Unusual Clinical and Molecular-Pathological Profile of Gerstmann-Sträussler-Scheinker Disease Associated With a Novel PRNP Mutation (V176G)

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IMPORTANCE Here we describe the unusual clinical and molecular-neuropathological profile of a case of Gerstmann-Sträussler-Scheinker disease associated with a novel prion protein (PRNP) gene mutation.

OBSERVATIONS This case report from the Australian National Creutzfeldt-Jakob Disease Registry concerns a 61-year-old British-born woman with no history of neurodegenerative disorder in first-degree relatives. Rapidly progressive dementia, altered behavior, and cerebellar ataxia dominated the clinical picture in the period immediately following minor elective surgery, with death 1 month later in an akinetic-mute state. Brain histopathological examination revealed neuronal loss, scant foci of spongiform change, and diffuse multicentric amyloid plaques, selectively immunoreactive for prion protein, within the cerebral and cerebellar cortices and deep gray matter. Tau immune-reactive neurofibrillary tangles and neuritic threads were present in the cerebral cortex. PRNP sequencing demonstrated a valine to glycine mutation at codon 176, with valine homozygosity at polymorphic codon 129. Western-blot analysis of frozen brain tissue displayed a nonclassic protease-resistant prion protein banding pattern, with a prominent approximately 8-kDa protease-resistant fragment.

CONCLUSIONS AND RELEVANCE Reported is a proband with a novel PRNP mutation associated with neuropathologically confirmed Gerstmann-Sträussler-Scheinker disease displaying a somewhat unusual constellation of clinicopathological features, which overall subserve to further broaden an already diverse phenotypic spectrum.

Gerstmann-Sträussler-Scheinker (GSS) disease is a rare, almost exclusively genetically determined prion disease, typically characterized clinically by progressive cerebellar ataxia and dementia and neuropathologically by diffuse, prion protein (PrP)-containing amyloid deposits.1 Although the P102L mutation in the prion protein (PRNP) gene is the most commonly observed in GSS disease,2 numerous insertion, missense, and point mutations, including P105L, A117V, H187R, F198S, D202N, Q212R, Q212P, Q217R, and Y218N, have been detected, probably contributing to the notable clinical and pathological diversity.2,3 Typically, GSS disease follows an autosomal-dominant inheritance pattern4 and manifests a prolonged clinical course, with median illness duration of 39 months reported in large patient series.5 In addition to progressive cerebellar ataxia and dementia, clinical findings in GSS disease may include myoclonus, extrapyramidal features, and evidence of pyramidal tract dysfunction.3 Cognitive decline usually follows cerebellar ataxia as a later symptom in the clinical course, especially in P102L GSS disease,4 and may be absent in patients with certain mutations such as Q212R5; however, early prominent dementia without cerebellar ataxia has been described in some less-common mutations such as G131V.7,8 Neuropathological findings are also variable but generally there is evidence in the cerebrum and cerebellum of widespread, PrP immunopositive, unicentric and/or multicentric amyloid plaques, associated with differing degrees of spongiform change, neuronal loss, and astrocytic gliosis; tau-positive neurofibrillary tangles may also be observed.6,9–11

Herein, we report a patient with neuropathologically confirmed GSS disease associated with a novel PRNP mutation, displaying an unusual combination of clinical and molecular-pathological features, which overall subserve to further broaden an already diverse phenotypic spectrum.
Methods

The proband was a white, British-born woman whose first definite neurological symptoms occurred at age 61 years, around the time of elective surgery for removal of a dislodged breast implant. However, during a period of years prior to surgery, the patient had become increasingly estranged from all relatives. Infrequent, irregular involvement with family precluded confident delineation of any specific neurological or psychiatric symptoms and their chronological evolution but a sister maintained some contact, describing 6 months of "low mood" and possible memory problems. In addition, posthumously, the sister of the proband became aware of some apparently unusual behavior, such as buying inappropriately sized underwear and the likely hoarding of large supplies of cleaning products, although in contrast, financial affairs and housework had been maintained in good order. In the immediate postoperative period, the patient developed what was thought to be an acute confusional state with delusional ideation, for example, claiming that she had swallowed a 5-cent coin and that staff members had been forcing her to swallow jewelry. The proband's sister affirmed that the postoperative demeanor was completely uncharacteristic.

