SHORT REPORT

# Prion-related peripheral neuropathy in sporadic Creutzfeldt-Jakob disease

Simone Baiardi,[1] Veronica Redaelli,[2] Paolo Ripellino,[3] Marcello Rossi,[4] Alessia Franceschini,[4] Maurizio Moggio,[5] Patrizia Sola,[6] Anna Ladogana,[7] Paolo Fociani,[8] Anna Magherini,[9] Sabina Capellari,[1,4] Armin Giese,[10] Byron Caughey,[11] Paola Caroppo,[2] Piero Parchi[4,12]

► Additional material is published online only. To view please visit the journal online (http://dx.doi.org/10.1136/jnnp-2018-319221).

For numbered affiliations see end of article.

**Correspondence to**
Professor Piero Parchi, IRCCS Istituto delle Scienze Neurologiche, Bologna 40139, Italy; piero.parchi@unibo.it

Received 17 July 2018
Revised 4 September 2018
Accepted 28 September 2018
Published Online First 24 October 2018

## ABSTRACT

**Objective** To assess whether the involvement of the peripheral nervous system (PNS) belongs to the phenotypic spectrum of sporadic Creutzfeldt-Jakob disease (sCJD).

**Methods** We examined medical records of 117 sCJDVV2 (ataxic type), 65 sCJDMV2K (kuru-plaque type) and 121 sCJDMM(V)1 (myoclonic type) subjects for clinical symptoms, objective signs and neurophysiological data. We reviewed two diagnostic nerve biopsies and looked for abnormal prion protein ($PrP^{Sc}$) by western blotting and real-time quaking-induced conversion (RT-QuIC) in postmortem PNS samples from 14 subjects.

**Results** Seventy-five (41.2%) VV2-MV2K patients, but only 11 (9.1%) MM(V)1, had symptoms or signs suggestive of PNS involvement occurring at onset in 18 cases (17 VV2-MV2K, 9.3%; and 1 MM(V)1, 0.8%) and isolated in 6. Nerve biopsy showed a mixed predominantly axonal and demyelinating neuropathy in two sCJDMV2K. Electromyography showed signs of neuropathy in half of the examined VV2-MV2K patients. Prion RT-QuIC was positive in all CJD PNS samples, whereas western blotting detected $PrP^{Sc}$ in the sciatic nerve in one VV2 and one MV2K.

**Conclusions** Peripheral neuropathy, likely related to $PrP^{Sc}$ deposition, belongs to the phenotypic spectrum of sCJDMV2K and VV2 and may mark the clinical onset. The significantly lower prevalence of PNS involvement in typical sCJDMM(V)1 suggests that the PNS tropism of sCJD prions is strain dependent.

## INTRODUCTION

Sporadic Creutzfeldt-Jakob disease (sCJD), the most common human prion disease, is a heterogeneous neurodegenerative disorder including six major clinical–pathological subtypes largely correlating with the genotype at the polymorphic codon 129 (methionine, M; or valine, V) in the prion protein gene (*PRNP*) and the type (1 or 2) of abnormal prion protein ($PrP^{Sc}$) accumulating in affected tissues (ie, MM1, MV2, VV2, etc).[1]

Patients with sCJD usually present with neurological signs of central origin reflecting the predominant $PrP^{Sc}$ accumulation in the central nervous system (CNS). However, the application of techniques with improved sensitivity allowed the demonstration of $PrP^{Sc}$ deposits also in peripheral tissues,[2–4] including autonomic and dorsal root ganglia,[5 6] and trigeminal[7] and peripheral nerves.[8] Despite this progress, studies specifically addressing the prevalence and type of peripheral nervous system (PNS) signs in sCJD are lacking, and neuropathy only anecdotally reported.[9–12]

In this study, we investigated whether and to what extent (1) PNS involvement features in the phenotypic spectrum of the most common sCJD subtypes and (2) $PrP^{Sc}$ accumulates in peripheral nerves from patients with sCJD.

