

Comprehensive clinical, radiological, pathological and biochemical analysis required to differentiate VV1 sporadic Creutzfeldt-Jakob disease from suspected variant CJD

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ABSTRACT

Background A diagnosis of variant Creutzfeldt-Jakob disease (vCJD), the zoonotic prion disease related to transmission of bovine spongiform encephalopathy, can carry enormous public health ramifications. Until recently, all vCJD clinical cases were confined to patients displaying methionine homozygosity (MM) at codon 129 of the prion protein gene (*PRNP*). The recent diagnosis of vCJD in a patient heterozygous (MV) at codon 129 reignited concerns regarding a second wave of vCJD cases, with the possibility of phenotypic divergence from MM vCJD and greater overlap with sporadic CJD (sCJD) molecular subtypes.

Method and results We present a case of CJD with clinico-epidemiological and radiological characteristics creating initial concerns for vCJD. Thorough case evaluation, including data provided by genetic testing, autopsy and neuropathological histological analyses, provided a definitive diagnosis of the rare VV1 molecular subtype of sCJD.

Conclusion Distinguishing vCJD from sCJD is of vital public health importance and potentially more problematic with the development of non-MM vCJD cases. The patient described herein demonstrates that in addition to the clinico-epidemiological profile, combined supplementary pathological, biochemical and critical radiological analysis may be necessary for confident discrimination of sCJD, especially rare sub-types, from vCJD.

INTRODUCTION

Human prion diseases, the most common of which is sporadic Creutzfeldt-Jakob disease (sCJD), are rare neurological disorders, with a global incidence between 1 and 2 cases per million population.¹ Prion diseases are caused by PrP^{Sc}, misfolded conformers of the cellular prion protein. Despite their rarity, due to the unique resistance of PrP^{Sc} to inactivation by standard sterilisation techniques, and its transmissible nature, prion diseases pose ongoing challenges to healthcare delivery and public health.

Prion disease diagnosis is further complicated by the existence of different prion 'strains', resulting in the heterogenous clinico-pathological manifestations seen in humans. Broadly, prion strain is determined by both the genotype at the polymorphic codon 129 of the prion protein (*PRNP*) gene (either methionine (M) or valine (V)), and the PrP^{Sc} glycoform (type 1 or type 2, following the most widely adopted nomenclature).² Glycoform is established by biochemical analyses and is based on the electrophoretic mobility of the protease-resistant C-terminal PrP^{Sc} core fragment, and the relative abundance of the 3 PrP^{Sc} glycoform bands. Glycoforms 1 and 2 are both seen in sCJD.

The outbreak of prion disease in cattle (bovine spongiform encephalopathy, BSE) in the UK and Europe in the 1980s resulted in zoonotic transmission to humans through dietary exposure and a distinct new human prion strain, named variant CJD (vCJD). In contrast to sCJD, vCJD is characterised by a younger age and prominent psychiatric or sensory symptoms at onset. A distinctive type 2B glycoform, denoted by a high representation of the diglycosylated PrP^{Sc} glycoform, is pathognomonic for vCJD. Importantly, unlike sCJD, in vCJD there is evidence of disease-associated prions in tissues of the lymphoreticular system and blood, and blood-borne human-human secondary transmission of vCJD,³ substantially increasing the public health implications of this diagnosis.

Until recently, all patients with vCJD were methionine homozygotes (MM) at the polymorphic codon 129 of the *PRNP* gene. However, in 2017 the first case of symptomatic vCJD was confirmed in a codon 129 heterozygote (MV).⁴ The patient was young



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and presented with an illness differing from typical vCJD. Brain MRI was akin to classical sCJD and lacked the ‘pulvinar sign’ considered diagnostic of vCJD.⁵ This patient did not meet vCJD diagnostic criteria, although did fulfil criteria for probable sCJD; it was not until neuropathology and biochemical analysis of PrP^{Sc} that a vCJD diagnosis was confirmed. This patient’s MV status supported the hypothesis that the vCJD incubation period may be longer in non-MM individuals, raising concerns of a second wave of vCJD cases. This hypothesis also receives support from similar observations of prolonged incubation periods in MV individuals in Kuru, another orally transmitted acquired human prion disease,⁶ and the finding of likely subclinical vCJD in VV individuals.⁷ This first clinical MV vCJD case underscored the importance of brain autopsy to accurately identify and classify suspected CJD.

