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ORIGINAL ARTICLE

A Second Case of Gerstmann-Sträussler-Scheinker Disease Linked to the G131V Mutation in the Prion Protein Gene in a Dutch Patient

Casper Jansen, MD, Piero Parchi, MD, PhD, Sabina Capellari, MD, Rosaria Strammiello, PhD, Elise G.P. Dopper, MD, John C. van Swieten, MD, PhD, Wouter Kamphorst, MD, and Annemieke J.M. Rozemuller, MD, PhD

Abstract

A rare case of Gerstmann-Sträussler-Scheinker disease in a 36-year-old Dutch man is reported. The clinical phenotype was characterized by slowly progressive cognitive decline, later followed by ataxia and parkinsonism. Neuropathologic findings consisted of numerous amyloid plaques in the cerebellum, which showed positive staining for the abnormal prion protein (PrP^{Sc}). In addition, there were tau accumulations around numerous amyloid deposits in the cerebral cortex, striatum, hippocampal formation, and midbrain. There was no spongiform degeneration. Western blot analysis showed the co-occurrence of 2 distinct abnormal prion protein species comprising an unglycosylated, protease-resistant fragment of approximately 8 kd, which was found to be truncated at both N- and C-terminal ends by epitope mapping, and a detergent-insoluble but protease-sensitive form of full-length PrP^{Sc}. Sequence analysis disclosed a mutation at codon 131 of the prion protein gene (*PRNP*), resulting in a valine-for-glycine substitution (G131V). The patient was heterozygous at the polymorphic codon 129 and carried the mutation on the methionine allele. To our knowledge, this is the second family worldwide in which this mutation has been identified. Gerstmann-Sträussler-Scheinker disease should be considered in patients with a clinical diagnosis of familial frontotemporal dementia.

Key Words: Genetic Creutzfeldt-Jakob disease, Gerstmann-Sträussler-Scheinker disease, Prion protein, Tau.

INTRODUCTION

Prions cause largely transmissible neurodegenerative diseases associated with accumulation of PrP^{Sc}, a misfolded and aggregated form of the cellular prion protein PrP^{C} (1). Gerstmann-Sträussler-Scheinker (GSS) disease arises owing to mutations in the prion protein gene (*PRNP*), which encodes PrP^{C}. In its classic form, GSS disease is characterized by a progressive cerebellar syndrome accompanied by extrapyramidal and pyramidal signs and cognitive decline, which may evolve into severe dementia (2). However, there is considerable heterogeneity in age at onset, disease duration, and clinical manifestations, both within and between families with the same mutation. Neuropathologic features associated with GSS disease always include multicentric amyloid plaques composed of PrP^{Sc}, but in some patients, neurofibrillary tangles and/or spongiform changes may also be present (2). To date, approximately 15 point mutations in *PRNP* have been associated with GSS disease, including both missense and nonsense mutations. Among these mutations, a point mutation at codon 102 occurs relatively frequently, whereas mutations at other codons are rarer. In addition, several insertional mutations in the N-terminal octapeptide repeat region of *PRNP*, resulting in a GSS disease-like phenotype, have been described (3).

In 2001, Panegyres et al (4) reported a novel G131V mutation in *PRNP* associated with GSS disease. Since then, no other patients with a similar mutation have been identified. Here, we describe the clinical, neuropathologic, and molecular findings in a Dutch patient with the G131V mutation in *PRNP*.

MATERIALS AND METHODS

Case History

The patient, a male of Indo-Asian descent, presented with a change in personality, forgetfulness, and concentration problems at the age of 36. He showed delusions of grandeur, especially related to his work, and recklessly spent large amounts of money. He became restless and aggressive, both verbally and physically. His family noted that he had become increasingly apathetic and showed flattening of affect and loss of intimacy. His clinical history was otherwise unremarkable. Neurological examination did not reveal any focal signs. Bradyphrenia and confabulations were noted on neuropsychologic evaluation. He also showed short-term memory impairment, was highly distractible, and was disoriented in time and

From the Dutch Surveillance Centre for Prion Diseases (CJ, AJMR), University Medical Centre Utrecht, Utrecht, the Netherlands; Dipartimento di Scienze Neurologiche (PP, RS), Università di Bologna, Bologna, Italy; Department of Neurology (EGPD, JCVS), Erasmus University Medical Center, Rotterdam; Netherlands Brain Bank (WK, AJMR), Netherlands Institute for Neuroscience, Amsterdam; and Department of Pathology (AJMR), VU University Medical Centre, de Boelelaan, Amsterdam, the Netherlands.