## MATERIALS AND METHODS

### Study design and data collection

We reviewed medical charts and analysed clinical and neurophysiological data of 277 subjects with a 'definite' diagnosis of sCJD MM(V)1 (n=121), VV2 (n=91) and MV2K (n=65), and 26 cases fulfilling the criteria for 'probable' sCJD[13] and carrying VV at codon 129. Exclusion criteria included (1) the lack of comprehensive medical records, (2) clinical features suggestive of the VV1 subtype in the 'probable' group and (3) a positive history for a concomitant cause of neuropathy (online supplementary figure 1).

We recorded the clinical findings suggesting a PNS involvement and evaluated the results of neurophysiological tests (electromyography, motor and somatosensory evoked potentials). To rule out a possible 'central' origin of sensory symptoms and signs, we limited the analysis to those involving the extremities symmetrically and did not count those with an asymmetrical distribution, affecting either one or both limbs of the same side. Similarly, we considered limb weakness of PNS origin only in the absence of pyramidal signs.

### Prion detection in peripheral nerves

We searched for $PrP^{Sc}$ seeding activity in 21 nerve samples collected post mortem from 12 definite CJD cases and two non-CJD controls by the real-time quaking-induced conversion (RT-QuIC) assay. In most cases, we also looked for $PrP^{Sc}$ by western blotting (WB) after various purification steps. We carried out RT-QuIC and WB analyses as previously described,[14 15] with only minor modifications. Details about the samples analysed and the protocols for RT-QuIC, $PrP^{Sc}$ purification and WB are provided in the online supplementary materials.



**To cite:** Baiardi S, Redaelli V, Ripellino P, *et al*. *J Neurol Neurosurg Psychiatry* 2019;**90**:424–427.

**Table 1** Clinical and neurophysiological findings in the most common sporadic Creutzfeldt-Jakob disease subtypes

| Clinical findings | VV2, n=117 | MV2K, n=65 | MM(V)1, n=121 |
|---|---|---|---|
| Symptoms | | | |
| Limb weakness* | 10 (8.5) | 7 (10.8) | – |
| Numbness/paraesthesia/dysesthesia† | 7 (5.9) | 10 (15.4) | 3 (2.5) |
| Cramps/myalgia | 1 (0.8) | 1 (1.5) | 1 (0.8) |
| Signs | | | |
| Absent or reduced deep tendon reflexes† | 35 (29.9) | 18 (27.7) | 6 (4.9) |
| Absent or reduced vibratory sense† | 9 (7.7) | 2 (3.1) | – |
| Absent or reduced touch, thermic or pick sense† | 4 (3.4) | 5 (7.7) | – |
| Positive Romberg's test | 7 (5.9) | 6 (9.2) | 1 (0.8) |
| Fasciculation | 10 (8.5) | 4 (6.1) | 1 (0.8) |
| Muscular atrophy | 3 (2.6) | 4 (6.1) | - |
| **Electromyographic findings** | **VV2, n=28** | **MV2K, n=12** | **MM(V)1, n=7** |
| Axonal polyneuropathy‡ | 3 (10.7) | 3 (25.0) | – |
| Demyelinating polyneuropathy§‡ | 1 (3.6) | 2 (16.6) | 1 (14.3) |
| Mixed axonal and demyelinating polyneuropathy‡ | 1 (3.6) | – | – |
| Polyradiculoneuropathy | 2 (7.1) | – | – |
| Mononeuropathy | 1 (3.6) | 2 (16.6) | – |
| Unspecified neuropathy¶‡ | 3 (10.7) | 2 (16.6) | – |
| Fasciculation, myokymia, fibrillation, positive waves | 14 (50.0) | 1 (8.3) | – |
| Subacute denervation signs | 6 (21.4) | – | – |
| Chronic denervation signs | 3 (10.7) | – | – |
| Normal | 6 (21.4) | 3 (25.0) | 6 (85.7) |

Data are expressed as number of patients (n) and percentage (%).
*Without pyramidal signs.
†With distal and symmetrical distribution.
‡Sensorimotor involvement in 5 cases, pure sensory in 4 and motor in 4.
§In one case with conduction block.
¶Not specified whether with axonal or demyelinating pattern of damage.

