An expanded GGGGCC hexanucleotide repeat in a gene on chromosome 9, C9ORF72, is a common genetic cause of frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS), the general class of disorders referred to as c9FTD/ALS.1,2 Pathogenic C9ORF72 expansions have been reported infrequently in patients with clinically diagnosed Alzheimer disease3 and rarely in autopsy-confirmed Alzheimer disease,4 as well as clinically probable dementia with Lewy bodies,5 Parkinson disease,6 and corticosubcortical or ataxia syndromes.6,7 Psychiatric presentations have also been reported, including psychosis and depression.8 Moreover, depression may be more frequent in c9ALS than in sporadic ALS.8 In addition to system-specific neuronal loss and gliosis, the hallmark neuropathologic feature of c9FTD/ALS is presence of neuronal inclusions immunopositive for ubiquitin and ubiquitin-binding proteins,10 some of which also contain dipeptide repeat polymers11 possibly generated by repeat-associated non-ATG (methionine) translation. Dipeptide repeat polymers can be detected with the c9FTD/ALS disease-specific antibody, C9RANT.12 The clinicopathologic spectrum of C9ORF72-related disease and discussion of disease mechanisms have recently been reviewed by Cruts and colleagues.13

We present clinicopathologic findings of 2 patients with depression clinically thought to have dementia, where the differential diagnosis included depressive pseudodementia, a controversial term for cognitive deficits thought to be due to a treatable, often psychiatric condition.14 The prognosis of depressive pseudodementia is contentious, with some studies reporting conversion to irreversible organic dementia and other studies reporting no conversion, but rather a disease course resembling the primary underlying psychiatric disorder.15,16 In the present study, we describe patients in whom there is no overt evidence of neurodegeneration, based on macroscopic and microscopic studies with routine histologic and fluorescence microscopy. We screened 31 cases meeting these criteria and found 2, both with a family history of neurodegenerative disease, which had dipeptide repeat lesions characteristic of c9FTD/ALS but lacked significant brain atrophy or neuronal loss in neuronal populations usually vulnerable to c9FTD/ALS. This study illustrates that the C9ORF72 repeat expansion should be considered in patients with depressive pseudodementia, especially if they have a family history of neurodegenerative disease.
Methods

We screened a consecutive series of 31 cases from the brain bank for neurodegenerative disorders at Mayo Clinic in Jacksonville, Florida, for evidence of C9RANT immunoreactive inclusions using immunohistochemistry of cerebellar sections with a previously characterized antibody (Rb5823, 1:5000). The demographic features of the cases are summarized in the Table. The mean age of the cohort was 77 years (range 50-102 years). The mean brain weight (determined by doubling the weight of the fixed hemibrain) was 1285 g for men and 1079 g for women. Cases were included in the study if routine neuropathologic evaluation assessing macroscopic atrophy and microscopic evidence of neuronal loss and gliosis was negative. They also had to have an antemortem diagnosis of dementia, depression, or both. Three cases had a clinical antemortem history or diagnosis of depression, and 22 cases had a clinical history or diagnosis of both dementia and depression. They had to have minimal or no Alzheimer-type changes as assessed with thioflavin-S fluorescence microscopy and no α-synuclein lesions, or at most, sparse Lewy bodies consistent with incidental Lewy body disease, on α-synuclein immunohistochemistry. They also could not have significant cerebrovascular disease that would be consistent with vascular ischemic dementia.

Brains were obtained from patients from whom autopsies were performed after written informed consent by the legal next of kin. Clinical information was obtained by review of available medical records supplied to the brain bank, which operates under protocols approved by the Mayo Clinic Institutional Review Board, in accordance with Health Insurance Portability and Accountability Act guidelines.

