Published in final edited form as:
J Neurol Neurosurg Psychiatry. 2013 February ; 84(2): 163–169. doi:10.1136/jnnp-2012-303507.

Cognitive decline and reduced survival in *C9orf72* expansion Frontotemporal degeneration and Amyotrophic lateral sclerosis

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Abstract

Background—Significant heterogeneity in clinical features of frontotemporal lobar degeneration (FTLD) and amyotrophic lateral sclerosis (ALS) cases with the pathogenic *C9orf72* expansion (C9P) have been described. To clarify this issue, we compared a large C9P cohort with carefully matched non-expansion (C9N) cases with a known or highly-suspected underlying TDP-43 proteinopathy.

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Contributors DJI: data analysis and interpretation and drafting and critical revision of the manuscript. CTM, JB, JP, DJL, JB, KR, JBT, AB, KC, EMW, LS, JHW, PAC, DAW, VMV, LFM, LE: acquisition and analysis of data and interpretation, administrative support and critical revision of the manuscript. SEA, VML, JQT and MG: obtained funding for support and provided critical revision of the manuscript and MG provided the concept and design and overall supervision of the study.

Ethics approval Ethics approval was provided by the Perelman School of Medicine at the University of Pennsylvania.

Data Sharing Statement

Clinical data used in this study is available in the CNDR integrated neurodegenerative disease database at the Perelman School of Medicine at the University of Pennsylvania. (Please see Xie S, et al ; Alzheimers Dement. 2011 Jul;7(4):e84-93).

Methods—A retrospective-cohort study using available cross-sectional and longitudinal clinical and neuropsychological data, MRI voxel-based morphometry (VBM) and neuropathological assessment from 64 C9P cases (ALS=31, FTLD=33) and 79 C9N cases (ALS=36, FTLD=43).

Results—C9P cases had an earlier age of onset (p=0.047), and in the subset of deceased patients, an earlier age of death (p=0.014) than C9N. C9P had more rapid progression than C9N: C9P ALS cases had a shortened survival (2.6±0.3 years) compared to C9N ALS (3.8±0.4 years; log-rankλ^2=4.183,p=0.041), and C9P FTLD showed a significantly greater annualized rate of decline in letter fluency (4.5±1.3words/year) than C9N FTLD (1.4±0.8words/year, p=0.023). VBM revealed greater atrophy in the right fronto-insular, thalamus, cerebellum and bilateral parietal regions for C9P FTLD relative to C9N FTLD, and regression analysis related verbal fluency scores to atrophy in frontal and parietal regions. Neuropathologic analysis found greater neuronal loss in the mid-frontal cortex in C9P FTLD, and mid-frontal cortex TDP-43 inclusion severity correlated with poor letter fluency performance.

Conclusions—C9P cases may have a shorter survival in ALS and more rapid rate of cognitive decline related to frontal and parietal disease in FTLD. *C9orf72* genotyping may provide useful prognostic and diagnostic clinical information for ALS and FTLD patients.

Keywords

Frontotemporal dementia; Amyotrophic lateral sclerosis; *C9orf72*; neuropsychological tests; neuroimaging

INTRODUCTION

Over the past decade, significant evidence emerged supporting a clinicopathological continuum between amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD).[1] This includes cognitive impairment in up to 50% of ALS patients,[2] subclinical motor-neuron disease in FTLD,[3] the presence of neuronal and glial inclusions composed of the RNA-binding protein TDP-43 in both diseases,[4] and families with members afflicted with one or both disorders linked to a locus on chromosome 9.[5]

The recently discovered hexanucleotide-expansion in the *C9orf72* gene appears to be the most common genetic cause of familial[6,7] ALS and FTLD, and is also found in a number of apparently sporadic cases.[8] Due to the high prevalence of this mutation, information on the natural history and clinical features of this hereditary degenerative condition is very important for prognostic and diagnostic considerations. Previous reports find considerable heterogeneity in the clinical phenotype and demographic features of expansion-positive cases (C9P) as compared with non-autopsy-confirmed sporadic expansion-negative (C9N) cases.[9–11] C9P cases have been shown to have an earlier age of onset[8,9,12–14] and to further assess the hypothesis that C9P cases may progress more rapidly, we evaluated longitudinal clinical data in C9P compared to C9N cases with known or highly-suspected TDP-43 neuropathology. Comparative studies using C9N FTLD cases with underlying TDP-43 neuropathology are essential to identify specific characteristics of this expansion, as care must be taken to minimize tauopathies (FTLD-tau) or atypical presentations of Alzheimer's disease (AD) that may contaminate a clinically-derived FTD reference cohort.[15]

METHODS

Patients

Patients were initially seen at the University of Pennsylvania Frontotemporal Degeneration Center (FTDC), ALS Center (ALSC), or Alzheimer's Disease Center (ADC). The clinical

diagnosis of ALS was made using the El Escorial-revised criteria[16] and FTLD clinical phenotypes using established clinical criteria.[17,18] Cognitive impairment in two ALS patients not meeting criteria for FTD was diagnosed clinically as non-amnestic mild cognitive impairment (ALS-MCI) and grouped with ALS cases for analyses below. Family history was assessed using a modified Goldman score.[19] Briefly, score 1= autosomal dominant (≥ three people in two generations with any combination of FTD or ALS with one person being a first-degree relative (FDR) of the other two), 2= familial aggregation (≥three relatives with dementia or ALS and criteria for autosomal dominant inheritance were not met), 3= a single affected FDR with dementia or ALS (age ≤65), 3.5= a single affected FDR with dementia or ALS (>65), and 4= no contributory family history or unknown family history. All procedures were performed in accordance with the Institutional Review Board at the University of Pennsylvania using an approved written consent procedure.