Following surgery, gait unsteadiness was also noteworthy and 2 falls occurred while she was in the hospital. Mental status assessment in the postoperative period confirmed cognitive impairment (Mini Mental State Examination score, 20 out of 30), especially defective short-term recall and mild expressive and nominal dysphasia. Neurological examination revealed cerebellar ataxia with bilateral intention tremor, generalized hyperreflexia, and myoclonus. The patient never left institutional care following her surgery, experiencing rapid, steady cognitive and gross motor decline and dying in an akinetic-mute state around 1 month following surgery.

There was no known family history of neurodegenerative disease in first-degree relatives (Figure 1), with the proband's mother dying aged 88 years of cancer and the father aged 92 years as a consequence of a head injury sustained from a fall. Scant, imprecise information existed for nonfirst-degree relatives, especially in the patrilineage, but there was a history of a maternal aunt dying (age unknown) in a mental hospital after an illness of only a few months' duration. The patient had migrated to Australia at the age of 37 years, around the time of onset of the United Kingdom bovine spongiform encephalopathy epidemic. There was no history of recognized risk factors for iatrogenic prion disease.

Routine blood test results were unremarkable. Cerebrospinal fluid test results were normal aside from the presence of 14-3-3 proteins. Magnetic resonance imaging of the brain showed mild generalized atrophic changes (consistent with age) and a few, nonspecific, deep white matter hyperintensities in the cerebral hemispheres, but there were no findings more typical of sporadic Creutzfeldt-Jakob disease such as areas of gray matter restricted diffusion nor increased signal on fluid-attenuated inversion recovery sequences (Figure 2). Electro-
encephalography findings showed attenuated alpha rhythms with frequent diffuse rhythmic delta activity but no periodic complexes.

Results

Neuropathological Findings
Routine hematoxylin and eosin stains of frontal, temporal, and occipital cortical sections showed numerous (densely eosinophilic) amyloid plaques (Figure 3A), neuronal loss, and astrocytic gliosis. The amyloid plaques, sometimes appearing multicentric, were extensively deposited, most marked in the lower laminae, with immune-peroxidase studies showing reactivity with both 3F4 and 12F10 anti-PrP monoclonal antibodies (Figure 3B); plaques did not demonstrate amyloid-β immunoreactivity. Many of the amyloid plaques showed an associated microglial response but did not resemble florid plaques (Figure 3A). The basal ganglia showed a similar prominent deposition of closely packed, granular deposits, again immunoreactive with 3F4 and 12F10 anti-PrP monoclonal antibodies. Scant spongiform change was seen focally in the cerebral cortex, basal ganglia, and cerebellar cortex. Hippocampal sections displayed numerous tau immunoreactive neurofibrillary tangles in association with extensive neuropil thread formation and areas of neuritic plaque formation (Figure 3C).

In the cerebellum, there was extensive immunopositive prion protein deposition within the molecular layer, patchy Purkinje cell loss, and a moderately intense microglial response within the cortex. This extensive PrP-positive amyloid deposition in association with prominent tau pathology was felt to be most consistent with a neuropathological diagnosis of GSS disease.

PRNP Genotyping
Appropriate consent for genetic examination was obtained. Genomic DNA was extracted from the brain, and the PRNP open reading frame was sequenced in the forward and reverse orientations with overlapping primers (PrP106 and PrP46 sequences described previously12; PrPF2 5′-CCGAGTAAGC-CAAAAACCAAC-3′ and PrPR2 5′-TCACTGCCGAAATGTATGATG-3′). Sequencing revealed the patient to be homozygous for valine at the polymorphic codon 129. A guanine to adenine nucleotide substitution was found in a 5′ noncoding region, at position -21 relative to the start codon, and a synonymous adenine to guanine substitution was seen at the third position of codon 117, transitions both previously described as PRNP polymorphisms.13 A thymine to guanine transversion was detected at the second position of codon 176, resulting in a predicted amino acid change from a valine to glycine. This mutation has not previously been reported. A diagrammatic representation of these sequence variations is shown in Figure 4A.

