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-amyloid cardiomyopathy.

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 of 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 on PRNP codon 129 and human-to-human transmission of vCJD through blood transfusion have raised concern about atypical clinical features and alternative distribution of PrPSc in vCJD (5). We report on the novel clinicopathologic characteristics of vCJD as prion-amyloid cardiomyopathy in 1 of 3 macaques inoculated with BSE.

The Study

In 2002, three rhesus macaques were inoculated with BSE intraperitoneally (10 mL of a 10% homogenate of brain from BSE-diseased cattle). As controls, 2 rhesus macaques received saline (10 mL) and 1 was untreated. All procedures involving rhesus macaques were performed at the Institute of Neuropathology, University Medical Center Hamburg-Eppendorf (Hamburg, Germany), in accordance with the German Animal Welfare Act and the Council Directive 86/609/EEC (Permit 33.42502/08–08.02 LAVES, Lower Saxony, Germany). Animals were observed for clinical signs of prion disease and, when signs of terminal prion disease became evident, were euthanized and underwent autopsy. In all 3 BSE-challenged macaques and none of the controls a progressive neurologic disease developed 49, 59, and 61 months postinoculation. Examination of brain by using hematoxylin and eosin staining showed typical neuropathologic features of vCJD (data not shown) and abundant deposits of PrPSc in the cortex, basal ganglia, and cerebellum in paraffin-embedded tissue blots performed as described by using 12F10 monoclonal antiprion antibody (6) (Figure 1, panel A). The mobility of the unglycosylated PrPSc band and the glycoform ratio of proteinase K–digested PrPSc were similar to those in BSE when assessed by Western blot analysis using monoclonal POM-1 antiprion antibody as described (7) (Figure 1, panel B).

Besides lymphoreticular tissues, the muscular compartment is targeted by prions (7,8). Thus, we assessed presence of PrPSc in skeletal and heart muscle by Western blot analysis with sodium phosphotungstic acid precipitation for enrichment of PrPSc and protein misfolding cyclic amplification by using published protocols (3). We could not detect substantial amounts of PrPSc in skeletal muscle (Figure 2, panel A). One macaque showed abundant PrPSc (=1/100 of PrPSc found in brain) in heart in Western blot and protein misfolding cyclic amplification (Figure 2, panels A, B). Paraffin-embedded tissue blot analysis of this heart showed PrPSc as amyloid, occupying considerable stretches of heart tissue, mainly in the septum (Figure 2, panel C), whereas no PrPSc could be seen in hearts of other macaques (data not shown). 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

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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/19/6/12-0906-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 primate that preexisting pathology, such as spontaneous cardiomyopathy or inflammation of the heart, might have contributed to cardiac PrP<sup>Sc</sup>, 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 PrP<sup>Sc</sup> 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 PrP<sup>Sc</sup> in vCJD is well studied (3). We were surprised by the amount and deposition type of PrP<sup>Sc</sup> 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 PrP<sup>Sc</sup> (<1/1000 that found in brain), and PrP<sup>Sc</sup> in muscle is virtually impossible to detect by in situ methods (6,8,13). To our knowledge, PrP<sup>Sc</sup> has not been detected in heart of vCJD-diseased persons or in patients with systemic amyloidosis, although primates orally exposed to BSE show profuse deposition of PrP<sup>Sc</sup> 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 PrP<sup>Sc</sup> in brain of BSE-infected monkeys with incubation times of 49, 59, and 61 mpi. PrP<sup>Sc</sup>-type is as expected for BSE prions, and no major differences in PrP<sup>Sc</sup> 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 <i>PRNP</i> 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 <i>PRNP</i> 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 PrP<sup>Sc</sup> 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 PrP<sup>Sc</sup>, 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 PrP<sup>Sc</sup> 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 PrP<sup>Sc</sup> in vCJD is well studied (3). We were surprised by the amount and deposition type of PrP<sup>Sc</sup> 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 PrP<sup>Sc</sup> (<1/1000 that found in brain), and PrP<sup>Sc</sup> in muscle is virtually impossible to detect by in situ methods (6,8,13). To our knowledge, PrP<sup>Sc</sup> has not been detected in heart of vCJD-diseased persons or in patients with systemic amyloidosis, although primates orally exposed to BSE show profuse deposition of PrP<sup>Sc</sup> 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 PrP<sup>Sc</sup> in brain of BSE-infected monkeys with incubation times of 49, 59, and 61 mpi. PrP<sup>Sc</sup>-type is as expected for BSE prions, and no major differences in PrP<sup>Sc</sup> load were detected. All samples were proteinase K–digested; loading amount was 0.5 and 0.1 mg fresh wet tissue for each sample.

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


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**etymologia**

**Shewanella haliotis**

[shoo-"nələ hā-li-o-'tis]

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**

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BSE-associated Prion-Amyloid Cardiomyopathy in Primates

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 PrP Sc, 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 C t 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'-ATGTTCGCTGATGGGTTGAA-3'</td>
</tr>
<tr>
<td>GAPDH R</td>
<td>5'-TGAGTCCCTTCAGGATACCA-3'</td>
</tr>
<tr>
<td>ANP F</td>
<td>5'-TCTCCACCACATGCTGAGC-3'</td>
</tr>
<tr>
<td>ANP R</td>
<td>5'-GGGCACGACCTCATCTTCTA-3'</td>
</tr>
<tr>
<td>TNFα F</td>
<td>5'-TCAGGCTCTTCTTCTTCTTCTG-3'</td>
</tr>
<tr>
<td>TNFα R</td>
<td>5'-CTTGGGGTGAGAAGATGA-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 hypertension 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 hypertension, 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 PrPSc (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.