Prion Protein Accumulation in Eyes of Patients with Sporadic and Variant Creutzfeldt-Jakob Disease

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PURPOSE. Creutzfeldt-Jakob disease (CJD) primarily affects the brain. This study was conducted to assess the possible involvement of the eye in sporadic and variant CJD by testing for the presence of the disease-associated, protease-resistant isof orm of the prion protein (PrPSc) in ocular tissue.

METHODS. Human eyes from donors with CJD and non-prion neurodegenerative disease control eyes were studied. In situ hybridization and Western blot analysis were used to determine the normal pattern of cellular prion protein (PrPC) expression. Western blot analysis and immunohistochemistry were then used to determine the localization, abundance, and iso type of PrPSc in eyes in CJD.

RESULTS. PrPSc was expressed in the nuclear layers of the retina. In both the sporadic and variant forms of CJD, PrPSc accumulated throughout the synaptic layers of the retina. The levels of PrPSc found in the retina were comparable with those found in the brain. Lower levels of PrPSc could be found in the optic nerve, but no PrPSc was detectable in other ocular tissues. The glycoform ratio of PrP was not detected in the retina did not correspond to that found in the brain.

CONCLUSIONS. Presumptive centrifugal spread of PrPSc from the brain through the optic nerve occurs in two major types of CJD. PrPSc is a marker of CJD infectivity. Given that routine decontamination may not remove PrPSc from surgical instruments, a careful risk assessment should be made of possible iatrogenic spread of sporadic and variant CJD after surgery to the retina or optic nerve. (Invest Ophthalmol Vis Sci. 2003;44:342–346) DOI:10.1167/iovs.01-1273

Creutzfeldt-Jakob disease (CJD) is a rare fatal neurodegenerative disease characterized by neuronal loss, gliosis, and spongiform change in the brain. According to the prion hypothesis, the agent responsible for CJD is an altered form (PrPSc) of a host-encoded glycoprotein (PrPC) which is highly aggregated and partially resistant to proteolysis. CJD occurs in idiopathic, familial, and acquired forms. The most common of these is the idiopathic form, sporadic Creutzfeldt-Jakob disease (sCJD) which occurs worldwide at a rate of one per million persons per annum and primarily affects the elderly.1 The acquired forms include Kuru and iatrogenic Creutzfeldt-Jakob disease (iCJD). Most cases of iCJD result from medical exposure to either growth hormone extracted from human cadaveric pituitary glands or from human dura mater grafting during neurosurgical operations.2 Nevertheless, one definite and two probable cases of iCJD have been attributed to corneal grafting, including the first ever reported case of iCJD.3–5 Iatrogenic exposure can result in very long incubation periods, measured in years in growth hormone recipients, although the incubation periods after exposure through corneal grafting were short in two cases.2 These data imply that CJD infectivity resides in ocular tissue and that transplantation of infected ocular tissue offers a direct route for entry of the agent into the brain. Direct confirmation of the former comes from the transmission to primates of disease from pooled CJD eye tissue.6

The appearance of a new variant of Creutzfeldt-Jakob disease (vCJD) in the United Kingdom7 has refocused clinical interest in CJD. vCJD differs from sCJD in a number of important respects, including earlier age at onset, longer disease duration, and presence of peripheral disease and infectivity in elements of the lymphoreticular system.8–10 This has necessitated a reappraisal of the potential for iatrogenic spread of CJD, including those risks posed by the transplantation of ocular tissues.11–13 Unfortunately, there is a dearth of primary investigative studies of ocular disease in CJD, even though neuroophthalmic features are common14 and retinal degeneration has been described in an animal model of CJD.15

We have taken advantage of a rare opportunity to examine histologically and biochemically eyes of patients with variant and sporadic CJD for the accumulation of the disease-associated form of PrP to better understand the pathogenesis of CJD and provide data for risk assessment of iatrogenic transmission.

