PrP<sup>res</sup> deposition in the retina is a common finding of sporadic, familial and iatrogenic Creutzfeldt-Jakob diseases (CJD)

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Text

Creutzfeldt-Jakob disease (CJD) is clinically characterized by progressive dementia and neuropathologically characterized by deposits of a protease-resistant isoform of the prion protein (PrP<sup>res</sup>) in the central nervous system. PrP<sup>res</sup> deposits in the neural retina were identified in the outer and inner plexiform layers (OPL and IPL) in a limited number of sporadic Creutzfeldt-Jakob diseases (sCJD) and two variant CJDs [1, 2]. However, the presence of PrP<sup>res</sup> in the neural retina remains unknown in other types of CJDs. Therefore, we analyzed 16 prion cases from our brain bank, including sporadic, familial, and iatrogenic CJDs by using retinal sections [3].

At the time of autopsy, full permission was obtained from each patient’s next-of-kin. The posterior portion of the eye ball was removed with a scalpel, leaving the cornea and lens for funereal purposes. The following cases were available: nine cases of sCJD (MM1), two cases of sCJD (MM1 + 2, MM1 > 2), one case of sCJD (MM2), three cases of familial CJD (fCJD) (two of V180I and one of M232R), and one case of iatrogenic CJD (cadaveric dura mater graft, dCJD). We classified sCJDs based on the Parchi’s methodology [4, 5]. We also used four autopsy-confirmed neurological cases as controls (Table 1). For immunohistochemical studies to detect PrP<sup>res</sup>, formalin-fixed and formic acid-treated sections of the retina were immunolabeled with monoclonal antibodies specific to prion proteins 3F4 (109–112) (1:200, Biolegend, USA) and 12F10 (142–160) (1200, Bertin Bioreagent, France). The retinal sections were processed by using a Ventana Discovery automated immunos- tainer. Evaluations of 3F4 and 12F10-immunoreactive deposits (PrP<sup>res</sup>-irs) of the outer and inner plexiform layers were performed by using both antibodies in a semi-quantitative manner: 0 = none, 1 = positive and scattered, 2 = positive (Table 1). PrP<sup>res</sup>-irs staining was weak or focal in the outer and inner nuclear layers (ONL and INL), as well as in the ganglion cell and nerve fiber layers (GCL andNFL); thus, we calculated the frequency of cases with PrP<sup>res</sup>-irs staining, separated on the basis of each anatom- ical region of the neural retina.

In all CJD cases, 3F4 and 12F10-irs were consistently and clearly observed in the OPL and IPL of the neural retina (Fig. 1a). In our series, 12F10-irs staining was stronger than 3F4-irs. PrP<sup>res</sup>-irs staining exhibited granular and fine synaptic patterns in the OPL and IPL, respectively. Although PrP<sup>res</sup>-irs staining was always present in both OPL and IPL, PrP<sup>res</sup>-irs staining was stronger in sCJD (MM2), fCJD, and dCJD cases than in sCJD (MM1) cases (Fig. 1a).

In some instances, fine-dot PrP<sup>res</sup>-irs staining was observed in the INL, ONL, GCL and NFL. More consistent findings were observed in sCJD (MM2), fCJD, and dCJD cases (Fig. 1b). No PrP<sup>res</sup>-irs staining was present in the photoreceptor cell layer. In addition, there was no amyloid-beta (4G8), p-tau (AT8), p-synuclein, or TDP-43-irs staining in the retina. No PrP<sup>res</sup>-irs staining was observed in retinas from control cases. There was no clear PrP<sup>res</sup>-irs staining in the optic nerves.

Clinical characteristics, such as age at onset, duration, gender, and initial presentation were not associated with the presence or absence of PrP<sup>res</sup>-irs in the retina. Our methodology analyzing the posterior portion of the eye
ball accurately reflected the pathologic condition of the neural retina in prion diseases. Indeed, a previous study showed that PrP res-irs were not prominent in the anterior portion of the neural retina [2].

Our study is the first to describe PrP res-irs within the retina in a series of cases of sCJD (MM1), sCJD (MM2, MM1 + 2), fCJD, and dCJD. Although we did not quantitatively evaluate the amount of PrP res in each case, PrP res-irs in the OPL and IPL may be more prominent in fCJD and dCJD cases. In addition, PrP res-irs were occasionally observed within layers of neural retina other than the OPL and IPL.

Protease-sensitive normal cellular PrP was identified in the neural retina of healthy controls [1]. Because no 3F4-irs and 12F10-irs were present in control cases, we suspect that 3F4-irs and 12F10-irs were present in the OPL and IPL may be more prominent in fCJD and dCJD cases. In addition, PrP res-irs were occasionally observed within layers of neural retina other than the OPL and IPL.

