SHORT REPORT

Familial prion disease in a Hungarian family with a novel 144-base pair insertion in the prion protein gene

T Kovács, J A Beck, M I Papp, P L Lantos, Z Arányi, I G Szirmai, M Farsang, A Stuke, A Csillik, J Collinge

J Neurol Neurosurg Psychiatry 2007;78:321–323. doi: 10.1136/jnnp.2006.104372

About 15% of human prion diseases are inherited, and are associated with point or insertional mutations of the prion protein gene (*PRNP*). Four families with six octapeptide repeat insertions (OPRI) in the *PRNP* gene have been described in the literature so far. Here we report two cases in a Hungarian family with a new six OPRI (R1R2R2R3R2R3gR3R2R2R3R4) in the *PRNP* gene. The clinical features (progressive ataxia, dementia and anosmia), the age of onset and the duration of disease were almost identical. In addition to the cerebellar and parahippocampal pathological changes already described, we also found deposits of pathological prion protein in the olfactory system.

Human prion diseases are acquired, iatrogenic and hereditary degenerative disorders of the central nervous system.¹ About 15% of these disorders are inherited and are associated with point or insertional mutations in the prion protein (PrP) gene (*PRNP*) on chromosome 20.² Insertional mutations consist of one to nine extra octapeptide repeats of the *PRNP* gene, which normally has one nonapeptide (R1) and four octapeptide (R2, R2, R3, R4) repeats between codons 51 and 91.^{1,2} One, two or four octapeptide repeat insertions (OPRIs) usually cause rapidly progressing dementia similar to classic Creutzfeldt–Jakob disease (CJD), whereas the clinical phenotype in five to nine OPRIs is heterogeneous and different phenotypes have been reported even within the same family.³ Neuropathologically, the degree of spongiform change is variable and PrP^{Sc} deposition is mainly restricted to the molecular layer of the cerebellum, where elongated diffuse deposits perpendicular to the leptomeningeal surface are characteristic in four to seven repeats and plaques in eight or nine repeats.⁴

Here we describe two affected members of a Hungarian family with a new six OPRI. The clinical phenotype was strikingly similar in the two affected family members and neuropathological examination showed cerebellar, parahippocampal and olfactory tract PrP^{Sc} deposits.

METHODS

Neuropathology

After limited autopsy, the brain of patient 1 was fixed in 10% formol-saline. Sections of 7 µm thickness were cut (from tissue blocks of cortical areas of all four lobes, the basal ganglia, thalamus, brain stem, cerebellum, olfactory bulbs and tracts) and stained by conventional histological techniques and by immunohistochemistry for PrP^{Sc} (monoclonal antibody 12F10, dilution 1:250, German Primate Center, Göttingen, Germany) and for glial fibrillary acidic protein (dilution 1:1500, DAKO, Glostrup, Denmark).

DNA amplification and sequencing

PRNP open reading frame was amplified by polymerase chain reaction (PCR) of genomic DNA extracted by standard methods from peripheral blood leucocytes. Nested sequencing primers were used to sequence sense and antisense DNA. Characterisation of the OPRI and verification of its size were performed by TA cloning and size fractionation by agarose electrophoresis, respectively, of a small exonic PCR amplicon that included the *PRNP* octapeptide repeat region. Sequencing of cloned DNA was performed using PCR primers. Details of PCR primers, conditions and TA cloning have been reported previously.⁵

Case reports and the family (fig 1)

Patient 1 was a man with an uneventful medical history. His first complaint developed at the age of 33 years, when he experienced loss of memory of recent events. At the age of 35 years, movement became slow and clumsy, an unsteady gait developed and his behaviour became aggressive. On first medical examination at the age of 35 years, no focal neurological signs were observed. Magnetic resonance imaging of the brain showed slight generalised atrophy. The patient was diagnosed as having Alzheimer's disease and was referred to our department for assessment 6 months after the initial examination. During this 6 months, his cognitive function rapidly deteriorated. Neurological examination disclosed

Figure 1 Family tree. Patient 1 had neuropathological confirmation of prion disease. Patient 2 had genetic testing of the *PRNP* gene at the age of 42 years and no OPRI was found. Patient 4 may have carried the OPRI mutation and may have been affected by the disease.