MATERIALS AND METHODS

Pairs of eyes from three autopsy-proven cases of CJD, one sCJD (S) and two vCJD, were examined (V1 and V2). Two pairs of eyes from neurologic control cases were also analyzed. One was an autopsy-proven case of Alzheimer disease (C1) and the other a case of corticobasal ganglionic degeneration (C2). Full permission was obtained for research, and the study protocol conformed with the provisions of the Declaration of Helsinki. In all five cases, frozen and fixed eye and frozen cerebral cortex (CC) was available for study. After enucleation, the left eye of each pair was dissected into the following components; cornea, lens, vitreous body, neural retina, choroid and retinal pigmented epithelium, sclera, and optic nerve. Due to the nature of the eye after death, complete separation was not possible. This was particularly true for the posterior segment tissues, and it is certain that cross contamination occurred between neural retina and the overlying vitreous body and the underlying pigmented epithelium. The right eye of each pair was fixed whole. Two additional control eyes, which had not been decontaminated with formic acid, were used for the in situ hybridization analysis. Both were enucleations, one due to a recurrence of melanoma of the iris (C3) and the other due to optic pain resulting from rubecitic glaucoma (C4).

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In situ hybridization to localize PrP mRNA was performed with digoxigenin-labeled, PCR-generated, single-stranded sense and antisense RNA probes, visualized with alkaline phosphatase and an nitroblue tetrazolium/5-bromo-4-chloro-3-indoly phosphate (NBT/BCIP) chromogen as previously described. The protease-resistant core fragment (PrPsc) of disease-associated PrPsc was detected by Western blot analysis with the monoclonal antibody 3F4, as previously described. Initially, all eye samples were prepared and analyzed as protease-treated 10% (wt/vol) tissue homogenates. In samples in which PrPsc was initially found to be low or undetectable, it was concentrated (Hil1003, Herts, UK). The normal cellular protease-sensitive prion protein PrPc (model GS700, with Quantity One software; Bio-Rad Laboratories, Herts, UK). The normal cellular protease-sensitive prion protein PrPsc was detected by Western blot analysis with the monoclonal antibody KG9, as outlined earlier. After visualization with DAB, sections were lightly counterstained with hematoxylin and eosin (HE) stained, and positive labeling was seen in all nuclear layers (B). A hematoxylin and eosin–stained section is shown (A) for orientation with the retinal pigmented epithelium at the top and the vitreal surface at the bottom. A region of (B) is shown at higher magnification in (D), and the retinal layers are indicated. pr, retinal pigmented epithelium; prs, photoreceptor segments; onl, outer nuclear layer; opl, outer plexiform layer; inl, inner nuclear layer; ipl, inner plexiform layer; gcl, ganglion cell layer; nfl/ilm, nerve fiber layer/inner limiting membrane. Scale bar, 50 μm.

Western blot analysis of non-CJD control eyes (C1 and C2) shows the presence of PrPsc in the neural retina (Fig. 2). The PrPsc detected in the neural retina was of a mobility similar to that seen in the corresponding brain sample (Fig. 2A). Signals were also seen in lens samples but these were of lower molecular weight. We noted that the concentration of soluble protein in the vertebrate lens far exceeded that in any other organ, including the brain, and that most of these lens proteins are the crystallins, which cluster in the 16- to 30-kDa mass range. Given that our Western blot analyses were loaded with an equal tissue weight equivalent, it seems likely that 3F4 binds to extremely abundant crystallins in the lens lanes. The signals in retina and brain were lost entirely by treatment of the samples with proteinase K, indicating that the PrP detected in the retina, similar to that of the brain, was the protease-sensitive normal cellular form of the protein PrPsc (see Fig. 5).

Immunohistochemical staining for PrP by a protocol optimized for PrPsc detection did not produce a positive reaction in the control non-CJD eye (Fig. 3A). In contrast, immunostaining of the sCJD and vCJD eyes all showed strong positive staining for PrPsc (Figs. 3B–E). The staining in each case was largely restricted to the inner plexiform layer (IPL) and outer plexiform layer (OPL), in a uniform distribution around the retina. The staining in the OPL was of a coarse granular pattern, whereas the INL staining tended to be of a finer “synaptic” type (Fig. 3C). No positive staining was seen in the photoreceptor cells (PRS) or other neuronal cell bodies. Doubling labeling for
GFAP and PrP Sc showed GFAP-positive astrocytic end feet in the vCJD retina (Fig. 3D). The cornea, iris, lens, ciliary body, choroid, sclera, and optic nerve sheath all showed a negative reaction. Positive staining was seen in sections of the optic nerve of donors with sCJD and vCJD (Fig. 3F) and in one of the vCJD donor eyes was in an obvious quadrantic distribution. The positive staining seemed to be associated with astrocytic cell bodies, as confirmed by immunocytochemistry for GFAP in the areas where axon loss and secondary myelin loss was accompanied by astrocytosis (data not shown). No evidence of spongiform change or formation of amyloid plaque was observed in the retina or optic nerve on routine stains or on immunohistochemistry for PrP. However, individual neuronal processes showed vacuolation. The retina in sCJD and vCJD showed variable loss of ganglion cells and photoreceptors, some of which may be age-related in the donor with sCJD. No other specific morphologic features were identified in the retina or in the optic nerve.