Send correspondence and reprint requests to: Casper Jansen, MD, Dutch Surveillance Centre for Prion Diseases, University Medical Centre Utrecht, Heidelberglaan 100, PO Box 85500, 3508 GA, Utrecht, the Netherlands; E-mail: c.jansen@umcutrecht.nl

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place. He did not have any insight into his functioning. An electroencephalogram showed diffuse hypofunctional changes but no periodic synchronous wave complexes. A computed tomograhic scan of his brain showed mild atrophy of the cerebral cortex and cerebellum. The clinical diagnosis was frontotemporal dementia. During the next years, his cognition further deteriorated. His spontaneous speech was reduced, and he developed perseverations and stereotypes, resulting in admission to a nursing home 3 years after disease onset. During his stay, he developed apraxia, ataxia, compulsive laughing, tremor, incontinence, and myoclonus. Neurological examinations revealed a masked face, bradykinesia, rigidity of arms and legs, with cog wheeling of the arms, and a tendency to fall. He died of a recurrent bronchopneumonia 15 years 8 months after the disease onset at the age of 52.

The patient's father had died of a similar dementia at the age of 44. He had experienced personality changes, restlessness, loss of decorum, perseverations, disorientation, parkinsonism, dysarthric speech, and inability to write. The autopsy report mentioned severe frontal and cerebellar atrophy with plaque-like structures in the cerebellum. Unfortunately, neither the slides nor the tissue blocks could be retrieved for this study.

Neuropathology

All tissues were obtained from the Netherlands Brain Bank (NBB), Netherlands Institute for Neuroscience, Amsterdam. A written informed consent for brain autopsy was available, and the use of the material and clinical information for research purposes had been obtained by the NBB. Both frozen and formalin-fixed tissues were sampled within 8 hours after death. Diagnostic tissue blocks were obtained from all areas of the cerebral cortex, basal ganglia, thalamus, subthalamic nucleus, limbic area, hippocampus and parahippocampal gyrus, mesencephalon including substantia nigra, colliculus inferior, locus coeruleus and base of pons, medulla oblongata, and cerebellum, including dentate nucleus. Histopathologic examination was performed on 5-μm-thick sections of formalin-fixed, paraffin-embedded brain tissue blocks. Hematoxylin and eosin, Bodian silver, methenamine silver, and combined Luxol fast blue–periodic acid–Schiff reaction stains were performed using standard techniques. Congo red stains were performed on several sections of the cerebral and cerebellar cortex. Monoclonal antibody 3F4 (1:400; Signet Laboratories, Dedham, MA) was used for immunohistochemical detection of the disease-associated form of the prion protein after pretreatment with proteinase K. Other immunohistochemical stains included antibodies against β-amyloid ($A\beta_{1-17}$, 1:400; Dako, Glostrup, Denmark), phosphorylated tau (AT8 1:250; Innogenetics, Gent, Belgium), glial fibrillary acidic protein (1:800; Dako), HLA-DRβ (1:400; Dako), and ubiquitin (1:500; Dako).

Genetic Analysis

Genomic DNA was used to amplify the coding region of *PRNP*. The codon 129 genotype was examined by digestion with the restriction endonuclease Nsp1. After screening with denaturing high-pressure liquid chromatography, the *PRNP* open reading frame was sequenced as described (5).

