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# Prion Diseases and their Prpsc-Based Molecular Diagnostics

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## Abstract

Prion diseases, also known as Transmissible Spongiform Encephalopathies (TSEs), are fatal neurodegenerative disorders with characteristic sponge-like microscopic appearance in the infected brain. They are caused by a protein-only particle consisting of an abnormal isoform (PrP<sup>Sc</sup>) of the normal ubiquitous cellular prion protein PrP<sup>C</sup>. Prion diseases affect both human and animals, and can cause interspecies Corbière. In humans, there are six different phenotypes of prion diseases, including kuru disease, Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler-Scheinker (GSS) syndrome, Fatal Familial Insomnia (FFI), variant Creutzfeldt-Jakob disease (vCJD) and variably protease sensitive prionopathy (VPSPr). There are five well-studied prion diseases in herbivorous animals and carnivorous animals, including scrapie, Bovine Spongiform Encephalopathy (BSE), Chronic Wasting Disease (CWD), Transmissible Mink Encephalopathy (TME), and Feline Spongiform Encephalopathy (FSE). As the pathogen of the diseases, PrP<sup>Sc</sup> accumulates in tissues and body fluids of the affected individuals, and serves as the most reliable marker for diagnosis of prion diseases. The bioassay, immunoblotting, immunohistochemistry (IHC), and enzyme-linked immunosorbent assay (ELISA) tests have been used in prion disease diagnosis and surveillance, but they are not sufficient for the preclinical diagnosis. The newly improved *in vitro* PrP<sup>Sc</sup> amplification methods, such as protein misfolding cyclic amplification (PMCA), amyloid seeding assay (ASA), and real-time quaking-induced conversion (RT-QUIC), showed greatly increased sensitivity. Recently, RT-QUIC and PMCA were applied to noninvasive tests targeting PrP<sup>Sc</sup> in urine or nasal swab, making early diagnosis and surveillance of prion diseases become more practical.

**Keywords:** Prion; Diagnostics; TSEs; PrP<sup>Sc</sup>; PrP<sup>C</sup>; Transmissible Spongiform Encephalopathies

## Introduction

Prion diseases are rare but deadly conditions of central nervous system, manifesting neurodegenerative symptoms such as convulsions, dementia, ataxia and behavioral changes. Because of their transmissibility feature and the characteristic

