Prion diseases: New considerations

Abstract

The transmissible spongiform encephalopathies, which include Creutzfeldt-Jakob disease, are fatal neurodegenerative disorders caused by the pathological accumulation of abnormal prion protein. The diagnosis of Creutzfeldt-Jakob disease is complex. The electroencephalogram, magnetic resonance imaging, lumbar puncture and genetic testing findings can help in the differential diagnosis of rapidly progressive dementia. There has recently been considerable debate as to whether proteins involved in the development of neurodegenerative diseases should be regarded as prions or only share prion-like mechanisms. Two recent reports described the detection of abnormal prion protein in the nasal mucosa and urine of patients with Creutzfeldt-Jakob disease. These findings raise major health concerns regarding the transmissibility of human prion diseases. We set out to address this neurological hot topic and to draw conclusions on the basis of what is known in the literature thus far.

Keywords: Creutzfeldt-Jakob disease; prion protein; diagnosis; neurodegenerative diseases; transmissibility

1. Introduction

Human prion diseases, also known as transmissible spongiform encephalopathies, are a group of rare, invariably fatal progressive neurodegenerative disorders that affect mammalian species [1]. With the present review, we intended to address several aspects of the human prion diseases.

One of the aims was to survey the possibilities of the diagnosis of Creutzfeldt-Jakob disease (CJD), and to present a concise description of recent studies that detected pathological prion protein in the nasal mucosa and urine of CJD patients. These findings raise new health care concerns. We additionally set out to address a hot topic in neurology, i.e. whether or not neurodegenerative diseases (NDs) should be regarded as prionopathies.

2. Aetiology

Human prion diseases can be divided into three aetiological groups: sporadic, genetic and acquired. Sporadic CJD (sCJD), the most frequent form of prion diseases, has an annual mortality rate of 1 to 1.5 per million [2]. The genetic prion diseases, which are related to mutation of the prion protein gene (PRNP) on human chromosome 20, account for approximately 5-15% of all prion diseases worldwide [3]. On the basis of their clinicopathological features they can be divided into genetic CJD (gCJD), Gerstmann-Straussler-Scheinker disease and fatal familial insomnia [4]. The acquired forms of prion diseases include iatrogenic CJD (iCJD), due to medical interventions, variant CJD (vCJD), related to bovine spongiform encephalopathy (BSE), and kuru [5]. They are responsible for 2-5% of all prion diseases.

3. The prion protein

The word prion is short for proteinaceous infectious particle, implying that prions contain only amino acids [6]. Under physiological conditions, prion protein is found on the surface of cells (this form is abbreviated as PrP^c), attached to the outer part of the bilipid layer of the cell membranes through a glycophosphoinositol (GPI) anchor [7]. The precise physiological function of PrP^c remains to be elucidated. Studies have suggested that, through signal transductional pathways, it might have a prominent role in embryogenesis, the activation and differentiation of lymphocytes, the reproduction of haematopoetic stem cells, neuritogenesis and neuronal differentiation [8-15]. In structure, PrP^c is comprised mainly of alpha-helices

[16]. On the other hand, the pathological form of the protein (PrP^{sc}, where sc stands for scrapie) consists mostly of beta-sheets [17]. The misfolding of PrP^{c} to PrP^{sc} has a devastating effect on the central nervous system (CNS). The initial misfolding of the physiological protein can occur spontaneously (in sCJD) or it can be inherited (in gCJD). PrP^{sc} can also enter the CNS through protein ingestion (via the consumption of the meat of cattle with BSE) or by iatrogenic means (i.e. dura mater or corneal grafts). Once developed, PrP^{sc} serves as a template and can change the conformation of the physiological PrP^{c} in such a way that it will be misfolded into PrP^{sc}. This process is known as autocatalytic conversion [18]. Abnormal prions can aggregate and form soluble oligomers [19]. Increasing quantities of PrP^{sc} attach to one another and form insoluble polymers, which then make up amyloid fibres. The polymers may also break, thereby providing more nuclei for PrP^{c} to misfold into PrP^{sc}. **Figure 1** schematically illustrates the misfolding of PrP^{c} into PrP^{sc}.

The accumulation of aberrant prion proteins inside neurons will eventually lead to programmed cell death (apoptosis). Misfolded proteins are normally degraded in cells via proteosomes or autolysomes [19]. However, PrP^{sc} can evade these clearance pathways and accumulate in cells. It remains unclear exactly how PrP^{sc} aggregates exert neurotoxic effects. A small number of mechanisms have been proposed which, individually or acting in parallel, can lead to programmed cell death [20]. PrP^{c} has been demonstrated to have a neuroprotective effect [21]. The conformational change of the protein may therefore lead to increased levels of oxidative and endoplasmic reticulum stress. Moreover, overloading and the consequent dysfunction of the ubiquitin-proteosome and endosome-lysosomal systems by accumulated PrP^{sc} could induce apoptosis. Another theory postulates that the loss of function of PrP^{c} might play a significant role in the synaptic alterations and dendritic atrophy observed in transmissible spongiform encephalopathies. The exact signal transductional pathways and proteins involved in the above-mentioned apoptosis-inducing mechanisms have not yet been elaborated.

PrP^{sc} has the further characteristic attribute that it can propagate from cell to cell. Autopsies on patients who died from transmissible spongiform encephalopathy have demonstrated that PrP^{sc} is found throughout the brain. The pathological form of the protein can be transmitted between individuals, and also between species. The consumption of the meat of cattle infected with BSE caused a major epidemic in Great Britain not too long ago [22].
The main characteristics of prion proteins are summarized in **Table 1**.

