

SHORT REPORT

Type 1 protease resistant prion protein and valine homozygosity at codon 129 of *PRNP* identify a subtype of sporadic Creutzfeldt-Jakob disease

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Abstract

A man was studied with sporadic Creutzfeldt-Jakob disease (sCJD) who had serial cortical syndromes evolving over 15 months without significant ataxia, prominent myoclonus, or periodic complexes on EEG examinations. This clinical phenotype correlated with a predominantly cortical and striatal distribution of lesions and accumulation of protease resistant prion protein with relative sparing of the brainstem or cerebellum. No amyloid plaques were seen and prion protein (PrP) immunohistochemistry only demonstrated very faint granular deposits in the cerebral cortex. Molecular analysis showed homozygosity for valine at codon 129 in the prion protein gene (*PRNP*) and protease resistant prion protein type 1 deposition. The comparison of molecular and clinicopathological features of the present case with those previously reported in sCJD, indicates that valine homozygosity at codon 129 and type 1 protease resistant prion protein are associated with a distinct phenotypic variant of sCJD. The data also support the view that the *PRNP* codon 129 polymorphism and the physicochemical properties of the protease resistant prion protein are major determinants of phenotypic variability in sCJD.

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Sporadic Creutzfeldt-Jakob disease (sCJD), the most common human prion disease, includes distinct phenotypes or variants, which differ in clinical signs at onset, type, and distribution of lesions, and pattern of cerebral deposition of the abnormal, protease resistant, isoform of the prion protein (PrP-res), the hallmark of these disorders.^{1,2} At the molecular level, phenotypic heterogeneity largely correlates with the genotype at codon 129 of the prion protein gene (*PRNP*), the site of a common methionine (M)/valine (V) polymorphism, and the physicochemical properties of

PrP-res.² Two types of PrP-res with distinct relative molecular masses, and four distinct phenotypes have been described in a series of 19 patients with sCJD.² PrP-res type 1, the most common isoform, was only seen in patients homozygous for methionine at codon 129 (MM1). This combination was found in patients with a typical CJD phenotype, characterised by a short duration of illness with prominent early dementia and abnormal periodic sharp wave complexes (PSWCs) on EEG; whereas the rarest PrP-res, type 2, was found in association with three distinct phenotypes and any of the three codon 129 haplotypes: MM2, MV2, and VV2.²

More recently, PrP-res type 1 was found in association with VV and MV at codon 129.³ Here we report the detailed study of clinical and pathological features of one patient with PrP-res type 1 and VV. Our results indicate that this novel molecular combination is linked to a distinct clinicopathological variant of sCJD.

Case history

The patient was a 49 year old restaurateur who presented with a 4 month history of progressive disorganisation and confusion.

In February 1996, he was first noted to have difficulty reading clocks. Over the ensuing 4 months, he developed insidious personality changes, confusion, general disinterest in activities, and temporal and spatial disorientation. He was admitted to the psychiatry service in July 1996.

He had no history of medical or psychiatric illness, surgery, or hormone therapy. There was no family history of dementia, psychiatric, or neurological disease.

On examination he was alert with preserved language skills, social interactiveness, and humour. He appeared distractible, loquacious, and labile. There was a snout reflex but no other frontal release signs, neglect, or ideomotor apraxia. Elements of Gerstmann syndrome included acalculia, finger agnosia, and poor right-left differentiation. There were signs of constructional apraxia, map apraxia, topographical agnosia, and bilateral agraphesthesia. Memory difficulties fluctuated; at times he showed nearly complete amnesia with confabu-

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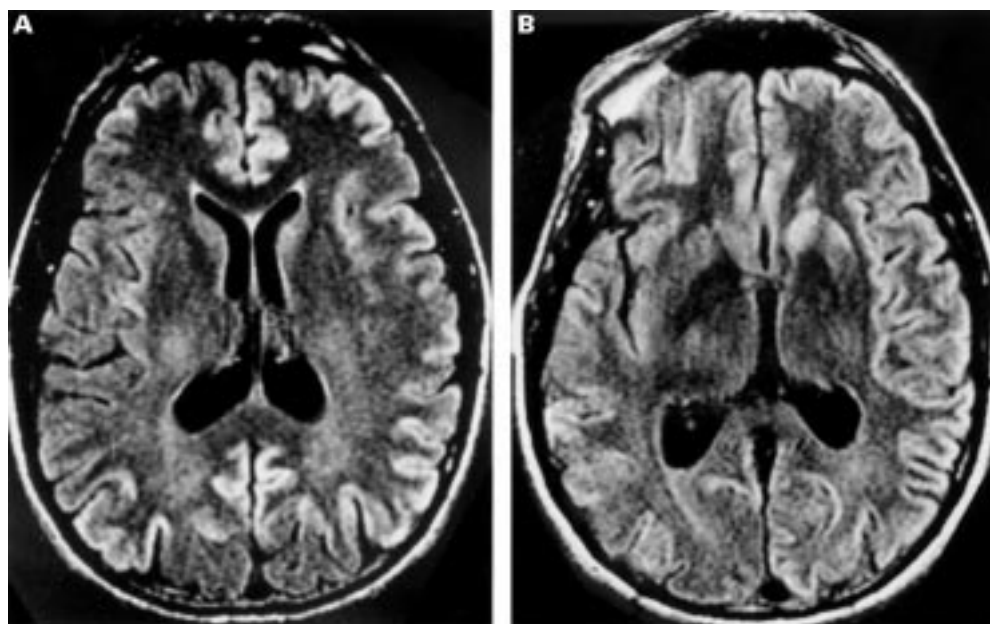