Abbreviations: OPRI, octapeptide repeat insertions; PCR, polymerase chain reaction; PrP, prion protein

Figure 2 Neuropathology of patient 1. (A) Scarce spongiosis in the cerebellum. Haematoxylin-eosin (HE), bar 300 µm. (B) Perpendicular prion protein (PrP^{Sc}) deposits in the molecular layer of the cerebellum. PrP^{Sc}-IH and HE counterstaining. Bar 150 µm. (C) Parahippocampal gyrus with patches of PrP^{Sc} deposition. PrP^{Sc}-IH and haematoxylin counterstaining. Bar 150 µm. (D) Diffuse deposits of PrP^{Sc} in the olfactory tract. PrP^{Sc}-IH and haematoxylin counterstaining. Bar 15 µm.

anosmia, gaze-evoked horizontal nystagmus, hypomimia, dysarthria, rigidity, hypokinesia, ataxia of the limbs, dysidiadochokinesis and cerebellar tremor. Myoclonus was not observed. Furthermore, frontal release signs, perseveration, asomatognosia, finger agnosia, apraxia, agraphia, alexia, anomic aphasia, severe short-term and long-term memory problems and mirror movements of the left hand were seen.

Electroencephalography showed diffuse slow waves. Cerebrospinal fluid examination (including protein 14–3–3) was negative. Magnetic resonance imaging of the brain disclosed severe generalised atrophy. Hyperintensity on T2-weighted images in the basal ganglia was not seen. Positron emission tomography (¹⁸F-deoxyglucose) found severe hypometabolism in all cortical areas and in the cerebellum.

During the following 6 months, the patient's condition further deteriorated and he died at the age of 36 years from pneumonia, after a disease duration of 3 years.

Patient 3 was born in 1938. His first symptom at the age of 35 years was also forgetfulness. Soon afterwards, his gait became unsteady and he started to behave aggressively with members of his family. Pneumoencephalography showed diffuse atrophy. His condition quickly deteriorated and he died at the age of 38 years. On autopsy, diffuse atrophy of the brain was described. Apart from neuronal loss in various cortical areas, no microscopical changes were mentioned in the report. Unfortunately, flooding destroyed the material and thus it was not possible to re-examine the tissue samples.

Patient 4 died in World War II, at the age of 35 years. Relatives described his behaviour as strange and aggressive and said that he had started to drink heavily. He has not been examined.

RESULTS

Molecular genetics

Complete sequencing of the *PRNP* open reading frame of patient 1 was performed and this showed an OPRI mutation on

one allele. The codon 129 genotype was methionine homozygous. Size fractionation of a small PCR amplicon encompassing the octapeptide repeat region confirmed an extra six octapeptide repeats on the mutant allele. TA cloning and sequencing of this allele showed this to be a novel insertion (R1R2R2R3R2R3 gR3R2R2R3R4).

Neuropathology

On autopsy, the brain of patient 1 weighed 1310 g and the brain stem with the cerebellum weighed 140 g. Mild dorso-lateral and dorsomedial bilateral frontal atrophy and enlarged ventricles were found on coronal slices. No focal changes were seen.

On light microscopy, neuronal loss was seen in all cortical areas. Spongiform change (fig 2A) and astrogliosis on glial fibrillary acidic protein-IH were very mild in the cerebellar molecular layer. On PrP^{Sc}-IH, the molecular layer of the cerebellum (fig 2B) contained numerous diffuse deposits perpendicular to the leptomeningeal surface. Apart from a few diffuse plaques in the parahippocampal gyrus (fig 2C), other cortical areas were free of PrP^{Sc} immunoreactivity. In addition, strong diffuse PrP^{Sc} immunoreactivity was detected in the olfactory tract and in the granular layer of the bulb (fig 2D).

DISCUSSION

Four families with six OPRI in the *PRNP* have been described in the literature. The largest family reported by Poulter *et al*⁶ with the octapeptide repeat sequence of R1R2R2R2R3R2R3 gR2R2R3R4, has multiple affected members with age of onset ranging from 22 to 53 years and duration of disease of 1 to 18 years. The clinical picture and neuropathology were heterogeneous.⁷ Nicholl *et al*⁸ described two cases where the clinical picture and the course of the disease were strikingly different. The order of repeats in this family was R1R2R2R3R2R3 gR2R3gR2R3R4. Oda *et al*⁹ described four cases; three of them had a similar clinical picture and disease course. The order of

the repeats was R1R2R2R3 gR2R2R3 gR2R2R3R4. Capellari *et al*¹⁰ studied a Basque family with the repeat sequence of (R2)₆. The three affected members had a variable clinical course.

