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## **Pathological and biochemical investigation of a woman diagnosed with genetic Creutzfeldt-Jakob disease shortly after parturition**

Creutzfeldt-Jakob disease (CJD) is a rare fatal neurodegenerative condition that most commonly occurs in a sporadic form, predominantly in the elderly. Genetic and acquired forms of CJD also occur, and these may affect younger age groups, including women of childbearing age. The transmissible nature of CJD makes horizontal secondary transmission a concern generally; however a foetus born to a mother carrying a pathogenic mutation associated with CJD might be at risk of developing CJD as a result either of genetic inheritance or potential intrauterine exposure to infectivity. Here we report on the pathological investigation of a woman who developed a severe neuropsychiatric illness shortly after giving birth and who was diagnosed with genetic CJD.

Human prion diseases are uniformly fatal neurodegenerative conditions that occur in idiopathic, acquired and genetic forms. All are transmissible, including the genetic forms, of which CJD E200K-129M is the most common [1]. There are analogous diseases in animals, such as scrapie in sheep and the epidemiology of scrapie suggests both horizontal and vertical transmission [2], but the exact mechanism of maternal transmission in scrapie is a matter of debate, with evidence for *in utero* exposure in addition to infection via the oral route around the time of birth [3].

The infectious agent in human and animal prion diseases is thought to be a misfolded and aggregated form of the prion protein (PrP) that undergoes a self-perpetuating conformational transition from the normal cellular isoform (PrP<sup>C</sup>) to the disease associated isoform (PrP<sup>Sc</sup>)

that deposits in the brain and, in some prion diseases, at lower levels in peripheral tissues. The expression of PrP<sup>C</sup> within a tissue is therefore considered a precondition for both PrP<sup>Sc</sup> accumulation and prion replication. The presence of PrP<sup>C</sup> in human blood [4] and human reproductive tissues, notably the placenta, has been documented [5,6] and is suggestive of a possible route of exposure.

Evidence of vertical transmission in human prion disease is absent, but the evidence base is not large. A priori, variant CJD might be supposed to present the greatest risk of maternal transmission because of the younger age distribution of affected individuals, the disseminated nature of PrP<sup>Sc</sup> and infectivity in peripheral tissues and the transmission of variant CJD infectivity by blood or blood products on five separate occasions (<http://www.cjd.ed.ac.uk/TMER/TMER.htm>). Despite the comparatively large numbers of parents with variant CJD known to the National CJD Research & Surveillance Unit, including nine women with clinical variant CJD while pregnant, no evidence of vertical transmission has been found, although further follow up was recommended considering the potentially lengthy incubation periods involved [7]. Nevertheless, it remains important to consider the possibility of secondary transmission in all forms of CJD, including the possibility of maternal transmission in the rare circumstances of a pregnancy in a person with clinical or pre-clinical CJD.

The patient presented at the age of 32 years, after she gave birth at term with no complications. The baby subsequently showed developmental delay; investigations for a possible underlying metabolic disorder are being undertaken. The first symptoms were somewhat nonspecific comprising anxiety attacks, insomnia, psychotic symptoms and irritability during her pregnancy, which grew worse and resulted in admission to a psychiatric hospital. Before admission to the hospital she had experienced a seizure. Within weeks her state deteriorated: severe insomnia, cognitive decline and progressive gait imbalance was

observed; she was unable to communicate properly with the physicians, or to read. Myoclonic jerks were noted in all extremities. Cranial CT did not reveal atrophy or a space-occupying lesion, however, cranial MRI performed one month after her admission revealed symmetric high signal intensity in the caudate nucleus and putamen. EEG showed periodic sharp waves including those with a triphasic morphology. A search for neoplastic disease and paraneoplastic antibodies did not show positive results. Immunoblotting of the cerebrospinal fluid sample for protein 14-3-3 showed strong positivity (there was no blood contamination or elevated cell count), while genetic analysis of the *PRNP* revealed an E200K mutation and MM homozygosity at codon 129. Further progression to akinetic mutism was observed and the patient died four months after the onset of clinical symptoms.