Two cases were found to have C9RANT immunoreactive inclusions. In addition to routine studies, for these 2 cases, sections of cortex, hippocampus, amygdala, basal forebrain, thalamus, medulla, pons, and cerebellum were studied with immunohistochemistry for phospho-tau (CP13, 1:1000), p62 (p62 Ick ligand, 1:250), and TDP-43 (pS409/410, 1:5000) as previ-
Previously described. Frozen cerebellar tissue was processed for repeat-primed polymerase chain reaction (PCR) and Southern blot as previously described.1

Results

Neuropathology of Cases With Evidence of C9RANT
Two of the 31 cases had neuronal cytoplasmic inclusions (NCI) in the cerebellum with C9RANT immunohistochemistry. As expected, given the inclusions criteria, neither of the 2 cases had macroscopic evidence of cortical atrophy and no foci of softening or discoloration (Figure 1A and B). The calculated brain weights (1380 g and 1360 g) were within normal limits. Blood vessels at the base of the brains had no atherosclerosis. In patient 1, the frontal and temporal horns of the lateral ventricle were not enlarged (Figure 1C), and in patient 2, there was only mild widening (Figure 1D). In both individuals, the cerebral gray mantle had normal thickness and distribution. The hippocampal formation, amygdala, basal ganglia, thalamus, midbrain, pons, and medulla were unremarkable. The substantia nigra and locus ceruleus had visible pigmentation (Figure 1E and F).

Microscopically, all regions examined revealed normal neuronal populations and no gliosis. Specifically absent was evidence of motor neuron disease in cortex and brainstem nuclei. There was also no cortical spongiosis and no hippocampal or amygdala neuronal loss. Neither patient had hippocampal sclerosis. Thioflavin-S fluorescent microscopy did not reveal any senile plaques or amyloid angiopathy, and only isolated neurofibrillary tangles in the neocortex (patient 1), hippocampus (patients 1 and 2), entorhinal cortex (patient 1), and basal forebrain (patients 1 and 2). In patient 1, no inclusions were detected with α-synuclein immunohistochemistry. In patient 2, immunohistochemistry with α-synuclein revealed sparse Lewy neurites in the substantia nigra, raphe and periaqueductal gray, mesopontine tegmentum, and medullary tegmentum. Isolated Lewy bodies were detected in the basal forebrain, hypothalamus, and amygdala, but not in the basal ganglia. No cortical Lewy bodies were detected in the cerebral cortex. Given the absence of neuronal loss in areas with α-synuclein lesions, the findings were interpreted as incidental Lewy bod-
ies. Tau immunohistochemistry revealed only mild age-related medial temporal pretangles and neuropil threads. A few neurons in the well-populated hippocampal dentate fascia had NCI with TDP-43 immunohistochemistry. Sparse NCI were also present in the entorhinal cortex, occipitotemporal cortex, and amygdala of patient 1. There were no TDP-43 lesions in basal ganglia or in any of the vulnerable brainstem nuclei. Isolated TDP-43-immunoreactive NCI and dystrophic neurites were found in the frontal and temporal cortices of patient 2, with rare NCI found in the basal ganglia and substantia nigra. The density of the TDP-43 lesions was not much greater than detected in a subset of neurologically healthy individuals.17

Neuronal inclusions immunoreactive with C9RANT and p62 were more numerous than with TDP-43 immunohistochemistry in both brains. There were C9RANT- and p62-positive NCI in the dentate fascia and the pyramidal layer of the hippocampus (Figure 2B). C9RANT and p62 inclusions were detected in the neocortex, amygdala, basal ganglia, and thalamus, with the highest burden in the cerebellum (Figure 2B). There was no neuronal loss or gliosis in the cerebellar cortex.
or deep nuclei. C9RANT and p62 lesions were more numerous in patient 2. Additional neuropathologic documentation is included in eFigures 1 and 2 in the Supplement.

**Genetics**

Molecular genetic studies with repeat-primed PCR and Southern blot analysis for both patients demonstrated the characteristic C9ORF72 saw-tooth, stutter amplification-repeat pattern on PCR (Figure 2A) and high molecular weight bands on Southern blot at approximately 8.5 kilobases, which corresponds to 1000 to 1400 hexanucleotide repeat units. This expansion size is comparable to positive c9FTD controls (Figure 2C).

**Report of Cases**

**Patient 1**

A 66-year-old right-handed man presented first for neuropsychological evaluation at the age of 57 with a 1-year history of toe numbness. He was a retired junior high school teacher. He did not smoke, and drank minimal amounts of coffee and alcohol. He had a medical history of hypertension and hypercholesterolemia. Family history was notable for parkinsonism and depression in his deceased father. He also had a 5-year history of depression with stable appetite and weight. Physical and neuropsychological examination findings were normal, except for mild distal sensory loss, suggestive of an early polyneuropathy; however, results of a nerve conduction study was within normal limits. Toe sensation and numbness gradually improved. Six months later, he had worsening depression. His Lexapro (escitalopram oxalate) dosage was increased, and Wellbutrin (bupropion hydrochloride) was added. Magnetic resonance imaging (MRI) revealed findings suggestive of an old left basal ganglia lacunar infarct and small vessel ischemic changes. Carotid ultrasonography revealed minimal plaque formation.