4. The prion hypothesis of neurodegenerative diseases

Parkinson's disease (PD), degenerative parkinsonisms, Alzheimer's disease (AD), Huntington's disease (HD) and amyotrophic lateral sclerosis (ALS) are all NDs, and they share some common pathomechanisms. These include glutamate-induced excitotoxicity, impaired Ca^{2+} homeostasis, the production of reactive oxygen species, a mitochondrial dysfunction, neuroinflammation and an altered tryptophan metabolism [23-26]. Our research group has investigated the roles of neuroactive kynurenines in various NDs. The key components of the tryptophan degradation pathway include kynurenic acid (KYNA), 3-hydroxy-L-kynurenine and quinolinic acid. KYNA has been demonstrated to have neuroprotective effects, whereas the latter two are neurotoxic compounds [27]. An unfavourable shift in the degradation pathway of tryptophan can therefore promote neurotoxicity and exert deleterious effects on the neurones.

The accumulation of aberrant, misfolded proteins in the CNS can also play a pivotal role in the development of NDs. Recent findings provide evidence that the proteins responsible for the above-mentioned diseases share prion-like mechanisms [28-32]. In this paragraph, we did not aim to give a critical opinion, instead we set out to summarize the research done in this topic.

4.1 Parkinson's disease

The pathological hallmark of PD is the presence of misfolded α-synuclein (α-syn) aggregates in certain areas of the brain. Similarly to PrP^{c}, α-syn is normally present in an α-helix-rich form, but misfolds into a structure characterized by β-sheets [33,34]. The phosphorylated and ubiquitinated forms of α-syn aggregate via autocatalytic conversion and form Lewy bodies [35]. Braak et al. demonstrated that the Lewy bodies are initially present in the anterior olfactory nucleus and dorsal motor nucleus of the vagal nerve [36,37]. The pathology then spreads towards the rostral areas of the brain, including the substantia nigra, and by the end stage of the disease, α-syn aggregates have even developed in certain cortical areas.

The propagation of α-syn pathology raises the question of whether α-syn can spread from cell to cell in a PrP^{sc}-like manner. Autopsy studies have revealed that, 12-16 years after transplantation, Lewy bodies are present in the grafted-embryonic mesencephalic neurones in PD patients [28,29,38]. This unequivocally indicated that α-syn has prion-like cell-to-cell transmissibility. Furthermore, after injecting three different brain areas (the substantia nigra,

the striatum or the entorhinal cortex) of wild-type mice with recombinant α-syn fibrils, Masuda-Suzukake et al. found that propagation of the phosphorylated α-syn pathology affected different brain regions in direct or indirect contact with the initial injection site [39]. These observations support the hypothesis that α-syn spreads between interconnected brain areas through axonal transport. The possible mechanisms of transmission of α-syn are still unclear, but Mohamed et al. described several transmission routes for tau protein [40], which might also apply to α-syn.

4.2 Alzheimer's disease

Dementia is most commonly characterized by the presence of extracellular β-amyloid (Aβ) fibrils and intracellular hyperphosphorylated tau polymers forming neurofibrillary tangles. Tau has a significant role in other NDs, i.e. progressive supranuclear palsy and corticobasal degeneration, and it has been demonstrated that tau protein also participates in prion-like cell-to-cell propagation [30,41]. Other experiments revealed that inoculation with abnormal human tau or Aβ resulted in the presence of misfolded protein aggregates in the brains of transgenic mice [42,43].

One somewhat astonishing finding has supported the link between AD and prionopathy. Under experimental conditions, extracellular soluble Aβ oligomers activated PrP^c on the surface of hippocampal neurones [44]. PrP^c bound Aβ oligomers as part of a multiprotein receptor complex that includes low-density lipoprotein receptor-related protein-1 as a co-receptor and requires the integrity of cholesterol-rich lipid rafts that contain the receptor complex in the cell membrane [45]. PrP^c activation led to an increased function of Fyn kinase, which induced tau hyperphosphorylation and also phosphorylation of the NR2B subunit of the NMDA receptor, through signal transduction pathways [45-47]. Consequently, the NMDA receptor was activated and glutamate excitotoxicity became more pronounced. This eventually led to cell death and a synaptic dysfunction. Although these findings are fascinating, they have not been inconclusively proven. Some experiments led to contradictory results regarding the connection between Aβ and PrP^c [48-50]. It has additionally been demonstrated that Aβ displays neurotoxic effects in the absence of PrP^c.

PrP^c has beneficial, neuroprotective effects. It can inhibit both the NMDA receptor and β-secretase, thereby reducing excitotoxicity and the formation of Aβ [51,52]. α-Cleavage of PrP^c is carried out directly or indirectly by the ADAMs (a disintegrin and metalloproteinases), members of the zinc-dependent metalloproteinase family (more specifically ADAM8, ADAM9, ADAM10 and ADAM17) [53,54]. α-Cleavage produces two fragments, N1 and C1.

The N1 fragment can bind to Aβ and reduce aggregation and propagation of the aberrant protein [55]. It also has a neuroprotective effect by inhibiting p53-mediated cell death [56]. Moreover, the C1 fragment can inhibit the formation of PrP^{sc} [57].

These findings (similarly as in PD) support the hypothesis that tau and Aβ can spread between cells. The available findings allow the conclusion that tau and Aβ share prion-like mechanisms.

4.3 Other neurodegenerative diseases

A number of studies have demonstrated that the protein huntingtin exhibits prion-like behaviour in HD, as does superoxide dismutase-1 in ALS. These proteins are also prone to misfold, convert normal structured proteins to abnormal ones, escape cellular clearance pathways and aggregate [31,32,58-60]. Cell-to-cell propagation has likewise been demonstrated. **Tables 2 and 3** summarize the characteristics and prion-like attributes of the proteins involved in the development of NDs.