Formalin fixed, paraffin-embedded tissue blocks were evaluated from several cortical areas; hippocampus, basal ganglia, thalamus, brainstem and cerebellum. In addition to Haematoxylin and Eosin, Luxol Fast Red, and Bielschowsky silver staining, the following monoclonal antibodies were used for immunohistochemistry: anti-PrP 3F4 (epitope: aa. 106-112; 1:1,000, Signet/Covance, Berkeley, CA, USA), anti-PrP 12F10 (epitope: aa. 142-160; 1:1,000, Cayman Chemical, Ann Arbor, MI, USA), anti-PrP 6H4 (epitope: aa. 144-152; 1:500, Prionics, Schlieren, Switzerland), anti-tau AT8 (pS202/pT205, 1:200, Pierce Biotechnology, Rockford, IL, USA), anti-phospho-TDP-43 (pS409/410, 1:2,000, Cosmo Bio, Tokyo, Japan), anti- $\alpha$ -synuclein (1:2,000, clone 5G4, Roboscreen, Leipzig, Germany), anti-A $\beta$  (1:50, clone 6F/3D, Dako, Glostrup, Denmark), anti-p62 (1:1,000, BD Transduction, Lexington KY, USA), The DAKO EnVision<sup>®</sup> detection kit, peroxidase/DAB, rabbit/mouse (Dako, Glostrup, Denmark) was used for visualization of antibody reactions. In addition the internal organs (spleen, heart, skeletal muscle, liver, lung, kidney, uterus and ovary) were also immunostained for PrP. The most striking alteration was the presence of spongiform change in the neuropil, which was accentuated in the deeper layers of the cortex. This was associated

with an unusually high degree of neuronal loss and gemistocytic type of reactive astrogliosis in all examined neocortical areas (Figure 1A). Importantly, the CA subregions of the hippocampus and the brainstem nuclei were relatively preserved. The thalamus and the basal ganglia showed reactive gliosis and neuronal loss but only moderate or mild spongiform change. The cerebellar molecular layer showed only mild spongiform degeneration, however there was loss of Purkinje cells with Bergmann gliosis (Figure 1B). The severe degree of cortical and subcortical reactive astrogliosis and neuronal loss was somewhat unusual as compared to other E200K patients from Hungary. Immunostaining for PrP showed diffuse/synaptic immunoreactivity in all examined regions (Figure 1C), without neuronal, perineuronal or perivacuolar accentuation. There was lack of kuru type plaques, florid plaques or PrP plaques. We observed diffuse PrP immunoreactivity in the cerebellar cortex (Figure 1D), but not the stripe-like pattern, which is seen frequently, at least in the cohort of Hungarian patients [1]. The internal organs did not show PrP immunostaining. In addition, there was a lack of depositions of different neurodegenerative disease-related proteins, which often concur with the E200K mutation [1].

Frozen tissue specimens were available from the brain, spleen, heart, skeletal muscle, liver, lung, kidney, uterus and ovary. Western blot analysis for protease-resistant (disease associated) prion protein (PrP<sup>res</sup>) showed readily detectable PrP<sup>res</sup> in the cerebral cortex (frontal lobe) and in the cerebellum (Figure 2A,B). The electrophoretic mobility of the non-glycosylated PrP<sup>res</sup> (lowest band) was around 21kDa and had the same electrophoretic mobility as type 1 in a reference case of sporadic CJD of the MM1 subtype. The ratio of the three glycoforms in both frontal cortex and cerebellum in this case was dominated by the upper two (mono- and di-glycosylated) forms and was intermediate in ratio between the sporadic CJD (type 1A) and variant CJD (type 2B) reference standards. The pattern is classified by us as type 1A/B, and it is typical of genetic CJD associated with the E200K-