Western blot analysis for PrPSc confirmed the results of the immunohistochemical analysis. PrPSc was detectable in the retina from the case of sCJD and in both cases of vCJD. The level of PrPSc in the vCJD retina was similar to that seen in the corresponding sample of brain, with lower levels present in optic nerve (Fig. 4A). Longer exposure of the Western blot showed all three glycoforms (di-, mono-, and non-glycosylated PrPSc) present in the brain, neural retina, and optic nerve (Fig. 4B). Low molecular weight, presumptive crystallin, and degradation products were seen in the lens sample. Weak reactions to a protein of approximately the same mobility as diglycosylated PrPSc were also seen in otherwise negative lanes. We observed this band in all heavily exposed Western blot analyses of proteinase K-treated samples and when proteinase K (molecular weight, ~29 kDa) was analyzed in the absence of tissue extracts. Our working assumption is that this band represents weak cross-reactivity between the primary or secondary antibody and proteinase K.

Concentration of any PrPSc in the samples by a factor of 20 with centrifugation confirmed the positive signal from the

![Figure 2](image2.png)

**Figure 2.** Western blot analysis of PrP in cornea (Co), lens (Le), vitreous body (Vi), neural retina (NR), retinal pigmented epithelium and choroid (PE), sclera (Sc), and optic nerve (ON) in the non-CJD eye. (B) Case C1; (A) case C2. Cerebral cortex (CC) from case C2 was included as a positive control for PrP (A). Because of the abundance of PrP in the cerebral cortex, one fifth of this sample was analyzed, compared with the eye sample. Optic nerve was not available for C1.

![Figure 3](image3.png)

**Figure 3.** Immunohistochemical localization of PrPSc in retina (A–E) and optic nerve (F) from control, C1 (A); sCJD (B, C); and vCJD, V1 (D) and V2 (E, F). Sections were immunostained for PrP with monoclonal antibody KG9 and diaminobenzidine (brown) and counterstained with hematoxylin (blue). Double labeling for GFAP with red stain is shown for the retina of V1 (D). Scale bars, 50 μm.
neural retina and optic nerve, but a weaker signal also became evident in retinal pigmented epithelium (RPE; Fig. 4C). The centrifugal concentration step clearly indicates the dramatic difference in the level of PrPres between positive tissues (retina and optic nerve) and negative tissues (cornea, lens, vitreous body, and sclera). It also serves to confirm that the positive signals in retina and optic nerve are in fact PrP Sc, in that they are insoluble in nondenaturing buffers in addition to being protease resistant, reacting with the antibody 3F4, and having the expected molecular weight of di-, mono-, and non-glycosylated PrPres. The tissue distribution of PrPres in the sCJD eye resembled that found in the vCJD eyes (i.e., restricted to the retina), although frozen optic nerve was not available for study in this case. The level of PrPres in the retina in the case of sCJD was similarly high and exceeded that found in the corresponding brain sample (Fig. 5). Analysis of control (C1) brain and retina in parallel shows that the abundant PrP in these tissues was completely sensitive to proteolytic degradation (PrP sens), and therefore it corresponds to the normal cellular form PrP C (Fig 5). Glycoform ratios of PrPres in the sCJD and vCJD eyes were not identical with those in the corresponding brain samples. Instead, each exhibited a strong shift toward increased abundance of the monoglycosylated form in retina. This was particularly noticeable in the cases of vCJD in which the retinal glycoform ratio was no longer typical of vCJD, but more closely resembled that usually associated with sCJD (Fig. 6).

