Characterization of a Family With c9FTD/ALS Associated With the GGGGCC Repeat Expansion in C9ORF72

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Background: The hexanucleotide repeat in the chromosome 9 open reading frame 72 (C9ORF72) gene was recently discovered as the underlying genetic cause of many families with frontotemporal dementia (FTD) and/or amyotrophic lateral sclerosis (ALS) linked to chromosome 9 (c9FTD/ALS). We report the clinical, neuropsychologic, and neuroimaging findings of a family with the C9ORF72 mutation and clinical diagnoses bridging the FTD, parkinsonism, and ALS spectrum.

Objective: To characterize the antemortem characteristics of a family with c9FTD/ALS associated with the GGGGCC repeat expansion in C9ORF72.

Design: Clinical series.

Setting: Tertiary care academic medical center.

Patients: The members of a family affected by the mutation with features of FTD and/or ALS.

Main Outcome Measures: Clinical, neuropsychologic, and neuroimaging assessments.

Results: All 3 examined subjects had the hexanucleotide expansion detected in C9ORF72. All had personality/behavioral changes early in the course of the disease. One case had levodopa-unresponsive parkinsonism, and 1 had ALS. Magnetic resonance imaging showed symmetric bilateral frontal, temporal, insular, and cingulate atrophy.

Conclusions: This report highlights the clinical and neuroimaging characteristics of a family with c9FTD/ALS. Further studies are needed to better understand the phenotypic variability and the cliniconeuroimaging-neuropathologic correlations.


FRONTOTEMPORAL DEMENTIA (FTD) is a neurodegenerative disorder characterized by significant abnormalities of frontal and/or temporal lobes that result clinically in personality/behavioral changes and/or progressive aphasias. Frontotemporal dementia may occur in a sporadic or familial form. The first major gene associated with frontotemporal dementia with or without parkinsonism was microtubule-associated protein tau (MAPT), mutations of which lead to the cascade of hyperphosphorylated tau. Mutations in the gene encoding progranulin (PGRN) also cause FTD with or without parkinsonism, with ubiquitin-immunoreactive and TAR DNA-binding protein 43–immunoreactive inclusions being the pathologic hallmark. Many kindreds with FTD with or without amyotrophic lateral sclerosis (ALS) with TDP-43-immunoreactive inclusions were linked to chromosome 9, and the hexanucleotide repeat in the chromosome 9 open reading frame 72 (C9ORF72) gene was recently discovered as the underlying genetic cause. Such cases are now collectively termed under the c9FTD/ALS terminology. We report the clinical, neuropsychologic, and neuroimaging findings of a family with the C9ORF72 mutation and clinical diagnoses bridging the FTD, parkinsonism, and ALS spectrum.

METHODS

SUBJECTS

Three subjects underwent clinical evaluations at other clinics, and then were referred to our institution and enrolled in the Mayo Alzheimer Disease Research Center—a Mayo Foundation institutional review board–approved program. The subjects and/or their spouses provided written consent for participation. All additional data from affected relatives were collected and analyzed.
CLINICAL AND NEUROPSYCHOLOGIC DATA

Age at onset of dementia was the age at which the subject first demonstrated behavioral/personality and/or cognitive changes as noted by themselves, their relatives, or their clinicians. Age at onset of ALS was the age at which any symptom reflecting upper motor neuron and/or lower motor neuron dysfunction was noted by themselves, their relatives, or their clinicians. Survival (in years) was based on the number of years from onset of any neurologic symptoms to death. All neurologic clinical data were reviewed. The presence or absence of the following clinical features were recorded: behaviorality/personality changes (self-explanatory), executive dysfunction (defined as impairment in sustained attention, multitasking, decision making, problem solving, etc.), memory impairment (defined as forgetfulness for details of recent events or upcoming plans and poor delayed word list recall), and aphasia (defined as difficulties with object or person naming or receptive or expressive language functioning). Parkinsonism was defined as some combination of masked facies, stooped posture, shuffling gait, rest tremor, bradykinesia, rigidity, and postural instability. Features of upper motor neuron and lower motor neuron dysfunction were considered present when specifically recorded in the clinical record. Neuropsychologic testing was performed using standard measures. Clinical diagnoses were based on published criteria for FTD, progressive nonfluent aphasia, semantic dementia, amyotrophic lateral sclerosis, etc, and each case was reclassified using the updated behavioral variant FTD and primary progressive aphasia criteria. Patients with ALS were classified according to published criteria.