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Table 1  PrP immunoreactivity of the retina in 16 cases of Creutzfeldt-Jakob disease (CJD)

| Case number | AAD (y) | Duration (months) | Sex | Initial symptom | Diagnosis | Codon 129 | Western blot analysis of PrPres | OPL | IPL |
|-------------|---------|-------------------|-----|-----------------|-----------|-----------|-------------------------------|-----|-----|
|             |         |                   |     |                 |           |           | 3F4-irs | 12F10-irs | 3F4-irs | 12F10-irs |
| Case 1      | 72      | 18                | F   | visual acuity, color agnosia | sCJD      | MM        | Type 1 | 1       | 2       | 2       |
| Case 2      | 67      | 51                | F   | amnesia         | sCJD      | MM        | Type 1 | 1       | 2       | 1       |
| Case 3      | 75      | 17                | F   | palilalia       | sCJD      | MM        | Type 1 | 2       | 1       | 2       |
| Case 4      | 74      | 6                 | M   | metamorphopsia  | sCJD      | MM        | Type 1 | 1       | 1       | 0       |
| Case 5      | 73      | 2                 | M   | amnesia         | sCJD      | MM        | Type 1 | 2       | 2       | 2       |
| Case 6      | 82      | 5                 | F   | sensory disturbance | sCJD      | MM        | Type 1 | 1       | 2       | 1       |
| Case 7      | 62      | 42                | M   | loss of motivation | sCJD      | MM        | Type 1 | 1       | 2       | 1       |
| Case 8      | 68      | 8                 | M   | visuospatial disturbance | sCJD      | MM        | Type 1 | 2       | 2       | 2       |
| Case 9      | 85      | 7                 | F   | communication disturbance | sCJD      | MM        | Type 1 | 2       | 2       | 2       |
| Case 10     | 67      | 30                | M   | amnesia         | sCJD      | MM        | Type 2 | 1       | 2       | 1       |
| Case 11     | 62      | 10                | F   | dizziness, ataxia | sCJD      | MM        | Type 1 + 2 | 2       | 2       | 2       |
| Case 12     | 72      | 21                | F   | visual hallucination | sCJD      | MM        | Type 1 > 2 | 2       | 2       | 2       |
| Case 13     | 94      | 90                | F   | hearing disturbance | V180I     | MV        | Type 2 equivalence | 2       | 2       | 2       |
| Case 14     | 93      | 46                | F   | communication disturbance | V180I     | MV        | Type 2 equivalence | 2       | 2       | 2       |
| Case 15     | 70      | 19                | M   | dementia        | M232R     | MM        | Type 1 equivalence | 2       | 2       | 2       |
| Case 16     | 74      | 7                 | M   | dementia        | dCJD      | MM        | Type 1 equivalence | 2       | 2       | 2       |
| Control 1   | 95      |                   | F   |                 | AD+DLB    |           | 0       | 0       | 0       |
| Control 2   | 69      |                   | M   |                 | ALS       |           | 0       | 0       | 0       |
| Control 3   | 53      |                   | F   |                 | ALS       |           | 0       | 0       | 0       |
| Control 4   | 61      |                   | M   |                 | MSA       |           | 0       | 0       | 0       |

Typing of prion protein was performed on the basis of biochemical analysis of the frontal cortex. Diagnosis was performed on the basis of neuropathology and molecular biology analyses.

Semi-quantification: 0 = none, 1 = positive and scattered staining, 2 = positive and consistent staining in both outer and inner plexiform layers.

Abbreviations: AAD age at death, M male, F female, sCJD sporadic CJD, fCJD familial CJD, dCJD cadaveric dura mater graft CJD, AD Alzheimer's disease, DLB dementia with Lewy body disease, ALS amyotrophic lateral sclerosis, MSA multiple system atrophy, MM methionine/methionine, VV valine/valine, −irs -immunoreactive deposits
Fig. 1 A. Representative images of PrP immunohistochemistry of retinas in Creutzfeldt-Jakob disease. 3F4 and 12F10 immunoreactive deposits are present in the OPL and IPL. 12F10 immunoreactive deposits stain more strongly than those of 3F4. In particular, cases of MM2, MM1 + 2, V180I, M232R, and dCJD show 3F4 and 12F10 immunoreactive fine deposits in the INL, ONL, GCL, and NFL. B. Frequency of PrP immunoreactivity of each anatomical region in the retina. Fine-dot PrPres-irs staining was occasionally observed in the INL, ONL, and NFL. Staining was more consistent in cases of MM2, fCJD, and dCJD

Abbreviations
CJD: Creutzfeldt-Jakob disease; dCJD: Cadaveric dura matter graft Creutzfeldt-Jakob disease; fCJD: Familial Creutzfeldt-Jakob disease; GCL: Ganglion cell layer; INL: Inner nuclear layer; IPL: Inner plexiform layer; -irs: -Immunoreactive deposits; NFL: Nerve fiber layer; ONL: Outer nuclear layer; OPL: Outer plexiform layer; PrP: Prion protein; PrP res: Protease-resistant isoform of the prion protein; sCJD: Sporadic Creutzfeldt-Jakob disease

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Availability of data and materials
All data generated or analyzed during this study are included in this published article.

Authors’ contributions
MT: conceptualization, methodology, autopsy, investigation (neuropathological analysis), and writing of the manuscript; HK and BM: conceptualization of clinical study; TK: Genetic and biochemical analysis. All authors read and approved the final manuscript.

Ethics approval and consent to participate
All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study was approved by the institutional review board of Mihara Memorial Hospital (084-02). We obtained written informed consent from the deceased relatives for autopsy and further neuropathological analysis, and all subjects were registered with our brain bank for future research. The brain bank was approved by the Ethics Committee of Mihara Memorial Hospital for neuropathological analysis (072-01, 078-01, 085-01).

Consent for publication
We obtained written informed consent from the deceased relatives for publication.

Competing interests
The authors declare that they have no competing interests.

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