Biochemical Studies

PrP^{Sc} was extracted from several brain regions (frontal, temporal, parietal, and occipital cortices, cingulate gyrus, striatum, thalamus, and cerebellum) and characterized by Western blotting on crude homogenates (6) or after partial purification of the protein in sarkosyl (7). The following mouse monoclonal antibodies recognizing different human PrP epitopes were used: 3F4 (residues 106–110) (8), 12B2 (residues 89–93) (9), Sha31 (residues 145–152) (10), and SAF60 (residues 157–161) (all from Signet Laboratories) (10). An antiserum against the N-terminal end (anti-N, residues 29–41, provided by Dr B. Ghetti, Indiana University, Indianapolis, IN) was also used. For PrP deglycosylation, denatured proteins were incubated with peptide N-glycosidase F (PNGase F) following the instruction from the manufacturer (New England BioLabs, Inc, Beverly, MA).

RESULTS

Neuropathology

The brain (weight, 1,460 g) showed severe atrophy of the frontal cerebral cortex and mild atrophy of the cerebellum. Microscopic examination revealed numerous congophilic plaques and diffuse deposits distributed in varying degrees throughout most gray matter structures of the cerebrum, cerebellum (Figs. 1A, B), and midbrain (Table). There was no evidence of spongiform degeneration. Amyloid deposition was most marked in the molecular layer of the cerebellum, Ammon horn (CA1 region), subiculum and entorhinal cortex, followed by the thalamus and midbrain (Table). The transentorhinal region, pons, and medulla oblongata were relatively spared. The amyloid plaques stained intensely with anti-PrP antibodies. Immunohistochemistry for PrP also showed widespread diffuse positivity in the cerebral cortex and striatum, mainly in a reticular or linear pattern (Figs. 1C-E). Amyloid deposits were surrounded by microglial cells and abnormal neurites.

Neurofibrillary tangles were found in the Ammon's horn of the hippocampus and in the entorhinal cortex but not in the temporal neocortex or striate area of the neocortex, which would correspond to Braak stage III in Alzheimer disease (11). Pretangles were noted in the striatum and medial nucleus of the thalamus (Fig. 1G). In addition, tau-positive neuropil threads were found in all areas of the cortex, striatum, thalamus (medial nucleus), Ammon horn, entorhinal region, mesencephalon, and cerebellum (Figs. 1F, H). The tau deposits were most numerous in the areas of the brain that showed the most severe PrP amyloid deposits and appeared mainly to be colocalized with the amyloid deposits. The absence of Aβ pathology excluded Alzheimer disease.

Genetic Analysis and Biochemical Studies

Restriction enzyme analysis showed heterozygosity for methionine and valine at codon 129 of *PRNP*. DNA analysis revealed a G131V mutation on the methionine allele. Western blot analysis of brain homogenates revealed 2 major PrP^{Sc} forms comprising an ~8-kd unglycosylated, protease-resistant fragment and a detergent-insoluble but protease-sensitive form of full-length PrP^{Sc} (Fig. 2). The 8-kd fragment was recognized