CASE REPORT

A 34-year-old right-handed UK-born male electrician developed rapidly progressive dementia over 3 months. Initially, social withdrawal, anorexia and paucity of speech were diagnosed as depression in the primary care setting. Several episodes of near-electrocution necessitated his taking leave from work.

Medical history was significant for a rare childhood right orbital rhabdomyosarcoma diagnosed by surgical biopsy and successfully treated with chemotherapy and stereotactic radiotherapy, resulting in right orbital rim hypoplasia. After migration to Australia when he was aged 7, he underwent artificial ocular lens insertion for radiation-induced cataract. He received no blood transfusions or dural grafts at any time.

At presentation the patient was disoriented to place, time and person. All memory domains were grossly impaired: he could not recall his age, children’s names or any biographical details. His affect was markedly apathetic. Examination revealed lower limb hyperreflexia with non-sustained ankle clonus bilaterally.

Electroencephalography was abnormal with near-continuous left frontotemporal 3–4Hz delta slowing, at times seen synchronously and at lower amplitude in the right frontotemporal region. MRI demonstrated extensive geographic areas of abnormal diffusion restriction of the cerebral cortex, predominantly in the left cerebral hemisphere (figure 1). Bilateral caudate, lentiform and pulvinar hyperintensity and abnormal diffusion restriction was seen, the latter more pronounced on the left. The hippocampi were not convincingly abnormal.

Cerebrospinal fluid 14-3-3 protein was elevated. Brain biopsy showed florid spongiform degeneration involving the cerebral cortex, concentrated in laminae II, III and IV. Blood was sent for genetic analysis: no *PRNP* mutation was detected; the patient was homozygous for valine (VV) at codon 129.

Within 5 months the patient displayed akinetic mutism, startle myoclonus, spasticity and appeared to

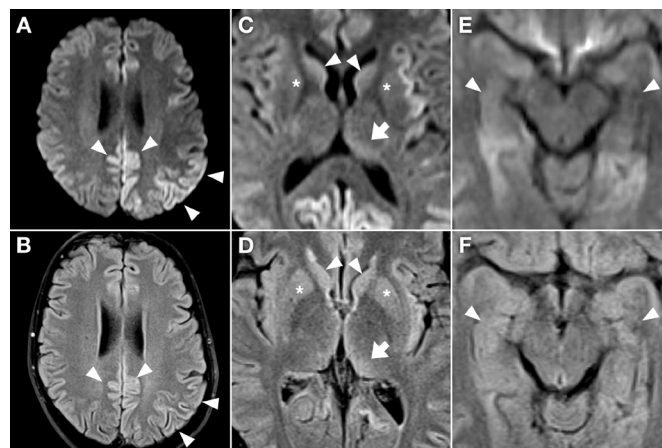


Figure 1 Diffusion weighted imaging B=1000 (A, C, E) and matching fluid-attenuated inversion recovery (B, D, F) images. Geographic regions of cerebral cortical abnormal diffusion restriction (A; arrowheads) with more subtle increase in T2 signal (B, arrowheads) most marked in the left hemisphere. Abnormal diffusion restriction (C) and increased T2 signal (D) involving the caudate nucleus heads (arrowheads), left posteromedial thalamus—pulvinar (arrows) and to a lesser degree bilateral lentiform nuclei (asterisks). The hippocampi demonstrate no convincing abnormal diffusion restriction (E; arrowheads) or T2 signal abnormality (F; arrowheads).

be responding to internal stimuli. Sleep–wake cycle was erratic. Eleven months later, he died at home. Duration of illness from onset of symptoms to death was 20 months.