## RESULTS

The prevalence of symptoms/signs suggestive of PNS involvement was significantly higher in the VV2 (n=46, 39.3%) and MV2K (n=29, 44.6%) subjects than in the MM(V)1 group (n=11, 9.1%) (table 1).

Symptoms occurred in association with objective signs of PNS dysfunction in 23 patients (VV2, n=13; MV2K, n=9; MM(V)1, n=1) and isolated in 11 (VV2, n=4; MV2K, n=5; MM(V1), n=2). Conversely, 52 subjects (VV2, n=29; MV2K, n=15; MM(V)1, n=8) had objective signs in the absence of subjective complaints.

While only one MM(V)1 patient presented limb paraesthesia early in the course, nine VV2 (7.7%) and eight MV2K (12.3%) subjects had putative PNS symptoms at disease onset, which occurred isolated in six. Notably, an initial diagnosis of peripheral neuropathy was formulated in most of these cases, while CJD was not considered a possibility at this time in any of them. Indeed, two of them (both sCJDMV2K) even underwent a sural nerve biopsy that revealed fibre loss with some admixed demyelination, but no inflammatory infiltrates (figure 1A–E).

In support of the peripheral origin of sensory symptoms, the prevalence of thalamic MRI abnormalities did not significantly differ between patients with or without sensory complaints (46.1% vs 42.1%).

Nine (7.7%) VV2 patients also presented a cranial nerve dysfunction affecting the oculomotor (n=1), trigeminal (n=1), abducens (n=3) and facial (n=4) nerves.

Forty VV2-MV2K patients underwent electromyography. Among them, 13 had signs of polyneuropathy, with either a sensorimotor (n=5) or pure motor (n=4) or sensory (n=4) impairment, and 2 of polyradiculoneuropathy. Moreover, a mononeuropathy was diagnosed in three additional subjects and a not-better-specified neuropathy in two. Spontaneous muscle fibre activity was recorded in 15 patients, especially of the VV2 subtype, associated with signs of subacute or chronic denervation in 9. Interestingly, electroneurography was unremarkable in most of these cases (n=9).

Somatosensory and motor evoked potentials were abnormal in 7 out of 17 tested VV2-MV2K (VV2: 5 out of 12, 41.6%; MV2K: 2 out of 5, 40%).

In the MM(V)1 group, the neurophysiological examinations were invariably unrevealing, except for a mild sensorimotor demyelinating polyneuropathy in one patient.

### $PrP^{Sc}$ detection in peripheral nerve

Western blotting demonstrated PK-resistant $PrP^{Sc}$ fragments in two enriched (P3) samples of the sciatic nerve (VV2, n=1; MV2K, n=1) (figure 1F) and all total homogenates of cranial nerves analysed (two trigeminal and one vagus nerves from two sCJDVV2 cases) (online supplementary table). By serial sample dilution and densitometric analysis of WB signals, we estimated the amount of $PrP^{Sc}$ in peripheral nerves being approximately $10^{-4}$-fold lower than in the frontal cortex of a typical sCJDVV2 subject (data not shown).

All nerve samples from patients with sCJD showed a full positive seeding activity in RT-QuIC (all four loaded wells crossed the established threshold), while none of the controls showed any seeding activity (figure 1G–H and online supplementary table).