5. Contradictory findings to the prion hypothesis of neurodegenerative diseases

Tables 1 and 3 reveal that the significant difference between prions and the proteins involved in NDs is that prions can be transmitted. There is other evidence that prionopathies and NDs are two different entities, e.g. the utterly different courses of the diseases. In CJD, the health of the patients deteriorates rapidly and death follows within months after the onset of the symptoms. In NDs, on the other hand, the disease course demonstrates a slow progression. It takes years or even decades even for NDs to be fatal.

Moreover, the pathological findings vary greatly. Prionopathies are characterized by spongiform changes, presenting as microvacuoles and confluent vacuoles in the neuropil [20,61]. Reactive astrocytosis, microglia accumulation and atrophy are also present. Spongiform changes are not hallmark findings in NDs. Various brain regions are affected in different NDs. In PD, for instance, the affected brain areas are the olfactory nuclei, the brain stem including the substantia nigra, the basal prosencephalon, the mesocortex and eventually the neocortex [36,37]. In AD, abnormal protein accumulation is most apparent in the entorhinal cortex, the limbic cortex and finally the isocortical areas [62,63]. Since different brain regions are involved in each disease, the various diseases are characterized by different symptoms and diagnostic findings. In consequence of their great variety, a detailed discussion of these symptoms and findings is beyond the scope of this article.

6. Diagnosis of Creutzfeldt-Jakob disease

6.1 Present practice

The diagnosis of CJD is complex. It is often based on the exclusion of other, more frequent causes of rapidly progressive dementia. The definite diagnosis is neuropathological; in most cases, the diagnosis can be established with the use of a series of diagnostic procedures [64,65].

Besides the rapidly progressive dementia, the suspicion of the diagnosis of CJD should always be raised by the early development of myoclonus, the visual or cerebellar disturbances, the pyramidal or extrapyramidal features and akinetic mutism [66].

The electroencephalogram (EEG) may support the diagnosis, but the EEG findings alone are not specific for CJD. Frontal intermittent non-peaked rhythmical delta activity is generally seen in the early phase of sCJD [67]. Periodic synchronous bi- or triphasic sharp-wave complexes are found in the terminal stage of sCJD, with a sensitivity of 64% and a specificity of 91% [68].

In the clinical diagnosis of sCJD, magnetic resonance imaging (MRI) is essential. Meissner et al. specified the cerebral cortical regions, the hippocampus, the basal ganglia, the thalamic nuclei and the cerebellum as the frequently affected anatomical regions of the brain [69]. Zerr et al. later concluded that high signal intensities in the caudate nucleus and putamen or in at least two cortical regions (temporal-parietal-occipital) in the FLAIR or DWI sequences are the MRI diagnostic criteria for sCJD [64]. These criteria are accepted and recommended by the European MRI-CJD Consortium. The sensitivity and specificity of MRI has been found to be 96% and 93%, respectively [70].

The 14-3-3 protein concentration of the cerebrospinal fluid (CSF) is usually elevated following neuronal damage. The WHO guidelines suggest a positive CSF 14-3-3 protein concentration as a criterion for probable sCJD [71]. An increased CSF 14-3-3 protein level has high sensitivity and low specificity for the diagnosis of sCJD and gCJD cases [72].

Genetic prion diseases are associated with numerous disease-, geographic- and frequency-specific PRNP mutations [65]. The E200K mutation is the most frequent mutation worldwide, while other mutations (P105L, N171S or V180I) are rare [4]. The polymorphism of codon 129

influences the phenotype [1,73]. As a positive family history is absent in a high proportion of the cases, PRNP analysis can be used to differentiate between certain sCJD and all genetic prion disease cases, and it may allow the early or pre-symptomatic diagnosis of genetic prion diseases [4]. **Table 4** summarizes the steps involved in the diagnosis of CJD.

6.2 Novel findings

In the past few years, possible novel methods have been put forward for the detection of CJD, and two papers have raised public health concerns regarding the transmissibility of the disease.

The real-time quaking-induced conversion (RT-QuIC) is a method that allows the detection of small amounts of PrP^{sc} through the formation of thioflavin T (ThT) fluorescent amyloid fibrils. This method was first used for the PrP^{sc} detection in CSF with high sensitivity and specificity for the diagnosis of sCJD [74-76].

Orrú et al. have demonstrated that the olfactory epithelium can be of vital importance in the detection of sCJD and gCJD [77]. A study was conducted in which 2 samples were taken from each participant, one from the olfactory epithelium and one from the CSF. The samples were then analysed by RT-QuIC.

Positive RT-QuIC reactions were observed in 30 of 31 patients diagnosed with sCJD or gCJD. In the control groups, no positive reactions were found. That study therefore implies an estimated sensitivity of 97% and an estimated specificity of 100% for the RT-QuIC of nasal brushing samples. In contrast, the corresponding figures for the CSF samples were 77% and 100% respectively. Moreover, the ThT detection of PrP^{sc} amyloids from olfactory samples required 50 hours, whereas CSF sampling needed 90 hours [77]. It may therefore be concluded that nasal brushing is a superior sampling technique for the detection of CJD, rather than examinations on the CSF. However, further studies are warranted to establish whether this novel technique is advantageous for the clinical diagnosis of the various forms of CJD.