129M [1,8]. Sodium phosphotungstic acid (NaPTA) precipitation in advance of Western blotting was used to enrich for PrP<sup>Sc</sup> from larger volumes of tissue homogenates of peripheral tissues from this case to determine whether PrP<sup>res</sup> is present at levels lower than those found in the brain. Recovery of brain PrP<sup>res</sup> from this case spiked into non-CJD brain and non-CJD spleen homogenate was found to be efficient and functions as a positive control for NaPTA precipitation and Western blotting. However, no specific signal denoting the presence of PrP<sup>res</sup> was observed in samples of heart, skeletal muscle, spleen, liver, lung, kidney, uterus or ovary from this case (Figure 2C,D) suggesting that if PrP<sup>Sc</sup> (and by implication prion infectivity) is present in these tissues it must be present only at very low levels.

To our knowledge this report constitutes the first detailed pathological investigation of a patient with genetic CJD who developed severe neuropsychiatric symptoms shortly after the birth of a child. All of the available clinical, genetic, pathological and biochemical data indicate that this patient had CJD typical of that associated with the E200K-129M haplotype [1]. The absence of detectable PrP<sup>res</sup> in peripheral tissues is consistent with pathological changes originating in the brain with only limited centrifugal spread to the periphery (as is found in sporadic CJD), compared to variant CJD in which there is clear evidence of peripheral nervous and lymphoreticular tissue involvement [9,10]. The situation pertaining to ovary and uterus bear further comment: PrP<sup>res</sup> has been demonstrated in uterus and ovary in a patient with variant CJD who was not pregnant whilst ill [11], whereas no evidence could be found of the presence of PrP<sup>res</sup> in the uterus, placenta or amniotic fluid from a gravida with sporadic CJD of the VV1 subtype [12]. Taken together these data and ours indicate that in genetic CJD (of at least the E200K type) the involvement of the female reproductive tract more closely resembles that of sporadic rather than variant CJD. Although this may offer the foetus a degree of protection from intrauterine exposure, it does not obviate the genetic risk.

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## **Author contributions**

H.Y. performed the Western blot analysis. M.W.H. supervised the biochemical analysis. K.T. performed the autopsy and the pathomorphological investigation, collected histological samples, collected data; E.K., and C.R. collected data and performed investigations, G.G.K. performed the neuropathological examination and collected the data. The manuscript was drafted by M.W.H., G.G.K. and J.W.I., and approved by all authors.

## **Conflict of interests**

None

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## Figure legends

Figure 1. Histopathology of the reported case. A: Severe astrogliosis in the frontal cortex (x400). B: Mild spongiform change in the cerebellar molecular layer and loss of Purkinje cells with Bergmann gliosis (x400). C: Diffuse/synaptic PrP immunoreactivity (12F10) in the frontal cortex (x200). D: Diffuse/synaptic immunoreactivity in the cerebellar cortex (12F10) (x200).

Figure 2. Biochemical investigation of the reported case. Conventional diagnostic Western blot analysis of frontal cortex (A) and cerebellum (B) 10% w/v brain homogenate, loaded as 5ul without proteinase K digestion (-), or as 0.5ul (0.5), 5ul (5), or 50ul collected by centrifugation ([50]) after proteinase K digestion. Molecular mass standards (M) and ~5ul of 10% w/v proteinase K digested frontal cortex from sCJD MM1 subtype (1A), sCJD VV2A subtype (2A) and vCJD (2B) are shown for reference. (C,D) shows NaPTA precipitation, proteinase K digestion and Western blot analysis of 500ul of 10% w/v homogenates [500 NaPTA] of heart (H), muscle (M) and spleen (S), liver (Li), lung (Lu), kidney (K), uterus (U) and ovary (O). For NaPTA Western blot analysis additional controls were performed in which 500ul of negative brain (B-) and 500ul of negative spleen (S-) homogenates were spiked with 5ul of 10% w/v frontal cortex homogenate from this case (B+) and (S+) respectively, prior to NaPTA precipitation, proteinase K digestion and Western blot analysis. Lanes marked above the figure show analysis of samples from the case in question, whereas those marked below show the analysis of reference standards.

