Abnormal prion protein in the retina of the most commonly occurring subtype of sporadic Creutzfeldt-Jakob disease

M W Head, A H Peden, H M Yull, D L Ritchie, R E Bonshek, A B Tullo, J W Ironside


Background: Involvement of the eye has been reported in patients with variant Creutzfeldt-Jakob disease (vCJD), but there is disagreement on whether retinal involvement occurs in sporadic Creutzfeldt-Jakob disease (sCJD).

Methods: Western blotting, paraffin embedded tissue blotting, and immunohistochemistry were used to test whether the abnormal form of the prion protein (PrPSc) accumulates to detectable levels in the eye in a case of the most common subtype of sCJD (MM1).

Results: Low levels of PrPSc were detectable in the retina, localised to the plexiform layers of the central retina. PrPSc was not detectable in other ocular tissues.

Conclusions: The abnormal form of the prion protein is present in the retina in the most common sCJD subtype (MM1), albeit at levels lower than those found previously in vCJD and in sCJD of the VV2 subtype.

Conclusions:

Western blot analysis for PrPSc was carried out using the method described previously.

RESULTS

Western blot analysis using the standard method showed that type 1 PrPSc was abundant in the brain (frontal cortex) from this case of sCJD. In contrast, PrPSc was undetectable in a sample of retina (fig 1A). We then re-sampled the ocular tissues and prepared tissue homogenates using a modified tissue dispersion step employing a FastPrep tissue homogeniser (Qbiogene, Cambridge, UK) used in combination with a preparatory step that employs sodium phosphotungstate (Qbiogene, Cambridge, UK) and sodium phosphotungstate (Qbiogene, Cambridge, UK), and the manufacturer’s instructions (Invitrogen Life Sciences, Paisley, UK). Western blots were probed with the monoclonal antibody 3F4 (Dako, High Wycombe, UK), horseradish peroxidase conjugated anti-mouse secondary antibody, and the signal captured by enhanced chemiluminescence (ECL+) using x ray film (all from Amersham Biosciences, Bucks, UK). In some experiments a modified method described previously is available to them. We found, however, that PrPSc was detectable at levels similar to those found in the brain and distributed uniformly throughout the plexiform layers in the retina from the eye of a patient with sCJD. One possible explanation for this discrepancy is the known diversity of clinical and pathological phenotype in sCJD. This depends partly on the PrPSc type present (either type 1 or type 2) and on the methionine/valine polymorphism at codon 129 of the PRNP gene. The eye which we previously examined was from a patient who had type 2 PrPSc in the brain and was homozygous for valine at codon 129 of PRNP (that is, VV2). We have recently had the opportunity to readdress this issue by examining both fixed and frozen eye tissues from a 67 year old man referred to the National CJD Surveillance Unit with suspected CJD. Autopsy examination confirmed the diagnosis of sCJD, and the biochemical and genetic tests identified this to be of the most commonly occurring MM1 subtype. Consent was given for tissue retention and research use.

Materials and methods

Western blot analysis using the standard method showed that type 1 PrPSc was abundant in the brain (frontal cortex) from this case of sCJD. In contrast, PrPSc was undetectable in a sample of retina (fig 1A). We then re-sampled the ocular tissues and prepared tissue homogenates using a modified tissue dispersion step employing a FastPrep tissue homogeniser (Qbiogene, Cambridge, UK) used in combination with a preparatory step that employs sodium phosphotungstate (Qbiogene, Cambridge, UK) and sodium phosphotungstate (Qbiogene, Cambridge, UK), and the manufacturer’s instructions (Invitrogen Life Sciences, Paisley, UK). Western blots were probed with the monoclonal antibody 3F4 (Dako, High Wycombe, UK), horseradish peroxidase conjugated anti-mouse secondary antibody, and the signal captured by enhanced chemiluminescence (ECL+) using x ray film (all from Amersham Biosciences, Bucks, UK). In some experiments a modified method described previously is available to them. We found, however, that PrPSc was detectable at levels similar to those found in the brain and distributed uniformly throughout the plexiform layers in the retina from the eye of a patient with sCJD. One possible explanation for this discrepancy is the known diversity of clinical and pathological phenotype in sCJD. This depends partly on the PrPSc type present (either type 1 or type 2) and on the methionine/valine polymorphism at codon 129 of the PRNP gene. The eye which we previously examined was from a patient who had type 2 PrPSc in the brain and was homozygous for valine at codon 129 of PRNP (that is, VV2). We have recently had the opportunity to readdress this issue by examining both fixed and frozen eye tissues from a 67 year old man referred to the National CJD Surveillance Unit with suspected CJD. Autopsy examination confirmed the diagnosis of sCJD, and the biochemical and genetic tests identified this to be of the most commonly occurring MM1 subtype. Consent was given for tissue retention and research use.