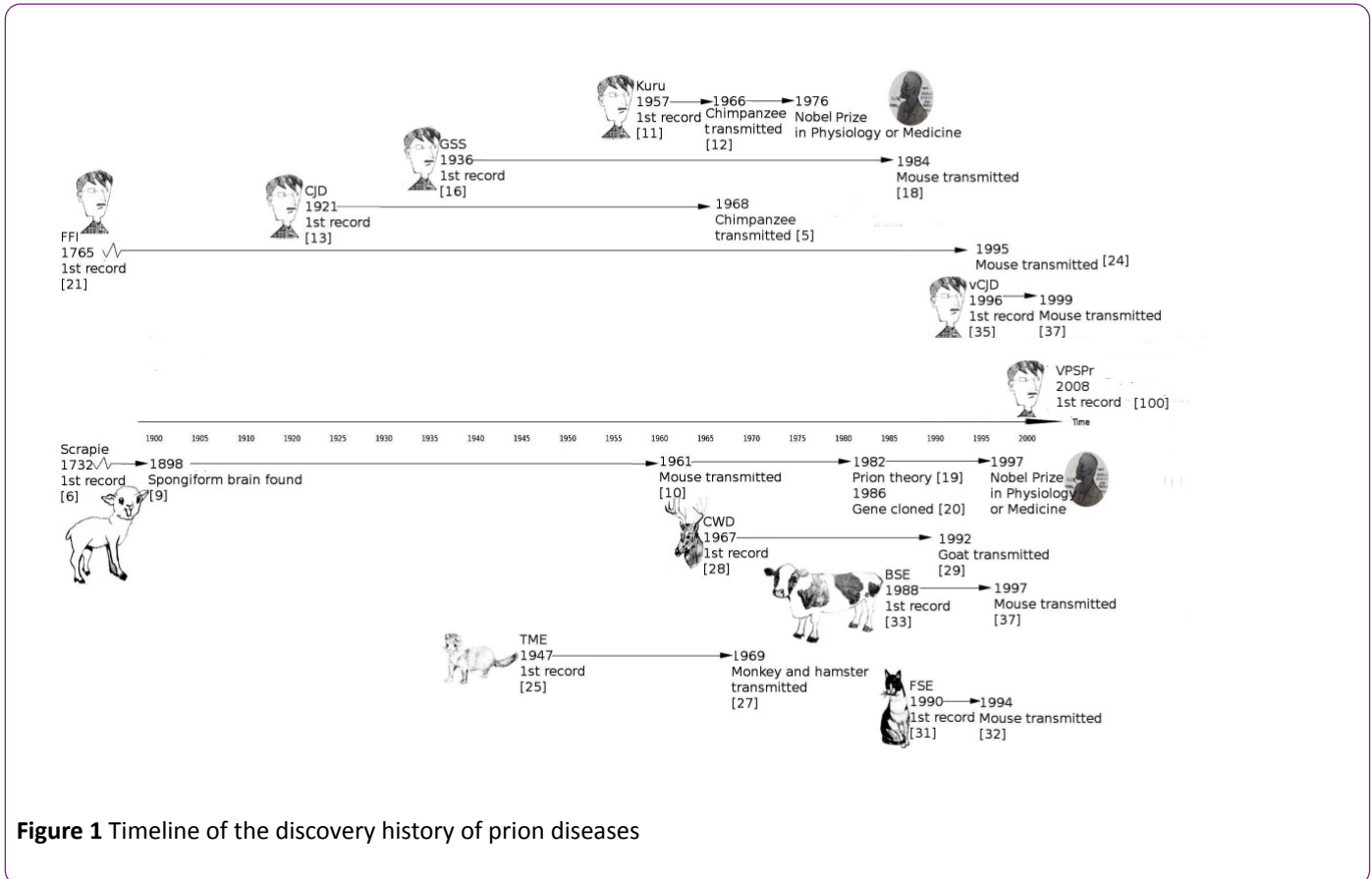
sponge-like microscopic appearance in the affected brain tissue, they are also called Transmissible Spongiform Encephalopathies (TSEs). The incubation period for prion disease is long, but once the clinical signs appear, the disease progresses fast and rapidly results in death. Although symptom relief treatments exist, no survival benefit-conferring drug is available for the treatment of human prion diseases [1,2]. The variant Creutzfeldt-Jakob disease (vCJD) crisis among humans in 1996 in The United Kingdom was caused by the consumption of Bovine Spongiform Encephalopathy (BSE) infected cattle, testifying that the potentially prevalent prion diseases could spread from food animals to human beings, and therefore the crisis raised deep concerns about human health and economy [3]. It is also dangerous to omit those infected people without significant clinical signs, because the time from being infected to showing signs of the disease can be up to 50 years [4], and during this period of incubation time the infected people can be pathogen carriers and may spread the prion diseases through medical/dental instrument contamination, transfusion or transplantation [5]. Therefore, it is important to develop sensitive techniques for the diagnosis of prion diseases not only for the patients' symptom relief per se, but also for the protection of public health. Traditionally, prion diseases were usually diagnosed by clinical signs and confirmed by post-mortem examination, but there was no sufficient method for early stage diagnosis of the diseases. Recently, the newly improved *in vitro* amplification diagnosis methods may serve as a promising solution to this shortage, and may provide the potential noninvasive screening tools for disease surveillance. In this review, we give a brief overview of the histories of prion diseases' discovery, pathogenesis and the molecular diagnosis, and in particular we summarized the fast and accurate noninvasive screening tests developed recently, in order to shed some light on the early diagnosis and surveillance of prion diseases.

## Discovery History of Prion Diseases

In human beings, there are 6 phenotypes of prion diseases: kuru disease, Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler-Scheinker syndrome (GSS), Fatal Familial Insomnia (FFI), variant Creutzfeldt-Jakob disease (vCJD) and variably protease sensitive prionopathy (VPSPr); In herbivorous ungulate animals, there is scrapie in sheep and goats, Bovine Spongiform Encephalopathy (BSE, also known as "mad cow disease") in cattle, Chronic Wasting Disease (CWD) in deer and elk; In carnivorous animals, there is Transmissible Mink

Encephalopathy (TME) in mink, and Feline Spongiform Encephalopathy (FSE) in cat and cheetah. It is very likely that more species carry this type of disease. All these prion diseases share the common features including the fatal neurodegenerative process, sponge-like brain, and disease

transmission ability. **Figure 1** shows the timeline of the discovery of the known prion diseases, and we summarized here the brief history of each type of them, from the first discovery to the well-established stage typified by the successful inoculation to model animals.



