CASE RECORDS of the MASSACHUSETTS GENERAL HOSPITAL



# Case 20-2021: A 69-Year-Old Man with Ataxia

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## PRESENTATION OF CASE

*Dr. Claudio M. de Gusmao:* A 69-year-old man was evaluated in the neurology clinic of this hospital because of progressively worsening ataxia.

The patient had been well until 9 years before this evaluation, when abnormal gait and impaired coordination developed. He was unable to walk in a straight line; he bumped into walls when walking down a hallway. He also had difficulty reading traffic signs while driving and noted a change in the quality of his voice. The patient was evaluated at the neurology clinic of another hospital and began physical therapy.

During the next several years, symptoms gradually worsened despite physical therapy. The patient was no longer able to drive or play golf, and he intermittently used a walker because of unsteadiness. He had frequent falls, including one that led to fractures in six ribs. His speech changes worsened, such that he could no longer sing. The patient was referred to the neurology clinic of this hospital for evaluation.

In the neurology clinic, the patient reported frequent bowel movements, approximately 10 per day. He had no vertigo, lightheadedness, hearing loss, tremor, erectile dysfunction, disturbance of sleep, or urinary incontinence. He reported a sense of visual disorientation with head movement and with changes in the direction of gaze. He had a history of hyperlipidemia. Medications included atorvastatin, cetirizine, and a multivitamin; there were no known drug allergies. The patient had recently moved from an urban area in the southeastern United States to a suburban area of New England. He drank one glass of wine nightly; additional alcohol use caused worsening of ataxia and dysarthria. He had previously smoked five cigarettes daily but had quit smoking 30 years before this evaluation; he did not use illicit drugs.

The patient's mother had died of heart failure at an elderly age, and his father had died after a stroke and myocardial infarction at 70 years of age. The patient had two adult children, who were healthy. His two brothers and one of his sisters had celiac disease; the sister also reportedly had a shuffling gait. The sister's daughter had received a diagnosis of Turner's syndrome and had been seen by a neurologist

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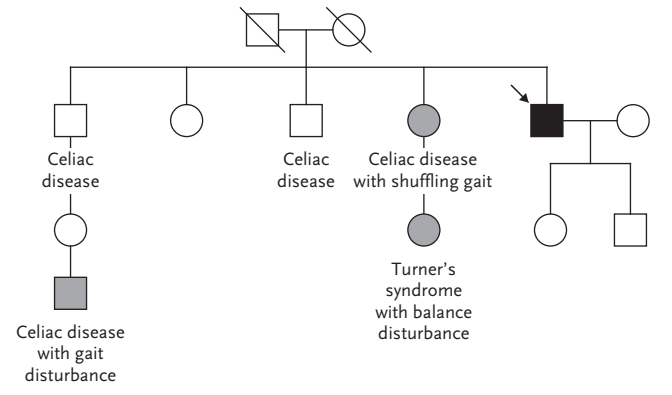



**Figure 1. Family History.**

Shown is a representation of the patient's family history obtained at presentation. The black square with the arrow indicates the patient. Shading indicates family members with a balance or gait disorder. Slashes indicate deceased family members.

for a balance disturbance. A brother's grandson also had celiac disease and a gait disturbance (Fig. 1).

The temperature was 36.6°C, the blood pressure 136/88 mm Hg, and the pulse 72 beats per minute. The patient was alert, oriented, and attentive and provided a detailed history. He had moderate dysarthria, with impairment in the rate, rhythm, and clarity of speech. An oculomotor examination revealed complete and conjugate gaze in all directions; he had intact smooth pursuit movements but had slow, dysmetric saccades. Results of a head impulse test were abnormal, with corrective saccades noted bilaterally. Normal tone and full strength were present in all major muscle groups of the arms and legs. Reflexes were 2+ and symmetric at the knees but absent at the ankles. Plantar responses were flexor bilaterally. Rapid tapping of the index fingers on the thumbs was dysrhythmic and uncoordinated. Finger–nose–finger testing revealed mild intention tremor. Heel-to-shin testing revealed symmetric jerking movements with lateral movements across the shins. Vibration and joint-position senses in the legs were profoundly decreased: the patient was unable to sense vibration at the toes, had a decreased ability to sense vibration at the knees, and had difficulty discerning the position of his big toe with his eyes closed. There was sway in his stance, regardless of whether his eyes were open, and he could not stand with the feet together. The patient walked without support. He had a wide-based gait with considerable staggering and jerky, stiff movements of the legs. He was unable to maintain balance in a tandem stance or to walk with a tandem gait.

The complete blood count and blood levels of electrolytes were normal, as were test results for kidney, liver, and thyroid function; antibodies to thyroid peroxidase and thyroglobulin were not detected. Blood levels of cyanocobalamin, methylmalonic acid, vitamin E, and glycated hemoglobin were normal; anti–glutamic acid decarboxylase antibodies and tissue transglutaminase antibodies were not detected. Results of serum protein electrophoresis were normal. Genetic testing revealed no mutations in genes associated with spinocerebellar ataxia types 1, 2, 3, 6, and 8. Clinical testing of otolith function revealed abnormal vestibular-evoked myogenic potentials on the left side. Nerve-conduction studies revealed absent or barely detectable responses in the bilateral sural nerves, left superficial peroneal nerve, left median nerve, left ulnar nerve, and right radial nerve. Results of motor nerve-conduction studies were normal, as were results of a modified barium-swallow evaluation. A previous workup at another hospital for chronic diarrhea had reportedly been normal, with no evidence of malabsorption.

