BSE-associated Prion-Amyloid Cardiomyopathy in Primates

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Prion amyloidosis occurred in the heart of 1 of 3 macaques intraperitoneally inoculated with bovine spongiform encephalopathy prions. This macaque had a remarkably long duration of disease and signs of cardiac distress. Variant Creutzfeldt-Jakob disease, caused by transmission of bovine spongiform encephalopathy to humans, may manifest with cardiac symptoms from prion-amylod cardiomopathy.

Human prion diseases are progressive neurologic disorders that include sporadic, genetic, and acquired forms of Creutzfeldt-Jakob disease (CJD) (1). A key step in disease initiation is conversion of PrPC into PrPSc, which is partially resistant to proteolytic digestion and an essential part of prion infectivity. Transmission of bovine spongiform encephalopathy (BSE) to humans has led to a novel form of acquired CJD, termed variant CJD (vCJD) (2). The pathogenesis of vCJD differs substantially from sporadic CJD with remarkable colonization of non-central nervous system regions with infectious prions and PrPSc (3).

Although risk reduction measures have been introduced to limit transmission from BSE-diseased cattle to humans, vCJD has occurred in several hundred instances (www.eurocjd.ed.ac.uk). Most clinically affected vCJD patients are homozygous for methionine on polymorphic codon 129 on the gene coding PrP (PRNP), and the clinical presentation of vCJD in these patients is uniform (4). The occurrence of atypical clinical features in persons with vCJD that encodes methionine and valine (≈1/100 of PrPSc found in brain) in Western blot analysis of this heart showed PrPSc as amyloid, occupying considerable stretches of heart tissue, mainly in the septum (Figure 2, panel C). These findings were confirmed by strong Congo red–positive patch-like deposits in cardiomyocytes in the heart of this monkey (Figure 2, panel D). The primate with cardiac PrPSc showed the longest disease duration (4 months, compared with 4 weeks for other BSE-infected monkeys), signs of cardiac affection.

1These authors contributed equally to this article.
when assessed by relevant makers of cardiac hypertrophy and of cardiac distress–associated inflammation, and only this macaque showed clinical signs of fatigue and signs of cardiac distress (i.e., venous congestion) on autopsy (Table, online Technical Appendix Table, wwwnc.cdc.gov/EID/article/19/6-0906-Techapp1.pdf). Histologic examination of heart tissue with hematoxylin and eosin staining and immunohistochemical stainings against B and T cells (CD20 [not shown] and CD3) did not provide evidence for toxic cardiomyopathy (i.e., fibrosis or vacuolization), nor did we find evidence for toxic cardiomyopathy or inflammation in the heart, might have contributed to cardiac PrPSc, and the fact that we did not find any evidence for toxic cardiomyopathy or inflammation in the primates does not exclude this possibility. Because the macaque with abundant PrPSc deposition in heart had longer disease duration, it is also possible that longer disease duration, which favors centrifugal spread of prions to peripheral tissues, contributed to cardiac affection in this primate (7). Peripheral deposition of PrPSc in vCJD is well studied (3). We were surprised by the amount and deposition type of PrPSc in heart, reaching 1/100 of the amount seen in brain and deposited as amyloid across large stretches of heart tissue. Skeletal muscle of prion-diseased patients and nonhuman primates routinely harbor minimal amounts of PrPSc (<1/1000 that found in brain), and PrPSc in muscle is virtually impossible to detect by in situ methods (6,8,13).

To our knowledge, PrPSc has not been detected in heart of vCJD-diseased persons or in patients with systemic amyloidosis, although primates orally exposed to BSE show no major differences in PrPSc load were detected. All samples were proteinase K–digested; loading amount was 0.5 and 0.1 mg fresh wet tissue for each sample.

**Conclusions**

Although the vCJD epidemic is declining, considerable concern exists that clinical characteristics of vCJD will shift.

The most important genetic risk factor for development of vCJD is homozygosity for methionine on PRNP codon 129, and all but 1 patient with clinical vCJD carry this polymorphism (5). Thus, future cases of vCJD with longer incubation times are likely to comprise more patients with alternative codon 129 polymorphisms than methionine homozygosity. Data from rodent experiments indicate that clinical features of vCJD may differ in these patients (9). Thus, the next decades may see a shift in vCJD phenotypes. Further uncertainty for atypical cases in humans results from the possibility of secondary transmission of vCJD through blood products from subclinical carriers, which may lead to development of nonclassical vCJD phenotypes (5).