In another recently published study, Moda et al. demonstrated that the urine of patients with vCJD also contains low amounts of PrP^{sc}, which can be detected even when the patients are in the presymptomatic phase of the disease [78]. The urine of healthy subjects and of patients with vCJD, sCJD, gCJD, or other neurodegenerative and non-degenerative neurological disorders, was analysed by a protein misfolding cyclic amplification (PMCA) method, followed by proteinase K treatment and Western blotting (WB). With the PMCA method, a

small amount of PrP^{sc} can be amplified (similarly as with RT-QuIC), allowing the detection of the aggregates by WB. 13 of the 14 urine samples from patients with vCJD proved to contain PrP^{sc}. No other positive reactions were observed. It was therefore concluded that with this technique PrP^{sc} can be unambiguously detected in the urine of patients with vCJD. PMCA and WB showed an estimated sensitivity of 93% and an estimated specificity of 100% in this study [78]. The peripheral tissues of patients with the infectious form of CJD were earlier demonstrated to contain more PrP^{sc} than those of patients with gCJD or sCJD [79-81].

7. Conclusions

Although proteins share prion-like mechanisms in NDs, as long as their transmissibility is not proven they should not be regarded as prionopathies. Clinical, diagnostic and pathological findings also support the distinction between NDs and prionopathies. However, this current hot topic in neurology should remain under scrutiny until sufficient evidence is available to demonstrate quite clearly either the prion-like or the non-prion-like behaviour of NDs.

The presence of PrP^{sc} in the olfactory mucosa raises the question of whether CJD can be transmitted between humans through nasal discharges. Animal models have shown that the nasal and airborne transmission of prion disease is possible [82-86]. However, no cases of the airborne spread of transmissible spongiform encephalopathies between humans have yet been reported. The iatrogenic transmission of prion diseases by instruments that have come into contact with the nasal mucosa is still a possibility that warrants more research [87,88]. Moreover, the presence of PrP^{sc} in the urine of patients with vCJD raises major health concerns as regards its transmissibility. Since the incubation period of vCJD can vary from years to decades [87], significantly more people than previously thought may well be infected, but still in the presymptomatic phase of the disease. At the present time, the documented cases of iCJD in the UK have been due to growth hormone replacement from cadavers, dura mater grafts, blood transfusion and plasma transfusion [89]. Furthermore, transmission of prion diseases was suspected after corneal transplantation and the use of contaminated neurosurgical instruments and EEG electrodes [90-92].

The presence of PrP^{sc} in numerous tissues of the body demands an answer to the question of whether prion disease can be transmitted through instruments during surgical and other invasive interventions. A possibly even more important question is whether CJD can be transmitted through airborne infection, direct contact with nasal discharge, urine, blood and other bodily fluids, or even with sexual contact, in addition to the already proven transmission

mechanisms of the acquired forms of prion diseases. In vCJD, it has been suggested based on animal models that after oral intake, prion proteins are transported through the intestinal mucosa via M-cells [93]. Prion proteins can penetrate into the enteric nervous system, Peyer's patches and from there into other organs of the lymphoreticular system [94,95]. Prions can then reach the brain via peripheral autonomic nerves: directly through the vagus nerve or by reaching the spinal cord via sympathetic nerves and then travelling cranially to the brain [96,97]. Banks et al. have suggested that the prion protein can cross the blood-brain barrier [98]. Therefore, after transfusion of blood from a CJD patient, there is a possibility that transfused prions can penetrate into the brain directly from the blood stream. Theoretically, based on animal models, it is also possible that prion proteins can appear in the skin of those who came in contact with bodily fluids of CJD patients [99]. From there, through cutaneous nerves or the bloodstream, prion proteins might be able to penetrate into the CNS. Similarly, in theory, airborne infection might be possible by accumulation of prions in the respiratory organs and then spreading into the CNS via blood vessels or autonomic nerves supplying the lungs, trachea, etc. During sexual contact, CJD patients might cause prion infection in the reproductive organs of the partner. From these organs, prion proteins might be able to reach the CNS via peripheral nerves or the blood stream. However, to date, no evidence of the occupational transmission of CJD has been reported among health care workers, and infection has not been detected after direct contact with body fluids [100]. Nonetheless, since the incubation period of acquired CJD can be so long, there well might be asymptomatic carriers amongst us. They could even be blood or organ donors who are spreading the disease without being aware of the potential devastating effect on others' lives. The presymptomatic detection of the disease and the screening of blood and organ donors is therefore clearly of vital importance. The two articles highlighted above give rise to the hope that medical science is on the verge of detecting prion diseases before the development of symptoms. The questions raised here could also apply to other NDs if their prion-like transmissibility is once proven. The previous/current and newly proposed transmission mechanisms of prion diseases are summarized in **Table 5**.

Acknowledgements

This work was supported by the MTA-SZTE Neuroscience Research Group of the Hungarian Academy of Sciences and the University of Szeged.

Conflicts of interest

The authors declare that they have no conflict of interest.

Abbreviations

α-syn: α-synuclein; Aβ: β-amyloid; AD: Alzheimer's disease; ADAMs: a disintegrin and metalloproteinases; ALS: amyotrophic lateral sclerosis; BSE: bovine spongiform encephalopathy; CJD: Creutzfeldt-Jakob disease; CNS: central nervous system; CSF: cerebrospinal fluid; EEG: electroencephalogram; gCJD: genetic Creutzfeldt-Jakob disease; GPI anchor: glycophosphoinositol anchor; HD: Huntington's disease; iCJD: iatrogenic CJD; KYNA: kynurenic acid; MRI: magnetic resonance imaging; NDs: neurodegenerative diseases; PD: Parkinson's disease; PMCA: protein misfolding cyclic amplification; PRNP: prion protein gene; PrP^{c}: physiological prion protein; PrP^{sc}: pathological prion protein; RT-QuIC: real-time quaking-induced conversion; sCJD: sporadic Creutzfeldt-Jakob disease; SOD-1: superoxide-dismutase-1; ThT: thioflavin T; vCJD: variant Creutzfeldt-Jakob disease; WB: Western blotting.