*Dr. McKinley Glover:* Magnetic resonance imaging (MRI) of the head (Fig. 2), performed without the administration of intravenous contrast material, revealed mild diffuse parenchymal volume loss and prominent cerebellar volume loss, most notably involving the vermis and superior cerebellar hemispheres. There was mild flattening of the superior margin of the midbrain, and the ratio of midbrain to ventral pons was mildly reduced at 0.51 (reference range, >0.52).[1] The anteroposterior diameter of the midbrain was 8.90 mm (reference range, >9.35).[1] There was no abnormal signal in the pons, and the middle cerebellar peduncles were normal. There was a chronic lacunar infarct of the right thalamus.

*Dr. de Gusmao:* Additional history was obtained, and a diagnosis was made.

## DIFFERENTIAL DIAGNOSIS

*Dr. Vikram Khurana:* I was involved in the care of this patient, and I am aware of the diagnosis in this case. The first step in developing a differential diagnosis for this patient with ataxia and balance abnormalities is to carefully consider the anatomical localization and time course of his disorder.

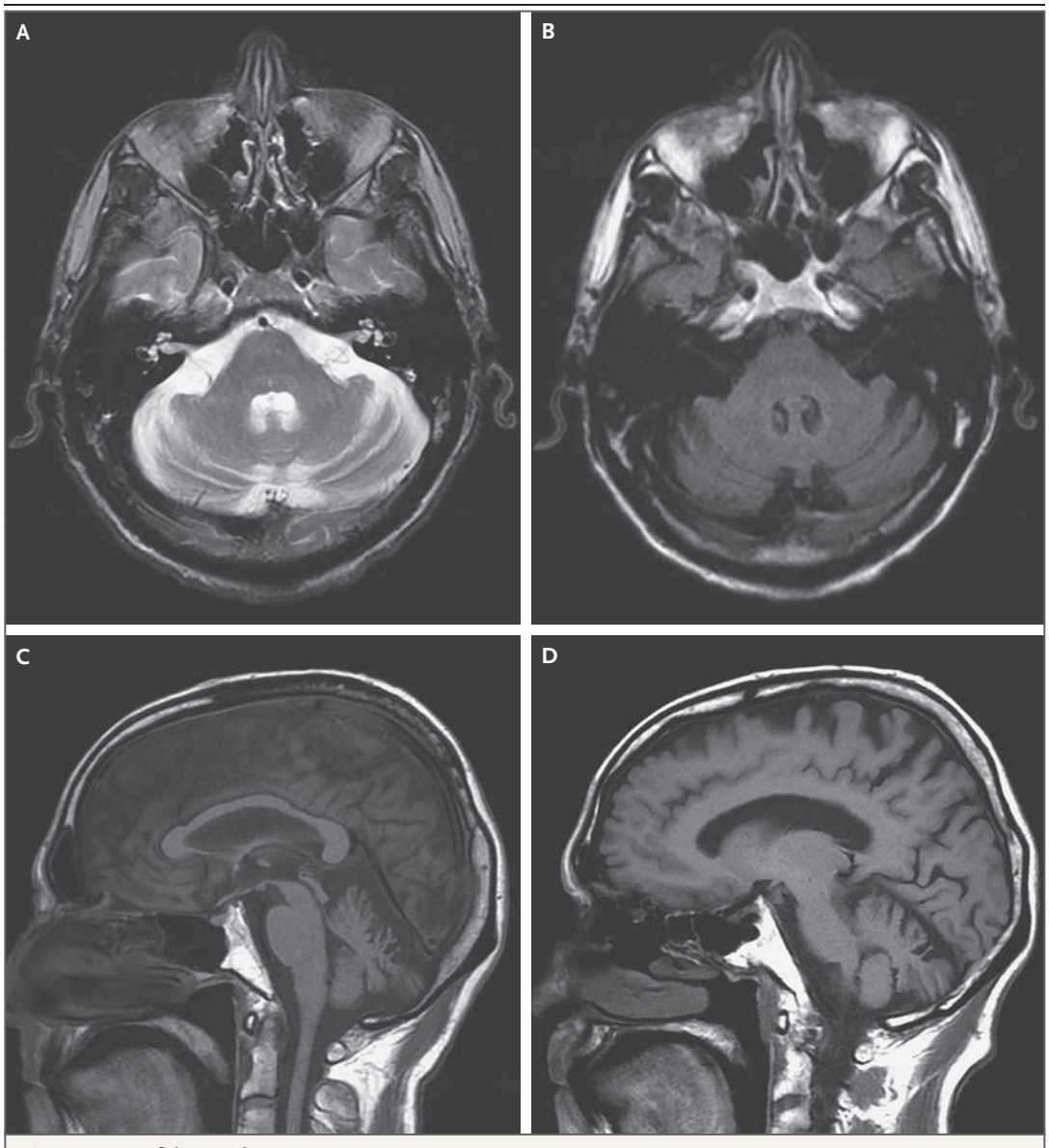



**Figure 2. MRI of the Head.**

An axial T2-weighted image (Panel A) and an axial fluid-attenuated inversion recovery image (Panel B) obtained at the level of the middle cerebellar peduncles show moderate cerebellar volume loss without focal signal abnormality. A sagittal T1-weighted image (Panel C) shows moderate volume loss in the superior vermis, mild flattening of the superior margin of the midbrain, and a ratio of midbrain to ventral pons of 0.51. A parasagittal T1-weighted image (Panel D) shows moderate volume loss in the cerebellar hemispheres, predominantly the superior aspects.

### ANATOMICAL LOCALIZATION

Balance, gait, and posture are dependent on three types of sensory inputs: proprioceptive, vestibular, and visual inputs. The integrator of these inputs is the cerebellum.

This patient's balance was affected because of impairment in multiple systems. There was a striking loss of proprioception in the legs. Nerve-conduction studies revealed absent or barely detectable responses in the bilateral sural nerves, left superficial peroneal nerve, left median nerve, left ulnar nerve, and right radial nerve, findings that suggest a non–length-dependent sensory abnormality, such as dorsal root ganglionopathy. In addition, the head impulse test was positive bidirectionally, which suggests additional involvement of the vestibular system. Finally, there was evidence of impairment in cerebellar function,

with cerebellar dysarthria (disordered rate, rhythm, and clarity of speech) and ocular dysmetria.

### TIME COURSE

The differential diagnosis can be narrowed by considering the temporal development of the symptoms. The 9-year time course of this patient's illness rules out acute causes, such as vascular, infectious, parainfectious, and toxic metabolic processes. The timing is also not a good match for subacute causes, such as inflammatory and infiltrating diseases, paraneoplastic diseases, nutritional diseases, or prionopathies. In rare cases, mutations in *PRNP* can cause an atypical prionopathy with slowly progressive ataxia, diarrhea, and neuropathy,[2] but this patient did not have associated features such as cognitive decline or seizures. The prolonged history of symptoms in this patient is most consistent with a chronic degenerative ataxia.