## DISCUSSION

The results of the present study demonstrate that symptoms and signs suggestive of PNS involvement, likely related to

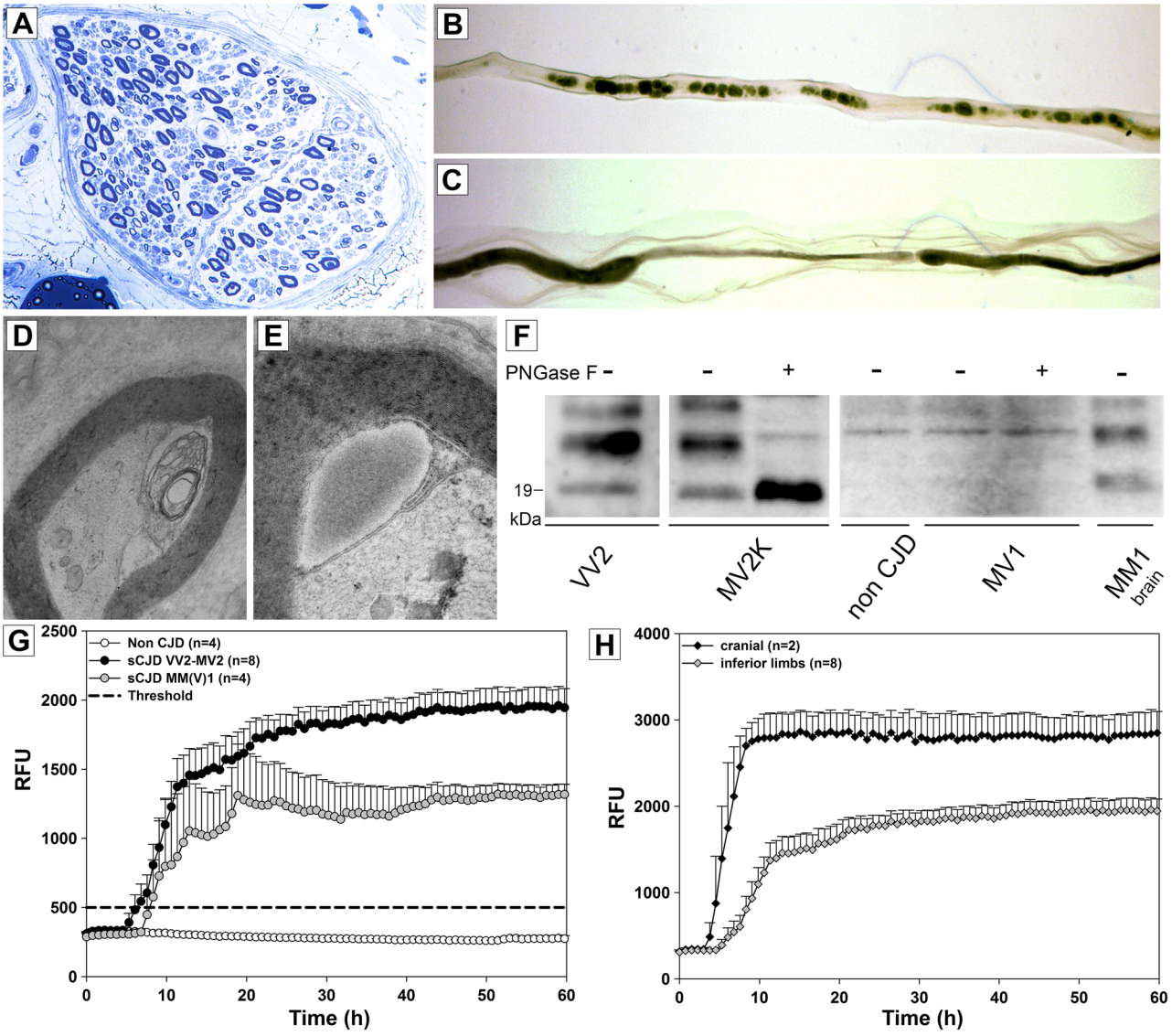


**Figure 1** Histopathological findings and prion detection in peripheral nerves of patients with sporadic Creutzfeldt-Jakob disease (sCJD). Sural nerve biopsy findings in a patient with sCJDMV2K (A–E). Marked loss of fibres (A), toluidine-blue-stained plastic sections ×400 (fibre density=1200 ff/mm$^2$; normal range=4800 ff/mm$^2$). Teased fibre analysis showing predominant signs of axonal damage with Wallerian degeneration ('myelin balls') (B, ×200) and segmental demyelination (C, ×200). Electron microscopy of large myelinated fibres showing vacuolation of uncompact myelin in the adaxonal space (D, ×12 000) and accumulation of electrondense material with a fibrillar structure (E, ×20 000), slightly resembling amyloid fibrils. PrP$^{Sc}$ detection and typing by western blotting (F). Enriched P3 samples were resolved in 7 cm long gels and probed with the primary antibody 3F4. Relative molecular masses are in kilodaltons. PrP$^{Sc}$ type 2 was detected in sciatic nerves of sCJDVV2 and MV2K cases. Immunoblotting was negative in both sCJDMV1 (sciatic nerve) and non-CJD (lumbosacral roots) cases. A cortical sample from a sCJDMM1 case (P3 diluted 1:500) was included as positive control. For deglycosylation, samples were treated with PNGase F. PrP$^{Sc}$ detection by real-time quaking-induced conversion assay (G, H). Thioflavin-T (ThT) fluorescence traces of peripheral nerves from 12 definite CJD cases and 2 non-CJD cases (G). Threshold was indicated as dashed line (500 relative fluorescent units (RFU)). Data are expressed as mean±SEM. Both VV2 and MV2K samples (sCJD V2 group, n=8) showed an earlier lag phase (sCJD V2: 12±1 hours; sCJDMM(V)1: 19±5 hours) and a higher ThT maximum response (sCJD V2: 2200±90; sCJDMM(V)1: 1840±240) than those from MM(V)1 cases (n=4). Comparison of ThT curves of peripheral nerves from different anatomical sites (cranial vs inferior limbs) in cases belonging to the V2 strain group (H). Cranial nerves showed a mean earlier lag phase (5.8±1.3 vs 11.9±1 hours) and a mean higher ThT value (2950±210 vs 2200±90 RFU) than the other peripheral nerves.

PrP$^{Sc}$ deposition, characterise about 40% of patients with sCJDVV2 and MV2K, and occur at disease onset in 9.3% of them. Neurophysiological studies revealed signs of neuropathy in about 50% of examined cases and 10.9% of collected VV2-MV2K cases overall, a number which likely underestimates the real prevalence of PNS electrophysiological abnormalities, given that electromyography was performed in only ~20% of our VV2-MV2K cohort.