Abbreviations: sCJD, sporadic Creutzfeldt-Jakob disease; vCJD, variant Creutzfeldt-Jakob disease

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homogeniser. Standard Western blot analysis again failed to detect PrP Sc in the retina even on maximal exposure (fig 1B). The remaining FastPrep tissue extracts (500 μl) were then used in a sodium phosphotungstic acid precipitation assay. Using this method to selectively precipitate PrPSc before Western blot analysis, a positive signal was seen in the retina sample (fig 1C). No PrP Sc was detectable in the cornea, lens, iris, vitreous body, sclera, or extraocular muscle in the standard assay or in the phosphotungstic acid precipitation experiments. A faint signal was obtained from the sample of optic nerve (data not shown).

The paraffin embedded tissue blot method was then used to assess the regional distribution of PrPSc in the retina. Positive staining was seen in some (but not all) preparations of the retina. Where present, this was in the form of a bi-layer when viewed at low magnification (fig 2A). Immunohistochemistry showed that this pattern corresponded to the plexiform layers of the neural retina in which focal granular PrP staining is seen (fig 2B). The intensity of immunohistochemical staining for PrPSc was less overall than that seen in the vCJD eye, and it diminished towards the more anterior parts of the neural retina. No obvious relationship was identified between the distribution of PrPSc and other anatomical structures in the central retina. Positive immunostaining for PrPSc was not evident in the optic nerve or any ocular tissues other than neural retina by the paraffin embedded tissue blot method or by immunohistochemistry.

DISCUSSION

The results presented here go some way to resolving the discrepancy between the two published studies of ocular involvement in cases of sCJD. Sporadic CJD is thought to originate from a chance conversion of the normal form of the prion protein to the abnormal form (PrPSc) in the brain in affected individuals. The presence of PrPSc outside the brain has been interpreted as centrifugal spread from the brain to the periphery, in the case of the eye, via the optic nerve with subsequent accumulation being a characteristic of regions of high synaptic density such as the plexiform layers of the retina.

Figure 1 Western blot analysis of protease resistant prion protein in the retina from a patient with sporadic CJD. (A) Analysis of brain (lane 3) and retina (lane 4) from the patient, flanked by samples of a standard sporadic CJD brain (lane 2) and a standard sample of variant CJD brain (lane 5). Molecular weight markers are shown in lanes 1 and 6 with their molecular weights indicated in kDa. (B) Analysis of a second sample of retina from the patient (lane 3), alongside a standard sample of variant CJD brain before (lane 1) and after (lane 2) treatment with proteinase K. (C) 500 μl of a 10% (w/v) retinal extract from this patient is shown after precipitation with phosphotungstic acid (lane 3) and the position of the positive signal is marked (*). Lanes 1 and 2 show 1 μl of a 10% (w/v) standard homogenate of variant CJD brain before (lane 1) and after (lane 2) digestion with proteinase K. All blots were probed with the antibody 3F4.

Figure 2 Localisation of PrPSc in the retina from a patient with sporadic CJD by (A) paraffin embedded tissue blotting with the antibody 3F4 and (B) immunohistochemistry with the antibody 12F10. The retinal layers are indicated: outer nuclear layer (onl), outer plexiform layer (opl), inner nuclear layer (inl), inner plexiform layer (ipl), ganglion cell layer (gcl).
PrPSc positivity is known from animal studies to progress at a slow but defined rate (~1 mm/day) along neural pathways. If it follows that ocular involvement could be considered to be a function of the presence of PrPSc in the relevant region of the brain, the time during which it can spread via the optic nerve to the retina, and the opportunity that it has to accumulate in the synaptic layers. The mean duration of illness in sCJD is shorter than that of vCJD, and this is particularly true of the duration of the MM1 subtype of sCJD which progresses very rapidly. The cases of sCJD that we have reported are typical of their subtypes. The duration of the illness in the VV2 sCJD case we reported previously was 9 months, whereas the MM1 patient reported here had a duration of illness of only 1 month. We would predict that any larger study of ocular involvement in CJD might find a correlation between the level and extent of PrPSc accumulation in the retina and disease duration. A similar argument has recently been advanced to explain why some (but not all) cases of sCJD have detectable PrPSc in spleen and skeletal muscle.

Irrespective of whether this general explanation is found to be correct, this study clearly indicates that the retina should be considered a potential source of transmissible infection when ophthalmic surgery is performed on any patient suffering from neurological symptoms consistent with a diagnosis of CJD. The results also underscore the importance of localisation studies in addition to sensitive biochemical tests when analysing tissues that might be heterogeneous in their accumulation of PrPSc.

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