A brain-only autopsy was performed. Sections through the cerebral cortex showed atrophy with significant neuronal loss and astrocytic gliosis. Numerous neurons contained eosinophilic cytoplasmic inclusions. The basal ganglia and thalamus showed prominent spongiform encephalopathy with neuronal loss and gliosis. The cerebellum showed a focal spongiform encephalopathy associated with significant Purkinje cell loss and Bergman layer gliosis with other areas of sparing. PrP immunoreactivity showed very sparse faint peri-vacuole immunoreactivity in areas of spongiform change.

Frozen post-mortem brain sampled from the frontal cortex underwent PrP^{Sc} glycoform analysis by polyacrylamide gel electrophoresis and western blotting. Based on the electrophoretic mobility and relative abundance of the PrP^{Sc} bands, the patient’s glycoform was determined to be type 1 (figure 2), with the final molecular subtype designated VV1.

DISCUSSION

sCJD is a heterogeneous disease which can be subtyped molecularly according to the polymorphism at codon 129 of *PRNP* and the PrP^{Sc} glycoform. Of the six molecular subtypes defined through the most widely adopted nomenclature, the VV1 type—identified in our patient—is the rarest, comprising <1% of sCJD cases.² Both vCJD and the VV1 sCJD subtype are characterised by young age at disease onset, early psychiatric manifestations and

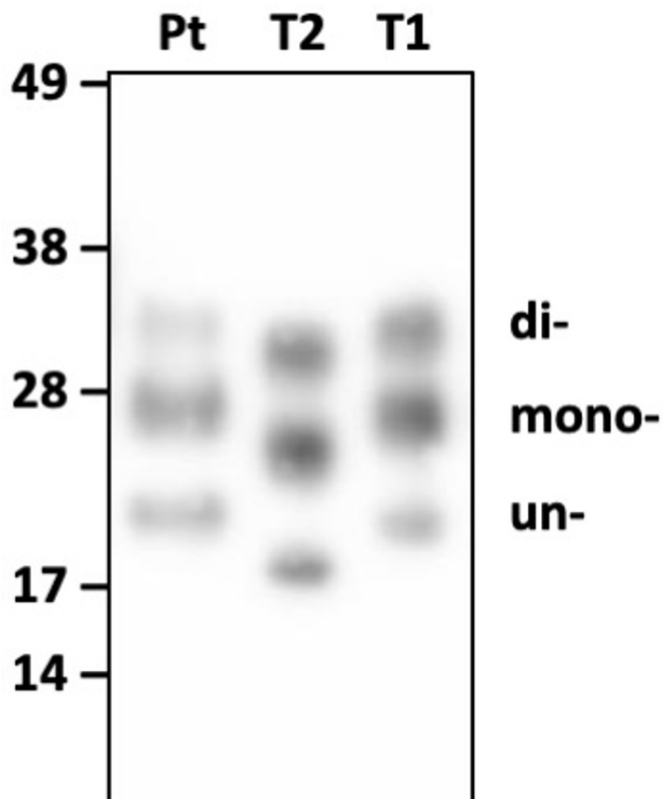


Figure 2 PrP^{Sc} glycotyping of the patient's brain. Proteinase K digested patient (Pt) and glycotype control (T1, T2) brain homogenates were analysed. The patient's unglycosylated band (un-) resolves at approximately the same mobility as the T1 control, and the diglycosylated band (di-) is appreciably under-represented compared with the monoglycosylated and unglycosylated bands. Relative molecular weights are indicated.

prolonged disease duration, making it challenging to distinguish between the two pre mortem.

The largest study of patients with VV1 sCJD comprises a series of nine individuals.⁸ Seeking to establish criteria to support a VV1 sCJD diagnosis, the authors concluded that elevated 14-3-3 cerebrospinal fluid (CSF) protein in conjunction with cerebral cortical, rather than pulvinar, signal increase on MRI is the criterion that best supports the diagnosis of VV1 sCJD, as opposed to vCJD. Further, the authors suggest that hippocampal signal increase on MRI might be a specific feature of VV1 sCJD. Our patient's MRI findings were initially considered somewhat suggestive of vCJD: prominent bilateral thalamic pulvinar T2 hyperintensity exceeding that evident in the cerebral cortex and/or basal ganglia is cited as a highly specific criterion for vCJD.⁴ Cerebral cortical signal intensity was present, however the postulated VV1 sCJD-specific finding of hippocampal involvement was not. Basal ganglia hyperintensity was rare in the aforementioned case series, occurring in two of seven patients, both in the caudate nucleus. As far as we are aware, our case represents only the second published instance of a positive pulvinar signal in a patient with VV1 sCJD.⁹ Our patient's pattern

of pulvinar, lentiform and caudate involvement, plus the expected cerebral cortical changes, represents a new pattern of MRI changes in VV1 sCJD.