Genetic testing for the *C9orf72* hexanucleotide-repeat was performed in 222 patients (except one obligate carrier) with a clinical or neuropathological diagnosis of ALS and/or FTLD (67 C9P, 155 C9N).

After exclusion of cases with minimal clinical data and those likely to have neuropathologies other than TDP-43 in the C9N group,[15,18,20–23] the final cohort consisted of sixty-four C9P cases and seventy-nine C9N (Figure 1). All C9P cases were unrelated except for two pairs. In the C9N group there were seven unrelated cases with known pathogenic progranulin (GRN) mutations[24] (GRN c.348A>C (p.Ser116Ser, predicted to cause splice site mutation), p.Arg110X, p.Arg418X, c.1179+2T>C (predicted to cause p.Val395TyrfsX29), p.Ser226TrpfsX28, p.Thr272SerfsX10, p.Asp441HisfsX4) known to have underlying TDP-43 neuropathology.[23] Since our main outcome measures involve cognition and survival, patients were divided into two groups according to cognitive status: FTLD (FTLD/ALS-FTLD) and ALS without dementia (ALS; Table 1).

Genetic Analysis

DNA was extracted from peripheral blood or brain tissue and genotyped for the *C9orfF72* hexanucleotide-repeat using a modified repeat-primed polymerase-chain reaction[7] as previously described.[25] *GRN* mutation testing of the cohort had been done previously.[24]

Neuropsychological Testing

Neuropsychological test scores were selected within six months of neuroimaging or, in the absence of neuroimaging, at the earliest testing visit. Longitudinal analysis included data from the most complete subsequent testing visit > six months after baseline (median=13.8, range=6.2–43.4 months). Tests included the Mini-Mental State examination (MMSE),[26] and letter-guided verbal fluency. We focused on verbal fluency because of earlier reports demonstrating difficulty on executive measures in C9P.[27,28] Verbal fluency scoring was determined by the total number of novel “F words” generated in one minute, with the exclusion of proper nouns and numbers. An annualized decline score was calculated by dividing the change in score between visits by the time interval (years) between evaluations. Additional cognitive measures available in smaller numbers of subjects are summarized in Supplementary Materials.

MRI Acquisition and Analysis

A subset of FTLD patients (n=41) underwent a structural T1-weighted MPRAGE MRI acquired from a SIEMENS 3.0T Trio scanner with an 8-channel coil using the following parameters: repetition time=1620 msec; echo time=3 msec; slice thickness=1.0 mm; flip angle=15°; matrix=192×256, and in-plane resolution=0.9×0.9 mm. Whole-brain MRI volumes were preprocessed using PipeDream (https://sourceforge.net/projects/

neuropipedream/) and Advanced Normalization Tools (http://www.picsl.upenn.edu/ANTS/), as described[2930] and resampled to $2mm^3$ voxels. We analyzed cortical thickness[31] for the cerebrum. We performed a separate grey matter (GM) density analysis of the cerebellum because of the distinct anatomic characteristics of this region.

Analyses were performed using FSL's randomise module (www.fmrib.ox.ac.uk/fsl/randomise). Group differences were evaluated using an explicit mask to constrain voxelwise comparisons to regions of GM and we used permutation-based (N=5000) methods for each test. For each patient group compared to healthy seniors, we report clusters that survive a $p<0.05$ (FDR-corrected) threshold and contain a minimum of 50 adjacent voxels. For direct comparison of C9P and C9N, we report clusters that survive a $p<0.01$ (uncorrected) threshold and contain a minimum of 50 adjacent voxels. We additionally performed a nonparametric linear regression analysis using FSL's randomise module to relate verbal fluency performance in imaging subjects to regions of significant GM disease. We constrained our analysis to regions of disease using an explicit mask generated from the direct comparisons of patient groups, since interpretation of a result in a region without disease would otherwise be difficult. For example, a significant brain-behavior relationship in areas that did not differ between groups could be attributed to a variety of non-specific factors.

Neuropathological Examination

Autopsy was performed on a subset of cases (n=64) using standard techniques as previously described.[15] To evaluate the severity of neurodegeneration, hematoxylin-and-eosin stained slides were graded by trained examiners (DJI, JB) blinded to C9 status using a semi-quantitative scoring system (0=none, 1=mild, 2=moderate, and 3=severe) for neuronal loss and gliosis in four cortical regions, thalamus and granule layer of the cerebellum. A random-sample of 20% of regions were re-rated independently by examiners (DJI, JBT) to evaluate inter-rater and intra-rater reliability, and Spearman's correlations confirmed high reliability ($r=0.84$; $p<0.001$ and $r=0.81$; $p<0.001$, respectively). Ubiquilin (UBQLN) neuropathology was also examined using techniques as previously described.[25]

Statistical Analysis

Between-group comparisons for clinical data were performed using independent-sample t-tests, one-way and repeated-measure ANOVAs and χ^2 analyses, as appropriate. We report mean values ± standard error. Survival was calculated based on interval from the reported onset of symptoms to death and analyzed using a Kaplan-Meier log-rank analysis. Non-normally distributed semi-quantitative neuropathologic scores and annualized neuropsychological decline scores were compared between groups using non-parametric Mann-Whitney U rank sum tests. Correlations between clinical and neuropathologic variables were performed using Spearman correlations. We performed two-sided tests with a $p<0.05$ level of significance using SPSS 19.0 (SPSS, Chicago, Ill).

