Iatrogenic and sporadic Creutzfeldt-Jakob disease in 2 sisters without mutation in the prion protein gene

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ABSTRACT. Human genetic prion diseases have invariably been linked to alterations of the prion protein (PrP) gene PRNP. Two sisters died from probable Creutzfeldt-Jakob disease (CJD) in Switzerland within 14 y. At autopsy, both patients had typical spongiform change in their brains accompanied by punctuate deposits of PrP. Biochemical analyses demonstrated proteinase K-resistant PrP. Sequencing of PRNP showed 2 wild-type alleles in both siblings. Retrospectively, clinical data revealed a history of dural transplantation in the initially deceased sister, compatible with a diagnosis of iatrogenic CJD. Clinical and familial histories provided no evidence for potential horizontal transmission. This observation of 2 siblings suffering from CJD without mutations in the PRNP gene suggests potential involvement of non-PRNP genes in prion disease etiology.

KEYWORDS. Prion, prion diseases, Creutzfeldt-Jakob disease, PRNP, gene

ABBREVIATIONS. CJD, Creutzfeldt-Jakob disease; FFI, fatal familial insomnia; gCJD, genetic/familial Creutzfeldt-Jakob disease; GSS, Gerstmann-Sträussler-Scheinker disease; iCJD, iatrogenic CJD; PCR, polymerase chain reaction; PRNP, PrP gene; PK, proteinase K; PrP, prion protein; PrPSc, scrapie (disease-associated) prion protein; sCJD, sporadic CJD; TSEs, transmissible spongiform encephalopathies

Transmissible spongiform encephalopathies (TSEs) or prion diseases are inevitably fatal neurodegenerative diseases that result from the seeded propagation of the scrapie prion protein PrPSc onto the cellular prion protein PrPC. Genetic TSEs in humans account for 10–15% of all cases and comprise genetic/familial Creutzfeldt-Jakob disease (hereafter referred to as gCJD), Gerstmann-Sträussler-Scheinker disease (GSS), and fatal familial insomnia (FFI), all of which follow an autosomal dominant pattern of inheritance. Only one previous report has shown the co-incidence of CJD in 2 siblings without aberrations in the human PrP coding
To date, however, all reported genetic prion diseases have been exclusively ascribed to mutations in PRNP. We present the case of 2 sisters carrying wild-type PRNP, both of whom were diagnosed at autopsy with CJD.

Patient 1 was a 52-year-old woman who was admitted to the hospital because of progressive abnormal behavior, intermittent vision disorders and fatigue for over 6 months. In addition, she had developed anorexia and listlessness accompanied by obliviousness and occasional confusion during the last 2 weeks. At admission, she complained about dizziness, prefrontal headaches and expressed paranoid ideas. Furthermore, she was unable to stand and to walk safely. Focal lesions were excluded by magnetic resonance imaging. Empiric therapy with ceftriaxone and acyclovir was immediately initiated after sampling for virological and microbiological testing. All analyses of blood and cerebrospinal fluid were negative, except for a positive PCR for varicella zoster virus. In addition to the latter, cerebrospinal fluid samples tested repeatedly positive for the 14-3-3 protein. A brain biopsy was performed, and Western blot analysis of the biopsy was in line with CJD. During the course of disease the patient had to be intubated because of worsening neurology. A follow-up magnetic resonance imaging indicated new occipito-temporo-basal brain lesions on the right side. She developed paracentral bilateral pulmonary embolism in spite of thromboprophylactic treatment. Because of the diagnosis and poor prognosis, therapy was discontinued and the patient was extubated. She died in palliative care around 7 months after her first symptoms had occurred.

Patient 2 was a 73 y old female patient who presented at admission with fluctuating memory deficits, paranoid delusions, visual hallucinations and unattended personal hygiene. According to her relatives, cognitive dysfunction and social isolation had worsened rapidly over the last 3 months, when she still was able to manage book keeping at home. Moreover, the patient showed pronounced amnestic aphasias as well as severe apraxia such as not knowing how to sit down on a chair. Within two weeks after admission, she exhibited increasing swallowing difficulties, painful myoclonia on the forearms, tactile sensory dysfunction of the lower extremities, positive pyramidal signs including Babinski, gait and static ataxia, as well as emotional lability. The patient developed a formal thought disorder as indicated by incoherent speech, became increasingly bedbound and exhibited behavioral symptoms, characterized by mood-incongruent euphoria and partly mutistic states. Sixteen days after admission she was transferred to a palliative care ward where she finally died 14 y after the death of her sister, with a disease duration of approximately 5 months.

Both patients came from a family of 4 sisters, with patient 1 being the youngest and patient 2 the oldest sibling, respectively. Patient 1 was married and had one healthy son. Their father died from chronic myeloid leukaemia, their mother was mildly demented at age 98 or 99. Patient 1 had a dura mater graft at an operation for prolactinoma in 1978, 22 y before death. Unfortunately no detail on the source of the graft is now available. There was neither a history of blood transfusions nor organ donations or other possible horizontal cross-contamination between the 2 sisters. The two sisters lived 30.5 km apart from each other in linear distance, and the relationship between both was described as close and harmonic. Until the death of her sister, patient 2 spent the night at her apartment at least once a week, accompanied by a shared lunch and dinner. For around 12 years, both sisters had regularly spent their holidays in Greece together.