NEUROIMAGING

Magnetic resonance images (MRI) were performed either at 1.5 T or 3 T (GE Healthcare). At 1.5 T, a 3-dimensional high-resolution spoiled gradient recalled acquisition in steady state and at 3 T, magnetization-prepared rapid gradient echo acquisition were used for the high-resolution T1-weighted images. A fluid-attenuated inversion recovery sequence was performed at both 1.5 and 3 T. To visualize the patterns of gray matter atrophy in individual subjects, we created z score maps or Structural Abnormality due to NeuroDegeneration maps, which indicate the atrophy in terms of z scores relative to a group of cognitively normal control subjects in 120 brain regions. Statistical Parametric Mapping software version 5 was used for tissue segmentation and normalization of each of these scans to a custom template to assess atrophy patterns at the group level. Voxel-wise image differences in gray matter density between the 2 patients in whom MRI was available and age, sex, and field strength-matched cognitively normal subjects were assessed using 2-sided t tests within the general linear model framework of SPM. The voxel-level analyses were corrected for multiple comparisons using family-wise error at p < .05 and a cluster threshold of 20 voxels.

GENETIC ANALYSES

Genomic DNA was extracted from peripheral blood samples using standard procedures. For each subject, genomic DNA was screened for the presence of the expanded hexanucleotide repeat in C9ORF72 using the repeat-primed polymerase chain reaction method, as previously described. Genetic analysis and DNA sequencing for C9ORF72, MAPT, and PGRN were performed as previously described.

RESULTS

CLINICAL, NEUROPSYCHOLOGIC, AND NEUROIMAGING DATA

Case III.2

The pedigree is shown in Figure 1. The proband developed increased slowness and decreased spontaneous speech as well as difficulties with ambulation at 62 years of age. After 3 to 4 months from the onset of symptoms, the patient reported slowness in motor functioning and cognition, difficulties with activities of daily living, and weakness in the right lower limb, and the patient was forced to use a wheeled walker to walk unassisted. The patient was evaluated at another institution and underwent an electromyogram that showed moderate fibrillation potentials in the right gastrocnemius, right peroneus longus, left gastrocnemius, and upper rectus abdominis muscles, prompting the suspicion of a diagnosis of ALS. The patient was prescribed carbidopa/levodopa to treat bradykinesia without any significant benefit. On our first encounter, the patient had a total score of 22 of 38 on the Kokmen Short Clinical and Neuropsychologic Assessment. Additional findings included pseudobulbar affect, apraxia, poor mirror imaging, right/left confusion, and decreased prosody. There was generalized hyperreflexia with the Babinski sign present on the right, as well as weakness only in the proximal and distal right lower extremity. Gait was bradykinetic and apraxic, with freezing and decreased ability to turn. There was spasticity in the right greater than left upper and lower extremity. Sensory examination was normal. Neuropsychologic assess-
consume multiple meals, and fecal and urinary incontinence functional dependence, the tendency to overeat and genetically mediated disorder was suspected. With the knowledge of the positive family history, a gestures led to the diagnosis of behavioral variant FTD, and structures were preserved.

The parietal, occipital, and posterior cingulate cortices were normal. L indicates left; R, right.

Figure 2. Fluorodeoxyglucose F 18 positron-emission tomographic scan of case III.3, with representative images in the axial (A) and coronal (B) planes. Note that the degree of hypometabolism is quite mild and primarily involves the frontotemporal regions, which is slightly more apparent in the left cerebral hemisphere. The parietal, occipital, and posterior cingulate cortices were normal. L indicates left; R, right.

The parietal, occipital, and posterior cingulate cortices were slightly more apparent in the left cerebral hemisphere. The parietal, occipital, and posterior cingulate cortices were normal. L indicates left; R, right.

The clinical, neuropsychologic, and neuroimaging features led to the diagnosis of behavioral variant FTD, and with the knowledge of the positive family history, a genetically mediated disorder was suspected.