While sural biopsies suggested a pattern characterised by prominent signs of axonal damage with secondary demyelination, electromyographic findings did not reveal a specific neurophysiological pattern of abnormalities.

Notably, needle electromyography revealed the occurrence of spontaneous activity in about a third of patients, especially in the VV2 group. These abnormal muscular activities were often associated with other subacute or chronic signs suggesting a

lower motor neuron dysfunction. However, in a few cases, the spontaneous activity occurred in the absence of any other sign of denervation, indicating peripheral nerve hyperexcitability, as previously reported in two sCJDVV2 patients.[16]

The not irrelevant prevalence of electrophysiological signs of neuropathy in the middle-aged or elderly control population and the potential difficulty to distinguish between a peripheral and central cause of symptoms such as paraesthesia and weakness raise the question of the specificity of our findings both in terms of aetiology (prion-related vs incidental) and their origin (peripheral vs central). The timing of appearance of symptoms/signs always matching the disease onset, the lack of other causes of neuropathy in our selected population, the worsening of symptoms and signs during the disease course in some cases, and the demonstration of $PrP^{Sc}$ deposition in the peripheral nerve are all in support of a $PrP^{Sc}$-related aetiology. Regarding the origin of symptoms, although we cannot rule out that a dysfunction of central pathways to the sensory and motor symptoms played a role, especially in cases without electrophysiological assessment, it is noteworthy that we excluded any sensorimotor signs or complaints with an asymmetrical or hemibody distribution, which would be more indicative of a CNS origin. Furthermore, the absence of an increased prevalence of thalamic abnormalities at brain MRI in patients with sensory complaints further corroborated the idea of a peripheral origin of these symptoms.

