderate (case 1) (b) TDP-43 positive neuronal cytoplasmic inclusions in the dentate gyrus. (both 400x).

Figure 5. Hippocampal dentate gyrus of case 5 with frequent cytoplasmic inclusions labeled with p62 (a) and with TDP-43 (b) (both 400x). Note that fewer are labeled with TDP-43 than with p62.

Figure 6. Synaptic TDP-43 labeling, CA2-4 region of the hippocampus. Sparse (case 5, CA2-3 region) (a) and moderate (case 4, CA3-4 region) (b) synaptic labeling in CA2-4 region of the hippocampus.

Figure 7. Frequent TDP-43 positive fine dystrophic neurites in CA1 of case 5 with *C9ORF72* mutation (a) and case 10 without mutation, FTLT-TDP type A (b) (400x).

Figure 8.

Cerebellar granular neurons with p62 positive cytoplasmic inclusions (case 2) (a); same field showing fewer ubiquitin positive cytoplasmic inclusions (b) (both 600x). A rare p62 positive intranuclear inclusion in a cerebellar granular neuron (arrow) (case 3) (c) (600x). Cerebellar molecular layer with p62 positive cytoplasmic inclusions in basket cells (arrows) and one Purkinje cell (asterisk) (case 3) (d) (200x).

Figure 9. Hippocampal CA3 region of case 2 showing several p62 positive cytoplasmic inclusions, some star-shaped (arrows), and numerous intranuclear dot-like inclusions (asterisks) (400x).

Figure 10. p62 positive star-shaped cytoplasmic inclusion in pyramidal neuron of frontal cortex of case 5 (600x).

Table 1

CASE #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
C9orf72 mutation	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TDP pathology																									
TDP-43 type A B C	B	B	B	B	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	B	B	C	C
NJLs <i>a</i>	0	0	1	0	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hippocampus/dentate gyrus TDP CI score <i>b</i>	5	4	2.5	5	5	4	5	4	3	1.5	5	4	4	5	3	4	4	5	5	5	5	2	4	5	5
CA1-subiculum TDP thin DNS score <i>b</i>	0.5	0.5	6	0	0	2	0	0	1	6	1	0	0	0	0	0	0	1	3	0	1	0	0	1	3
CA2-CA4 TDP "synaptic" pathology score <i>b</i>	0.5	0.5	0	4	2	2	1	2	3	0	5	0	0	0	0	0	0	3.5	0	0	0	0	0	0	0
p62+/TDP+ inclusions																									
cerebellar granular neuron inclusions	0.5	3	2	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
star-shaped inclusions, hippoc CA1-CA4	3	3	2	2	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ApoE	3,3	3,4	3,3	2,3	N/A	3,4	N/A	N/A	3,4	3,3	3,3	3,3	3,4	2,3	N/A	3,3	3,4	3,3	N/A	3,4	3,3	3,4	3,3	3,4	2,3
Demographics																									
Gender	M	F	M	F	M	F	M	F	F	M	M	M	F	M	M	M	F	M	M	M	F	F	F	F	F
Onset age	58	56	58	64	57	63	74	85	54	57	54	54	65	64	unk	59	43	65	71	62	65	60	55	63	52
Duration, yrs	3	3	7	6	5	5	10	2	4	8	6	4	3	2	unk	5	3	6	1	5	12	4	4	8	13
Clinical History																									
FF neurologic disorder	Y	unk	Y	Y	Y	N	unk	N	Y	Y	Y	Y	Y	N	N	Y	N	Y	Y	N	N	Y	N	N	Y
FF dementia/ALS, 1st or 2nd deg. relative	Y	unk	N	Y	Y	N	unk	N	Y	Y	N	N	Y	N	N	Y	N	Y	Y	N	N	N	N	N	Y
Motor neuron disease	Y	Y	N	N	N	N	N	N	N	N	Y	Y	Y	Y	Y	Y	Y	Y	N	N	Y	Y	Y	N	N
Extrapontal signs	N	Y	Y	N	N	N	unk	unk	Y	N	N	N	N	N	N	N	N	N	Y	Y	N	N	N	N	N
Psychiatric signs (incl. depression)	Y	Y	N	N	N	Y	unk	unk	Y	N	Y	Y	N	N	N	N	Y	Y	N	Y	Y	Y	Y	N	Y
Memory disorder	N	Y	Y	Y	Y	N	Y	unk	Y	N	Y	N	N	Y	N	N	Y	Y	Y	Y	N	N	N	Y	Y
Language disorder	Y	Y	Y	Y	N	Y	unk	Y	Y	Y	Y	N	Y	Y	N	Y	Y	Y	N	Y	N	N	Y	Y	Y
Behavioral/executive disorder	Y	Y	Y	Y	Y	Y	unk	unk	Y	Y	Y	Y	Y	Y	N	N	Y	Y	Y	Y	Y	N	Y	Y	Y
Initial clinical diagnosis	FTD-MND	CBS	PPA	FTD	FTD	PPA	dem	PPA	PPA	PPA	FTD	FTD-MND	PPA	FTD	ALS +dem	PPA	FTD-MND	FTD-MND	FTD-CBS	FTD	FTD	FTD-MND	FTD	FTD	FTD
Final clinical diagnosis	FTD-MND	CBS	FTD	FTD	FTD	FTD	dem	PPA	PPA	PPA	FTD-MND	FTD-MND	FTD-MND	FTD-MND	ALS +dem	PPA-ALS	FTD-MND	FTD-MND	FTD-CBS	FTD	FTD-MND	FTD-MND	FTD	FTD	FTD
Pathologic diagnosis	FTLD-MND	FTLD-MND	FTLD-TDP	FTLD-TDP	FTLD-MND	FTLD-TDP	FTLD-TDP	FTLD-TDP	FTLD-TDP	FTLD-TDP	FTLD-MND	FTLD-MND	FTLD-MND	FTLD-MND	FTLD-MND	FTLD-MND	FTLD-MND	FTLD-MND	FTLD-MND	FTLD-MND	FTLD-MND	FTLD-MND	FTLD-TDP	FTLD-TDP	FTLD-TDP
Gross pathology <i>C</i>																									
Brain wt	1400	1000	1000	1150	1040	970	1180	860	1100	1040	1280	unk	1340	1320	1480	1350	1150	1240	1240	1330	1200	1260	1220	970	810
Frontal atrophy	1	3	3	2	2	2	3	3	3	3	2	1	0	2	1	1	1	1	1	2	3	0	1	3	3
Temporal atrophy	0	2	2	2	2	1	3	3	2	2	1	1	0	1	0	1	0	1	0	1	1	0	0	3	3
Parietal atrophy	0	2	2	1	2	1	3	2	3	2	1	1	0	0	0	1	0	1	0	1	1	0	0	1	3
Motor cortex atrophy	1	2	2	1	3	1	1	1	1	1	1	1	0	0	0	0	0	1	1	1	1	0	0	1	1
Caudate atrophy	0	0	1	2	2	0	1	2	3	3	0	0	0	1	1	0	0	1	0	2	0	0	0	3	3