**Figure 1** Timeline of the discovery history of prion diseases

### Scrapie in sheep and goat

Scrapie is the earliest prion disease described. The first record dates back to 1732, when sheep scrapie was described in Great Britain [6]. Scrapie can infect both sheep and goats, spread among them through contact, and cause infected animals scraping off wool or hair, losing weight, and displaying odd behaviors [7,8]. In 1898, the brain lesion in the scrapie-infected sheep was noticed, which is the first mention of the characteristics of the spongiform encephalopathy [9]. In 1961, Chandler discovered that scrapie could be given to mice, which accelerated the progress of scrapie research [10], and the method of transmission to a model animal was widely used on other prion disease studies since then. After the researchers found diseases with clinical signs due to the spongiform encephalopathy, they would study the diseases in model animals, which thereby quickened the pathogenesis research.

### Kuru, CJD, GSS, FFI and VPSPr in human beings

In 1957, Gajdusek reported kuru disease in the Fore tribe located in Papua, New Guinea [11]. The disease was named after “kuru” because this local word translates to “trembling from fear or cold”, well reflecting the unique tremor characteristics of the disease during the early stages. Gajdusek later determined the cause of kuru as the ritualistic

cannibalism in Fore tribe after he achieved the transmission of kuru to chimpanzees [12].

In 1920s, Creutzfeldt and Jakob found another fatal disease of spongiform encephalopathy, Creutzfeldt-Jakob disease (CJD), which leads to late-onset neurodegenerative dementia with a 6-months overall survival rate of 30% [13]. In 1968, Gibbs did the CJD transmission to the chimpanzee [5]. By 1972, Gajdusek and other researchers realized that scrapie, kuru and Creutzfeldt-Jakob diseases were all transmissible subacute spongiform encephalopathies, and they proposed that these diseases were caused by a slow virus [14]. In 1978, CJD was transmitted to the mouse model by Manuelidis [15].

GSS was firstly reported in 1936 [16], and was eventually found to share similar pathogenic process with CJD [17] and can be transmitted to rodent models [18]. In 1982, Prusiner found that prion was the actual cause of scrapie [19], and he later categorized the three degenerative human diseases similar to scrapie, kuru, CJD and GSS, as prion diseases [20]. Prusiner was awarded with the Nobel Prize in Physiology or Medicine for his discovery. FFI can be dated to 1765, when the first recorded death of a patient who had suffered from insomnia for more than a year [21]; however, it was identified in 1982 [22] and was included into the prion

disease category [23]. FFI was conformed transmissible to laboratory animals in 1995 [24].

VPSPr was first reported in 2008 [25]. It is a sporadic human prion disease that is heterogeneous in terms of protease sensitivity [26]. VASPr is not a well-studied prion disease yet and there are no published studies of its transmissibility to animal models.

### TME in mink, CWD in deer and elk, and FSE in cats

The first observations of transmissible mink encephalopathy were reported in 1947 [27,28]. Transmissible mink encephalopathy is a progressive, fatal disease of ranch mink. The disease has been experimentally transmitted to monkeys and hamsters [29]. In 1967, Chronic Wasting Disease (CWD) in North American cervids was first observed, and was found transmissible by intracerebral inoculation to cattle, sheep, and goats [30]. Surveillance efforts for CWD are more difficult, because the disease was not only found in captive cervids [31], but also in free-ranging cervids [32].

In 1990 FSE was firstly found in a cat, with a spongiform encephalopathy characterized principally by grey matter neuropil spongiosis [33], and this disease was transmitted to mice later [34]. More cases of FSE were found in captive wild cats including cheetahs, pumas, ocelots, tigers, lions and Asian golden cats [35].

### BSE in cow and vCJD in human

BSE and vCJD are introduced together since they are closely related. vCJD was found in human beings as a result of transmission of bovine prions from BSE-infected cattle to human. It was in 1980s that a British cow was diagnosed with BSE, also known as "mad cow disease". At the beginning, the spreading of BSE in British cattle raised the fear of transmission to humans, since people could accidentally consume BSE infected cows that carry the transmissible agent prion [36]. Unfortunately, the fears turned to become reality in 1990s when the variant Creutzfeldt–Jakob Disease (vCJD) found in humans was confirmed as a result of transmission of bovine prions from BSE-infected cattle [37–39]. vCJD was identified as the consequence of eating diseased cattle, and it tends to occur in younger people. vCJD causes prominent psychiatric or sensory symptoms and delayed the onset of neurological abnormalities, which are different with other CJDs [40].