While our patient's CSF was positive for the 14-3-3 protein, this finding is of limited diagnostic utility in excluding vCJD as it occurs in approximately 50% of patients with vCJD.¹⁰ Recent developments in CJD diagnostic testing include the implementation of the CSF RT-QuIC assay, which has higher sensitivity and specificity for sCJD compared with the 14-3-3 assay.¹¹ Of note, our patient's CSF was subjected to RT-QuIC analyses as part of the Australian National CJD Registry's development of the RT-QuIC test and was found to be negative. Some sCJD subtypes, including VV1, can produce a negative CSF RT-QuIC result, as does vCJD, again highlighting the diagnostic challenge of distinguishing some rare sCJD subtypes from vCJD pre mortem.

Personality change occurred in all patients with VV1 in the aforementioned case series: mainly aggression, regression to childlike behaviour and fear responses. Neurological signs including ataxia, myoclonus and hyper-reflexia occurred late, after a median of 7 months. Our patient lacked such personality change: instead, he exhibited early psychiatric features usually associated with vCJD including dysphoria, withdrawal and anergia.¹² Another unusual feature was early hyper-reflexia, a finding in keeping with vCJD, where neurological signs occur in approximately 57% of cases within 2 months of first symptoms.

There are few pathologic reports of sCJD with a VV1 subtype.^{2 8 13 14} The hallmark of the VV1 subtype, as seen in our case, is dissociation between the severe histological lesions and the faint PrP immunostaining. Previously described cases have shown relative sparing of the cerebellum and thalamus, although our case showed focal cerebellar spongiform encephalopathy with Purkinje cell loss and marked thalamic involvement.

Our patient's clinico-epidemiological profile was suspicious for vCJD. He presented young with prominent affective features, early neurological signs and had a putative prolonged disease duration on a background of meat consumption and neurosurgical instrumentation in the UK during the BSE epidemic. His MRI featured modest bilateral pulvinar hyperintensity initially furthering concerns regarding a vCJD diagnosis. In reaching our final diagnosis, we placed the greatest relative weighting on the objective evidence provided by subsequent brain autopsy, biochemical analysis of PrP^{Sc} and *PRNP* genotyping, which demonstrated findings more in keeping with the rare VV1 molecular subtype of sCJD.

CONCLUSION

A combination of clinical evaluation, neuroimaging, CSF analysis, *PRNP* genotyping, brain autopsy and immunoblot analysis are needed for the most accurate CJD diagnosis. Clinical and radiological characteristics alone are neither sensitive nor specific enough to discriminate

between rare sCJD subtypes and vCJD. While our patient was ultimately not the first case of vCJD diagnosed in Australia, this case underscores the importance of diagnostic accuracy when it comes to CJD and its subtypes given the public health implications of a vCJD diagnosis and the recent identification of an altered vCJD phenotype in a non-MM homozygote at codon 129 of the *PRNP* gene.

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Contributors SH, RW and HB were involved in the clinical care of the patient. FG performed and interpreted the neuroradiological study. VL and SJC conducted immunoblot analysis. SH prepared the initial draft of the manuscript which was critically revised for intellectual content by all authors, who approved the final draft.

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Competing interests None declared.

Patient consent for publication Consent obtained from next of kin.

Ethics approval This paper is a case report. Consent to produce the paper was sought from the patient's next of kin (his wife). The patient did not have capacity to consent to participation in this case report. After his death, his next of kin provided consent for this report to be written. Of note, the patient's next of kin is certain that the patient had expressed strong premorbid wishes for involvement in medical research if/when possible.

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