Case III.3

The proband’s sibling started to present symptoms at around 65 years of age. The family observed personality changes characterized by dampened emotions, reduced interest in family business matters, and lack of motivation. The patient had difficulty in performing calculations and taking care of finances without any significant impairment in short-term or long-term memory at this time. The family also noted word-finding difficulties and the tendency to speak more in common phrases instead of full sentences.

At age 67 years, the patient was seen by a local primary care physician as well as a neurologist and underwent neuropsychologic testing and MRI and positron-emission tomographic scans. The fluorodeoxyglucose F 18 positron-emission tomographic scan of the brain (Figure 2) showed mild frontotemporal hypometabolism, slightly more apparent in the left cerebral hemisphere. The parietal, occipital, and posterior cingulate cortices were preserved.

The clinical, neuropsychologic, and neuroimaging features led to the diagnosis of behavioral variant FTD, and with the knowledge of the positive family history, a genetically mediated disorder was suspected.

During the following year, the family noticed progressive functional dependence, the tendency to overeat and consume multiple meals, and fecal and urinary incontinence. At our first encounter with the patient at age 69 years, the Kokmen Short Test of Mental Status was 7 of 38, with significant impairment of language. Prominent agrammatism was present, responding only with “yes” and “no,” as well as apraxia of speech and nonverbal oral apraxia. There was also mild generalized hyperreflexia, pyramidal signs in the left more than right, and no definitive parkinsonian signs. On MRI, there was a generalized cerebral and cerebellar atrophy and moderate volume loss, especially in the bilateral frontal, insular, and temporal cortices (Figure 3). Confluent bifrontal T2-sequence white matter hyperintensity was noted. Two years later (at age 71 years), the MRI brain scan showed a progression of the atrophy, with the posterior cingulate more obviously involved. The subcortical white matter findings showed moderate progression as well.

She was prescribed treatment with memantine and rivastigmine transdermal patch. At the most recent visit at 71 years of age, the patient had generalized bradykinesia but was not exhibiting agitation or socially disinhibited behavior.

Case IV.1

The proband’s child developed symptoms at around 44 years of age, with verbal perseveration and difficulties remembering events and facts. After 1 year, the patient quit driving and needed help taking care of personal finances. Language was not compromised. The patient behaved inappropriately by touching people, talking with strangers, and being garrulous. The patient was functioning reasonably well in taking telephone orders for the family business. The patient did not show emotional lability but clearly had apathy. There was no hyperphagia or parkinsonian features.

At the initial neurologic evaluation at our institution at age 50 years, the patient scored 16 of 38 on the Kokmen Short Test of Mental Status, with difficulties in digit span, arithmetic, similarities, construction, and recall. The neurologic examination was otherwise within normal limits. Neuropsychologic testing showed impairment in category fluency, confrontation naming, psychomotor speed, divided attention, visuospatial functioning, and delayed recall. Initial MRI showed generalized cortical and cerebellar atrophy most marked in the frontal, temporal, and mesial parietal and occipital regions (Figure 3). The clinical diagnosis was behavioral variant FTD, likely familial, realizing the degree of impairment on visuospatial tasks and degree of atrophy in nonfrontal and temporal lobe structures were atypical.

By age 53 years, the patient had become more passive and apathetic and engaged in more compulsive behavior such as constantly tidying things up, making the bed, folding newspapers, and changing the channels on the television. The patient bought items excessively and the business failed, resulting in the need to quit working. The patient bathed and dressed independently but sometimes only changed clothing if prompted. Examination at this time revealed a score of 9 of 18 on the Kokmen Short Test of Mental Status, and the patient was more withdrawn, with the tendency toward verbal perseveration. There was a reduction of facial expression and in
speech volume. The neuropsychologic assessment showed global cognitive impairment. The MRI showed progression of atrophy in previously affected structures (Figure 3). Electromyogram results were normal.

OTHER RELATIVES

A parent of the proband had a diagnosis of dementia with onset at 62 years of age and died at age 64 years. This person’s 2 siblings exhibited dementia also—one at age 50 years and the other at age 85 years. Another sibling of the proband received a diagnosis of dementia at 55 years of age and died at age 81 years; no other details are known.