References

1. Prusiner SB (1998) Prions. Proc Natl Acad Sci U S A 95:13363-13383.
2. Ladogana A, Puopolo M, Croes EA, Budka H, Jarius C, Collins S, Klug GM, Sutcliffe T, Giulivi A, Alperovitch A, Delasnerie-Laupretre N, Brandel JP, Poser S, Kretzschmar H, Rietveld I, Mitrova E, Cuesta Jde P, Martinez-Martin P, Glatzel M, Aguzzi A, Knight R, Ward H, Pocchiari M, van Duijn CM, Will RG, Zerr I (2005) Mortality from Creutzfeldt-Jakob disease and related disorders in Europe, Australia, and Canada. Neurology 64:1586-1591.
3. Kovacs GG, Voigtlander T, Gelpi E, Budka H (2004) Rationale for diagnosing human prion disease. World J Biol Psychiatry 5:83-91.
4. Kovacs GG, Puopolo M, Ladogana A, Pocchiari M, Budka H, van Duijn C, Collins SJ, Boyd A, Giulivi A, Coulthart M, Delasnerie-Laupretre N, Brandel JP, Zerr I, Kretzschmar HA, de Pedro-Cuesta J, Calero-Lara M, Glatzel M, Aguzzi A, Bishop M, Knight R, Belay G, Will R, Mitrova E, Eurocjd (2005) Genetic prion disease: the EUROCJD experience. Hum Genet 118:166-174.
5. Will RG (2003) Acquired prion disease: iatrogenic CJD, variant CJD, kuru. Br Med Bull 66:255-265.
6. Prusiner SB (1982) Novel proteinaceous infectious particles cause scrapie. Science 216:136-144.
7. Stahl N, Borchelt DR, Hsiao K, Prusiner SB (1987) Scrapie prion protein contains a phosphatidylinositol glycolipid. Cell 51:229-240.
8. Cashman NR, Loertscher R, Nalbantoglu J, Shaw I, Kascsak RJ, Bolton DC, Bendheim PE (1990) Cellular isoform of the scrapie agent protein participates in lymphocyte activation. Cell 61:185-192.
9. Chen S, Mange A, Dong L, Lehmann S, Schachner M (2003) Prion protein as trans-interacting partner for neurons is involved in neurite outgrowth and neuronal survival. Mol Cell Neurosci 22:227-233.
10. Graner E, Mercadante AF, Zanata SM, Forlenza OV, Cabral AL, Veiga SS, Juliano MA, Roesler R, Walz R, Minetti A, Izquierdo I, Martins VR, Brentani RR (2000) Cellular prion protein binds laminin and mediates neuritogenesis. Brain Res Mol Brain Res 76:85-92.
11. Hajj GN, Lopes MH, Mercadante AF, Veiga SS, da Silveira RB, Santos TG, Ribeiro KC, Juliano MA, Jacchieri SG, Zanata SM, Martins VR (2007) Cellular prion protein interaction with vitronectin supports axonal growth and is compensated by integrins. J Cell Sci 120:1915-1926.
12. Kanaani J, Prusiner SB, Diacovo J, Baekkeskov S, Legname G (2005) Recombinant prion protein induces rapid polarization and development of synapses in embryonic rat hippocampal neurons in vitro. J Neurochem 95:1373-1386.
13. Malaga-Trillo E, Solis GP, Schrock Y, Geiss C, Luncz L, Thomanetz V, Stuermer CA (2009) Regulation of embryonic cell adhesion by the prion protein. PLoS Biol 7:e55.
14. Santuccione A, Sytnyk V, Leshchyns'ka I, Schachner M (2005) Prion protein recruits its neuronal receptor NCAM to lipid rafts to activate p59fyn and to enhance neurite outgrowth. J Cell Biol 169:341-354.
15. Zhang CC, Steele AD, Lindquist S, Lodish HF (2006) Prion protein is expressed on long-term repopulating hematopoietic stem cells and is important for their self-renewal. Proc Natl Acad Sci U S A 103:2184-2189.
16. Riek R, Hornemann S, Wider G, Billeter M, Glockshuber R, Wuthrich K (1996) NMR structure of the mouse prion protein domain PrP(121-231). Nature 382:180-182.
17. Pan KM, Baldwin M, Nguyen J, Gasset M, Serban A, Groth D, Mehlhorn I, Huang Z, Fletterick RJ, Cohen FE, et al. (1993) Conversion of alpha-helices into beta-sheets features in the formation of the scrapie prion proteins. Proc Natl Acad Sci U S A 90:10962-10966.