Chronic degenerative ataxias can be classified according to whether there is a known or an unknown cause.[3] In this patient, the presence of a sensory neuropathy allows for further differentiation (Table 1).

### CHRONIC DEGENERATIVE ATAXIAS

#### *Friedreich's Ataxia*

Chronic degenerative ataxias associated with sensory neuropathy that have a known cause are predominantly genetic disorders. The most common is Friedreich's ataxia, a multisystem autosomal recessive disorder associated with dorsal root ganglionopathy and cerebellar dysfunction. There are rare case reports of patients with a very late onset of Friedreich's ataxia, in whom symptoms developed after 40 years of age.[5] Among patients with late-onset disease, there may be a decreased incidence of typical features, such as areflexia, cardiomyopathy, and scoliosis. Genetic testing can be performed to evaluate for this condition.

#### *Nutritional Deficiencies*

The presence of a normal vitamin E level can rule out ataxia with vitamin E deficiency. This condition was considered in this patient because of his history of chronic diarrhea, although his previous workup for diarrhea had not revealed malabsorption. There was no history of excessive alcohol intake, nutritional deficiency, or mental status changes that would suggest Wernicke's encephalopathy or alcohol-related cerebellar degeneration.

#### *Celiac Disease*

This patient has a strong family history of celiac disease, which suggests the possibility of gluten ataxia, a controversial diagnosis that is loosely defined as ataxia associated with positive serologic tests for celiac disease (i.e., tests for IgA to gliadin and IgA or IgG to tissue transglutaminase).[6] Gluten ataxia classically causes gait and appendicular ataxia in conjunction with a large-fiber sensory neuropathy. The gluten trigger has been questioned because gluten ataxia can occur in the absence of enteropathy, and there are mixed reports regarding the efficacy of a gluten-free diet. Nevertheless, there are reports of cross-reactivity of the gluten antibodies with cerebellar antigens, and patients with "sporadic" cerebellar ataxia have a higher frequency of positive serologic tests for celiac disease than control populations in some studies.[6] Gluten ataxia remains a consideration in this case, although the poor correlation between ataxia and celiac disease in his family makes this an unlikely diagnosis.

#### *Other Genetic Ataxias*

Sensory ataxic neuropathy, dysarthria, and ophthalmoparesis (SANDO) is an autosomal recessive disorder that disrupts mitochondrial function and can sometimes develop later in life.[7] SANDO is a possible diagnosis in this patient because he had ganglionopathy, ataxia, and dysarthria. However, he did not have other characteristic features of SANDO, specifically ophthalmoparesis and episodic symptoms including seizures, migraines, and myoclonus.

This patient does not have a family history of an autosomal dominant ataxia, so he is unlikely to have one of these disorders, which include some of the most prevalent spinocerebellar ataxias associated with peripheral neuropathy (types 1, 2, 3, and 6).[8] Furthermore, genetic testing for these spinocerebellar ataxias was negative. His age at symptom onset and clinical features are not consistent with other hereditary ataxias associated with neuropathy, including two autosomal recessive disorders, ataxia with oculomotor apraxia and ataxia telangiectasia.