GENETIC FINDINGS

DNA was available for analysis in the 3 examined subjects, each of whom had the hexanucleotide expansion detected in C9ORF72. No mutation was present in MAPT or PGRN.

A summary of the key antemortem features in this kindred are shown in the Table.

COMMENT

To our knowledge, this is one of the first family descriptions of c9FTD/ALS associated with the GGGGCC intronic repeat expansion in C9ORF72. The phenotype is typically FTD, ALS, or a combination of both. All of the cases had personality/behavioral changes early in the course of the disease; they were characterized by pseudobulbar affect, obsessions (eg, collecting newspapers), social inappropriateness, and apathy. As previously reported, some cases may have parkinsonism. In the family in our study, the proband developed features of parkinsonism that were not levodopa responsive. Moreover, our proband manifested signs and symptoms of motor neuron disease. All the cases in the family reported a decline in memory and cognitive status either early or late in the course of disease. Two of the cases developed severe aphasia in the end stages of the disease, but prominent aphasia early in the course was absent.

The demographic and clinical heterogeneity is one of the features of c9FTD/ALS. The affected individuals may express the mutation with different phenotypes such as behavioral variant FTD, FTD/ALS, FTD/ALS/parkinsonism, ALS, or parkinsonism. It is also possible that some of the unexamined cases in this kindred had underlying Alzheimer disease and were thus phenocopies, but an Alzheimer disease–like phenotype has been identified with this mutation. Although there is growing knowledge of the role of the hexanucleotide repeat expansion in C9ORF72, the explanation for the variable phenotypical expression is not known. The age at onset and duration of disease are also highly variable for unclear reasons.
As the apparent mechanism of disease involves a hexanucleotide repeat expansion, it is intriguing to ponder whether the variable age at onset (including what may be anticipated in this and other kindreds) and/or variable duration of disease are owing in part to the degree of the expansion; this issue warrants further study.

All 3 of the examined patients were rather advanced in the illness by the time neuropsychologic testing was performed, thus insights into the early neuropsychologic profile of impairment cannot be inferred. However, the imaging findings are consistent with other recent analyses in C9FTD/ALS cases. 

<table>
<thead>
<tr>
<th>Feature</th>
<th>C9ORF72</th>
</tr>
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<tbody>
<tr>
<td>Demographics</td>
<td></td>
</tr>
<tr>
<td>With neurodegenerative syndrome, No.</td>
<td>7</td>
</tr>
<tr>
<td>With FTD with/without parkinsonism with/without ALS, No.</td>
<td>4</td>
</tr>
<tr>
<td>With hexanucleotide repeat in C9ORF72 identified, No.</td>
<td>3</td>
</tr>
<tr>
<td>Sex, No.</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>1</td>
</tr>
<tr>
<td>Female</td>
<td>6</td>
</tr>
<tr>
<td>Age at onset, median (range), y</td>
<td>62 (44-65)</td>
</tr>
<tr>
<td>Survival, median (range), y</td>
<td>12 (1-26)</td>
</tr>
<tr>
<td>Magnetic resonance imaging</td>
<td></td>
</tr>
<tr>
<td>Dorsolateral frontal/insular atrophy</td>
<td>+++</td>
</tr>
<tr>
<td>Parietal cortex atrophy</td>
<td>++</td>
</tr>
<tr>
<td>Temporal cortex atrophy</td>
<td>+++</td>
</tr>
<tr>
<td>Cerebellar atrophy</td>
<td>++</td>
</tr>
<tr>
<td>Hippocampal atrophy</td>
<td>0</td>
</tr>
<tr>
<td>Markedly focal or asymmetric atrophy</td>
<td>0</td>
</tr>
<tr>
<td>While matter signal changes</td>
<td>+</td>
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Abbreviations: ALS, amyotrophic lateral sclerosis; bvFTD, behavioral variant frontotemporal dementia; C9ORF72, chromosome 9 open reading frame 72; CBS, corticobasal syndrome; FTD, frontotemporal dementia; NOS, not otherwise specified; PPA, primary progressive aphasia.

As expected for a frontotemporal lobar degeneration–spectrum disorder, parietal, occipital, and cerebellar atrophy can also be present, as shown in our cases with imaging. The newly appreciated ubiquitin-positive inclusions in the cerebellum in C9FTD/ALS cases likely reflects cerebellar degeneration that can be detected using quantitative neuroimaging techniques.

