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# Prion-Seeding Activity is Widely Distributed in Tissues of human prion Disease Patients: Mini Review

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## Abstract

Human prion diseases are fatal neurodegenerative disorders caused by abnormally folded prion proteins (PrP-res). Accumulated PrP-res in the central nervous system can be detected using the Real-Time Quaking Induced Conversion (RT-QuIC) assay. The RT-QUIC assay allows for the detection of ≥ 1 fg of abnormal prion protein in diluted Creutzfeldt–Jakob Disease (CJD) brain homogenate.

Using this in vitro PrP-amyloid amplification assay, we quantified the seeding activity of affected human brains. End-point assay using serially diluted brain homogenates of sporadic CJD patients demonstrated that a 50% Seeding Dose (SD50) is reached in approximately 10/g brain tissue ($10^{(8.79–10.63)}$/g).

Historically, infectivity has been detected only in the central nervous system and lymphoreticular tissues of patients with sporadic CJD; however, recent reports suggest that the prion seeding activity of CJD prions accumulates in various non-neuronal organs including the liver, kidney, and skin. We re-analyzed autopsy samples collected from patients with sporadic and genetic human prion diseases and found that seeding activity exists in almost all digestive organs. Because the RT-QuIC assay is extremely sensitive compared with bioassay, we examined the distribution of the prion seeding activities in other organs, which we obtained from sporadic CJD patients at autopsy. Surprisingly, prion activity in the esophagus reached a level of prion seeding activity ($10^{7.98–8.38}$) close to that in the central nervous system in some CJD patients, indicating that the safety of endoscopic examinations should be reconsidered.

**Keywords**: RT-QUIC assay; Prion; Prion-seeding activity; Non-neural tissue

## Introduction

Prion diseases are characterized by the accumulation of protease-resistant Prion Protein (PrP-res) in the Central Nervous System (CNS), and this accumulation is thought to be the sole cause of these diseases. Human prion diseases present in various forms, including Creutzfeldt Jakob Disease (CJD), Gerstmann Sträussler Scheinker Syndrome (GSS), fatal familial insomnia, and kuru. CJD may be sporadic, genetic, or acquired [1-3]. PrP-res are thought to be solely composed of amyloid prion proteins. Sporadic CJD (sCJD) is a rapidly progressive neuropsychiatric syndrome with a fatal outcome, characterized by aggregations of the misfolded Prion Protein Scrapie (PrPSc) in the brain. Sporadic CJD is the most common form of human prion disease (about 90% of cases) with an incidence of around 1.5 to 2.0 per million person-years. The PrP genotype and the PrP-res type have a major influence on the disease phenotype in sporadic prion diseases. sCJD was classified the function of these two disease determinants. Based on the genotype at codon 129 on both PRNP alleles, the size of protease resistant PrP-res fragments and disease phenotype, we divide sporadic CJD into six subtypes: MM1/MV1, VV2, MV2, MM2 (MM2-cortical form and MM2-thalamic form) and VV1 [4,5].

Infectivity has not been detected outside of the brain in studies using animal models of sCJD, so infectious prion has been regarded as restricted to the CNS. However, in recent studies, PrP-res has been detected in the spleen from a patient with sCJD by western blot, and the level of PrPSc was lower by a factor of approximately 10-4 than that in brain tissue from the patient [6]. The recent development and clinical application of PrPSc amplification assays, such as protein misfolding cyclic amplification and Real-Time Quaking Induced Conversion (RT-QuIC) [7] have constituted major breakthroughs as aids for a more confident antemortem diagnosis of human prion diseases. Moreover, RT-QuIC studies demonstrate that RT-QUIC is more sensitive than bioassay. Although bioassay is the only tool currently available for determining the infectivity of human prion, in the future it will be possible to replace $LD_{50}$ (50% lethal dose) with $SD_{50}$ (50% seeding dose) [6]. We are capable of determining the distribution of infectivity in humans, we applied end-point RT-QUIC to evaluate human prion seeding activity in brains from patients with human prion disease [6].

## End-Point RT-QUIC Assay

Purification of Recombinant Human PrP (rHuPrP: residues 23-231, codon 129M) was performed as previously described [7]. After purification, rHuPrP was stored at -80°C. Brain homogenates (5 µl, 10% w/v) were serially diluted (10-fold) and suspended in 95 µl of RT-QUIC buffer, then loaded into each well of a 96-well plate. The assay was monitored for 53 h. Four to eight replicates of each diluted sample were measured. The $SD_{50}$ was calculated by the Spearman–

Kärber method [8]. We arbitrarily designated positive reactions as those with fluorescence intensities more than double that of the average of negative controls. Using this approach, we established the end-point RT-QUIC assay in human prion disease [9].

The end-point RT-QUIC assay enabled us to quantify human prion seeding activity in brains from patients with prion disease. There was a linear correlation between $SD_{50}$ and PrP-res levels in the brains of six patients with CJD-MM1 ($R^2$=0.8173). On the basis of estimation by dot-blot analysis, 1 $SD_{50}$ was equivalent to 0.1 fg of PrP-res, suggesting that our RT-QUIC detected PrP over a wider range than conventional western blotting or ELISA techniques. $SD_{50}$ from all samples (10 patients, including MM2-cortical, MM2-thalamic, MV2, and GSS-P102L) exhibited a low correlation with the level of PrP-res ($R^2$=0.7532), possibly because resistance to protease digestion of PrP is not always the same as seeding activity.