#### *Multiple System Atrophy and Progressive Supranuclear Palsy*

Chronic degenerative ataxias that have an unknown or poorly defined cause are considerations in this case. Two types have well-defined neuropathological features. Multiple system atro-

phy of the cerebellar type (MSA-C) is an α-synucleinopathy characterized by the accumulation of misfolded α-synuclein in oligodendrocytes.[9] Dysarthria is a common feature, but other characteristic features, including early autonomic dysfunction and concomitant parkinsonism, are absent in this patient. The cerebellar variant of progressive supranuclear palsy (PSP-C) is a tauopathy characterized by the accumulation of misfolded tau in neurons and glia.[10] This rare disorder typically manifests as frontotemporal dysfunction and parkinsonism. Of note, the cardinal feature of progressive supranuclear palsy — supranuclear gaze palsy — may not be present initially or, in rare cases, may never develop in patients with the cerebellar variant.[11-13] On imaging studies, PSP-C is associated with disproportionate atrophy of the midbrain as compared with the pons, whereas MSA-C is associated with atrophy of the pons and middle cerebellar peduncles.[1] PSP-C, like MSA-C, can be associated with dysarthria and spasticity. PSP-C can also be associated with prominent falls and a jerky and stiff gait, which were present in this patient. However, sensory neuropathy is not characteristic of either disorder, nor is vestibulopathy.

### *Cerebellar Ataxia, Neuropathy, and Vestibular Areflexia Syndrome*

When I evaluated this patient, a new syndrome had recently been described, comprising progressive cerebellar ataxia, peripheral vestibular dysfunction, and the absence of sensory nerve action potentials, which suggests a non–length-dependent sensory deficit. By that time, the syndrome was named cerebellar ataxia, neuropathy, and vestibular areflexia syndrome (CANVAS).[14] Patients with CANVAS were described as having marked sensory ganglionopathy affecting dorsal root ganglia and vestibular, trigeminal, and facial ganglion cells, along with Purkinje cells in the cerebellum.[15,16] More recent data confirmed a loss of large and small myelinated fibers without active axonal degeneration.[17] Video oculography was described as the method of choice for the detection of both the abnormal vestibulo-ocular reflex with corrective saccades and the deficient smooth pursuit movements and optokinetic responses bilaterally. Other features described in association with this syndrome included chronic cough, oscillopsia, dysesthesia, dysphagia, and orthostatic hypotension. Although the cause of CANVAS was unknown at that time, an early report describing two pairs of affected siblings had suggested the possibility of autosomal recessive inheritance.[14]

In this patient with the triad of cerebellar ataxia, neuropathy, and bilateral vestibular dysfunction, the clinical syndrome of CANVAS is the most likely diagnosis. Given the patient's family history of a shuffling gait and celiac disease, immune and genetic causes of this syndrome should be ruled out.

## DR. VIKRAM KHURANA'S DIAGNOSIS

Cerebellar ataxia, neuropathy, and vestibular areflexia syndrome.

## ADDITIONAL HISTORY

*Dr. de Gusmao:* At this hospital, the patient was initially treated with varenicline, which was subsequently discontinued because apathy and nightmares developed. During the next 3 years, the gait and coordination impairments, dysarthria, and dysphagia worsened in severity. The patient became dependent on a walker and then required a wheelchair. MRI of the head performed 2 years after the initial study did not reveal any radiographically significant changes in cerebellar volume loss; concurrent MRI of the cervical and thoracic spine revealed degenerative disk disease without a signal change in the spinal cord. Cough, progressive diplopia, generalized muscle cramping, increased urinary frequency, and spasticity in the legs developed, as did mild weakness in the right leg. There was slowing of saccades despite full excursions of eye movements in all directions. A modified barium-swallow evaluation revealed a new impairment of the swallow reflex.

Additional genetic testing did not reveal any pathogenic mutations or repeat expansions in the genes *FXN*, *POLG1*, *APTX*, *SETX*, *TTPA*, and *SIL1* or in mitochondrial genes associated with the disorders MERRF (myoclonic epilepsy with ragged-red fibers), NARP (neuropathy, ataxia, and retinitis pigmentosa), and MELAS (mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes). Further genetic testing was negative, including testing for pathogenic mutations or repeat expansions in genes associated with spinocerebellar ataxia types 5, 7, 10, 13, 14, and 17 and with DRPLA (dentatorubral–pallidoluysian atrophy). Whole-exome sequencing of the patient,