DISCUSSION

The presence of PrPSc remains the sole, although possibly a surrogate, marker of CJD infectivity currently available. Although this study was not designed to directly address questions of relative infectivity in CJD eye tissues, the finding of PrPSc in the eyes of three individuals who died of CJD, though not unexpected, is clearly a cause for concern. Transmission of CJD by intracerebral inoculation of nonhuman primates with CJD eye tissue has been shown previously, but the material used was pooled, and it was therefore not possible to say which ocular components harbored the infectivity. Our results using both immunohistochemistry and Western blot analysis clearly implicate the retina. Although CJD has been transmitted inadvertently by corneal transplantation, PrPSc was not detectable in the cornea in any of the eyes examined in this study. It is well known that bioassay, even across a species barrier has a greater sensitivity than any currently available assay for PrPSc. From this we infer that our inability to detect PrPSc in the cornea, sclera, and lens cannot be taken as evidence for the presence of PrPSc in these tissues.
absence of infectivity in these tissues. Although the level of infectivity found in the retina is very much greater than that found in the cornea in a rodent model of scrapie, corneal transplantation failed to transmit disease in monkeys. The issues surrounding the relative levels of CJD infectivity and how this relates to the relative levels of PrPSc in human ocular tissues can be resolved only by transmission studies using primary human diseased eye tissue.

The agent(s) that cause CJD and related transmissible spongiform encephalopathies are notoriously difficult to decontaminate. Therefore the presence of readily detectable levels of PrPRes in the retina at levels equivalent to those found in the brain suggest that retinal surgery may present the same risk and therefore necessitates the same precautions as are taken in neurosurgery. The finding of PrPRes in the sCJD eye, however, indicates that this risk is not new and predates the appearance of vCJD. To date, there is no evidence that any form of CJD has been transmitted by intraocular surgery, although surgical manipulation of the retina has been undertaken in a significant number of cases only in the past 25 years.

Our data are in agreement in many respects with a recent publication reporting use of Western blot analysis to detect PrPRes in the retina and optic nerve of single case of vCJD. This brings the total number of reported cases of vCJD in which retinal positivity has been demonstrated to three, and we would anticipate this to be a general phenomenon. Our estimations of the relative levels of PrPRes in retina and optic nerve are different from the report that the optic nerve contains 10 times more PrPRes than in retina. The two cases of vCJD reported in this study were investigated by Western blot analysis and immunohistochemistry, are internally consistent and show a greater presence of PrPRes in the retina than in the optic nerve, where staining was less intense and less consistent. A further discrepancy is the absence of PrPRes from any eye tissue in the case of sCJD analyzed and the abundant PrPRes detected in the case of sCJD in this report. Given that our methods appear to be of equivalent sensitivity, it may be that sCJD is heterogeneous in retinal involvement. It may be premature to speculate on the basis of two cases, but there is convincing evidence that the scrapie agent strain and mouse host genotype are known to determine whether ocular involvement occurs in experimental rodent models of scrapie. In this light, it is of interest to note that the sCJD case reported and the sCJD case we report herein differ in both PRNP codon 129 genotype and PrPRes isotype.

The finding that PrPRes can be detected in the CJD optic nerve and accumulates in the retina in both sCJD and vCJD is consistent with centrifugal spread from the brain, presumably through the optic nerve. Although other factors may be involved, the accumulation of PrPRes in the plexiform layers is consistent with the pattern of expression of PrPSc in the neural retina (this report) and the known synaptic location of PrP in neurons.

Lastly, glycosylation site occupancy has been proposed as a marker of the vCJD agent strain, and this appears to be a consistent feature in the vCJD brain. However, PrPRes glycosylation occupancy is consistently higher in vCJD lymphoepithelial tissues than in brain. In this study, we report the first examples of vCJD PrPRes glycoform ratios (found in the retina of both cases of vCJD) that resemble the PrPRes glycootype that characterizes sCJD rather than vCJD. The tendency toward monoglycosylation was also evident in the retina from the case of sCJD. This indicates that tissue differentiation, even within the central nervous system can dominate over agent strain in specifying the PrPSc glycootype.

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References