RESULTS

Clinical Features and Neuropsychological Testing

The C9P group consisted of sixty-four patients (31 ALS, 33 FTLD) and the C9N group contained seventy-nine patients (36 ALS, 43 FTLD) (Figure 1). There were no significant differences in gender, percentages of clinical phenotypes, education and race between groups. There was a non-significant trend for a lower Goldman score (more substantial family history) in C9P than C9N ($p=0.088$). C9P cases had a significantly earlier age of onset ($p=0.047$), and in a subset of non-living cases, earlier death ($p=0.014$) compared to

C9N (Table 1). Subsequent analyses were performed separately on FTLD and non-demented ALS subgroups.

The majority of the C9P FTLD clinical phenotype was behavioral-variant FTD (bvFTD) (17/24), with one case of semantic variant primary progressive aphasia (svPPA) and four cases of non-fluent/agrammatic variant PPA (naPPA). Two C9P FTLD cases were clinically diagnosed with probable AD[32] during life and had a neuropathological diagnosis of FTLD-TDP. The C9N FTLD group had a similar predominance of bvFTD (23/33). C9P FTLD cases had no significant difference in the age of onset, death or onset-death interval compared with the C9N FTLD group (Table 1).

Neuropsychological data were available for twenty-eight C9P FTLD patients and forty-one C9N FTLD patients. Baseline testing was performed at approximately the same age and stage of illness relative to reported onset of symptoms for both groups (Table 2). There was no significant difference between cross-sectional and longitudinally studied cases in race, education, gender, or clinical phenotype.

C9P and C9N FTLD patients had similar mean baseline MMSE (C9P=23.3, C9N=24.7) and verbal fluency (C9P=6.2, C9N=6.4) scores (Table 2). A subset of these patients had follow-up testing data for MMSE (C9P=15, C9N=24) and verbal fluency (C9P=10, C9N=16), with similar time intervals between evaluations. There was a significantly greater decline in mean annualized letter fluency in C9P FTLD (4.5 words/year) compared to C9N FTLD (1.4 words/year; p=0.023). There was also a trend for a greater annualized decline in MMSE scores in C9P FTLD (p=0.078; Table 2). Repeated-measure ANOVAs found significant interaction of C9 status with MMSE [$F(1,37)=5.2$, p=0.028] and C9 status with verbal fluency [$F(1,24)=6.6$, p=0.017] scores between visits, suggesting more rapid decline on measures of global (MMSE) and executive-mediated (letter fluency) cognition for C9P FTLD compared with C9N. Individual case analysis found the majority of C9P FTLD with ≥3 word/year decline in letter fluency and ≥4 points/year in MMSE (Supplementary Table1). Neither second visit MMSE or verbal fluency scores, nor annualized rates of decline for these tests correlated with the interval between testing. Additional neuropsychological testing showed confrontation naming and other executive impairments in both C9P and C9N FTLD at baseline and at follow-up assessment, which was available for a minority of patients (Supplementary Table 2).

C9P ALS patients had a younger age of onset (p=0.044), death (p=0.003) and shorter onset-death interval (p=0.035) relative to C9N ALS cases. Additionally, Kaplan-Meier log rank analysis found a significantly shorter survival in C9P ALS (Figure 2; log-rankλ^2=4.183, p=0.041). Site of onset data was available for twenty-six C9P ALS patients, and this group consisted of similar numbers of bulbar (7/26), cervical (8/26) and lumbar (10/26) onset cases, with one thoracic-onset patient and no significant difference in onset site relative to C9N ALS.

Thirty-six C9P ALS and twenty-four C9N ALS patients had neuropsychological data available. Mean baseline MMSE (29.3 C9P, 29.4 C9N) and verbal fluency (11.9 C9P, 12.0 C9N) were similar between C9P and C9N ALS groups. Longitudinal data was available for MMSE (C9P=5, C9N=6) and verbal fluency (C9P=9, C9N=11). Although C9P ALS cases had a greater mean annualized decline in letter fluency (1.8 words/year) compared to C9N ALS (−0.1 words/year), no statistical differences in annualized decline were found in these small groups of ALS cases (Supplementary Table 2).

Neuroimaging Analysis

MRI data were available for forty-one FTLD cases (14 C9P, 27 C9N). There were no significant demographic differences between patient subgroups and elderly controls (Supplementary Table 3). Permutation analysis of GM thickness found significant bilateral GM thinning for both C9P and C9N relative to elderly controls (Supplementary Table 4). Direct comparison of GM thickness revealed significantly greater thinning in C9P relative to C9N cases in right frontal, fronto-insular, cingulate, occipital and thalamic regions and bilateral parietal cortex (Figure 3A, Supplementary Table 4). The reverse comparison revealed no significant GM thinning for C9N relative to C9P patients. An analysis of the cerebellum found two significant clusters of reduced GM density in C9P compared to C9N (Figure 3B, Supplementary Table 4) with no significant clusters in the reverse analysis.

Regression analysis revealed a significant relationship between poor baseline verbal fluency performance and GM thinning in right fronto-insular, inferior-parietal and thalamic regions (Figure 3C, Supplementary Table 4). We observed no relationship between verbal fluency and GM density in the cerebellum.

Neuropathological Examination

Twenty-five FTLD patients from our cohort were followed to autopsy (C9P=13, C9N=12). Cases did not differ by post-mortem interval or brain weight (Supplementary Table 5). Similar to neuroimaging findings of GM thinning in C9P FTLD, semi-quantitative analysis found more severe neuronal loss and gliosis in mid-frontal cortex ($p=0.022$, $p=0.005$, respectively) and trends for thalamus and angular gyrus in C9P FTLD compared with C9N FTLD (Table 3). As we described previously,[25] there was no appreciable granule cell loss or gliosis observed in the cerebellum. C9P and C9N FTLD did not differ significantly in TDP-43 severity or cortical distribution. However, we found a predominance of TDP harmonized[33] subtype B (10/13; A=2/13, C=1/13), and hippocampal sclerosis was present in only one case (Supplementary Table 5). UBQLN inclusion burden was more severe in C9P FTLD for all cortical regions sampled and also in the granule layer of the cerebellum where there was almost exclusive involvement in C9P cases.