The $LD_{50}$ of brain tissues from patients with sCJD-MM1 was within the range 107-9 $LD_{50}$/g according to a previous report [10]. These findings suggest that $SD_{50}$ could be 10-100 times more sensitive than $LD_{50}$, because similar differences between $SD_{50}$ and $LD_{50}$ were seen [11,12]. However, we are currently analyzing more samples to verify these findings (Table 1).

| | Patient 1 | Patient 2 | Patient 3 | Patient 4 |
|---|---|---|---|---|
| Age at onset (years) | 69 | 70 | 59 | 62 |
| Brain (mean ± S.D.) | 10.08 ± 0.12 | 9.42 ± 0.12 | 9.17 ± 0.42 | 10.00 ± 0.35 |
| Spleen | £5.83 | 6.17 ± 0.31 | N.D. | 5.92 ± 0.12 |
| Kidney | 5.5 | £6.25 | £5.92 | £6.08 |
| Lung | £5.58 | £6.08 | N.D. | 6.83 ± 0.12 |
| Liver | £5.92 | N.D. | £6.33 | 6.92 ± 0.24 |
| Adrenal grand | £5.13 | 7.42 ± 0.11 | 6.42 ± 0.51 | 6.5 ± 0.20 |
| Esophagus | ≤ 6.70 | 8.38 ± 0.16 | 7.98 ± 0.39 | N.E. |
| Stomach | ≤ 6.50 | 7.1 ± 0.14 | ≤ 6.57 | ≤ 6.80 |
| Duodenum | N.E | 7.1 ± 0.70 | 6.24 ± 0.25 | 8.31 ± 0.20 |
| Jejunum | ≤ 6.67 | 7.2 ± 0.12 | 6.44 ± 0.10 | ≤ 6.67 |
| Terminal ileum | 7.07 ± 0.26 | 7.74 ± 0.25 | ≤ 6.90 | N.D. |
| Transverse colon | N.E. | ≤ 6.14 | ≤ 7.00 | 6.5 ± 0.11 |
| Sigmoid colon | ≤ 6.12 | N.D. | N.E. | 7.6 ± 0.17 |

**Table 1:** Prion seeding activity of all organs in four sporadic Creutzfeldt-Jakob disease patients.

Prion seeding activity in tissues of sCJD patients was evaluated by end-point RT-QuIC, which detected an unexpectedly wide distribution of prion in all patients we tested. $SD_{50}$ reached around $10^6$/g in non-neural organs. We verified that RT-QuIC has approximately $\times 10^4$ higher sensitivity than bioassay using knock-in mice expressing a human mouse chimeric PrP, although $SD_{50}$ has been reported to be 100 times higher than $LD_{50}$ in a study of 263,000 hamster prions. For example" here implies that the measurement is from one example patient, but it appears to be a rang that prion seeding activity in the kidney of sCJD patients was $10^{5.5–6.25}$, infectivity ($LD_{50}$) could be $10^{1.5}$/g in the organ. This would be an extremely low level of infectivity compared with that in the CNS; however, the fact that prion activity can be detected in peripheral organs should not be neglected because human prion disease can develop even after 30–40 years of incubation. Expression of physiological PrP in the human body has been well studied. PrPC is expressed in almost all tissues; mRNA expression levels are highest in the CNS, and levels in the spleen and liver are 1/20 of those in the cortex, the lungs are 1/10, and the kidneys and adrenal glands are 1/5 [13,14].

In addition, we confirmed that $SD_{50}$ levels in the digestive system of sCJD and gCJD patients are slightly increased compared with control levels. The $SD_{50}$ in the transverse colon and cecum of a patient with GSS was $10^{8.46}$ and $10^{8.33}$, respectively, and that in the gallbladder and stomach of a patient with genetic human prion disease was $10^{8.84}$ and $10^{8.21}$, respectively [15]. We assume that these levels of infectivity are lower than those in the CNS based on experimental infections in animals. The esophagus and appendix showed the highest titer refe. In patients with variant CJD, bovine spongiform encephalitis prion infection can be caused by oral intake of contaminated foods, and it is well documented that bovine spongiform encephalitis prions accumulate in the appendix and Peyer's patches of the small intestine. However, the etiology of sCJD differs from that of variant CJD, and there is no evidence for oral infection of sCJD. Recent research has shown that RT-QuIC testing of olfactory epithelium samples obtained from nasal brushings accurately diagnoses CJD. Therefore, in patients with sCJD, secreted prions or nasal tissue might be repeatedly swallowed with resultant reabsorption of the prions in the gut [15].

PrP-res have been detected in the spleen and muscles of some sCJD patients by western blot analysis when PrP-res in the samples were concentrated by PTA [16]. However, we cannot ignore the possibility that seeding activities detected in peripheral tissues are a result of infectious agents overflowing from the CNS, because studies have reported that kidneys and adrenal glands can be infected and produce abnormal PrP *in situ*.

## Conclusion

### Future challenges and perspectives

RT-QuIC has to be more widely ultilized, but the protocols of RT-QuIC assay need to be unified. On that premise, past studies using peripheral tissue need to be validated with regard to important differential diagnoses, and more candidate tissues need to be evaluated.

RT-QuIC testing of olfactory epithelium samples obtained from nasal brushings has been shown to accurately diagnose CJD. Therefore, in patients with sCJD, secreted prions or nasal tissue might be repeatedly swallowed with resultant reabsorption of the prions in the gut. Diagnosis of sCJD might thus be possible by RT-QuIC testing of the upper digestive tract obtained from biopsy (GIS) from the esophagus, stomach, or duodenum.

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## Conflicts of Interest

The authors declare no conflict of interest.

## Authorship Declaration

All authors are in agreement with the content of the manuscript.

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