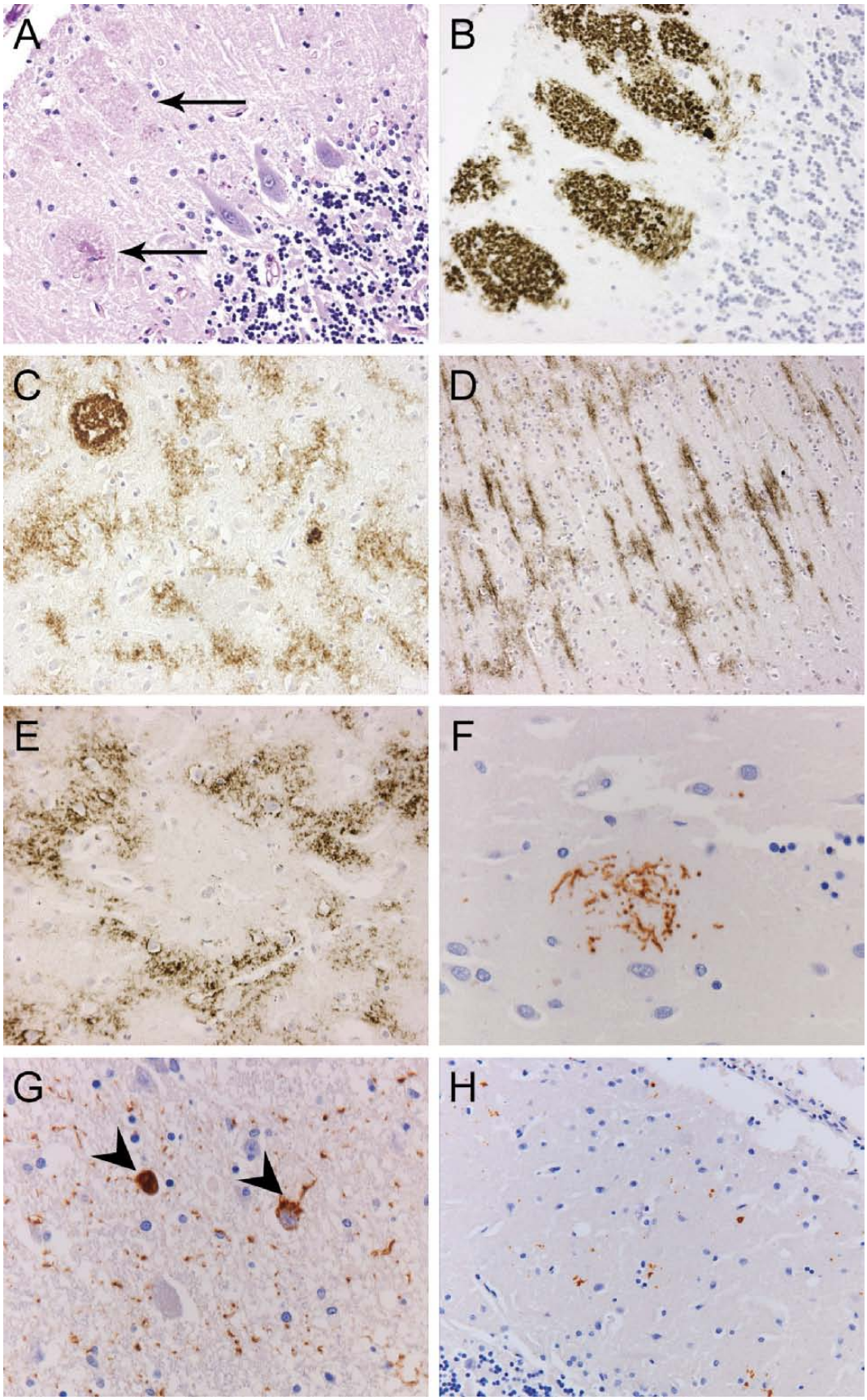

TABLE. Regional Distribution of Spongiosis, Gliosis, Tau Accumulation, and PrP Deposits

	PC	FC	TC	OC	Str	Th	Hipp	Entorh	Midbr	Pons	Med	Amygd	Cblm
Spongiosis	−	−	−	−	−	−	−	−	−	−	−	−	−
Gliosis	+	+	+	+	+	+ +	+ + +	+ + +	+ +	−	−	+	+ + +
Tau deposition	+	+	+	+	+	+ +	+ + +	+ + +	+ +	−	−	+	+ + +
PrP deposits/plaques	+	+	+	+	+	+ +	+ + +	+ + +	+ +	−	−	+	+ + +

Each anatomic region was scored semiquantitatively as follows: not detectable (−), mild (+), moderate (+ +), and severe (+ + +). Numbers of PrP-positive multicentric plaques were counted per high-power field and scored as follows: not detectable (−), 1 to 4 plaques (+), 5 to 9 plaques (+ +), and 10 to 15 plaques (+ + +).

Amygd, amygdala; Cblm, cerebellum; Entorh, entorhinal cortex; FC, frontal cortex; Hipp, hippocampal formation; Med, medulla oblongata; Midbr, midbrain; OC, occipital cortex; PC, parietal cortex; Str, striatum; TC, temporal cortex; Th, thalamus.

by antibodies that recognize epitopes spanning between residues 89-111 (12B2, 3F4), but was not detected by antibodies against the N-terminus (anti-N) or the C-terminus beyond residues 144 (SAF60, SHA131); thus, this fragment is truncated at both the N- and C-terminal ends (Fig. 2A).