## Pathogenesis of Prion Diseases

### Early studies on the unconventional “viruses” and the success on stopping kuru

The observation on scrapie showed that birthing fluids and placenta from infected females contained scrapie agent and caused disease transmission to healthy newborn lambs. Those newborn lambs that shared the same lambing pen were

extremely susceptible to infection. Back then, the known causes of transmissible diseases were limited to certain bacterial, viral, fungal, or parasitic agents. Wilson started to postulate that the agent was virus [41]. Many early scrapie researchers agreed because they found the agents of scrapie were very small, which was consistent with the postulation of virus [42]. Gajdusek in his work on kuru also proposed that the kuru agent should be a kind of transmissible virus [43]. However, Alper noticed that ultra-violet irradiation of suspensions of infected mouse brain extracts did not affect the infection of the extracts to other mice, therefore concluded that the agent probably did not depend on nucleic acids for its ability to replicate since UV could have killed the virus [44]. When Gajdusek transmitted the kuru, CJD and scrapie diseases to squirrel monkeys, he assumed that the agents of the 3 diseases were all “slow virus”, which means the virus might slowly develop from very residual amount that survived the environmental damaging factors [45]. Despite the fact that the suspected slow virus had never been identified, after the stopping of cannibalism in 1960, kuru cases decreased and finally had been completely eradicated. Because of his contribution on kuru research, Gajdusek won the 1976’s Nobel Prize in Physiology or Medicine.

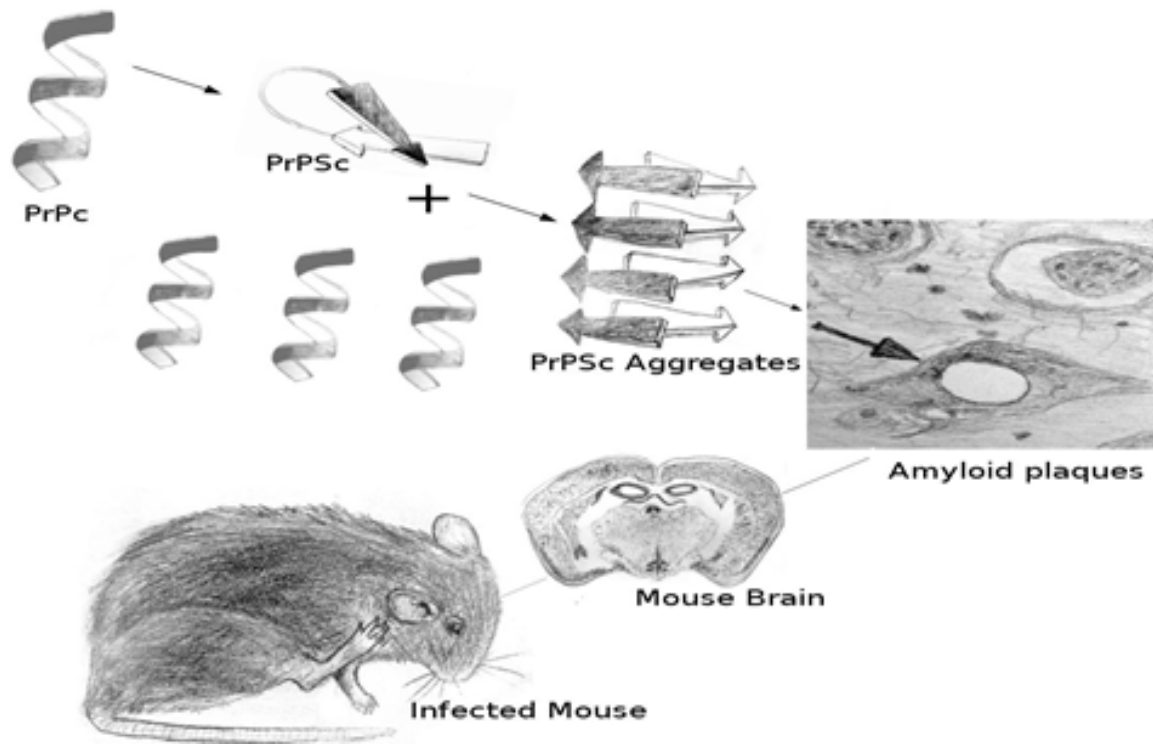
### Finding out prion as the agent and the establishment of the prion theory

In 1982, after a series of studies of the characteristics of the agents of these diseases, Prusiner found evidences to indicate the diseases’ agent is associated with a protein [46,47]. He termed a proteinaceous infectious agent, “Prion”, to explain the disease pathogenesis [48]. There are many debates against prion theory in the scientific community from the beginning [49], to the time after Prusiner was awarded the Nobel Prize in Physiology or Medicine for his discovery of prion in 1997, and even to very recent days [50–52]. However, prion theory was eventually well established and based on this theory, a new class of infectious diseases, “prion diseases”, had been categorized.