CASE #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Hippocampal atrophy	0	2	3	2	3	0	3	3	0	1	0	0	0	1	0	1	0	0	1	0	2	1	0	1	1
Ventricular dilation	0	3	3	2	3	1	3	2	3	3	1	0	1	2	0	2	1	2	1	1	1	0	0	2	3
Microscopic pathology ^c																									
Frontal neuronal loss/gliosis	2	3	3	1	3	1	2	2	3	3	1	0	0	0	1	1	2	0	1	2	3	1	2	1	2
Temporal neuronal loss/gliosis	0	2	2	1	2	1	1	2	2	2	0	0	0	0	0	1	1	0	0	1	1	1	1	3	2
Parietal neuronal loss/gliosis	0	2	1	0	2	1	1	1	3	3	0	0	0	0	0	1	1	0	0	1	0	1	1	1	1
Motor cortex neuronal loss/gliosis	2	2	2	1	3	1	0	1	1	1	1	1	1	1	1	1	1	0	1	0	2	1	1	1	1
Medial prefrontal cortex atrophy	1	1	0	0	1	0	0	0	0	0	2	1	2	1	2	1	3	1	3	0	1	3	1	0	1
Hypoglossal neuronal loss/gliosis	1	2	0	0	1	0	0	0	0	0	1	3	3	2	1	1	3	1	1	1	2	3	3	0	0
Hypoglossal Basaloid bodies	1	2	0	1	1	0	0	0	0	0	2	1	1	0	2	1	1	2	0	1	3	1	0	0	0
Striatal neuronal loss/gliosis	1	0	1	2	2	0	1	1	2	0	0	0	0	1	1	0	0	0	0	1	0	0	0	2	3
Hippocampal CA1 neuronal loss/gliosis	0	0	3	3	3	0	3	3	0	0	0	0	0	2	0	0	0	3	1	0	0	0	0	0	3
Substantia nigra neuronal loss/gliosis	2	2	3	1	3	2	1	2	2	3	2	2	3	2	2	2	3	3	0	2	0	2	3	1	0
Hippocampal Sclerosis ^{a,b}	0	0	1	1	1	0	1	1	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	1

N/A = not available; unk = unknown; Y=yes; N=no; NIs=neuronal intranuclear inclusions; CIs= cytoplasmic inclusions; DNs= dystrophic neurites; FTD-MND=frontotemporal dementia with motor neuron disease; CBS=corticobasal syndrome; PPA=primary progressive aphasia; FTD=frontotemporal dementia; dem=dementia, non-specific; ALS=amyotrophic lateral sclerosis; FTLD-TDP=FTLD with TDP proteinopathy

^a0=absent, 1=present;

^b see Materials & Methods for scoring method;

^c0=absent, 0.5 = rare, 1=mild, 2=moderate, 3=severe