A second band migrating at approximately 17 kd and other bands at higher molecular weight were also evident in the Western blots obtained with proteinase K–treated samples. Despite the change in molecular weight, however, all of these bands showed the same antibody recognition pattern as the 8-kd band. In particular, they were not recognized by SHA131 and SAF60 (Fig. 2A). This strongly suggests that they represent dimers or multiple aggregated forms of the 8-kd fragment.

The analyses of partially purified (P3) PrP^{Sc} preparations in sarkosyl showed that the 8-kd fragment, as well as the 17-kd band, were already visible in proteinase K–untreated preparations and that their electrophoretic mobility did not change after N-linked deglycosylation (Fig. 2B). Finally, the latter analyses demonstrated that the affected brain also contains full-length, detergent-insoluble but protease-sensitive PrP^{Sc} (Fig. 2B).

DISCUSSION

We describe the clinical, neuropathologic, and biochemical findings in a 36-year-old patient with a G131V mutation in *PRNP*. To our knowledge, this mutation has been described only once before by Panegyres et al (4) in a white male who was homozygous for methionine at codon 129 of *PRNP*. The clinical phenotype in both patients was remarkably similar and was characterized by an initial presentation of dementia followed by ataxia or parkinsonism. Both patients died at approximately the same age (52 and 51 years, respectively).

On neuropathologic analysis, both brains showed numerous PrP amyloid plaques in the molecular layer of the cerebellum; the frontal, parietal, and temporal cortices; the striatum; and the thalamus. These were accompanied by hyperphosphorylated tau depositions but without spongiform degeneration. The results of immunoblotting were consistent with an absence of spongiosis, in both cases showing smaller protease-resistant ~8-kd unglycosylated fragments truncated at both the C-terminal and N-terminal end, thus lacking the glycosylphosphatidylinositol anchor, which is typical for GSS (2, 7, 12), in the absence of the 19- to 21-kd truncated fragments that are typical of Creutzfeldt-Jakob disease (CJD) or GSS P102L with spongiform changes (7).

However, there were also minor differences between the 2 patients. In contrast to the patient described by Panegyres et al, our patient had a positive family history of neurodegenerative disease, consistent with an autosomal dominant inheritance pattern. In addition, the disease duration in our patient was substantially longer (approximately 15 years), and the neuropathologic findings were more severe. PrP amyloid plaques were present in more areas of the brain, including the occipital cortex, midbrain, and amygdala. Tau deposits were also more numerous, that is, they were not just restricted to the Ammon horn and entorhinal cortex (4) and largely following the distribution of the amyloid plaques. Although the longer disease duration in our patient is the most likely cause of the more extensive PrP deposits, the haplotype of the nonmutated allele (methionine vs valine at codon 129 of *PRNP*) may have played a role as well.

A striking pathologic feature in our case was the association of PrP-positive amyloid plaques with dystrophic neurites. Tau pathology is relatively uncommon in most forms of familial prion disease but is a prominent feature in patients harboring the P105L-129V, A117V-129V, F198S-129V, D202N-129V, Q217R-129V, and, more recently, the Y218N haplotypes (12–17). Furthermore, induction of tau phosphorylation has been described in other PrP cerebral amyloidoses, including PrP cerebral amyloid angiopathy associated with nonsense mutations in *PRNP* (18) and variant CJD (19). The tau accumulations in these diseases almost invariably cluster around PrP amyloid deposits, suggesting a relationship between amyloid fibrils and the accumulation of hyperphosphorylated tau. Recently, however, tau deposits have also been described in patients with sporadic CJD (20), E200K genetic CJD (21), and fatal familial insomnia (22) in the absence of PrP^{Sc} amyloid plaques or coexisting Aβ amyloid.