Our report highlights the clinical and neuroimaging characteristics of a family with C9FTD/ALS. Further studies are needed to better understand the possible anticipation mechanism, the phenotypical variability, and cliniconeuroimaging-neuropathologic correlations in other patients affected by the C9ORF72 hexanucleotide expansion.


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Financial Disclosure: Dr Knopman serves as deputy editor for Neurology; has served on a data safety monitoring board for Eli Lilly and Company; has served as a consultant for Elan/Janssen Alzheimer Immunotherapy; and is an investigator in clinical trials sponsored by Elan/Janssen Alzheimer Immunotherapy, Baxter International Inc, and Forest Laboratories Inc. Dr Knopman also receives research support from grants R01 AG011378 (co-investigator), P50 AG016574 (coinvestigator), U01 AG006786 (coinvestigator), AG029550 (coinvestigator), AG032306 (coinvestigator), and U01 096917 (coinvestigator) from the National Institutes of Health. Ms DeJesus-Hernandez and Dr Rademakers have patent applications pending on the discovery of the C9ORF72 repeat expansion. Dr Rademakers also receives research support grants R01 NS065782 (principal investigator), R01AG026251 (coinvestigator), and P50 AG016574 (principal investigator, project 2) from the National Institutes of Health; as well as support from the ALS Association, the ALS Therapy Alliance, and the Consortium for Frontotemporal Degeneration Research. Dr Rademakers has also received honoraria for lectures or educational activities not funded by industry and serves on the medical advisory board of the Association for Frontotemporal Degeneration. Dr Jack serves on scientific advisory boards for Elan/Janssen Alzheimer Immunotherapy, Eli Lilly and Company, GE Healthcare, and Eisai Inc; and he receives research support from Baxter International Inc, Allon Therapeutics Inc, and Pfizer Inc. Dr Jack has also received grants R01 AG011378 (principal investigator) and P50 AG016574 (coinvestigator) from the National Institutes of Health/National Institute on Aging, as well as research support from the Alexander Family Alzheimer’s Disease Research Professorship of the Mayo Foundation. He also holds stock/stock options in Johnson and Johnson. Dr Lowe serves on scientific advisory boards for Bayer Schering Pharma and GE Healthcare and...
receives research support from GE Healthcare, Siemens Molecular Imaging, the National Institutes of Health (National Institute on Aging and National Cancer Institute), the MN Partnership for Biotechnology and Medical Genomics, and the Leukemia & Lymphoma Society. Dr. Petersen serves on the safety monitoring committees for Elan Pharmaceuticals and Pfizer and as a consultant for Elan Pharmaceuticals and GE Healthcare. He also receives royalties from the publication of a book titled *Mild Cognitive Impairment* (Oxford University Press, 2003) and receives research support grants P50 AG016574 (principal investigator), U01 AG006786 (principal investigator), R01 AG011378 (co-investigator), and U01 AG024904 (co-investigator) from the National Institutes of Health. Dr. Boeve serves as an investigator for clinical trials sponsored by Cephalon Inc, Allon Pharmaceuticals, and GE Healthcare and receives royalties from the publication of a book titled *Behavioral Neurology of Dementia* (Cambridge Medicine, 2009). He also receives research support grants P50 AG016574 (co-investigator), U01 AG006786 (co-investigator), and R01 AG032306 (co-investigator) from the National Institutes of Health as well as research support from the Mangurian Foundation.

**Funding/Support:** This work was supported by the Mayo Alzheimer’s Disease Research Center (P50 AG016574), the Identifying Mechanisms of Dementia: Role for MRI in the Era of Molecular Imaging (R01 AG011378), the ALS Association (Dr. Rademakers), and the Robert H. and Clarice Smith and Abigail Van Buren Alzheimer’s Disease Research Program of the Mayo Foundation. Dr. Rademakers’ work is also funded by grants R01 NS057782 and R01 AG026251 from the National Institutes of Health.

**Additional Contributions:** We thank the patients and their families for participating in aging and neurodegenerative disease research. We appreciate the assistance of Samantha Wille, BS, in generating the magnetic resonance images.

**REFERENCES**