**Table 1. Differential Diagnosis of Ataxia in This Patient.***

| Diagnosis | Features | | Key Clinical Tests |
|---|---|---|---|
| | Present in This Patient | Absent in This Patient | |
| Genetic cause | | | |
| Friedreich's ataxia | Dorsal root ganglionopathy and cerebellar dysfunction; some atypical cases can have a late onset and preserved reflexes | Cardiomyopathy, diabetes, scoliosis, pes cavus, optic atrophy, and hearing loss | Test for GAA repeat expansion or point mutation in *FXN* |
| Common spinocerebellar ataxias (types 1, 2, 3, and 6) | Worsening bulbar symptoms (characteristic of types 1, 2 and 3) and slow saccades (characteristic of type 2) | Clear generation-to-generation family history; extrapyramidal symptoms, facial fasciculations, and bulging eyes (characteristic of type 3); and dystonia and blepharospasm (characteristic of type 6) | Test for CAG repeat expansion in *ATXN1, ATXN2, ATXN3,* and *CACNA1A* |
| Genetic ataxia with vitamin E deficiency | Sensory ganglionopathy and ataxia | Retinitis pigmentosa and early onset | Test for a low vitamin E level in the presence of normal lipoprotein levels and fat absorption; test for mutations in *TTPA*; lipid profiling and blood smear may indicate abetalipoproteinemia, which is an important mimic |
| SANDO and ataxia and neuropathy spectrum disorders | Ganglionopathy, ataxia, and dysarthria | Ophthalmoparesis, cognitive decline, neuropsychiatric symptoms, seizures, migraines, myopathy, vision loss, and liver dysfunction | Test for mutations in *POLG1* or *TWNK* |
| Mitochondrial disorders (MELAS, MERRF, and NARP) | Ataxia; vestibulopathy can occur in rare cases | Early onset, oculomotor paresis, hearing loss, retinitis pigmentosa, muscle weakness or myopathy, migraines, seizures, intellectual disability, myoclonus, cardiomyopathy, and short stature | Test for an elevated lactic acid level in blood or CSF; MRI of the head; genetic testing for mutations or copy-number variants in the mitochondrial genome (*MT-ATP6, MT-TL1, MT-TI,* and *MT-TP*) |
| Gerstmann–Sträussler–Scheinker disease (prionopathy) | Ataxia and diarrhea | Positive family history, disease duration of <4 years (although longer cases have been reported), dementia, and extrapyramidal symptoms, as well as myoclonus, sleep disturbances, and seizures in some cases | Test for mutations in *PRNP* |
| *RFC1*-associated CANVAS | Cerebellar ataxia, vestibular dysfunction, and sensory neuropathy | Chronic cough (initially) and autonomic dysfunction (although the diarrhea could be related) | Head impulse test or video oculography for reduced or absent visually enhanced vestibulo-ocular reflex; electromyography or nerve-conduction studies for sensory neuropathy or neuronopathy with normal motor studies; targeted analysis for AAGGG repeat expansion in *RFC1*† |

| | | | |
|---|---|---|---|
| Acquired cause | | | |
| Gluten ataxia | Celiac disease | Improvement with gluten-free diet (controversial), neuropsychiatric symptoms, and seizures | Serologic tests for antibodies to deamidated gliadin and tissue transglutaminase |
| Alcoholic cerebellar degeneration | Gait ataxia | Midline ataxia greater than appendicular symptoms, postural tremor, and cognitive deficits if there had been a previous thiamine deficiency | History taking; MRI for cerebellar atrophy involving the anterior vermis |
| Nutritional vitamin E deficiency | Chronic diarrhea leading to fat malabsorption syndrome | Myopathy and retinitis pigmentosa | Tests for a low vitamin E level, normal lipoprotein levels, and steatorrhea |
| Unknown cause | | | |
| Multiple systems atrophy of the cerebellar type | Late onset of progressive ataxia | Dysautonomia (e.g., orthostatic hypotension, urinary urgency or incontinence, or impotence in men), extrapyramidal symptoms (parkinsonism or dystonia), rapid-eye-movement sleep behavior disorder, and inspiratory stridor | Autonomic function tests for central dysfunction; MRI of the head for atrophy of the putamen, pons, and middle cerebellar peduncles (i.e., the "slit" sign in the putamen, the "hot-cross bun" sign in the pons, and hyperintense signal in middle cerebellar peduncles); quantitative volumetric analysis is more sensitive[4]; other, supportive tests include the DaT scan and FDG-PET scan |
| Cerebellar variant of progressive supranuclear palsy | Gait disturbance and falls | Cognitive decline, supranuclear gaze palsy, and parkinsonism | MRI for flattening or upward concavity at the superior surface of the midbrain tectum (i.e., the "hummingbird" and other signs); other tests include the DaT scan and FDG-PET scan |

* CANVAS denotes cerebellar ataxia, neuropathy, and vestibular areflexia syndrome; CSF cerebrospinal fluid; DaT dopamine transporter; FDG-PET $^{18}$F-fluorodeoxyglucose positron-emission tomography; MELAS mitochondrial encephalomyopathy, lactic acidosis, and strokelike episodes; MERRF myoclonic epilepsy with ragged-red fibers; NARP neuropathy, ataxia, and retinitis pigmentosa; and SANDO sensory ataxic neuropathy, dysarthria, and ophthalmoparesis.
† At the time of this patient's evaluation, the association of CANVAS with *RFC1* expansions was not known.

his niece, and his grandnephew did not reveal any candidate variants.