Comparison of C9P (n=12) and C9N (n=27) ALS cases showed equally low levels of extra-motor cortical neuronal loss, gliosis and TDP-43 inclusions (data not shown), and a detailed report of UBQLN burden in ALS has been previously described.[25]

Due to our imaging finding of increased frontal, parietal, cerebellar, and thalamic atrophy in C9P FTLD cases we performed additional tests to see if these were related to pathological markers of neurodegeneration. UBQLN severity in the mid-frontal cortex and angular gyrus correlated with neuronal loss and gliosis in these regions (all $r>0.5$, $p<0.05$; angular gyrus/ neuronal loss $r=0.399$, $p=0.054$) and inversely correlated with overall post-mortem brain weight (all $r<-4.8$, $p<0.05$). TDP-43 pathology in these regions did not correlate with neuronal loss in the midfrontal cortex, angular gyrus, or post-mortem brain weight ($p>0.05$).

We also evaluated whether reduced verbal fluency in C9P FTLD was related to pathological markers of neurodegeneration in these regions. Baseline verbal fluency score inversely correlated with TDP-43 severity in the mid-frontal cortex ($r=-0.731$, $p=0.016$) and UBQLN in the angular gyrus ($r=-0.706$, $p=0.022$). Neither TDP-43 nor UBQLN severity in the thalamus or cerebellum correlated with verbal fluency ($p>0.05$).

DISCUSSION

A comprehensive, multi-modal evaluation of one of the largest reported C9P FTLD and ALS cohorts reveals that, relative to C9N, C9P FTLD cases had more rapid cognitive

decline in verbal fluency and the Mini-Mental State exam and C9P ALS cases had a shorter survival. In C9P FTLD, direct correlations of performance with imaging and autopsy studies appear to relate this most closely to disease in frontal and parietal brain regions. By selecting C9N cases highly likely to have underlying FTLD-TDP neuropathology, we can speculate that these results specifically reflect the intrinsic effects of the pathogenic *C9orf72* expansion.

The demographic characteristics of our C9P patients are similar to previous reports with a predominance of bvFTD clinical phenotype,[9–11192728] a small number of clinically diagnosed AD cases,[11193435] and many cases with significant family history.[6–1012–14192527283536]

Previous cross-sectional analyses found marked executive dysfunction[2728] in C9P FTLD. While our data showed poor baseline cognitive performance for both C9P FTLD and closely matched cases of C9N FTLD, quantitative longitudinal neuropsychological testing provides novel evidence to suggest an increased rate of cognitive decline on a measure of executive functioning and a measure of global cognitive functioning in the majority of C9P FTLD cases. Further prospective detailed neuropsychological analysis of C9P patients will help confirm these findings.

Our neuroimaging analyses indicate a likely neuroanatomic substrate for these clinical observations in C9P. Previous studies describe similar patterns of cortical[11192837] and subcortical[1937] atrophy in C9P FTLD, including prominent parietal lobe and cerebellar involvement, and some reports note hemispheric symmetry compared with other hereditary forms of FTLD.[192837] Our findings also show bilateral disease compared to controls (Supplementary Table4). However, we found more prominent right-sided disease in the direct comparison of C9P to C9N FTLD. Furthermore, there were no areas of increased atrophy in C9N relative to C9P FTLD, suggesting more severe disease burden in C9P FTLD. These selective differences in C9P may reflect in part our careful selection of C9N cases, as we minimized confounds associated with unrelated neuropathologies (i.e. FTLD-tau and AD) that can be found in clinically defined FTD (i.e. C9N) reference cohorts[1520] used in one previous comparative study.[37] Additionally, other studies limited comparisons largely to healthy controls[1928] or qualitative assessments alone.[1127] Significant posterior parietal disease is an atypical neuroimaging feature in both sporadic and other hereditary forms of FTLD,[37] suggesting a unique pathophysiology of C9P FTLD that could serve as a potentially useful biomarker for the *C9orf72* expansion. Although verbal executive measures like letter-guided naming fluency are mediated bilaterally,[3839] our regression analysis found associations with mainly right sided areas, including the right fronto-insular region. This may reflect in part the asymmetric burden of disease in our direct comparison of C9P and C9N FTLD.

The analysis of neuronal loss and gliosis in the autopsy cases compliments our neuroimaging findings, as these areas of GM atrophy were also evident in the frontal, parietal and thalamic regions of patients studied at autopsy. In addition, we found UBQLN inclusions to be more severe in C9P cases and this correlated well with measures of neurodegeneration. Furthermore, both TDP-43 inclusions in the frontal lobe and UBQLN severity in parietal regions correlated with poor verbal fluency performance. While several years intervened between clinical evaluation and death, the observation of similar findings in imaging studies obtained at the same time as cognitive measures supports the clinical-pathological association we found. Together, these correlations appear to implicate frontal and parietal regions in declining cognition observed in C9P FTLD. It is intriguing that UBLN pathology was more closely correlated to neuronal loss than TDP-43. This may indicate that UBLN pathology is a further downstream event in the neurodegenerative

process in C9P and may contribute to the areas of increased cortical thinning seen in the VBM analysis of C9P FTLD compared with C9N FTLD. Future detailed clinical-pathological correlations and cell- and animal-model experiments are required to clarify these interactions in the complex pathophysiology of C9P FTLD and ALS.

