Pathologic Prion Protein Spreading in the Peripheral Nervous System of a Patient With Sporadic Creutzfeldt-Jakob Disease

Alexandre Favereaux, PhD; Isabelle Quadrio, MD; Claude Vital, MD; Armand Perret-Liaudet, MD; Olivier Anne, MD; Jean-Louis Laplanche, MD; Klaus G. Petry, PhD; Anne Vital, MD, PhD

Background: Involvement of the peripheral nervous system in the pathogenesis of prion diseases is becoming increasingly evident. However, pathologic protease-resistant prion protein deposition in the peripheral nerves of patients with Creutzfeldt-Jakob disease has never been demonstrated, to our knowledge.

Objective: To determine whether mutated prion protein accumulation could be shown in the peripheral nervous system of patients with sporadic Creutzfeldt-Jakob disease.

Design: Autopsy study.

Patients: Three patients with sporadic Creutzfeldt-Jakob disease.

Interventions: Study of the brain, spinal cord, and sciatic and superficial peroneal nerves by immunohistochemistry and Western blot analysis.

Main Outcome Measure: Demonstration of protease-resistant prion protein accumulation.

Results: In all cases, protease-resistant prion protein accumulation was found in the brain and posterior horns of the spinal cord. In 1 case, protease-resistant prion protein deposits were also evidenced in the dorsal root ganglia and the superficial peroneal nerve.

Conclusions: Protease-resistant prion protein may be found in the peripheral nervous system of some patients with sporadic Creutzfeldt-Jakob disease. However, a larger series is required to assess the incidence of peripheral nervous system involvement and to discuss the diagnostic usefulness of peripheral nerve biopsy in sporadic Creutzfeldt-Jakob disease.

Arch Neurol. 2004;61:747-750
chemistry. Prion protein labeling was carried out with 3F4 and 12F10 monoclonal antibodies (1:2000) after protease K digestion (20°C, 10 minutes) and guanidine thiocyanate treatment (4M, 4°C, 2 hours); counterstaining was done with Mayer hemalum. To avoid misinterpretation, controls for each anatomic site were performed.

WESTERN BLOT ANALYSIS

Frozen specimens of frontal, occipital, and cerebellar cortex, as well as spinal cord and dorsal root ganglia, were homogenized at 10% (wt/vol) in a 5% glucose solution. Peripheral nerve homogenates were obtained by impact grinding method in a liquid nitrogen bath resulting in a fine powder of tissue. Homogenates were forced through a 0.5-mm-diameter needle before protease K treatment. Protease K digestion and Western blot process were performed as previously described.9

RESULTS

Histopathologic examination of the 3 sporadic CJD cases showed marked spongiosis in the brain, whereas the spinal cord, dorsal root ganglia, and peripheral nerves appeared unmodified. In the 3 cases, neuronal loss was marked in the cerebral cortex, mild in the cerebellum, and absent in the spinal cord and the dorsal root ganglia. Gliosis was marked in the cerebral cortex, mild in the cerebellum and the spinal cord, and absent in the dorsal root ganglia.

Results of PrP immunohistochemistry and Western blot process were performed as previously described.9

Involvement of both central nervous system and PNS in the pathogenesis of transmissible spongiform encephalopathy (TSE) is well documented.7,8 Both cell- and virus-like forms of PrPsc can be detected in the CNS, whereas PrPsc accumulations in the PNS is rare, except for a few sporadic descriptions.8,9

Table 1. Clinical and Histologic Data

<table>
<thead>
<tr>
<th>Patient No./Sex/Age, y</th>
<th>14-3-3 Protein</th>
<th>Codon 129</th>
<th>Cerebral Cortex</th>
<th>Striatum</th>
<th>Cerebellar Cortex</th>
<th>Spinal Cord</th>
<th>Sciatic and Superficial Peroneal Nerve</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/F/82</td>
<td>+</td>
<td>Met/met</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>2/M/68</td>
<td>−</td>
<td>NA</td>
<td>+++</td>
<td>+++</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>3/M/84</td>
<td>+</td>
<td>Met/met</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>

*Graded from marked (+++) to absent (−).