We showed that BSE infection of primates may occur as prion-amyloid cardiomyopathy. Because prion-amyloid cardiomyopathy developed in only 1 of 3 macaques, host-encoded factors, such as genetic makeup, probably influence development of this cardiac phenotype. All macaques are homozygous for methionine on PRNP codon 129; thus, prion-amyloid cardiomyopathy cannot be related to polymorphic codon 129 in our study (10). Cardiac involvement has been observed in a patient with sporadic CJD and is prominent in prion-diseased mice expressing PrPSc lacking its membrane anchor (11,12). We considered the possibility that preexisting pathology, such as spontaneous cardiomyopathy or inflammation of the heart, might have contributed to cardiac PrPSc, and the fact that we did not find any evidence for toxic cardiomyopathy or inflammation in the primate does not exclude this possibility. Because the macaque with abundant PrPSc deposition in heart had longer disease duration, it is also possible that longer disease duration, which favors centrifugal spread of prions to peripheral tissues, contributed to cardiac affection in this primate (7).

**Table. Characteristics of 3 rhesus macaques in study of BSE-associated prion-amyloid cardiomyopathy**

<table>
<thead>
<tr>
<th>Primate</th>
<th>Age at inoculation</th>
<th>Time to clinical disease, mo</th>
<th>Disease duration, wk</th>
<th>Cardiac PrPSc</th>
<th>Signs of cardiac distress at autopsy</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSE inoculated</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 y</td>
<td>49</td>
<td>4</td>
<td>Neg</td>
<td>Neg</td>
<td></td>
</tr>
<tr>
<td>5 y</td>
<td>59</td>
<td>18</td>
<td>Pos</td>
<td>Pos</td>
<td></td>
</tr>
<tr>
<td>1 y</td>
<td>61</td>
<td>4</td>
<td>Neg</td>
<td>Neg</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 mo</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>Neg</td>
</tr>
<tr>
<td>17 y</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>19 y</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>Neg</td>
<td>Neg</td>
</tr>
</tbody>
</table>

*BSE, bovine spongiform encephalopathy; Neg, negative; Pos, positive; NA, not applicable.*

**Figure 1.** PrPSc distribution and content in brain of bovine spongiform encephalopathy (BSE)–infected rhesus macaques. A) Paraffin-embedded tissue blot of striatum and cerebellum show a typical BSE-like deposition pattern of PrPSc with no differences between individual BSE-diseased monkeys at 49, 59, and 61 months postinoculation (mpi). Scale bars = 1 mm. B) Western blot analysis for PrPSc in brain of BSE-infected monkeys with incubation times of 49, 59, and 61 mpi. PrPSc-type is as expected for BSE prions, and no major differences in PrPSc load were detected. All samples were proteinase K–digested; loading amount was 0.5 and 0.1 mg fresh wet tissue for each sample.
very low amounts of cardiac PrPSc (8,14,15). The lack of cardiac PrPSc in vCJD may result from small cohorts investigated. Because the spectrum of vCJD is likely to change, broad application of current clinical criteria for vCJD in clinical practice may lead to underreporting of vCJD, missing atypical cases of vCJD.

In conclusion, we showed that BSE-infection of primates may lead to prion-amyloid cardiomyopathy. These data should be considered when vCJD surveillance is conducted.

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The overall study was conceived and designed by M.G., A.A., F.J.K., and S.K. Animal care, housing, and observation were conducted by F.J.K., W.B., and W.S.S. Experiments were performed by S.K., G.M., E.K., W.S.S., and M.N. Data were analyzed by S.K., G.M., M.B., A.A., and M.G. S.K. and M.G. wrote the paper with substantial contributions from G.M. and A.A.

Dr Krasemann is a research scientist at the Institute of Neuropathology of the University of Hamburg working on prion spread. Her primary research interests are factors involved in spread and clearance of prions.

References


Figure 2. Abundant PrPSc in heart of 1 bovine spongiform encephalopathy (BSE)–infected rhesus macaque. A) In sodium phosphotungstic acid precipitation of PrPSc, followed by Western blotting, highly abundant PrPSc was demonstrated in the heart of 1 BSE-infected primate. In this monkey, only the heart contained PrPSc. Controls include cardiac muscle spiked with minimal amounts brain of a healthy (−) and prion-diseased (+) primate. All analyses were prepared from 50 mg of tissue except the heart of 1 monkey 59 months postinoculation (mpi) (20 mg). PK, protease K. B) In protein-misfolding cyclic amplification, PrPSc was amplified only from the heart of 1 monkey 59 months postinoculation (mpi). As a positive control, brain tissue from a BSE-diseased monkey was used, and tissue from an uninfected control monkey served as a negative control. PK–digested hamster PrPSc (263 K) served as loading and digestion control for PrPSc. C) Paraffin-embedded tissue blotting of the entire heart of the 59 mpi monkey showed abundant deposition of PrPSc, mainly in the septum of the heart. Inset confirms the deposition pattern of PrPSc as amyloid. Scale bar = 0.25 mm. D) Histologic and immunohistochemical examination of heart tissue of the 59-mpi monkey by using hematoxylin and eosin (HE) staining and immunohistochemical staining against T-cell marker CD3 showed regularly configured cardiomyocytes and only single T-cells associated with blood vessels (arrow). Congo red staining showed Congo red–positive material in cardiomyocytes in a patch-like deposition pattern (arrows). Scale bar = 10 µm.
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Shewanella haliotis