PrP<sup>sc</sup> Analysis
Frozen post mortem brain specimens from the cerebellum and occipital pole were homogenized to 10% (weight/volume) in phosphate-buffered saline. Aliquots were analyzed by Western blot with or without proteinase K (PK) digestion, as previously described.12 Briefly, samples were digested with 100 μg/mL PK for 1 hour at 37°C, before mixing with the appropriate polyacrylamide gel electrophoresis (PAGE) sample buffer.
Samples (using double the volume of PK digested compared with undigested) were resolved on 16% Tris-glycine or 12% NuPAGE (Invitrogen) gels and transferred to polyvinylidene difluoride membrane (Millipore) before incubation with the appropriate primary and secondary antibody and visualization using enhanced chemiluminescence (ECL Plus; Amersham).

Initially, before PRNP sequencing results were known, samples were subjected to sodium dodecyl sulfate–PAGE and Western blot analyses of protease-resistant PrP (PrPres) following PK digestion alongside gly cope type 1-4 controls, using the commonly used 3F4 antibody as previously described.14 As seen in Figure 4B, the characteristic di-, mono-, and un-glycosylated triple banding pattern of PrP^res was not present in the proband. Rather, a prominent immunoreactive, PK-resistant fragment of less than 15 kDa was observed. Numerous additional minor bands, creating a ladder spanning from greater than 15 kDa to at least 50 kDa, often superimposed on subtle lane smearing, were also observed.

Epitope mapping using antibodies spanning the length of the prion protein (Figure 4C and Table) was carried out to further characterize the small fragment. As seen in Figure 4C, the PrP^res fragment, resolving at approximately 8 kDa, was not immunoreactive with antibodies to the far N-terminus (8B4) or those with epitopes beyond codon 143 (ICSM 18) toward the C-terminus of the prion protein, leading to the conclusion that the 8-kDa fragment resulted from combined N- and C-terminal truncation of PrPres. Based on its apparent molecular weight and the known antibody epitopes, we predicted the 8-kDa fragment resulted from PrP cleavage around residues 60 and 140. Furthermore, the higher molecular weight bands

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**Table: PRNP Open Reading Frame**

<table>
<thead>
<tr>
<th>Codon</th>
<th>Amino Acid</th>
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<tbody>
<tr>
<td>117</td>
<td>A/A</td>
</tr>
<tr>
<td>129</td>
<td>V/V</td>
</tr>
<tr>
<td>176</td>
<td>V/G</td>
</tr>
</tbody>
</table>

**Figure 4. Molecular-Genetic Characterization**

A. Schematic representation of the prion protein open reading frame and protein, with the nucleotide changes, codon positions, and resulting amino acid changes detected in the proband as indicated. B. Proteinase K (PK) digest and polyacrylamide gel electrophoresis/Western blot analysis (using 3F4 antibody) of frozen brain tissue from 2 brain regions of the proband (cerebellum [Ce] and occipital pole [OP]) compared with glycotype controls (T1, T2, T3, and T4: protease-resistant prion protein [PrPres] glycotypes 1, 2, 3, and 4).14 C. Characterization of the small amyloidogenic PrP^res fragment by polyacrylamide gel electrophoresis/Western blot epitope mapping, using anti-prion protein antibodies spanning the length of the protein as specified. Schematic representations of the small fragment and oligomeric species and of the potential N- and C-terminal truncation sites based on antibody epitopes are highlighted on the right and below, respectively; WT indicates wild type.
were thought to represent aggregation of the 8-kDa fragment into highly stable, higher-order species.

**Tau Analysis**
Cerebrospinal fluid was examined by enzyme-linked immunosorbent assay (Innotest hTau Ag and Phospho-Tau [181P], Innogenetics NV Belgium), according to the manufacturer’s instructions, and showed both total tau and tau phosphorylated at threonine 181 were elevated (total tau, 1409 [normal, <412 pg/mL]; phospho-tau, 126 [normal, <82 pg/mL]). Reference ranges were determined from 71 well-characterized, healthy elderly volunteers participating in the Australian Imaging Biomarker Lifestyle Study.6

The cerebellum and hippocampus were homogenized (10% [weight/volume] in phosphate-buffered saline) and analyzed by Western blotting for the different tau isoforms using 4% to 12% NuPAGE gels and probed with a rabbit anti-human tau antibody (1:3000; Dako). In the cerebellum (absence of tau pathology), there was no difference in the tau isoform profile when comparing the proband with age-matched control and Alzheimer disease brains. In the hippocampus (abundant tau pathology), the tau isoform profile of the proband did not resemble that seen in either the control or Alzheimer disease brain (data not shown).