Repeat testing for serum IgA to gliadin and tissue transglutaminase revealed mildly elevated titers. Gluten was eliminated from the diet, but there was no improvement in neurologic symptoms. Intravenous immune globulin was administered, and there was possible improvement in the strength of the right leg; however, therapy was stopped after 3 months because there was no decrease in ataxia. Treatment with riluzole was started.

Approximately 4 years after the initial presentation to this hospital and 13 years after the development of ataxia, the patient fell at home and was admitted to another hospital. He received a diagnosis of aspiration pneumonia. Because of his overall neurologic decline, the patient and his family elected to redirect care to comfort measures. The patient died 3 weeks after hospice care was initiated. At the time of death, the family granted permission for an autopsy limited to the brain and spinal cord.

## PATHOLOGICAL DISCUSSION

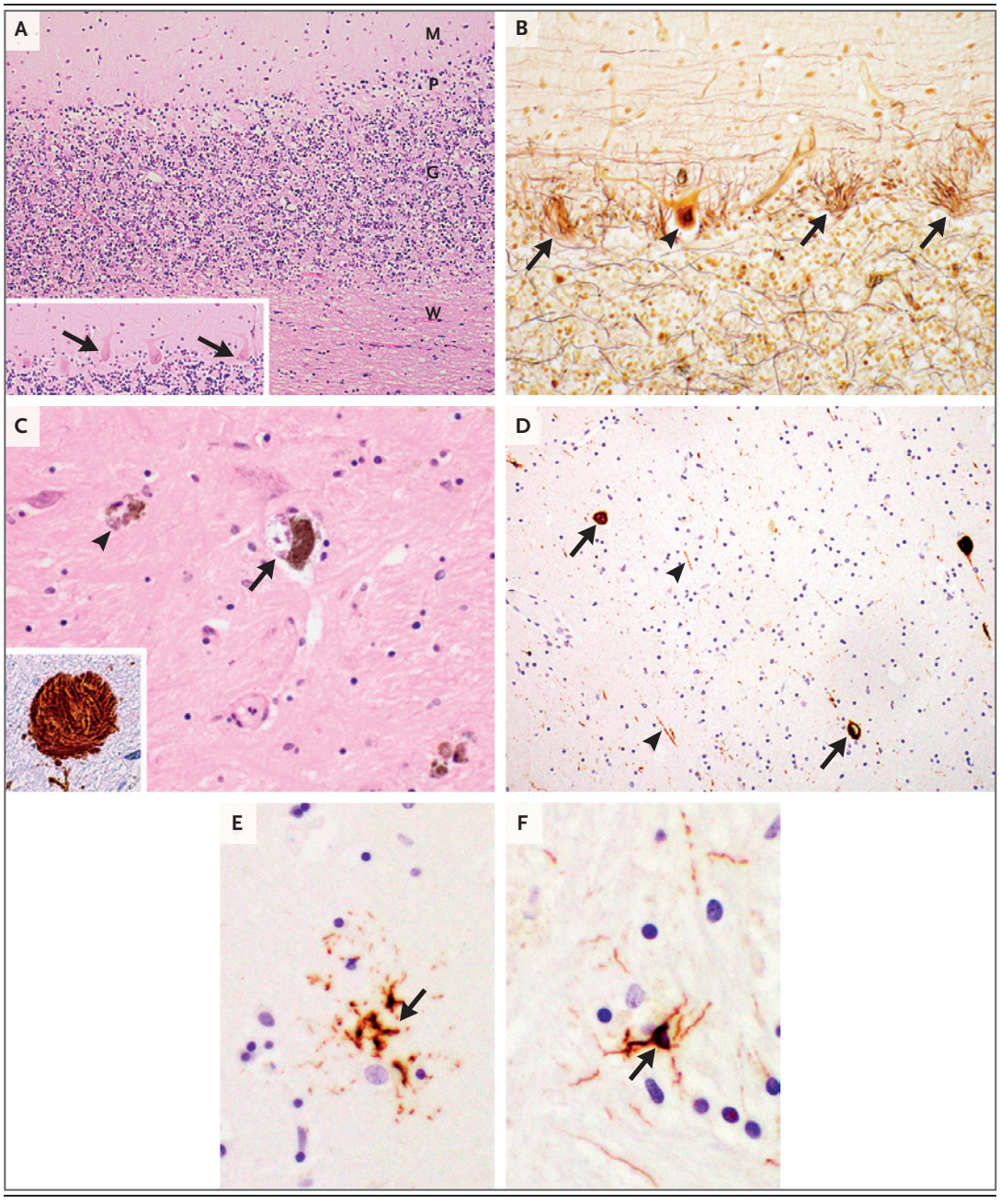
*Dr. Jeffrey Helgager:* Gross neuropathological examination of the brain was notable for marked atrophy of the cerebellum, most prominent in the superior aspect of the cerebellar vermis, as well as of the subthalamic nucleus. The substantia nigra was markedly pale, as was the locus ceruleus.