Figure 1

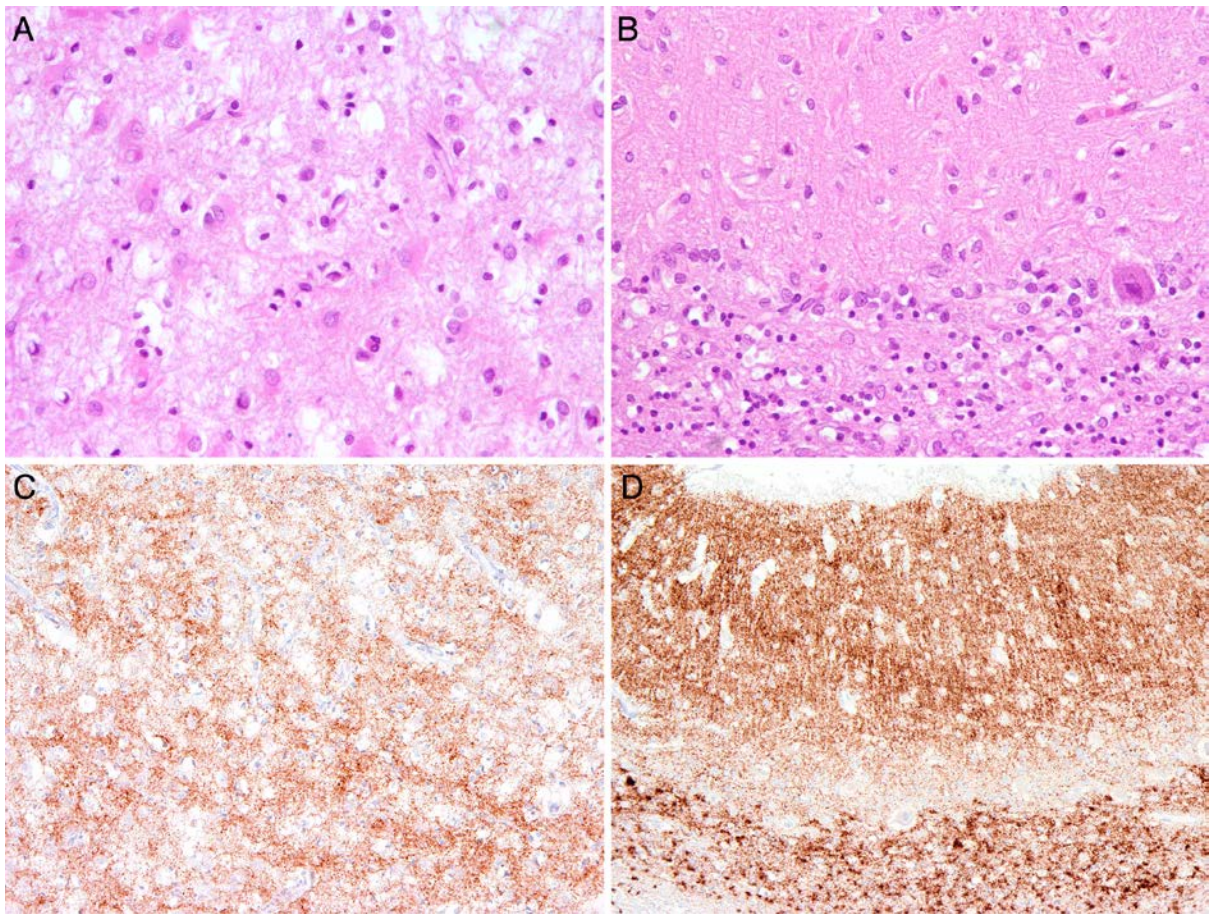


Figure 2