**Discussion**
Characteristically, GSS disease is inherited in an autosomal-dominant manner,4 therefore, usually occurs in the setting of a known family history of similar neurodegenerative disorder. No history of similar neurological disease was known among first-degree relatives; however, the absence of family history can occur in up to 30% of GSS disease pedigrees.2

Awareness of a maternal aunt dying in a mental hospital from a poorly characterized illness of apparently only months in duration is of uncertain but possible relevance.

Our proband manifested rapid cognitive and gross motor decline following her minor elective surgery, dying approximately 1 month after the procedure. The proband’s limited contact with her family prior to surgery rendered precise determination of presenting symptoms and their duration impossible. However, notwithstanding the long-standing estrangement from her family, the information available suggests a relatively brief duration of cognitive and behavioral changes for up to 6 months prior to hospital admission. Although limited survival after first symptoms has been reported in GSS disease, it is uncommon that durations of this length occur in patients with GSS disease.3,6,10,11 Typically, GSS disease follows a prolonged clinical course, evincing a median duration of more than 3 years.3 Our patient aligned more to the contrasting relatively short median survival observed in sporadic Creutzfeldt-Jakob disease.3 In addition, molecular pathological evaluation of our propositus using the 3F4 antibody demonstrated prominent, low-molecular-weight, protease-resistant PrP**res** bands on Western blots of the brain, a feature more usually associated with much longer survival.6,10 Similarly, although valine homozygosity at codon 129, as seen in our patient, has been reported in GSS disease, it is most unusual; furthermore, it also appears to usually correlate with prolonged symptomatic illness.6,10,11

Predominance of shorter (<15-kDa) PK-resistant PrP**res** fragments on Western blots has been observed in GSS disease in association with numerous mutations, including those clustered toward the C-terminus of the prion protein (H187R, F198S, D202N, Q212P, Q217R, and Y218M), as well as those situated more in the middle of the protein (P102L, P105L, A117V, G131V, and S132I).6,10,17 It remains to be determined how PrP-containing mutations within the C-terminus, including V176G, engender processing such that the core of amyloid deposits appear to be constituted by truncated species that do not actually harbor the mutation.10

Aligned to previous reports for the G131V, S132I, Y160X, H187R, F198S, D202N, Q217R, Y218M, and Q227X mutations,6,9,10,17 the presence of shorter, nonclassic PrP**res** fragments on Western blots in our proband was associated with the neuropathological profile of high amyloid plaque burden, neurofibrillary degeneration, and relative paucity of spongiform change in the brain; however, some mutations, such as P102L, P105L, and A117V, despite the presence of shorter PrP**res** fragments on Western blots and considerable amyloid deposition in the brain, do not consistently display neurofibrillary degeneration.17 It is noteworthy that those patients with GSS disease displaying predominance of longer 21-kDa to 30-kDa PrP**res** fragments on Western blots, as exemplified by some P102L carriers, generally have more prominent neuropil vacuolation, usually associated with synaptic-type PrP immunostaining rather than mainly amyloid plaque burden.10,13 The presence of tau-positive neurofibrillary tangles and neuropil threads, as frequently observed in GSS disease cases including our proband, underscores the likelihood that accumula-

### Table. Panel of Anti-PrP Antibodies Used for Epitope Mapping

<table>
<thead>
<tr>
<th>1st Antibody</th>
<th>1st Dilution</th>
<th>2nd Antibody (HRP Conjugated)</th>
<th>2nd Dilution</th>
<th>Supplier</th>
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<tr>
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<tr>
<td>EP1802Y (monoclonal)</td>
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<td>1:5000</td>
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</tr>
</tbody>
</table>
tion of aberrant tau in the brain may be a relatively nonspecific secondary consequence to certain primary cerebral amyloidoses, as typified by Alzheimer disease.

In summary, in addition to expanding the range of PRNP mutations found in neuropathologically confirmed prion disease, our case is of interest and informative through displaying a constellation of clinical, genetic, molecular, and pathological features, which although individually recognized across the spectrum of GSS disease, are unusual in their co-occurrence in a single person. Consequently, our proband serves to further broaden the already diverse genetic and phenotypic spectrum recognized for GSS disease.

REFERENCES