Our family with a new six OPRI of R1R2R2R3R2R3 gR3R2R2R3R4 has two affected members. In contrast with the other six-OPRI families, the clinical features, the age of onset and duration of disease were almost identical in our two patients. The neuropathology was similar to most of the cases in the English family,³ with very limited spongiform degeneration and absent PrP^{Sc} immunoreactivity in the cerebral cortex, except for the patches in the parahippocampal gyrus. PrP^{Sc} depositions in the cerebellar molecular layer were also of the typical, perpendicular type.^{3–4} However, this is the first reported patient (patient 1) with OPRI where the olfactory system contains PrP^{Sc}. The olfactory pathology correlated well with the clinical complaint of anosmia in the patient. Our case suggests that familial Creutzfeldt–Jakob disease with OPRI mutation may be similar to sporadic cases with respect to the pathology of the olfactory system.¹¹ Moreover, the olfactory pathogenesis (the olfactory system might be involved at an early stage because of the ongoing neurogenesis) proposed by Zanusso *et al*¹¹ may also apply to the genetic forms of prion diseases.

ACKNOWLEDGEMENTS

We thank the family for their support and help.

We also thank Mrs Réka Nagy and Mrs Eszter Bíró for their technical assistance.

Authors' affiliations

T Kovács, M I Papp, Z Arányi, I G Szirmai, M Farsang, A Csillik, Department of Neurology, Semmelweis University, Budapest, Hungary
J A Beck, J Collinge, MRC Prion Unit, Department of Neurodegenerative Disease, The National Hospital for Neurology and Neurosurgery, London, UK
P I Lantos, Department of Neuropathology, Institute of Psychiatry, London, UK
A Stuke, German Primate Center, Göttingen, Germany

Funding: The work of TK is supported by the Bolyai Fellowship of the Hungarian Academy of Sciences and by the Hungarian Scientific Research Fund (OTKA) grant T 046847.

Competing interests: None declared.

Informed consent was obtained from the patients for publication of their details in this paper.

Correspondence to: Dr T Kovács, Department of Neurology, Semmelweis University, Budapest, Balassa u. 6., H-1083 Hungary; tibor@neur.sote.hu

Received 12 August 2006

Revised 3 September 2006

Accepted 4 September 2006

REFERENCES

- 1 **Collinge J.** Prion diseases of humans and animals: their causes and molecular basis. *Annu Rev Neurosci* 2001;**24**:519–50.
- 2 **Collinge J.** Molecular neurology of prion disease. *J Neurol Neurosurg Psychiatry* 2005;**76**:906–19.
- 3 **King A,** Doey L, Rossor M, *et al.* Phenotypic variability in the brains of a family with a prion disease characterized by a 144-base pair insertion in the prion protein gene. *Neuropathol Appl Neurobiol* 2003;**29**:98–105.
- 4 **Vital C,** Gray F, Vital A, *et al.* Prion encephalopathy with insertion of octapeptide repeats: the number of repeats determines the type of cerebellar deposits. *Neuropathol Appl Neurobiol* 1998;**24**:125–30.
- 5 **Beck JA,** Mead S, Campbell TA, *et al.* Two-octapeptide repeat deletion of prion protein associated with rapidly progressive dementia. *Neurology* 2001;**57**:354–6.
- 6 **Poulter M,** Baker FM, Frith CD, *et al.* Inherited prion disease with 144 base pair gene insertion. 1. Genealogical and molecular studies. *Brain* 1992;**115**:675–85.
- 7 **Collinge J,** Brown J, Hardy J, *et al.* Inherited prion disease with 144 base pair gene insertion. 2. Clinical and pathological features. *Brain* 1992;**115**:687–710.
- 8 **Nicholl D,** Windl O, de Silva R, *et al.* Inherited Creutzfeldt–Jakob disease in a British family associated with novel 144 base pair insertion of the prion protein gene. *J Neurol Neurosurg Psychiatry* 1995;**58**:65–9.
- 9 **Oda T,** Kitamoto T, Tateishi J, *et al.* Prion disease with 144 base pair insertion in a Japanese family line. *Acta Neuropathol* 1995;**90**:80–6.
- 10 **Capellari S,** Vital C, Parchi P, *et al.* Familial prion disease with a novel 144-bp insertion in the prion protein gene in a Basque family. *Neurology* 1997;**49**:133–41.
- 11 **Zanusso G,** Ferrari S, Cardone F, *et al.* Detection of pathologic prion protein in the olfactory epithelium in sporadic Creutzfeldt–Jakob disease. *N Engl J Med* 2003;**348**:711–19.