Figure 1 (A) First MRI (July 1996): FLAIR image shows increased signal in the cortical ribbon. (B) Follow up (November 1996): progression of cortical abnormalities and interval development of abnormal FLAIR signal in the striatum more prominent on the right; MRI in sCJD is usually normal or shows non-specific atrophy although prominent basal ganglia or thalamic hyperintensities have been reported. Cortical abnormalities may correlate with clinical signs. In our patient, the abnormalities were seen only on FLAIR images and evolved in clinical correlation. The FLAIR abnormalities also helped guide the biopsy.

lation. Both experiential and historical time were disturbed. He had no hallucinations, visual field problems, visual distortions, or altered colour perception. There were no extrapyramidal features or pyramidal signs. Sensation, strength, coordination, and gait were normal. There was no myoclonus, amyotrophy, or startle response. Neuropsychological tests confirmed memory and constructional problems without impairment of language or abstraction. Cranial CT and MRI with and without gadolinium, and EEG were normal.

Fluid attenuated inversion recovery MRI (FLAIR) disclosed patchy areas of cortical high signal in the right temporal and both parietal lobes without abnormalities in the basal ganglia or thalamus (fig 1A). There was minimal involvement of the frontal and occipital lobes. Single PET demonstrated hypoperfusion in a similar pattern. Right temporal lobe biopsy confirmed spongiform encephalopathy.

Subsequently, he developed left sided neglect, perseveration, and word finding difficulty evolving into fluent aphasia and mutism. Later, he was hypokinetic with mild action induced myoclonus. He never had ataxia, visual or sensory complaints, or pyramidal or extrapyramidal signs. His EEGs became disorganised and slow without PSWCs. Sequential FLAIR MRI showed cortical and striatal abnormalities that evolved in parallel with neurobehavioural deficits (fig 1B). He died in May 1997, 15 months after the first symptoms.

PATHOLOGICAL FINDINGS

Histopathological studies of the biopsy showed severe cortical spongiform change sparing the white matter microscopically and ultrastructurally. No amyloid plaques were identified.

At necropsy, the brain weighed 1260 g with mild gross atrophy. Microscopic examination of the right hemisphere showed extensive

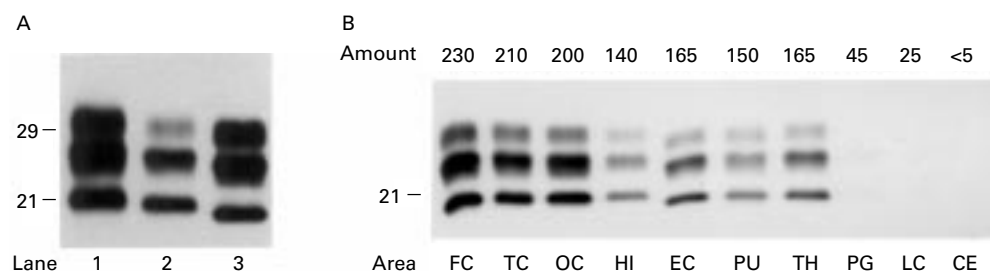


Figure 2 (A) Western blot immunodetection of prion protein after proteinase K digestion (PrP-res). Lane 1: cerebral cortex from a patient with sCJD, MM at codon 129 of PRNP, and with PrP-res type 1 (electrophoretic mobility of the PrP-res core ~21 kDa); lane 2: cerebral cortex from the present case; lane 3: cerebral cortex from a subject with sCJD, VV at codon 129 of PRNP and with PrP-res type 2 (electrophoretic mobility of the PrP-res core ~19 kDa). (B) Regional distribution of PrP-res: FC=frontal cortex; TC=temporal cortex; OC=occipital cortex; HI=hippocampus; EC=entorhinal cortex; TH=thalamus; PU=putamen; PG=midbrain periaqueductal grey; LC=locus coeruleus; CE=cerebellum. Molecular mass standards (kDa) are indicated at left, and the amounts of PrP-res (in arbitrary units) are indicated on the top. Quantification of PrP-res was performed after densitometric analysis as previously described.¹⁵ The relative amounts of PrP-res were expressed in numbers and referred to the highest value previously obtained in FFI patients,¹⁵ which was given the value of 100.

spongiform changes, neuronal loss, and gliosis throughout the full thickness of the grey matter in all neocortical areas although certain areas (motor strip, cingulum, and occipital lobe) were less involved. Ballooned neurons were seen in the cerebral cortex, particularly in the frontal and temporal lobes. Subjacent white matter had diffuse astrocytic gliosis. Subcortical pathology varied; it was extensive in the putamen and head of the caudate, moderate in the thalamus, and minimal in the globus pallidus, subthalamic nucleus, and nucleus basalis. There was relative sparing of the cerebellum, brainstem, and high cervical cord.