Based on this theory, the diseases are transmitted by a proteinaceous infectious particle, prion. On the molecular level, as shown in **Figure 2**, prion is the infectious abnormal conformation isoform (PrP<sup>Sc</sup>, PrP means prion protein, and Sc was named after scrapie) of a normal cellular prion protein (PrP<sup>C</sup>), while PrP<sup>C</sup> is primarily located on the cell surface of neurons within the central nervous system but its specific function is not completely understood. PrP<sup>Sc</sup> forms amyloids and is infectious because when touch PrP<sup>C</sup>, it converts PrP<sup>C</sup> into PrP<sup>Sc</sup> [53]. The accumulation of PrP<sup>Sc</sup> seriously damage cells, cause gliosis and neuronal loss. Furthermore, PrP<sup>Sc</sup> aggregates to make plaques known as amyloids and form vacuoles in the neurons, which cause the typical spongiform structure in the infected brain [54]. Since prion unit is a protein molecular, even electron microscope can only show the amyloids but not individual prions, which explained why the agents were deduced to be extremely small before. Prion is protein-only, which explained why the agents were resistant to procedures that usually break down nucleic acid and destroy

biological forms of other pathogens. Because prion is an abnormal form of a normal genetically encoded protein, it

causes the genetic feature of the familial prion diseases, and doesn't cause immune response in the host.



**Figure 2** Prion theory - pathogenesis of prion diseases.

It has been reported that transmissible prion like protein agent from a filamentous fungus can be generated *in vitro* [55], demonstrating the validity of the protein-only hypothesis. Although the prion based pathogenic mechanisms were first described in mammals, now they may have wider relevance since similar proteinaceous infectious particles were also found in fungi and yeast [56].

A PrPc shadow protein called Shadoo was found to have protective properties [57] to the disease, and the down-regulation of PrPc was an element of disease pathogenesis [58]. Recently, there are some inhibitors to the formation of PrPSc were found, raising hope of beneficial treatments of prion diseases [59,60]. Recent findings also suggested that the misfolding of prion enhance the formation of p-tau and aggregation of  $\alpha$ -synuclein and ubiquitin, which might lead to therapeutic modalities [61]. Prion disease vaccine possibility based on the significant progress on antibodies identifying PrPSc-specific epitope was also discussed [62,63]. A recent study found that heparanase plays a protective role in prion disease infection and progression by interfering the heparan sulfate involved in the pathogenesis of prion disease [64]. This *in vivo* study showed that the scrapie onset and progression were dramatically delayed in scrapie infected transgenic mice over-expressing heparanase, and thus revealed that heparanase is another potential treatment target. However, the basic mechanism on how PrPSc captures and recruits PrPc and converts it into PrPSc remains unclear till now. The discovery of the mechanism in this procedure not only may

lead to the development of new diagnostic and therapeutic approaches, but also may construct another milestone of basic biology science and culture the third Nobel Prize winner on prion diseases.

## Prpsc Detection Tools for Prion Disease Diagnosis

Prion diseases are traditionally diagnosed with clinical signs, electroencephalography and magnetic resonance imaging, and confirmed by post-mortem pathological examination. Molecular diagnosis generally can reach higher level of sensitivity and specificity, which are important for both diagnosis confirmation and preclinical screening. Since PrPSc is the agent of prion diseases, it becomes the only reliable molecular marker of prion diseases. There were molecular markers for prion disease, such as 14-3-3 protein, S100 protein and neuron-specific enolase (NSE), but those markers were not as good as PrPSc with respect to specificity. PrPSc-based diagnosis is not only the final pathological conformation test of the diseases, but also the first candidate for the preclinical screening [65,66]. In **Table 1**, we listed the classical PrPSc detecting methods, which are grouped into 3 categories, including *in vivo* amplification category, non-amplification category and *in vitro* amplification category. The *in vivo* amplification method is to use bioassay in laboratory animals. It is sensitive and reliable but time consuming (months or years), and sometimes hard to overcome species barriers. The



non-amplification methods have good specificity and they are traditionally used in clinical diagnosis, but in most cases post-mortemly. Only when some of them use enhanced techniques like capture enrichment and florescence, they can have high sensitivity for ante-mortem blood test. The *in vitro* amplification methods are sensitive enough to detect misfolded prion proteins in the asymptomatic stages, even in the noninvasive body fluid like urine.