Interestingly, PNS involvement in sCJD seems to be prion-strain dependent, which is also in support of a prion-specific origin of PNS dysfunction. Indeed, in patients with typical sCJD-MM(V)1, peripheral signs/symptoms were significantly less frequent and prion seeding activity in the analysed nerves lower than in sCJDVV2 or MV2K and rarely led to a neurophysiological assessment. However, the faster disease progression and the predominant cognitive symptoms at onset, affecting the ability of these patients to report symptoms and collaborate to neurological examination, might have partially concealed the PNS dysfunction in these cases.

Despite the lack of a standardised assessment of PNS involvement and the retrospective design, we demonstrated that a peripheral polyneuropathy, likely related to $PrP^{Sc}$ deposition, may occur in sCJD, even as presenting clinical manifestation of the disease. This unusual but likely underestimated onset may represent a significant diagnostic challenge for neurologists, who should be aware of this rare cause of neuropathy. This notion is also of importance for the biosafety and management of peripheral tissue specimens such as nerve and muscle biopsies.

Given the current availability of prion-specific cerebrospinal fluid assays with high diagnostic accuracy, CJD should be considered in the differential diagnosis of patients with polyneuropathy of recent onset especially when associated with initial signs of CNS involvement. The finding of a significantly higher prevalence of PNS involvement in sCJD subtypes related to the V2 strain (ie, VV2 and MV2K) than in typical MM(V)1 further corroborates the PrP-specific aetiology of the peripheral neuropathy and suggests a variable, strain-dependent, peripheral tropism of sCJD prions.

**Author affiliations**
[1]Department of Biomedical and Neuromotor Sciences, University of Bologna, Bologna, Italy
[2]Neurology and Neuropathology Unit, IRCCS, Foundation, Neurological Institute Carlo Besta of Milan, Milano, Italy
[3]Department of Neurology, Neurocenter of Southern Switzerland, Lugano, Switzerland
[4]IRCCS Istituto delle Scienze Neurologiche di Bologna, Bologna, Italy
[5]Neuromuscular and Rare Disease Unit, Department of Neuroscience, IRCCS Foundation Ca' Granda Ospedale Maggiore Policlinico, University of Milan, Milan, Italy
[6]Department of Biomedical, Metabolic and Neurosciences, University of Modena and Reggio Emilia, Modena, Italy
[7]Department of Neurosciences, Istituto Superiore di Sanità, Rome, Italy
[8]Department of Pathology, Luigi Sacco University Hospital, Milan, Italy
[9]Neurology Unit, Carlo Poma Hospital, Mantova, Italy
[10]Institut für Neuropathologie und Prion Forschung, Ludwig-Maximilians-Universität München, Munich, Germany
[11]LPVD, Rocky Mountain Laboratories, NIAID, NIH, Hamilton, Montana, USA
[12]Department of Experimental, Diagnostic and Specialty Medicine (DIMES), Università di Bologna, Bologna, Italy

**Acknowledgements** The authors wish to thank all the physicians who helped in the collection of clinical information and Barbara Polischi, M.Sc. for her valuable technical assistance.

**Contributors** Study design: SB, PP. Data acquisition: SB, VR, PR, MR, AF, MM, PS, AL, PF, AM, SC, AG, BC, PC, PP. Data analysis and interpretation: SB, MR, AF, PR, PP. Drafting of the manuscript: SB, PP. Study supervision: PP. All authors critically reviewed and approved the manuscript.

**Funding** The study was supported by the Italian Ministry of Health, grant RF2011-02351092, and by the Gino Galletti Foundation.

**Competing interests** None declared.

**Patient consent** Not required.

**Ethics approval** Ethical approval for the study was provided by the Local Ethics Committee (AUSL of Bologna, n. 16184/CE).

**Provenance and peer review** Not commissioned; externally peer reviewed.

**Data statement** The datasets analysed during the present study are available from the corresponding author and will be shared on reasonable request.

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