Slowly progressive or late onset C9P FTD patients[8,11,19,28,40] and cases with minimal cortical atrophy[10,11,28] have been described. Indeed, individual analysis of our C9P FTLD cohort reveals a wide range of age at onset (44–69) and survival (1.5–12.7 years). However, the majority of individual patients tested had substantial annualized decline in MMSE and letter fluency (Supplementary Table1), and all imaged patients had significant cortical atrophy on qualitative inspection. Thus, our results suggest that most C9P FTLD appear to clinically progress more rapidly than other forms of FTLD. Further understanding of the pathogenicity of expansion length and possible anticipation effect is necessary to fully resolve observations of phenotypic heterogeneity.

The observed shorter disease duration and survival in C9P ALS has been reported by some groups,[13,14,25] while others did not find this difference.[36] The distribution of the site of onset in the C9P ALS group in our study was similar to the general ALS population, as in some previous reports[9,13,14,28,34] and contrary to others that report a majority of bulbar-onset phenotype.[12,25,27,36] These discrepancies may be due in part to sample size and control patient selection. Indeed, we found marginal statistical significance in our analysis and these results will require confirmation in large prospective series. Some have also described more severe executive dysfunction in C9P ALS cases[14] corresponding to extra-motor frontal cortical atrophy on MRI[14] and high rates of co-morbid dementia.[12,13,36] Our cohort of C9P ALS only showed a trend for greater annualized decline in letter fluency compared to C9N ALS. This may reflect inclusion of ALS-FTLD in the FTLD subgroup analyses, the small number of ALS cases with cognitive testing, or the potential relatively rapid rate of motor progression in ALS. Further study with larger, prospective ALS datasets would be beneficial to clarify these observations.

Our study is limited due to the retrospective nature of data collection and small sample sizes in some subgroup analyses. While we attempted to limit the C9N cases to TDP-43 proteinopathies, there may be non-deceased patients with other, co-occurring neuropathologies, as TDP-specific biomarkers are currently lacking. Keeping in mind these limitations and others mentioned above, our findings suggest that C9P may confer more rapid cognitive decline in the majority of FTLD and reduced survival in ALS. Our observations have significant implications for clinical trial design, as C9P patients could influence outcome measures of cognition or survival.[14] Future prospective longitudinal study of C9P will be crucial to confirm our findings here and help further characterize the clinical heterogeneity of cases. The potential impact of the *C9orf72* hexanucleotide repeat on prognosis, and its occurrence in apparently sporadic cases, will be of utmost importance to elucidate, both for clinical practice and for the evaluation and implementation of emerging disease-modifying treatments.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We would like to thank the patients and their families for their kind contributions to make this study possible.

Funding This study was supported by the NIH P30AG010124, P01 AG017586, R01 NS44266, R01 AG15116, P01 AG32953, P01 NS53488, R21 NS06311 and the Wyncote Foundation. DJI is supported by the NIH T32-

AG000255, CTM is support by HD060406, JB is supported by a grant of the Deutsche Forschungsgemeinschaft DFG (AOBJ586910) and JBT is supported by a grant of the Alfonso Martín Escudero Foundation.

Competing interests DAW has received research support from NIH (R01 AG037376, R01 MH086492, K23 AG028018, P30 AG010124), GE Healthcare, and Pfizer, Inc. He has served as a consultant for GE Healthcare and Leclair Ryan. He received speaking honoraria from the American Academy of Neurology. SAE reports board membership for Cowan Group, Eli Lilly and BMS; Consulting services for Philadelphia district attorney's office and Bonner Kiernan Trebach & Cociata LLP; Grants from American College of Radiology Imaging Network, Pfizer, Alzheimer's Disease Neuroimaging Initiative, Johnson & Johnson, National Institute of Drug Abuse, Neuronetrix, National Institute of Mental Health, Eli Lilly; Payments for lectures at Harvard University, Rush University Medical Center, Trinitas Regional Medical Center, and University of Puerto Rico. VML and JQT report single consulting services to Pfizer, J&J, MetLife, and BMS; Royalty payments through Penn licenses; and research support from AstraZeneca and BMS. DJI, CTM, JB, DJL, AB, JP, JB, KC, EMW, JBT, LS, KR, JHW, PAC, VMV, LFM, LE and MG have no potential financial relationships of activities within the previous three years that could appear to have influenced the current work.