18. Acquatella-Tran Van Ba I, Imberdis T, Perrier V (2013) From prion diseases to prion-like propagation mechanisms of neurodegenerative diseases. Int J Cell Biol 2013:975832.
19. Brundin P, Melki R, Kopito R (2010) Prion-like transmission of protein aggregates in neurodegenerative diseases. Nat Rev Mol Cell Biol 11:301-307.
20. Kovacs GG, Budka H (2008) Prion diseases: from protein to cell pathology. Am J Pathol 172:555-565.
21. Roucou X, Gains M, LeBlanc AC (2004) Neuroprotective functions of prion protein. J Neurosci Res 75:153-161.
22. Anderson RM, Donnelly CA, Ferguson NM, Woolhouse ME, Watt CJ, Udy HJ, MaWhinney S, Dunstan SP, Southwood TR, Wilesmith JW, Ryan JB, Hoinville LJ, Hillerton JE, Austin AR, Wells GA (1996) Transmission dynamics and epidemiology of BSE in British cattle. Nature 382:779-788.
23. Sas K, Robotka H, Toldi J, Vecsei L (2007) Mitochondria, metabolic disturbances, oxidative stress and the kynurenine system, with focus on neurodegenerative disorders. J Neurol Sci 257:221-239.
24. Szalardy L, Klivenyi P, Zadori D, Fulop F, Toldi J, Vecsei L (2012) Mitochondrial disturbances, tryptophan metabolites and neurodegeneration: medicinal chemistry aspects. Curr Med Chem 19:1899-1920.
25. Zadori D, Klivenyi P, Plangar I, Toldi J, Vecsei L (2011) Endogenous neuroprotection in chronic neurodegenerative disorders: with particular regard to the kynurenines. J Cell Mol Med 15:701-717.
26. Zadori D, Klivenyi P, Vamos E, Fulop F, Toldi J, Vecsei L (2009) Kynurenines in chronic neurodegenerative disorders: future therapeutic strategies. J Neural Transm 116:1403-1409.
27. Vecsei L, Szalardy L, Fulop F, Toldi J (2013) Kynurenines in the CNS: recent advances and new questions. Nat Rev Drug Discov 12:64-82.
28. Brundin P, Li JY, Holton JL, Lindvall O, Revesz T (2008) Research in motion: the enigma of Parkinson's disease pathology spread. Nat Rev Neurosci 9:741-745.
29. Kordower JH, Chu Y, Hauser RA, Freeman TB, Olanow CW (2008) Lewy body-like pathology in long-term embryonic nigral transplants in Parkinson's disease. Nat Med 14:504-506.
30. Clavaguera F, Bolmont T, Crowther RA, Abramowski D, Frank S, Probst A, Fraser G, Stalder AK, Beibel M, Staufenbiel M, Jucker M, Goedert M, Tolnay M (2009) Transmission and spreading of tauopathy in transgenic mouse brain. Nat Cell Biol 11:909-913.
31. Busch A, Engemann S, Lurz R, Okazawa H, Lehrach H, Wanker EE (2003) Mutant huntingtin promotes the fibrillogenesis of wild-type huntingtin: a potential mechanism for loss of huntingtin function in Huntington's disease. J Biol Chem 278:41452-41461.
32. Munch C, O'Brien J, Bertolotti A (2011) Prion-like propagation of mutant superoxide dismutase-1 misfolding in neuronal cells. Proc Natl Acad Sci U S A 108:3548-3553.
33. Conway KA, Harper JD, Lansbury PT (1998) Accelerated in vitro fibril formation by a mutant alpha-synuclein linked to early-onset Parkinson disease. Nat Med 4:1318-1320.
34. Ferreon AC, Gambin Y, Lemke EA, Deniz AA (2009) Interplay of alpha-synuclein binding and conformational switching probed by single-molecule fluorescence. Proc Natl Acad Sci U S A 106:5645-5650.
35. Luk KC, Song C, O'Brien P, Stieber A, Branch JR, Brunden KR, Trojanowski JQ, Lee VM (2009) Exogenous alpha-synuclein fibrils seed the formation of Lewy body-like intracellular inclusions in cultured cells. Proc Natl Acad Sci U S A 106:20051-20056.
36. Braak H, Del Tredici K, Rub U, de Vos RA, Jansen Steur EN, Braak E (2003) Staging of brain pathology related to sporadic Parkinson's disease. Neurobiol Aging 24:197-211.
37. Braak H, Ghebremedhin E, Rüb U, Bratzke H, Del Tredici K (2004) Stages in the development of Parkinson's disease-related pathology. Cell Tissue Res 318:121-134.