At autopsy, neuropathology was very similar in both patients, showing typical spongiform degeneration in all areas of the cortex with small- to medium-sized, predominantly non-confluent vacuoles accompanied by neuronal cell loss and subsequent thinning of the cortical layer (Fig. 1A, upper panel). A marked gliosis was observed in both white and gray matter. Spongiform change was observed to a lesser extent also in the basal ganglia. The cerebellum showed signs of spongiform degeneration with small vacuoles in the molecular and granular cerebellar layers and accompanying neuronal cell loss, with relative sparing of Purkinje cells.
FIGURE 1. (A) Upper panel: microscopic analysis of the frontal cortex on H&E stains shows typical morphology of sporadic Creutzfeldt-Jakob disease (sCJD) with small and medium-sized vacuoles, neuronal cell loss and astrogliosis. Lower panel: Immunohistochemical analysis for pathological deposits of PrP using the antibody 3F4 demonstrates a synaptic pattern in the granular and molecular cell layers of the cerebellum. Scale bar = 100 μm. (B) Western blots with the monoclonal antibody POM15 of patient brain homogenates digested in the presence or absence of proteinase K (PK) revealed PK-resistant material with the lowest, unglycosylated band at 21 kDa, equivalent to resistant PrP Type I according to Parchi and Gambetti. Positive controls are taken from pathologically confirmed sCJD cases; negative controls are taken from a patient suffering from limbic encephalitis.
Immunohistochemical examination showed a diffuse and fine granular synaptic pattern of PrPSc deposition in the gray matter of the cortex and the molecular and granular layers of the cerebellum (Fig. 1A, lower panel).

Western blots of proteinase K-digested brain homogenates from the frontal cortex of both patients with mouse anti-human monoclonal anti-PrP-antibodies POM1 and POM2 showed 3 bands,\(^5\) with the lowest band corresponding to unglycosylated PrP at 21 kDa, consistent with proteinase K-resistant PrP Type I according to Parchi and Gambetti (Fig. 1B).\(^6\)

In both patients, PCR followed by Sanger sequencing of PRNP from DNA isolated from blood leukocytes showed no mutations in the entire coding sequence, with homozygosity for methionine codon at position 129. Hence histological, immunohistochemical, biochemical and genetic analyses are well in accordance with an MM/MV 1 histotype based on the consensus classification of sporadic CJD (sCJD) histotypes.\(^7\)

After a previous description of CJD in 2 siblings without mutations in PRNP,\(^4\) this second report of such a situation raises several questions and considerations. The first case in this report can be classified as iatrogenic CJD (iCJD) after a dural transplant; globally, more than 200 cases are on record after dural grafting.\(^8\) However, the second case cannot be classified other than sCJD, as no medical exposure risk or known genetic aberration was identified.

The chance co-occurrence of 2 rare conditions in siblings is a distinct possibility. Based on data from the Swiss CJD surveillance system, the probability of at least one of the 4 Swiss iCJD patients to have at least one sibling suffering from any kind of CJD is in the order of \(10^3\) (see derivations in Appendix). This is a small, but not vanishingly small, probability.

As CJD co-occurrence in sisters without PRNP aberrations has been now observed more than once, alternative explanations should be considered. Theoretically, both cases could have been exposed to a common prion source, as clinical, pathological and biochemical features were strikingly similar in these sisters, suggesting a similar or identical prion strain in both. Equally a remote but more intriguing possibility is consideration of potential horizontal transmission. Horizontal transmission of prion disease from one patient to another individual has been reported only for invasive medical procedures including blood transfusion or, historically, for cannibalistic Kuru. Only very few tissues, and instruments contaminated with these, are considered to have high prion infectivity titers, and have been implicated in iCJD.\(^8,9\) However, nothing was found in our cases suggesting a conceivable way of exposure. A similar situation has been reported previously in a husband and wife where either human-to-human transmission or chance co-occurrence of sporadic CJD were discussed.\(^10\)

Finally, the possibility of gCJD caused by aberrations in gene(s) other than PRNP might be considered. Most neurodegenerative diseases, including Alzheimer’s and Parkinson’s diseases as well as amyotrophic lateral sclerosis, can be caused by mutations in many different genes resulting in converging pathological phenotypes. Genetic prion diseases are exceptional in that they have only been linked to PRNP. However, it is very likely that additional factors (possibly including chaperones, heat-shock proteins, and disaggregases) may contribute to prion pathogenesis, and one would expect polymorphisms in these factors to affect genetic prion disease. A possible example for that is the description of apparently sporadic fatal insomnia in a fatal familial insomnia pedigree.\(^11\) The occurrence of families such as the one reported here may help identify such non-PRNP genes, thereby broadening our understanding of prion pathogenesis and, ideally, delivering additional therapeutic targets.

**DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST**

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SUPPLEMENTAL MATERIAL

Supplemental data for this article can be accessed on the publisher’s website.

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