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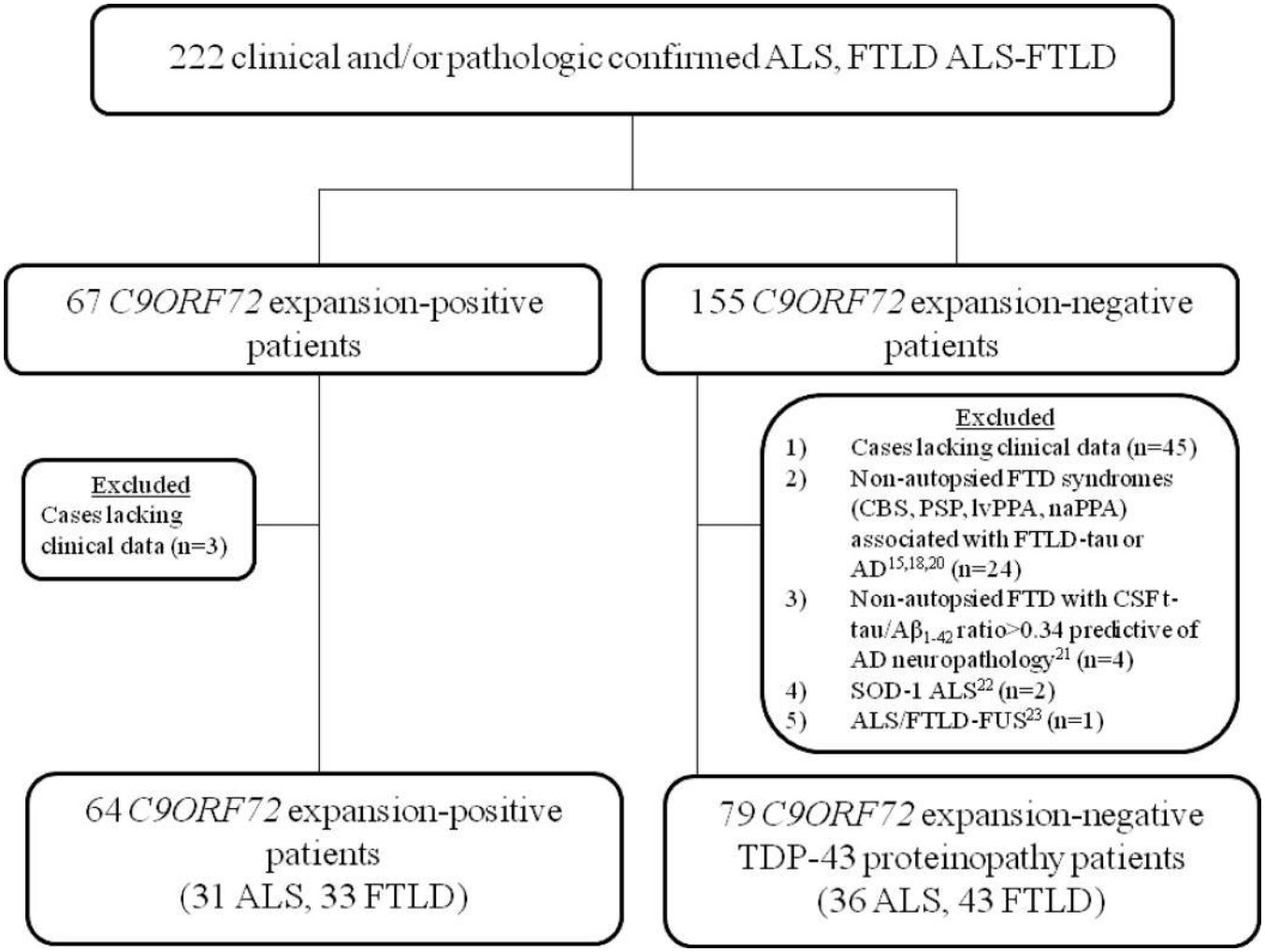

Figure 1. Flow chart of subjects included in analysis

Abbreviations: CBS corticobasal syndrome, PSP progressive supranuclear palsy, naPPA non-fluent/agrammatic variant-primary progressive aphasia, lvPPA logopenic variant PPA