### In vivo amplification detection of PrPSc

Bioassays that involve the use of laboratory animals are often used for the detection and titration of PrPSc, because PrPSc can convert PrPc to PrPSc *in vivo* and thus cause observable clinical signs and death. The titer can be measured by the incubation period in the experimental animals inoculated with serial dilutions of the test material [78], or determined by the median infective or lethal dose (ID50, LD50), which is the dose of prion that causes infection or fatal situation in 50% of the inoculated animals respectively [79]. Although they are extremely sensitive that can detect PrPSc in

blood [80,81], urine and saliva [67], they usually require long time of incubation and sometimes have species barrier problem. Transgenic mouse for the test is helpful to break through the species barrier and decrease the incubation time, but the cost is still high on time and money.

### Non-amplification detection of PrPSc

**Traditional immunological detections:** According to the fact that parts of PrPSc cannot be digested by proteinase, immunological detections of the proteinase K-resistant part of PrP were developed, including traditional Western blot [67], Immunohistochemistry (IHC) [69] and enzyme-linked immunosorbent assay (ELISA) [68]. IHC can show microscopic location of PrPSc but sometimes has antigen inactivation problems in the fixed tissues, so that antigen retrieval is needed in IHC [82]. Western blots can detect 1,000-fold dilution of scrapie-infected brain homogenate, while ELISA is more rapid than Western blot and is more convenient for antigen capturing enrichment

**Table 1** PrPSc-based diagnosis methods

Category	Method	Mechanism	Published	If detects Proteinase K-sensitive PrPSc?	Target Specimens	Notes	Commercial Kits
<i>In vivo</i> Amplification	Animal BioAssay	Measures ID50 or LD50 in inoculated animals	1961, Chandler [10]	Yes	Tissue, blood, urine, saliva	Amplified signs, but costly and sometimes has species barriers.	None
Non Amplification	Western Blot	PrP antibody stain	1983, McKinley [67]	No	Tissue	Can detect strain types.	Prionics-Check Western
	ELISA	PrP antibody stain	1986, Barry [68]	No	Tissue, blood	Convenient for antigen capture enrichment.	Prionics Check, Enfer TSEBio-Rad TeSeE, Roboscreen Beta Prion BSE, Institut Pourquier Speed'it BSE, Roche PrionScreen, IDEXX HerdChek BSE
	IHC	PrP antibody stain	1988, McBride [69]	No	Tissue	Can show locations in the tissue, but may miss antigen inactivated PrPSc.	Ventana IHC Systems
	CDI	Conformation dependent immunoassay using epitope protection	1998, Safar [70]	Yes	Tissue	Can detect strain types.	InPro CDI-5, Ceditec BSE
	DELFI A	Dissociation-Enhanced Lanthanide Fluorescent Immunoassay, measures percentage of insoluble PrPSc	2000, Barnard [71]	Yes	Tissue, Blood	Integrated time-resolved fluorescence signal.	PE DELFIA TRF

	MPD	misfolded protein diagnostic, attached excimer fires when the peptide converts to $\beta$ -sheet conformation	2005, Grosset [72]	Yes	Tissue, Blood	Simple steps without using any antibody.	None
	SOFIA	Surround optical fiber immunoassay	2009, Chang [73]	Yes	Tissue, Blood, Urine	Cooperating multiple antibodies.	None
<i>In vitro</i> Amplification	Cell BioAssay	Detects PrPSc in inoculated cells	2000, Bosque [74]	No	Tissue, Blood	Amplified signal, sometimes has species barriers.	None
	PMCA	Protein misfolding cyclic amplification	2001, Saborio [75]	Yes	Tissue, Blood, CSF, Urine	Amplified signal.	None
	ASA	Amyloid seeding assay, uses amyloid dye ThT	2007, Colby [76]	Yes	Tissue, Blood, CSF	Amplified signal, low background, needs precipitation step in seed preparation.	None
	RT-QUIC	Real-time quaking-induced conversion, uses amyloid dye ThT	2011, Atarashi [77]	Yes	Tissue, Blood, Nasal Swab	Amplified signal, has simple shaking step and low background.	None

ELISA can increase sensitivity significantly after capture enrichment so that it detect PrPSc in blood samples [83,84]. There is only one available commercial Western Blot kit, Prionics' Check Western, while there are several commercial ELISA kits for screening large numbers of animals, such as Prionics' Check serial, Enfer's TSEBio-Rad's TeSeE, Roboscreen Beta's Prion BSE, Institut Pourquier's Speed'it BSE, and Roche's PrionScreen.

Not all of the PrPSc were proteinase resistant [84,85], so the post-proteinase detection method is not sufficient for the proteinase sensitive PrPSc. Therefore, ELISA methods rely on antibodies or chemical media that specifically bind to PrPSc were used to detect proteinase K sensitive PrPSc [86,87]. The first commercial test based on PrPSc specific recognition is IDEXX HerdChek BSE Antigen EIA, which uses a polymeric ligand developed to capture PrPSc specifically [88].