On histologic examination (Fig. 3), there was severe global loss of Purkinje neurons throughout both the vermis and the lateral cerebellar hemispheres. The subthalamic nucleus and cerebellar dentate nucleus had evidence of gliosis and neuron loss. The substantia nigra and locus ceruleus had marked depletion of neurons. Immunohistochemical staining for tau and phosphorylated tau showed globose neurofibrillary tangles and neuritic threads within these two structures; neuronal and neuritic tau tangles and glial tau deposition were also detected throughout the caudate nucleus, putamen, globus pallidus, and subthalamic nucleus. Infrequent tau-immunoreactive threads were present in the cerebellar white matter. There was no evidence of Lewy bodies or other $\alpha$-synuclein disorders on immunohistochemical staining. Immunohistochemical staining for $\beta$-amyloid and p62 was negative, whereas TAR DNA-binding protein 43 (TDP-43) was present in a normal distribution. There were no ubiquitin-positive cerebellar inclusions. Immunohistochemical staining with the use of an anti–polyglutamine-expansion diseases marker antibody (1C2) was also negative. Sections of sural nerve showed mild depletion of large myelinated axons, and examination of latissimus dorsi muscle revealed marked variation in fiber size with numerous atrophic fibers, findings consistent with neurogenic atrophy. Examination of the spinal cord revealed mild axonal loss in sensory and motor tracts.

Overall, the neuropathological findings were consistent with cerebellar degeneration and an accompanying multisystem tauopathy. The cerebellar atrophy and loss of Purkinje neurons are consistent with findings previously described with CANVAS.[16] However, the neurodegenerative changes observed in the brain stem, cerebellar dentate nucleus, and subthalamic nucleus, along with the anatomical and cellular distribution of the multisystem tauopathy, are most reminiscent

**Figure 3 (facing page). Brain Specimens.**

On hematoxylin and eosin staining of a section of cerebellar cortex (Panel A), the Purkinje layer (P) is almost devoid of Purkinje neurons and shows Bergmann gliosis. The underlying cerebellar white matter (W) shows pallor and vacuolation (M indicates the molecular layer and G the granule-cell layer). These findings reflect a loss of Purkinje-neuron axons. The inset shows the Purkinje layer in an unaffected patient of a similar age, with a normal population of Purkinje neurons (arrows). On Bodian staining (Panel B), which highlights neurites, the Purkinje layer shows a lone remaining viable Purkinje neuron (arrowhead) and "empty baskets" (arrows), which are basket cells without corresponding Purkinje neurons; basket cells are interneurons that form a synapse with Purkinje neurons and frequently remain preserved, indicating foci of Purkinje-neuron loss. Hematoxylin and eosin staining of a section of substantia nigra (Panel C) shows marked neuron depletion with a rare remaining dopaminergic neuron (arrow) and surrounding foci of extraneuronal neuromelanin (arrowhead), findings that reflect degeneration of dopaminergic neurons. Similar findings were present in the locus ceruleus. The inset shows a globose neurofibrillary tangle detected on immunohistochemical staining for tau, a finding indicative of tauopathy; globose tangles were present in both the substantia nigra and the locus ceruleus. Immunohistochemical staining for phosphorylated tau (Panel D) shows tau inclusions in neuronal cell bodies (arrows) and in neurites (arrowheads). At higher magnification, there is a tufted astrocytic inclusion (Panel E, arrow) and an oligodendroglial inclusion (Panel F, arrow); similar astrocytic and oligodendroglial inclusions were found in the caudate nucleus, putamen, globus pallidus, subthalamic nucleus, and cerebellar white matter.




of progressive supranuclear palsy. Degeneration of the cerebellar cortex is not a feature typically seen in patients with a pathological diagnosis of progressive supranuclear palsy, and it does not appear to be a prominent feature on neuropathological examination in patients with the cerebellar variant (PSP-C).[9,10,13] Autopsy studies have not reported tauopathy as a component of CANVAS, but these studies are limited in number and scope and may not reflect all the neuropathological manifestations of the disease.

## PATHOLOGICAL DIAGNOSIS

Progressive supranuclear palsy or cerebellar ataxia, neuropathy, and vestibular areflexia syndrome.

## ADDITIONAL CONSIDERATIONS

*Dr. Khurana:* The histopathological diagnosis after autopsy was PSP-C, especially since there was no known molecular basis of CANVAS at that time. And yet, as the treating clinician, I thought