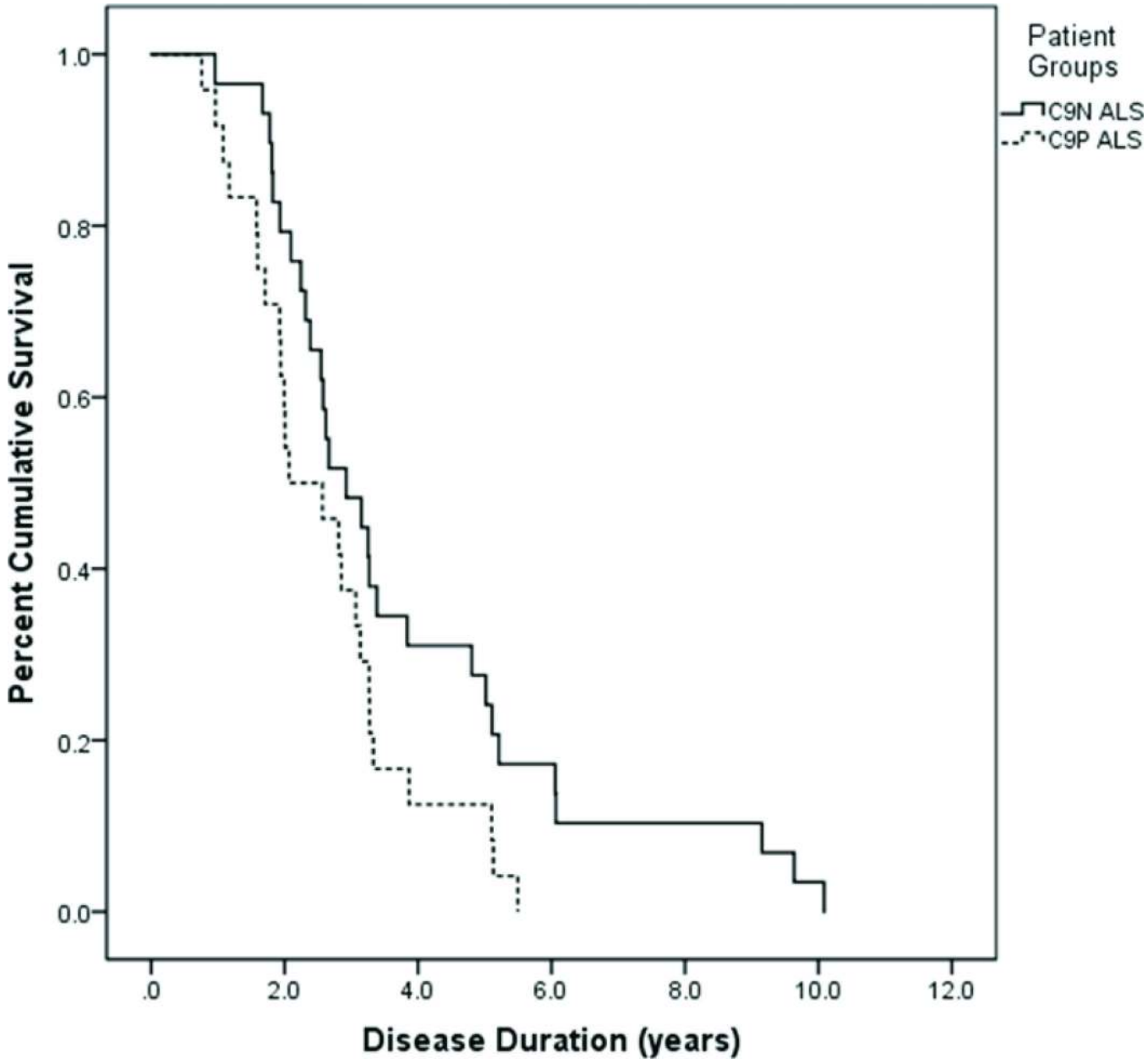

Figure 2.
Kaplan-Meier curve analysis of survival of ALS patients with (C9P=dashed line) and without (C9N=solid line) the pathogenic *C9orf72* hexanucleotide expansion (log-rank λ^2=4.183, p=0.041).

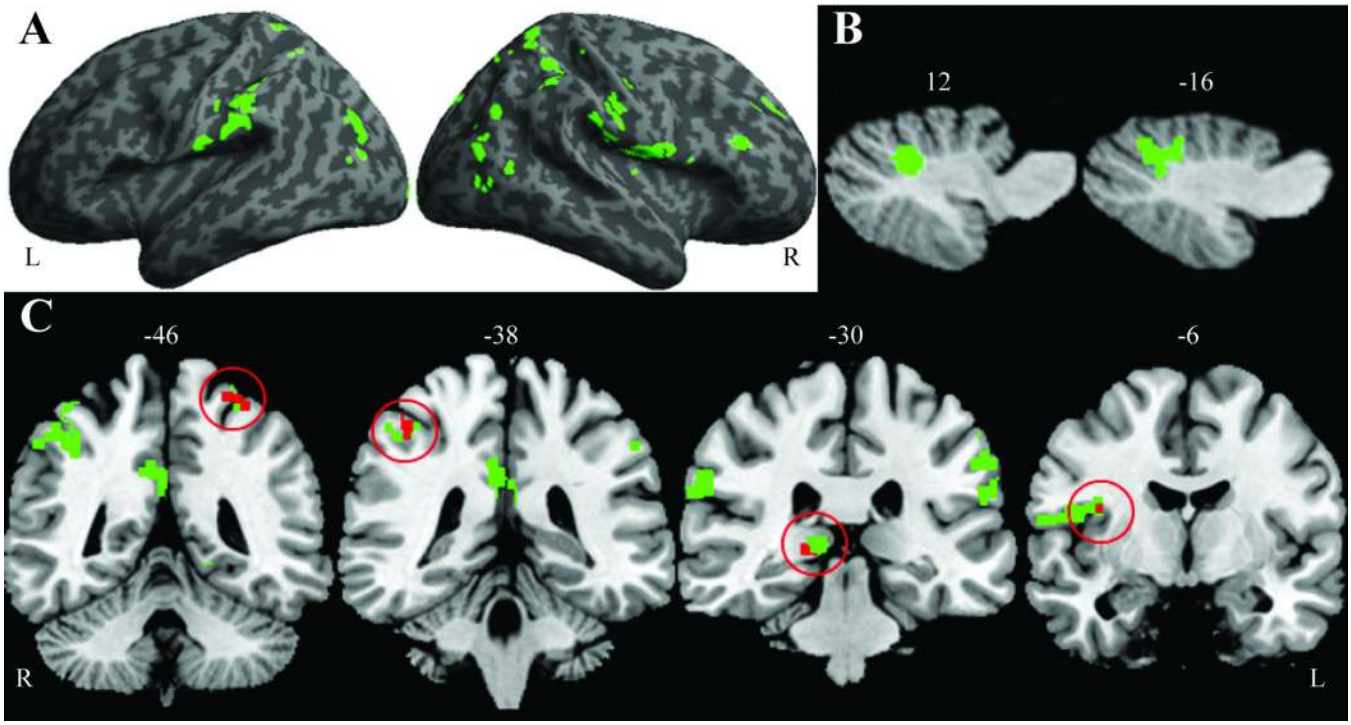

Figure 3.
Green regions illustrate areas of significant cortical atrophy in C9P compared directly to C9N FTLD for cerebrum (A) and cerebellum (B). Coronal slices (C) illustrate bilateral parietal and right frontal and thalamic regions (green) that have significant atrophy in C9P FTLD, and a subset (red) that correlate with poor verbal fluency performance. Numbers indicate sagittal (B) and coronal (C) slice axes location.