MOLECULAR FINDINGS

PrP-res was detected by western blot analysis as previously described.² The electrophoretic mobility and the ratio of the three glycosylated PrP-res isoforms were consistent with those of PrP-res type 1 (fig 2A).^{2,3}

The regional amount of PrP-res accumulation showed a direct correlation with the severity of spongiform degeneration. The highest amount of PrP-res was detected in the cerebral cortex, relatively moderate amounts were found in the hippocampus, putamen, and thalamus, whereas low to barely detectable concentrations of the protein were seen in the brainstem and cerebellum (fig 2B).

For molecular genetic analysis, DNA was extracted from blood and frozen brain using standard procedures. Direct sequencing of the *PRNP* open reading frame² showed no genomic mutations and valine homozygosity at codon 129.

Discussion

Phenotypic heterogeneity of sCJD parallels that of animal prion diseases such as scrapie or transmissible mink encephalopathy. In these diseases, phenotypic variation has been related to both variation of the agent strain and host genetic factors—namely, the *PRNP* genotype.^{4,5} Prion strains are distinguishable by the neuropathology they produce, the regional pattern of intracerebral PrP-res accumulation, and, to some extent, by differing physicochemical properties of PrP-res, such as size and glycoform ratio of the protein.⁴⁻⁸ These “signatures” are reproducible and characteristic of a given strain when examined within syngenic hosts,⁴⁻⁸ but they may change after transmission to hosts with a different *PRNP* genotype.^{5,9} Four distinct clinicopathological variants of sCJD have been recently identified in a series of 19 patients.² The typical CJD phenotype (myoclonic variant) and the Heidenhain variant were linked to MM at codon 129 and to PrP-res type 1. The ataxic variant and the variant with kuru plaques were linked to PrP-res type 2 with VV and MV at codon 129, respectively. Finally, a variant characterised by dementia of relatively long duration, with predominant pathology in the cerebral cortex, was linked to PrP-res type 2 and MM at codon 129.

The clinical course in our patient included serial cortical syndromes progressing for longer than 1 year without significant myoclonus,

ataxia, or PSWCs on EEG examinations. This clinical phenotype correlated with a predominantly cortical distribution of lesions and accumulation of PrP-res and relative sparing of the brainstem or cerebellum. Immunohistochemistry of PrP showed very faint granular deposits in the cerebral cortex. This phenotype differs from the typical CJD phenotype of the MM1 patients because of the longer duration of symptoms, absence of PSWCs on EEG examination, late appearance of myoclonus, and relative sparing of the cerebellum despite the long duration and the severe involvement of the cerebral cortex. The clinicopathological features of our patient are also different from those of the ataxic and kuru-plaque sCJD variants. Ataxia and cerebellar pathology, although more severe in the ataxic variant, are prominent features of VV2 and MV2 patients, and were not relevant features in our case. Additional characteristics of these variants lacking in our patient include widespread involvement of subcortical grey matter and brain stem structures and numerous focal, plaque-like PrP deposits. Finally, our case is clearly distinguishable from the MM2 patients molecularly and pathologically, despite some apparent similarities in clinical phenotype. The MM2 patients typically show a strong coarse or perivacuolar pattern of PrP-res deposition, and prominent involvement of the occipital lobe; neither were detected in our patient.

We have searched the CJD literature for cases that match the present one in clinicopathological features. Recent reviews of CJD delineated many clinicopathological phenotypes but did not include any description of cases similar to ours.^{1,10} Other clinical CJD series describe dementia with prolonged illness duration in patients of relative young age.^{11,12} These cases may be of specific relevance to ours given the possible clinical similarities. Unfortunately, no pathological information is available on these patients. It is of interest, however, that our patient shows striking phenotypic similarities to a familial subtype of CJD, designated CJD178, which has been linked to the D178N *PRNP* mutation, PrP-res type 1, and the valine codon at position 129.^{13,14} The clinical and pathological parallels between the phenotype of CJD178 and that of the present case and the similar size of PrP-res, suggest that the same prion strain may be involved in the two forms, and that our case does not represent a novel CJD phenotype.

In conclusion, our data indicate that this patient was affected by a distinct rare variant of sCJD, previously uncharacterised at the molecular and immunocytochemical level. Furthermore, they show another example of distinct clinicopathological phenotypes of sCJD in patients with the same *PRNP* genotype, which correlate with different physicochemical properties of PrP-res. This finding further suggests the existence of different strains of the CJD agent and that, at least in part, strain-specific properties of prions are encoded in the tertiary or quaternary structure of PrP-res.

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