At age 61, he began electroconvulsive therapy, with his family reporting benefits in his mood. Computed tomography and MRI at the time both revealed no significant changes. Three years later, he had worsening conditions and treatment-refractory depression, with lethargy, social withdrawal, and concerns about subjective memory. He was treated with Abilify (aripiprazole), Lamictal (lamotrigine), and Pamelor (nortriptyline hydrochloride) for his depression. He developed drug-induced Parkinsonism with hypomimia, intermittent resting leg tremor, decreased spontaneous movements in his right arm, bradykinesia, and minimal increased tone with facilitation in his arms. Rapid repetitive movements and upper extremity reflexes were normal. Lower extremity reflexes were absent. He had a normal gait, and he was able to tandem walk without difficulty. Findings from the Romberg test were negative. On neuropsychological testing, he scored 30 of 30 on the Mini-Mental State Examination (MMSE) and 93 of 100 on the Modified MMSE, with errors on timed naming, similarities, and delayed recall. Trail Making Tests A and B were completed in 30 and 55 seconds, respectively, both without errors.

At age 65, he was found in a catatonic state after becoming lost while wandering at night. He was unable to speak intelligently. Many of his medications were discontinued, and he was prescribed Risperdal (risperidone), Effexor XR (venlafaxine hydrochloride), Luvox (fluvoxamine maleate), and Cogentin (benztrapine mesylate). He had a slightly shuffling gait, paucity of movement, masked facies, and was withdrawn. Computed tomography and MRI revealed very mild cerebral atrophy and white matter changes. The patient died at age 66 with a final clinical diagnosis of possible dementia vs all psychiatric manifestations (pseudodementia inferred) and drug-induced parkinsonism.

**Patient 2**

A 71-year-old right-handed man first presented for neuropsychological evaluation at 66 years of age with depression and concerns about mild memory loss during the past year. He was a retired, college-educated banker who was working as a real estate sales associate. He was a pack-a-day smoker from age 19 to 59 and a frequent drinker who quit at the age of 70. He had a medical history of coronary artery disease with a bypass graft surgery at age 58. Family history was positive for an unspecified dementia in his deceased mother. He reported difficulties with short-term memory, rare word-finding difficulties, and social withdrawal. These issues did not impact sense of direction or work-related memory and cognition. He attributed these changes to a series of financial issues that caused stress and depression. Physical and neurologic examination findings were normal, and he scored a 28 of 30 on the MMSE.

He was prescribed escitalopram oxalate (10 mg/d) for his depression. Subsequent MRI revealed a normal brain with slight symmetric atrophy of the cerebral cortex.

Throughout the following year, he had some lingering difficulties with depression and a decrease in activity and motivation but reported improved mood. The escitalopram dosage was increased to 20 mg/d. Aricept (donepezil hydrochloride) was prescribed, but the patient did not tolerate it and it was discontinued after 3 months. The patient was subsequently prescribed topical Exelon (rivastigmine tartrate) (4.6 mg/d), which was tolerated. At age 67, he underwent neuropsychological evaluation in which he scored within acceptable limits in domains of executive function, had borderline deficits in attention and concentration, visuospatial skills, and motor processing speed, and more severe impairments in learning and memory. He scored 19 of 30 on the Montreal Cognitive Assessment (below normal cutoff), 7 of 10 on the Clock Drawing Test, 11 of 30 on the Geriatric Depression Scale, suggestive of mild depression, and 9 of 20 on the Geriatric Anxiety Inventory, indicating the absence of an anxiety disorder. He scored a best of 5 on the 10 word list and 0 on delayed recall. A diagnosis of probable Alzheimer disease was made at age 68.

The disease course was slowly progressive for 18 months with no changes in activities of daily living, but declining insight of cognitive deficits. Exelon became intolerable and he was prescribed Namenda (memantine hydrochloride). The Montreal Cognitive Assessment score was 14 of 30 at age 70.