Table 2. Results of Immunohistochemistry (IHC) and Western Blot (WB)*

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Cerebral Cortex</th>
<th>Cerebellar Cortex</th>
<th>Spinal Cord</th>
<th>Dorsal Root Ganglia</th>
<th>Sciatic Nerve</th>
<th>Superficial Peroneal Nerve</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IHC WB</td>
<td>IHC WB</td>
<td>IHC WB</td>
<td>IHC WB</td>
<td>IHC WB</td>
<td>IHC WB</td>
</tr>
<tr>
<td>1</td>
<td>+ Sc</td>
<td>++ Sc</td>
<td>++ Sc</td>
<td>+ Sc</td>
<td>− C</td>
<td>+ Sc</td>
</tr>
<tr>
<td>2</td>
<td>+++ Sc</td>
<td>+++ Sc</td>
<td>++ Sc</td>
<td>− C</td>
<td>− C</td>
<td>− C</td>
</tr>
<tr>
<td>3</td>
<td>++ Sc</td>
<td>+++ Sc</td>
<td>++ Sc</td>
<td>− C</td>
<td>− C</td>
<td>− C</td>
</tr>
</tbody>
</table>

*Positivity of prion protein immunohistochemistry was graded from marked (+++) to absent (−). Western blot permitted distinction between cellular (C) and scrapie (Sc) isoforms of prion protein.

Table 1. Clinical and Histologic Data

<table>
<thead>
<tr>
<th>Patient No./Sex/Age, y</th>
<th>14-3-3 Protein</th>
<th>Codon 129</th>
<th>Cerebral Cortex</th>
<th>Striatum</th>
<th>Cerebellar Cortex</th>
<th>Spinal Cord</th>
<th>Sciatic and Superficial Peroneal Nerve</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/F/82</td>
<td>+</td>
<td>Met/met</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>2/M/68</td>
<td>−</td>
<td>NA</td>
<td>+++</td>
<td>+++</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>3/M/84</td>
<td>+</td>
<td>Met/met</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>

Abbreviations: MEt, methionine; NA, not available.

In all sporadic CJD cases, Western blot analysis demonstrated that the PrP immunoreactivity observed on the brain and spinal cord paraffin sections was related to protease K–resistant PrP (PrPsc) accumulation (Table 2 and Figure 2). Brain and spinal cord specimens from control cases contained physiologic protease K–sensitive PrP (PrPc) (Table 2). In case 1, Western blot analysis confirmed that the PrP immunostaining observed in the spinal ganglia and the superficial peroneal nerve corresponded to PrPsc deposits (Figure 3), whereas PNS specimens from cases 2 and 3, as well as controls, contained PrPc (Figure 3). In accordance with immunohistochemistry findings, PrPc was detected in the sciatic nerve in case 1. For all the patients with sporadic CJD and in all tested areas, the electrophoretic mobility and the ratio of the 3 glycosylated PrPsc isoforms were consistent with PrPsc type 1 according to classification by Parchi et al.10

COMMENT

Involvement of both central nervous system and PNS in the pathogenesis of transmissible spongiform encephalopathy (TSE) is well documented.7,8 Both cell- and virus-like forms of PrPsc can be detected in the CNS, whereas PrPsc accumulations in the PNS is rare, except for a few sporadic descriptions.8,9

In all sporadic CJD cases, Western blot analysis demonstrated that the PrP immunoreactivity observed on the brain and spinal cord paraffin sections was related to protease K–resistant PrP (PrPsc) accumulation (Table 2 and Figure 2). Brain and spinal cord specimens from control cases contained physiologic protease K–sensitive PrP (PrPc) (Table 2). In case 1, Western blot analysis confirmed that the PrP immunostaining observed in the spinal ganglia and the superficial peroneal nerve corresponded to PrPsc deposits (Figure 3), whereas PNS specimens from cases 2 and 3, as well as controls, contained PrPc (Figure 3). In accordance with immunohistochemistry findings, PrPc was detected in the sciatic nerve in case 1. For all the patients with sporadic CJD and in all tested areas, the electrophoretic mobility and the ratio of the 3 glycosylated PrPsc isoforms were consistent with PrPsc type 1 according to classification by Parchi et al.10

©2004 American Medical Association. All rights reserved.