**Conformation-dependent immunoassay (CDI):** Studies found that the immunoreactivity of PrPSc is greatly enhanced by guanidinium thiocyanate denaturation [89], because the conformation of PrPSc changed and exposed the hidden epitopes during the guanidinium thiocyanate denaturation [90]. CDI uses the ratio of antibody binding to denatured/native PrP as a measure of the concentration of PrPSc, and tells the different strains according to the different positions on the plotted denatured/native PrP ratio graph [70]. The enhanced CDI can detect PrPSc in bodily fluids of CJD/vCJD patients [91].

**Dissociation-Enhanced Lanthanide Fluorescent Immunoassay (DELFI):** This method uses a two-site

immunoassay. It determines the PrP concentrations of two extracts processed with two concentrations of guanidine hydrochloride, and then measure the percentage of insoluble PrPSc as the diagnostic parameter. The assay is precise because it uses the very robust and sensitive time-resolved fluorescence kit that is commercially available from PE life science [71].

**Misfolded protein diagnostic (MPD):** MPD assay is a simple detection method based on the conversion of the synthetic prion peptide in the presence of PrPSc. The signal is generated from excimer formation of the fluorophore attached to the peptide. When the PrPSc in the sample convert the artificial peptide to  $\beta$ -sheet, the two attached fluorophore form an excimeric dimer to emit fluorescence [72].

**Surround optical fiber immunoassay (SOFIA):** In prion antibodies, there is a positive immunocooperativity phenomenon, which means that after one PrPSc specific monoclonal antibody binds to PrPSc, other PrPSc epitopes start to open for the other specific monoclonal antibody. Based on this phenomenon, SOFIA detection was developed. It consists an immunocapture step and a highly sensitive fiber optical-based laser-induced fluorescence detection step [73]. The high sensitivity of this assay is a good feature for preclinical PrPSc detection in biological fluids [92].

***In vitro* amplification detection of PrPSc:** Since PrPSc is a misfolding form of a protein and it has no specific maker on gene level, it cannot be detected through the traditional sensitive PCR-based methods. However, there are *in vitro*

systems for the amplification of PrPSc, conceptually analogous to PCR cycling, to increase the detection sensitivity. They rely on imparting very small amounts of misfolded protein seeds and amplifying them into larger amyloidogenic or proteinase-resistant protein aggregates.

**Cell bioassay:** Animal bioassay is a sensitive and reliable detection method based on *in vivo* PrPSc amplification, but it is expensive and time-consuming. High PrPSc infectivity cell lines provided good *ex vivo* models for PrPSc detection [74,93]. The cell-based bioassays usually use traditional Western blot and ELISA to detect the amplified PrPSc in the cell lines, and they had shorter incubation time while showed similar sensitivity with animal bioassay [94]. Like in the animal bioassays, there are species barriers in cell bioassays, but the overexpression of PrPc in the cell lines may overcome the species barriers [95,96]. Cell bioassay is an efficient method when the suitable immortalized cell line with certain DNA content is available.

**Protein misfolding cyclic amplification (PMCA):** PMCA uses *in vitro* cyclic amplification system that allows a rapid conversion of large excess recombinant PrPc into a proteinase-resistant PrPSc-like form in the presence of PrPSc template from the samples, and then detects the proteinase-resistant PrPSc by Western blot. In every cycle, converted PrPSc aggregate when the template PrPSc incubate with PrPc, and then the aggregates are disrupted by sonication to produce multiple PrPSc templates for the next cycle of amplification [75]. This method is sensitive enough for PrPSc detection in blood in the asymptomatic stages of prion diseases [97-100].

**Amyloid seeding assay (ASA):** Because PrPSc can convert PrPc into PrPSc and then cause amyloid formation, ASA uses PrPSc as seed to convert PrPc into amyloids, and then detects the amyloids by a fluorescence shift in the amyloid specific dye Thioflavin T [76]. This ASA reaction is an *in vitro* amplification system for PrPSc detection, and the low background fluorescence in the absence of amyloid helps to increase the sensitivity, which seems high enough to do the pre-clinical test. However the impurities in the sample tended to inhibit the amyloid formation, so ASA used Precipitation with phosphotungstic acid (PTA) [101] to partially purify the seed in the sample preparation.