Later that year his dementia began to worsen with delirium, increased anxiety, behavioral decompensation, and inappropriate outbursts. Computed tomography showed mild atrophy and white matter changes. Eight months later, delirium subsided and dementia began to stabilize. The patient died 5 months later at the age of 71 with a final clinical diagnosis of moderate Alzheimer disease.
Discussion

Both patients had unique clinicopathologic features for C9ORF72 repeat expansion carriers, most notably brains that ostensibly had no evidence of neurodegeneration, except for disease-specific neuronal inclusions with C9RANT immunohistochemistry and sparse NCI with TDP-43 immunohistochemistry. TDP-43 immunoreactive lesions, usually dystrophic neurites, are uncommon in healthy individuals and appear most often in advanced age.13 In these patients most of the abnormal TDP-43 was in NCI, not dystrophic neurites, and both men were much younger than subjects with incidental TDP-43 lesions. Nevertheless, TDP-43 lesions, including NCI, have been previously reported in the neocortex of individuals with psychiatric diseases,14 and given the mild burden of lesions in comparison to TDP-43 seen in frontotemporal lobar degeneration, it is reasonable that the TDP-43 seen in these two cases is within the limits of normal aging and psychosis. Conversely, they had abundant C9RANT immunoreactive inclusions that are specific to c9FTD/ALS and have not been detected in any other disorder or in healthy controls. While patient 1 had parkinsonism, the lack of substantia nigra or basal ganglia lesions is consistent with drug-induced parkinsonism. Patient 2 did not present with parkinsonism but had sparse Lewy bodies and neurites in vulnerable brainstem nuclei, basal forebrain, and amygdala. The extent of these lesions and the lack of clinical phenotype suggest that these lesions are merely coincidental and did not contribute to neuronal loss and, likely, the disease process. Despite neuroimaging findings of mild atrophy and white matter changes, no cerebrovascular lesions were observed during the neuropathological evaluation.

Notably, C9RANT immunoreactive inclusions were not found in the cerebella of 3 neuropathologically normal cases with dementia, 6 neuropathologically normal cases with depression, or 20 other cases with neuropathologic depressive pseudodementia in this series and in 97 cases from a previous series of neurologically healthy controls (unpublished data). The C9ORF72 expansion was found in 2 of the 22 pseudodementia cases (9.1%) in this series. Further screening of additional cases is needed to further establish the frequency of the C9ORF72 repeat expansion in pseudodementia.

Psychiatric clinical features have been reported in c9FTD/ALS15-19 and it is possible that C9ORF72 repeat expansion-related dysfunction is directly or indirectly related to depression in these patients. The most severe C9RANT lesions were in the cerebellum. The clinical significance of cerebellar lesions in c9FTD/ALS is unknown, but increasingly the cerebellum is thought to have roles in cognitive and affective functions. A study of resting-state functional connectivity in major depression suggests that the cerebellum should be considered as a possible node in the distributed disease-related brain network in depression.20 As previously noted, one study has reported a higher frequency of depression in c9ALS kindreds compared with sporadic ALS kindreds.2 Due to the high prevalence of depression, this phenotype may be unrelated to C9ORF72-associated lesions.

It is possible that these patients may have had prodromal or subclinical c9FTD/ALS and coincidental depressive pseudodementia. Alternatively, it is possible that the repeat expansion in these patients represents incomplete penetrance, which is relatively common in c9FTD/ALS and may explain some of the so-called sporadic cases of c9FTD/ALS. As in other neurodegenerative diseases, the present study raises questions about the significance of neuronal inclusions visible with ubiquitin, ubiquitin-binding proteins, and even C9RANT immunohistochemistry with respect to certain clinical manifestations of the C9ORF72 hexanucleotide repeat expansion. While the C9RANT lesions remain disease-specific, until the neurotoxic species is defined in c9FTD/ALS, these questions will remain open. More important, these cases broadened the range of clinicopathologic presentations of c9FTD/ALS and emphasized the need to consider C9ORF72 in patients with a family history of psychiatric disorders.

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Study concept and design: Bieniek, Dickson.
Acquisition, analysis or interpretation of data: All authors.

Drafting of the manuscript: Bieniek, Petrucelli.
Critical revision of the manuscript for important intellectual content: Bieniek, Blitterswijk, Baker, Rademakers, Dickson.

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REFERENCES


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