Table 1

Patient Demographic Information

	C9orf72 Expansion Positive	*C9orf72* Expansion Negative	P value
N (M/F)	64 (39M/25F)	79 (43M/36F)	0.435
Clinical Phenotype N(%)	• ALS 31 (48.4%)	ALS 36 (45.6%)	0.542
	ALS 30	ALS 35	
	ALS-MCI 1	ALS-MCI 1	
	• FTD 22 (34.4%)	• FTD 32 (40.5%)	
	bvFTD 17	bvFTD 23	
	svPPA 1	svPPA 7	
	naPPA 4	CBS 2	
	• ALS-FTD 9 (14.1%)	• ALS-FTD 10 (12.7%)	
	ALS-bvFTD 9	ALS-bvFTD 9 ALS-naPPA 1	
	• AD 2 (3.1%)[†];	• AD 1 (3.1%)[†]	
	AD-probable 2	lvPPA 1	
Family History Goldman Score N(%)			
1	10/62(16.1%)[‡]	5/72 (6.9%)[‡‡]	0.088
2	8/62 (12.9%)	5/72 (6.9%)	
3	10/62 (16.1%)	7/72 (9.7%)	
3.5	4/62 (6.5%)	11/72 (15.3%)	
4	30/62 (48.4%)	44/72 (61.1%)	
Age of Onset, years			
Mean (SEM)			
Total	55.8 (1.0)	58.8 (1.1)	**0.047**
ALS	55.1 (1.7)	60.3 (1.9)	**0.044**
FTLD	56.4 (1.2)	57.4 (1.2)	0.533
Age at Death, years			
Mean (SEM)[††]			
Total	60.1 (1.5) n=43	65.3 (1.4) n=49	**0.014**
ALS	58.0 (1.9) n=24	66.3 (1.9) n=29	**0.003**
FTLD	62.9 (2.3) n=19	63.9 (2.1) n=20	0.758
Disease Duration, years			
Mean (SEM)[††]			
Total	4.0 (0.4) n=43	4.6 (0.5) n=49	0.302
ALS	2.6 (0.3) n=24	3.8 (0.4) n=29	**0.035**
FTLD	5.7 (0.8) n=19	5.8 (0.9) n=20	0.897

[†]All AD clinical phenotype cases were pathologically confirmed FTLD with TDP-43 inclusions (FTLDTDP).

[††]Analysis performed on subset of deceased patients.

[‡]Positive family history data from two pairs of related individuals were omitted due to redundancy.

[##]Family history data from seven C9N *GRN* mutations cases were removed from the analysis.

Table 2

Neuropsychological test data in FTLD with and without the *C9orf72* repeat expansion.

	***C9orf72* Expansion Positive FTLD**	***C9orf72* Expansion Negative FTLD**	**P value**
Age at baseline testing, years	59.7 (1.4) N=28	61.2 (1.2) N=41	0.422
Onset-baseline testing interval, years	3.3 (0.5) N=28	3.8 (0.4) N=41	0.440
Baseline-follow up testing interval, months	14.2 (1.7) N=17	15.2 (1.6) N=25	0.695
MMSE baseline score **MMSE annualized decline**	23.3 (1.4) N=26 4.8 (1.2) N=15	24.7 (0.9) N=36 2.7 (1.1) N=24	0.505 0.078
Verbal Fluency baseline score **Verbal Fluency annualized decline**	6.2 (0.9) N=19 **4.5 (1.3)** **N=10**	6.4 (0.8) N=28 **1.4 (0.8)** **N=16**	0.777 **0.023**

Table 3

Pathologic analysis of neurodegeneration in FTLD with and without the *C9orf72* repeat expansion.

		C9orf72 Expansion Positive FTLD (N=13)	*C9orf72* Expansion Negative FTLD (N=12)	P value
Mid-Frontal Cortex	Gliosis	**2 (2,3)**	**1 (0.25,2)**	**0.022**
	Neuronal Loss	**2 (2,3)**	**2 (1,2)**	**0.005**
	TDP-43 *	3 (2,3)	2 (1.25,3)	0.284
	Ubiquilin-2 **	**2 (1,2)**	**0 (0,1)**	**0.001**
Superior-Mid Temporal Cortex	Gliosis	1 (0.5,2)	2 (1,2)	0.335
	Neuronal Loss	2 (1,2.5)	2 (1,2.75)	0.662
	TDP-43	3 (2,3)	3 (2.75, 3)	0.575
	Ubiquilin-2 *	**2 (1,2)**	**1 (0,1.75)**	**0.050**
Angular Cortex	Gliosis	1 (0.5,1.5)	0.5 (0,2)	0.688
	Neuronal Loss	2 (1,2)	1 (1,2)	0.771
	TDP-43	2 (1,3)	2 (1.25,3)	0.909
	Ubiquilin-2 *	**2 (1,2)**	**0 (0, 1.5)**	**0.002**
Cingulate Cortex	Gliosis	0 (0, 1.5)	1 (0.25,1.75)	0.298
	Neuronal Loss	1 (1,2)	1 (1,2)	0.953
	TDP-43 *	2.5 (1.25,3)	3 (2,3)	0.328
	Ubiquilin-2 *	**1.5 (1,2)**	**0 (0,1)**	**0.002**
Thalamus	Gliosis	1 (0,1)	0 (0,0.75)	0.198
	Neuronal Loss	1 (0,1)	0 (0,0.75)	0.109
	TDP-43	2 (0,2)	1 (0.25,2.0)	0.842
	Ubiquilin-2	0 (0, 0.5)	0 (0,0)	0.286
Granule Layer, Cerebellum	Gliosis	0 (0,0)	0 (0,0)	1.000
	Neuronal Loss	0 (0,0)	0 (0,0)	1.000
	TDP-43 *	0 (0,0)	0 (0,0)	0.317
	Ubiquilin-2	**3 (1.5,3)**	**0 (0,0)**	**0.001**

* Data missing for one case,

** Data missing for three cases