38. Li JY, Englund E, Widner H, Rehncrona S, Bjorklund A, Lindvall O, Brundin P (2010) Characterization of Lewy body pathology in 12- and 16-year-old intrastriatal mesencephalic grafts surviving in a patient with Parkinson's disease. Mov Disord 25:1091-1096.
39. Masuda-Suzukake M, Nonaka T, Hosokawa M, Kubo M, Shimozawa A, Akiyama H, Hasegawa M (2014) Pathological alpha-synuclein propagates through neural networks. Acta Neuropathol Commun 2:88.
40. Mohamed NV, Herrou T, Plouffe V, Piperno N, Leclerc N (2013) Spreading of tau pathology in Alzheimer's disease by cell-to-cell transmission. Eur J Neurosci 37:1939-1948.
41. Clavaguera F, Hench J, Goedert M, Tolnay M (2015) Invited review: Prion-like transmission and spreading of tau pathology. Neuropathol Appl Neurobiol 41:47-58.
42. Kane MD, Lipinski WJ, Callahan MJ, Bian F, Durham RA, Schwarz RD, Roher AE, Walker LC (2000) Evidence for seeding of beta -amyloid by intracerebral infusion of Alzheimer brain extracts in beta -amyloid precursor protein-transgenic mice. J Neurosci 20:3606-3611.
43. Meyer-Luehmann M, Coomaraswamy J, Bolmont T, Kaeser S, Schaefer C, Kilger E, Neuenschwander A, Abramowski D, Frey P, Jaton AL, Vigouret JM, Paganetti P, Walsh DM, Mathews PM, Ghiso J, Staufenbiel M, Walker LC, Jucker M (2006) Exogenous induction of cerebral beta-amyloidogenesis is governed by agent and host. Science 313:1781-1784.
44. Lauren J, Gimbel DA, Nygaard HB, Gilbert JW, Strittmatter SM (2009) Cellular prion protein mediates impairment of synaptic plasticity by amyloid-beta oligomers. Nature 457:1128-1132.
45. Rushworth JV, Griffiths HH, Watt NT, Hooper NM (2013) Prion protein-mediated toxicity of amyloid-beta oligomers requires lipid rafts and the transmembrane LRP1. J Biol Chem 288:8935-8951.
46. Roberson ED, Halabisky B, Yoo JW, Yao J, Chin J, Yan F, Wu T, Hamto P, Devidze N, Yu GQ, Palop JJ, Noebels JL, Mucke L (2011) Amyloid-beta/Fyn-induced synaptic, network, and cognitive impairments depend on tau levels in multiple mouse models of Alzheimer's disease. J Neurosci 31:700-711.
47. Um JW, Strittmatter SM (2013) Amyloid-beta induced signaling by cellular prion protein and Fyn kinase in Alzheimer disease. Prion 7:37-41.
48. Balducci C, Beeg M, Stravalaci M, Bastone A, Sclip A, Biasini E, Tapella L, Colombo L, Manzoni C, Borsello T, Chiesa R, Gobbi M, Salmona M, Forloni G (2010) Synthetic amyloid-beta oligomers impair long-term memory independently of cellular prion protein. Proc Natl Acad Sci U S A 107:2295-2300.
49. Calella AM, Farinelli M, Nuvolone M, Mirante O, Moos R, Falsig J, Mansuy IM, Aguzzi A (2010) Prion protein and Abeta-related synaptic toxicity impairment. EMBO Mol Med 2:306-314.
50. Kessels HW, Nguyen LN, Nabavi S, Malinow R (2010) The prion protein as a receptor for amyloid-beta. Nature 466:E3-4; discussion E4-5.
51. Griffiths HH, Whitehouse IJ, Baybutt H, Brown D, Kellett KA, Jackson CD, Turner AJ, Piccardo P, Manson JC, Hooper NM (2011) Prion protein interacts with BACE1 protein and differentially regulates its activity toward wild type and Swedish mutant amyloid precursor protein. J Biol Chem 286:33489-33500.
52. Khosravani H, Zhang Y, Tsutsui S, Hameed S, Altier C, Hamid J, Chen L, Villemaire M, Ali Z, Jirik FR, Zamponi GW (2008) Prion protein attenuates excitotoxicity by inhibiting NMDA receptors. J Cell Biol 181:551-565.
53. Liang J, Kong Q (2012) alpha-Cleavage of cellular prion protein. Prion 6:453-460.
54. McDonald AJ, Dibble JP, Evans EG, Millhauser GL (2014) A new paradigm for enzymatic control of alpha-cleavage and beta-cleavage of the prion protein. J Biol Chem 289:803-813.

55. Fluharty BR, Biasini E, Stravalaci M, Sclip A, Diomede L, Balducci C, La Vitola P, Messa M, Colombo L, Forloni G, Borsello T, Gobbi M, Harris DA (2013) An N-terminal fragment of the prion protein binds to amyloid-beta oligomers and inhibits their neurotoxicity in vivo. J Biol Chem 288:7857-7866.
56. Guillot-Sestier MV, Sunyach C, Druon C, Scarzello S, Checler F (2009) The alpha-secretase-derived N-terminal product of cellular prion, N1, displays neuroprotective function in vitro and in vivo. J Biol Chem 284:35973-35986.
57. Westergard L, Turnbaugh JA, Harris DA (2011) A naturally occurring C-terminal fragment of the prion protein (PrP) delays disease and acts as a dominant-negative inhibitor of PrPSc formation. J Biol Chem 286:44234-44242.
58. Grad LI, Guest WC, Yanai A, Pokrishevsky E, O'Neill MA, Gibbs E, Semenchenko V, Yousefi M, Wishart DS, Plotkin SS, Cashman NR (2011) Intermolecular transmission of superoxide dismutase 1 misfolding in living cells. Proc Natl Acad Sci U S A 108:16398-16403.
59. Munch C, Bertolotti A (2012) Propagation of the prion phenomenon: beyond the seeding principle. J Mol Biol 421:491-498.
60. Gunther MR, Vangilder R, Fang J, Beattie DS (2004) Expression of a familial amyotrophic lateral sclerosis-associated mutant human superoxide dismutase in yeast leads to decreased mitochondrial electron transport. Arch Biochem Biophys 431:207-214.
61. Kovacs GG, Budka H (2009) Molecular pathology of human prion diseases. Int J Mol Sci 10:976-999.
62. Braak H, Braak E (1995) Staging of Alzheimer's disease-related neurofibrillary changes. Neurobiol Aging 16:271-284.
63. Braak H, Braak E (1991) Neuropathological stageing of Alzheimer-related changes. Acta Neuropathol 82:239-259.
64. Zerr I, Kallenberg K, Summers DM, Romero C, Taratuto A, Heinemann U, Breithaupt M, Varges D, Meissner B, Ladogana A, Schuur M, Haik S, Collins SJ, Jansen GH, Stokin GB, Pimentel J, Hewer E, Collie D, Smith P, Roberts H, Brandel JP, van Duijn C, Pocchiari M, Begue C, Cras P, Will RG, Sanchez-Juan P (2009) Updated clinical diagnostic criteria for sporadic Creutzfeldt-Jakob disease. Brain 132:2659-2668.
65. WHO (2003) WHO manual for surveillance of human transmissible spongiform encephalopathies including variant Creutzfeldt-Jakob disease. World Health Organization Communicable Disease Surveillance and Response, Geneva, Switzerland
66. Rabinovici GD, Wang PN, Levin J, Cook L, Pravdin M, Davis J, DeArmond SJ, Barbaro NM, Martindale J, Miller BL, Geschwind MD (2006) First symptom in sporadic Creutzfeldt-Jakob disease. Neurology 66:286-287.
67. Hansen HC, Zschocke S, Sturenburg HJ, Kunze K (1998) Clinical changes and EEG patterns preceding the onset of periodic sharp wave complexes in Creutzfeldt-Jakob disease. Acta Neurol Scand 97:99-106.
68. Steinhoff BJ, Zerr I, Glatting M, Schulz-Schaeffer W, Poser S, Kretzschmar HA (2004) Diagnostic value of periodic complexes in Creutzfeldt-Jakob disease. Ann Neurol 56:702-708.
69. Meissner B, Kallenberg K, Sanchez-Juan P, Collie D, Summers DM, Almonti S, Collins SJ, Smith P, Cras P, Jansen GH, Brandel JP, Coulthart MB, Roberts H, Van Everbroeck B, Galanaud D, Mellina V, Will RG, Zerr I (2009) MRI lesion profiles in sporadic Creutzfeldt-Jakob disease. Neurology 72:1994-2001.
70. Vitali P, Maccagnano E, Caverzasi E, Henry RG, Haman A, Torres-Chae C, Johnson DY, Miller BL, Geschwind MD (2011) Diffusion-weighted MRI hyperintensity patterns differentiate CJD from other rapid dementias. Neurology 76:1711-1719.
71. WHO (1998) Human transmissible spongiform encephalopathies. Wkly Epidemiol Rec 73:361-365.