**Real-time quaking-induced conversion (RT-QUIC):** A quaking-induced conversion (QUIC) was developed to be *in vitro* amplification system similar to PMCA, but with a constant shaking incubation to replace the sonication cycles, which has the problem of varied delivery of vibrational energy to the samples [102]. There is an enhanced QUIC method in which immunoprecipitation is applied before the standard QUIC [103]. RT-QUIC is a method that combines QUIC with thioflavin T (ThT) fluorescence dye that is used in ASA, to do a real-time amyloid formation test [77]. RT-QUIC is sensitive enough for body fluid detection, and it can be used in noninvasive screening [104].

## Discussion

Since prion diseases are intra-species and inter-species transmissible, they are potentially prevalent in human through people's food consumption and iatrogenicity. Except for the majority of sporadic and genetic human prion disease cases, a few historic cases showed that their diseases were environmentally acquired through cannibalism, infected food animal consumption, and iatrogenicity. The cannibalism caused spreading was successfully terminated when the cannibalism was stopped. However, the more recent variant Creutzfeldt-Jakob disease (vCJD) crisis caused by consumption of bovine spongiform encephalopathy (BSE) infected cows, still reminds us that the infected food animals is a big threat to people. Prion infected patients without significant clinical signs remain as another threat, because the diseases may incubate in people's body for a long latent period and spread through medical or dental instrument contamination, transfusion or transplantation. Some sensitive and accurate prion disease diagnosis tools are very important not only for the early intervention for the patients' symptom relief, but also for the protection of public health against disease prevalence. Although currently the prion disease diagnosis tools are not sufficient for the clinical use or pre-clinical surveillance use, the recent advances of PrPSc detection technique provide the potential screening tools for the early diagnosis and disease surveillance.

There are mutual benefits between diagnosis studies and mechanism studies. Even before the establishment of the prion theory, the early diagnosis studies had revealed the characteristics of the disease and had suggested prevention approaches. For example, in early studies, the animal bioassay developed from scrapie diagnosis studies directly led to the determination of other prion diseases, and also consequently led to the termination of kuru disease. In later diagnosis studies after the setup of prion theory, some new findings, like the proteinase K insensitive PrPSc and positive immunocooperativity in the binding of the antibodies to epitopes, were very helpful for understanding the conformation differences in the prion strains. On the contrary, the prion theory discovered the pathogen behavior, and helped on identifying PrPSc as the most reliable marker for diagnosis of prion diseases. Based on PrPSc/PrPc transformation theory, more sensitive amplification detection methods were developed to diagnosis prion disease. Therefore, the recent breakthrough of the prion disease diagnostic studies, especially the newly modified methods that can detect body fluids, showed promising power to meet the clinical diagnosis needs. At the same time, the advanced diagnostic tools may also speed up the prion diseases pathogenesis studies, and even inspire therapeutic approaches.

In the diagnostic practice of prion diseases, including post-mortem confirmation, clinical diagnosis and pre-clinical screening, the high specificity and sensitivity are always crucial. Usually, antibody recognition meets the need of specificity, especially when the different monoclonal antibodies are used for recognizing different epitopes, they

can even tell the conformation differences in the prion strains. Only one method in our summarized method list, misfolded protein diagnostic method (MPD), does not use any antibody, but directly uses the recognition and binding ability between PrPSc and PrPc. As for increasing the sensitivity, capture enrichment in sample preparation, *in vitro* PrPSc amplification, and fluorescence application in the signal detection are the three main trends in prion diagnosis research. We introduced all the historical PrPSc detection methods even though some of them are not actively used, because an efficient way to improve the specificity and sensitivity in disease diagnosis is to combine the previous methods. The prion diagnosis history has showed that combinations often produce better output. For example RT-QUIC combined standard QUIC with ASA, and in enhanced QUIC, the standard QUIC was combined with the capture enrichment. There are many potential combinations in the current platforms such as combinations of CDI and DEFIA, PMCA and MPA, and so forth, which may turn out to be better diagnosis tools in future.

Since PrPSc is not evenly distributed and PrPSc involves different tissues in different prion diseases, it is critical to use the correct sampling strategy according to the development of a certain type of prion disease for the diagnostic tests. In the two recent non-invasive prion diagnosis studies in 2014, the RT-QuIC method found that the sensitivity of the olfactory mucosa-based test was higher than that of the cerebrospinal fluid-based test in the same sporadic CJD patients [103], while the PMCA method could only detected vCDJ in urine but not other human prion diseases [99]. Therefore, before the methods can be used diagnostically, further validation studies are needed to fully establish diagnostic sensitivities and specificities in clinical settings, such as tissue specific samples, pathogen types and strains, and stages of the prion diseases.

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