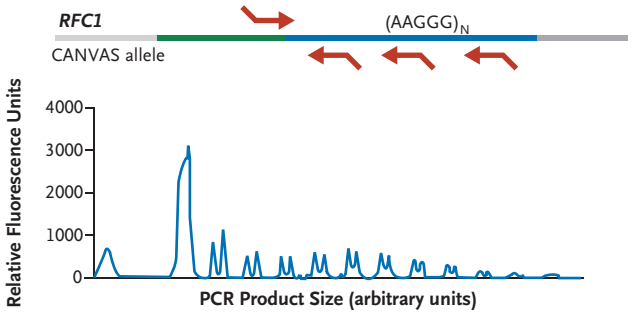



**Figure 4. Results of Diagnostic Testing for CANVAS.**

Shown are the results of a repeat-primed polymerase-chain-reaction (PCR) analysis for the detection of a mutation associated with cerebellar ataxia, neuropathy, and vestibular areflexia syndrome (CANVAS). A 5′ fluorescently tagged primer at the gene locus preceding the repeat expansion (flanking primer; arrow pointing to the right) is used in conjunction with a 3′ primer designed to detect the pathologic repeat expansion (repeat primer; arrows pointing to the left). Fluorescence quantitation yields a "ladder," which occurs when the repeat primer binds to complementary sequences at different positions along the repeat expansion, resulting in a family of PCR products. This technique identifies the presence of at least one expanded repeat but can provide only an estimate of the actual repeat size. The efficiency is higher for shorter repeats, which explains the higher peaks on the left side. A positive result is confirmed by conventional PCR analysis that shows the absence of any normal-sized gene products in the patient (not shown), indicating that the expansion is biallelic. In this case, the test was performed by the team of Dr. Brent Fogel at the University of California, Los Angeles.[20]

this diagnosis only partially explained the patient's clinical features, particularly the aggressive progression in the late phase of the illness. The features of the patient's initial presentation to this hospital were consistent with CANVAS. Vestibulopathy and sensory neuropathy are not clinical manifestations of PSP-C and remained unexplained by that diagnosis in my mind. There was no early cognitive dysfunction, and there was a vexing, if vague, family history. The patient even eventually had a chronic cough, an unusual feature that has been consistently described with CANVAS. Recognizing the limitations of whole-exome sequencing, we performed additional genetic analysis.

## ADDITIONAL DIAGNOSTIC TESTING

*Dr. de Gusmao:* In 2019, two different groups discovered that many cases of CANVAS were caused by biallelic expansions of a gene not previously associated with disease, *RFC1*.[18,19] In conducting genetic analyses in families that had two or more members with the CANVAS phenotype, both groups combined linkage analysis with next-generation sequencing. After identifying the suspected locus, the groups then adopted different bioinformatic and laboratory-based strategies to detect the expanded mutation.

*RFC1* is located on chromosome 4p14. In intron 2 of *RFC1*, there is a repeat stretch of five base pairs (a pentanucleotide). This is referred to as a short tandem repeat. The normal allele consists of a pentanucleotide of four adenine bases and one guanine base that repeats 11 times: $(AAAAG)_{11}$. The investigators found that patients with CANVAS had biallelic expansions of a mutated sequence, such that most patients had 400 to 2000 repeats of a pentanucleotide of two adenine bases and three guanine bases: $(AAGGG)_N$.[18]

We sent a sample of this patient's DNA to colleagues at the University of California, Los Angeles, for analysis of the repeat expansion. Using a combined approach that included long-range and repeat-primed polymerase-chain-reaction (PCR) analysis, they found that the normal allele was absent and at least one expanded allele was present; the findings were highly suggestive of a biallelic expansion in *RFC1* (Fig. 4). The patient's niece and grandnephew were found to be heterozygous carriers, with one expanded allele and one normal allele.

Although it has been estimated that 85% of disease-causing mutations reside within coding sequences of the genome, the accuracy of this estimate has been called into question.[20] The *RFC1* mutation is a repeat expansion in the noncoding region of the genome and thus was not identified by clinical whole-exome sequencing in this patient. Failing to arrive at a conclusive genetic diagnosis is not uncommon: variants are identified in approximately 50% of patients with suspected genetic ataxia who undergo whole-exome sequencing, and only half these variants are classified as causative (i.e., pathogenic or likely pathogenic), with the remainder classified as variants of unknown significance.[21] Whole-exome sequencing cannot identify repeat expansions or the intronic mutations or structural genetic changes that occur with intact chromosomal DNA sequences (e.g., copy-number variations, aneuploidy, or balanced translocations). Finally, mutations in mitochondrial DNA require separate analyses.

Whole-genome sequencing is a promising tool to clear these "blind spots," allowing for the

potential identification of mutations in noncoding regions, as well as copy-number variants. Repeat expansions such as the one found in this patient are more challenging to identify, requiring clinical assessment with arduous site-specific PCR analysis, although bioinformatic tools are emerging that will help to identify them with whole-genome sequencing.[19]

As we obtain more genetic data from our patients, the challenge of identifying variants that are relevant and cause disease will become more pressing. Both rigorous clinical phenotyping and the development of biologic tools that can stratify variants of unknown significance are likely to play a critical role in this effort.

*Dr. Khurana:* In this case, the identification of a recently described noncoding genetic lesion pinned down a specific genetic cause of CANVAS. *RFC1*-related ataxia should be considered in patients with a sensory ataxic neuropathy, especially when there are associated features of CANVAS such as vestibular and cerebellar dysfunction and chronic cough.[17] Future molecular genetic studies may shed light on the causative gene product and the implications of a heterozygous carrier state. The previously unreported finding of a tauopathy further underscores that additional neuropathological examination in patients with molecularly defined CANVAS will be important in defining the range of underlying neuropathological features of the disease. Discovery of the genetic lesion has thus paved the way for systematic histopathological studies and a logical path toward disease modeling and therapy.

## FINAL DIAGNOSIS

Cerebellar ataxia, neuropathy, and vestibular areflexia syndrome due to a biallelic expansion in *RFC1*.

This case was presented at Neurology Grand Rounds.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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