[shooʺə-nelʹə hāʺlĭ-oʹtĭs]

From the Greek halios (marine) and ōtos (ear), abalones, genus Haliotis, were first mentioned ≈2,500 years ago by Aristotle, who wrote of “the wild limpet (called by some the ‘sea ear’).” In D’Arcy Thompson’s translation of Aristotle, he notes that “wild limpet” is “commonly attributed to Fissurella graecea ... and conceals a forgotten name for Haliotis.” The “sea ear” was familiar to the Greeks and was named otia (little ear) by Pliny.

Shewanella haliotis, a species of rod-shaped, gram-negative, facultatively anaerobic bacteria, was first isolated from the gut microflora of abalones collected from the ocean near Yeosu, South Korea, by Kim et al. in 2007. The genus Shewanella had been previously named in 1985 by MacDonell and Colwell in honor of Scottish microbiologist James M. Shewan, for his work in fisheries microbiology.

Sources

1. Cox CW. California abalones, family Haliotidae. Sacramento (CA): The Resources Agency of California, Department of Fish and Game. Fish Bulletin no. 118; 1962.

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Technical Appendix

RNA Isolation and Analysis

Total RNA was extracted from 50 mg of heart tissue by using the SV Total RNA Isolation System (Promega, Madison, WI, USA) according to the manufacturer’s protocol. However, to denature PrPSc, lysis buffer containing 4 M guanidinium thiocyanate was added, and tissue homogenate was incubated 24 h at room temperature before clearing of debris by centrifugation and further processing as described in the protocol. The reverse transcription was performed by using the First strand cDNA synthesis kit (Thermo-Fisher Scientific former Fermentas, Schwerte, Germany) with oligo-dT primers from 100 ng RNA. Qualitative determination of macaque monkey mRNAs was performed with Phusion Hot Start II (Thermo Scientific former Finnzymes) polymerase and a touchdown PCR program between 60°–55°C. PCR products were visualized on 1%–2% agarose gels. Quantitative analysis of various macaca mRNAs was performed by real-time RT-PCR on a TaqMan ABI Prism 7900TH (Applied Biosystems, Foster City, CA, USA) machine using the Maxima SYBR Green/ROX qPCR Master Mix (Thermo Scientific former Fermentas), 900 nM primers and 1 µL cDNA (diluted 1:10). The mRNA levels of triplicates were analyzed with the comparative Ct method (2^-ΔΔCt) by using GAPDH as endogenous control. The specific primer pairs used are listed in the Table.

<table>
<thead>
<tr>
<th>Gene*</th>
<th>Primers</th>
</tr>
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<tbody>
<tr>
<td>GAPDH F</td>
<td>5'-ATGTTCGCTCAGGGTGTTGAA-3'</td>
</tr>
<tr>
<td>GAPDH R</td>
<td>5'-TGAGTCCTTCACGATAACCA-3'</td>
</tr>
<tr>
<td>ANP F</td>
<td>5'-TCTCCACCATCAGTGAGC-3'</td>
</tr>
<tr>
<td>ANP R</td>
<td>5'-GGGCACGACACCTCCTCTA-3'</td>
</tr>
<tr>
<td>TNFα F</td>
<td>5'-TCAGGCTCTTGTCTGAAGC-3'</td>
</tr>
<tr>
<td>TNFα R</td>
<td>5'-CTTGGGTTGAGAAGATGA-3'</td>
</tr>
</tbody>
</table>

*F, forward; R, reverse
Results

To investigate whether BSE-infected monkeys differ from controls in mRNA levels indicative of cardiac hypertrophy and of cardiac distress–associated inflammation, we carried out RT-qPCR. Because many rhesus sequences are only predicted by automated computational analysis, the specificity of the primers was tested by sequencing of the corresponding PCR products. The mRNA levels of atrial natriuretic peptide (ANP), a marker of hypertrophy, were lower in all 3 prion-diseased animals than in controls (Technical Appendix Figure, panel A). The mRNA levels of tumor necrosis factor-α (TNF-α), a marker of cardiac-distress associated inflammation, was increased in the BSE-infected monkey, with cardiac PrP^{Sc} (Technical Appendix Figure, panel B).

Technical Appendix Figure. Assessment of markers for hypertrophy and cardiac distress–associated inflammation in rhesus macaques. A, B) The mRNA levels of indicated marker proteins were determined in reverse transcription quantitative PCR with specific primers by using SyBR Green and GAPDH as endogenous control. Each sample was analyzed in triplicates. ANP, atrial natriuretic peptide; AU, arbitrary units.