FIGURE 1. Histologic and immunohistochemical findings. **(A)** The cerebellum shows occasional plaques in the molecular layer (arrows). Luxol fast blue–periodic acid–Schiff stain. **(B–E)** Immunostaining with the anti-PrP antibody 3F4 shows numerous large PrP deposits in the cerebellum **(B)**. Prion protein deposits are often arranged in a reticular or linear pattern with occasional plaques in the parietal cortex **(C)**, occipital cortex **(D)**, and striatum **(E)**. **(F–H)** Immunostaining for tau. In the striatum **(F)** and thalamus **(G)**, there are neuropil threads and pretangles (arrowheads) that are mainly associated with amyloid deposits. Neuropil threads and tau deposition were also found in the molecular layer of the cerebellum **(H)**. Original magnifications: **(A, B, C, E, H)** ×200; **(D)** ×100; **(F, G)** ×400.

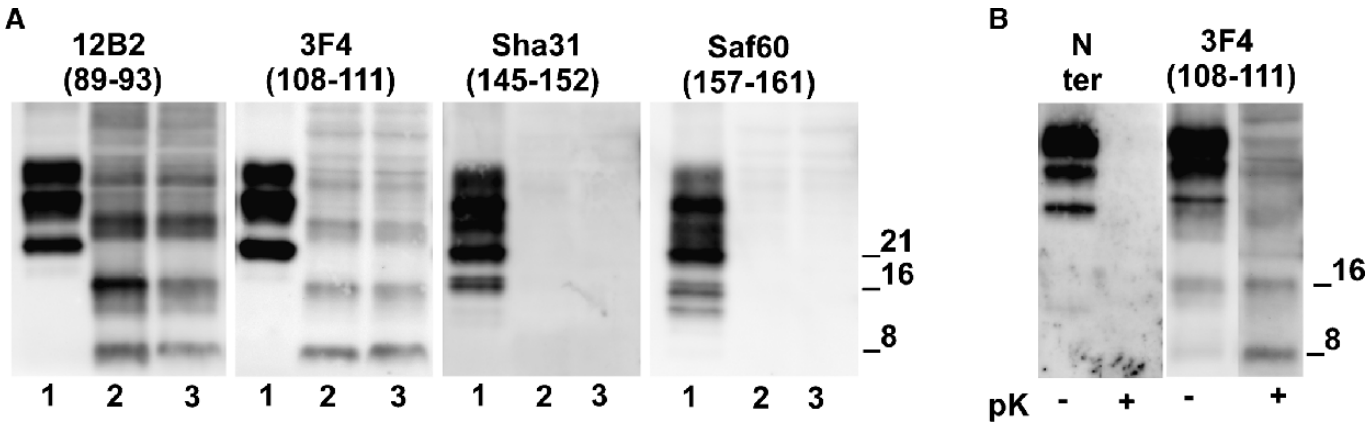

FIGURE 2. Western blot analyses. **(A)** Proteinase K (PK)–treated PrP^{Sc} extracts from crude homogenates of the frontal and occipital cortices (lanes 2 and 3, respectively). A sample of PK-treated PrP^{Sc} extracted from a subject with sporadic Creutzfeldt-Jakob disease MM 1 + 2C (lane 1) is included as a positive control. The results obtained with all 4 antibodies (12B2, 3F4, Sha31, and SAF60) and the epitopes recognized by them are shown. **(B)** Western blot analysis of partially purified abnormal PrP^{Sc} extracted from the frontal cortex. The results obtained with the anti-N antiserum (N-ter) and the 3F4 antibody on samples treated with (+) or without (−) PK are shown. Relative molecular masses are indicated in kilodaltons.

Although the exact mechanism remains to be elucidated, these findings seem to indicate that altered metabolism of mutated PrP interferes with the processing of other proteins and leads to their pathologic accumulation (21).

In conclusion, this patient with G131V inherited prion disease is the first to be described in the Netherlands and represents the second patient worldwide in whom this mutation has been identified. Our findings highlight the importance of autopsy investigation of atypical or familial dementias. In addition, GSS disease should be considered in patients with a clinical diagnosis of familial frontotemporal dementia.

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