Downloaded From: http://archneur.jamanetwork.com/pdfaccess.ashx?url=/data/journals/neur/13744/ on 04/01/2017
lopathies is becoming increasingly evident. Deposits of PrP have been shown, by immunohistochemistry, in the spinal cord from patients with either sporadic or growth hormone–associated CJD.8,11 Such PrP immunolabeling was also seen in 2 cases of Gerstmann-Straussler-Scheinker (GSS) syndrome associated with a missense mutation (proline to leucine) at codon 102 of the PrP gene8,12 and in new-variant CJD.13 Deposits of PrP in the posterior roots were reported in only 1 patient with GSS syndrome and 1 with CJD.8 A recent study demonstrated, by immunohistochemistry and Western blot analysis, PrPsc deposits in the olfactory cilia and central olfactory pathway in 9 patients with sporadic CJD.14 Several experimental studies have also demonstrated involvement of the PNS in the pathogenesis of prion diseases. Tissue-specific expression of PrPc in transgenic mice showed that PrPc-positive peripheral nerves are necessary and sufficient for successful infection of the brain after oral or intraperitoneal infection with scrapie.15 Glatzel and Aguzzi16 demonstrated that transgenic mice overexpressing PrPc undergo rapid PrPsc neuroinvasion on intraneur inoculation of prions. A transgenic mouse and
culture cell study established that PrPc could be expressed by Schwann cells in vivo and that PrPsc could be replicated in Schwann cell line.17

In our study, we found evidence, for the first time in the literature to our knowledge, of PrPsc deposits in the dorsal root ganglia and the superficial peroneal nerve from 1 of 3 patients with sporadic CJD. Immunohistochemistry permitted us to localize PrPsc accumulation likely to Schwann cells of both myelinated and unmyelinated nerve fibers. A suggested explanation for the discrepancy between the consistent immunolabeling observed in experimental models and the less frequent positivity in human prion disease is centripetal (experimental scrapie) vs centrifugal (sporadic and genetic human prion diseases) spreading of PrPsc, resulting in different patterns and amounts of PrPsc accumulation in the PNS. Thus, the absence of PrPsc detection in the sciatic nerve of patient 1 and in all PNS specimens from patients 2 and 3 might be related to the very small amounts of PrPsc accumulated in these tissues and to a lack of sensitivity of our methods. The use of protein misfolding cyclic amplification procedure18 in Western blot analysis of PNS specimens might be helpful in such cases. Although our series is not large enough to draw firm conclusions, the specificity of PrPsc testing in the PNS appears to be fair, because our 3 control cases showed negative results. Nevertheless, the sensitivity of our techniques needs to be improved, because 1 positive CJD case of 3 is still insufficient to suggest peripheral nerve biopsy as a useful tool in the diagnosis of CJD.

Accepted for publication December 3, 2003.

Author contributions: Study concept and design (Drs Favereaux, Perret-Liaudet, Petry, and A. Vital); acquisition of data (Drs Favereaux, Quadrio, Perret-Liaudet, Anne, Laplanche, and A. Vital); analysis and interpretation of data (Drs Favereaux, Quadrio, C. Vital, Perret-Liaudet, and A. Vital); drafting of the manuscript (Drs Favereaux and A. Vital); critical revision of the manuscript for important intellectual content (Drs Quadrio, C. Vital, Perret-Liaudet, Anne, Laplanche, Petry, and A. Vital); obtained funding (Dr Petry); administrative, technical, and material support (Drs C. Vital, Perret-Liaudet, Laplanche, Petry, and A. Vital); study supervision (Dr A. Vital).

Corresponding author: Alexandre Favereaux, PhD, Laboratoire de neurobiologie des affections de la myeline EA 2966, 146, rue Léo Saignant, BP 42, 33076 Bordeaux CEDEX, France (e-mail: alexandre.favereaux@neuropath.u-bordeaux2.fr).

REFERENCES