72. Hamlin C, Puoti G, Berri S, Sting E, Harris C, Cohen M, Spear C, Bizzi A, Debanne SM, Rowland DY (2012) A comparison of tau and 14-3-3 protein in the diagnosis of Creutzfeldt-Jakob disease. Neurology 79:547-552.
73. Alperovitch A, Zerr I, Pocchiari M, Mitrova E, de Pedro Cuesta J, Hegyi I, Collins S, Kretzschmar H, van Duijn C, Will RG (1999) Codon 129 prion protein genotype and sporadic Creutzfeldt-Jakob disease. Lancet 353:1673-1674.
74. Atarashi R, Sano K, Satoh K, Nishida N (2011) Real-time quaking-induced conversion: a highly sensitive assay for prion detection. Prion 5:150-153.
75. McGuire LI, Peden AH, Orru CD, Wilham JM, Appleford NE, Mallinson G, Andrews M, Head MW, Caughey B, Will RG, Knight RS, Green AJ (2012) Real time quaking-induced conversion analysis of cerebrospinal fluid in sporadic Creutzfeldt-Jakob disease. Ann Neurol 72:278-285.
76. Orru CD, Groveman BR, Hughson AG, Zanusso G, Coulthart MB, Caughey B (2015) Rapid and sensitive RT-QuIC detection of human Creutzfeldt-Jakob disease using cerebrospinal fluid. MBio 6.
77. Orru CD, Bongianni M, Tonoli G, Ferrari S, Hughson AG, Groveman BR, Fiorini M, Pocchiari M, Monaco S, Caughey B, Zanusso G (2014) A test for Creutzfeldt-Jakob disease using nasal brushings. N Engl J Med 371:519-529.
78. Moda F, Gambetti P, Notari S, Concha-Marambio L, Catania M, Park KW, Maderna E, Suardi S, Haik S, Brandel JP, Ironside J, Knight R, Tagliavini F, Soto C (2014) Prions in the urine of patients with variant Creutzfeldt-Jakob disease. N Engl J Med 371:530-539.
79. Brandner S, Whitfield J, Boone K, Puwa A, O'Malley C, Linehan JM, Joiner S, Scaravilli F, Calder I, M PA, Wadsworth JD, Collinge J (2008) Central and peripheral pathology of kuru: pathological analysis of a recent case and comparison with other forms of human prion disease. Philos Trans R Soc Lond B Biol Sci 363:3755-3763.
80. Head MW, Ritchie D, Smith N, McLoughlin V, Nailon W, Samad S, Masson S, Bishop M, McCardle L, Ironside JW (2004) Peripheral tissue involvement in sporadic, iatrogenic, and variant Creutzfeldt-Jakob disease: an immunohistochemical, quantitative, and biochemical study. Am J Pathol 164:143-153.
81. Wadsworth JD, Joiner S, Hill AF, Campbell TA, Desbruslais M, Luthert PJ, Collinge J (2001) Tissue distribution of protease resistant prion protein in variant Creutzfeldt-Jakob disease using a highly sensitive immunoblotting assay. Lancet 358:171-180.
82. Denkers ND, Hayes-Klug J, Anderson KR, Seelig DM, Haley NJ, Dahmes SJ, Osborn DA, Miller KV, Warren RJ, Mathiason CK, Hoover EA (2013) Aerosol transmission of chronic wasting disease in white-tailed deer. J Virol 87:1890-1892.
83. Denkers ND, Seelig DM, Telling GC, Hoover EA (2010) Aerosol and nasal transmission of chronic wasting disease in cervidized mice. J Gen Virol 91:1651-1658.
84. Haybaeck J, Heikenwalder M, Klevenz B, Schwarz P, Margalith I, Bridel C, Mertz K, Zirdum E, Petsch B, Fuchs TJ, Stitz L, Aguzzi A (2011) Aerosols transmit prions to immunocompetent and immunodeficient mice. PLoS Pathog 7:e1001257.
85. Kincaid AE, Bartz JC (2007) The nasal cavity is a route for prion infection in hamsters. J Virol 81:4482-4491.
86. Kincaid AE, Hudson KF, Richey MW, Bartz JC (2012) Rapid transepithelial transport of prions following inhalation. J Virol 86:12731-12740.
87. Brown P, Brandel JP, Sato T, Nakamura Y, MacKenzie J, Will RG, Ladogana A, Pocchiari M, Leschek EW, Schonberger LB (2012) Iatrogenic Creutzfeldt-Jakob disease, final assessment. Emerg Infect Dis 18:901-907.
88. Flechsig E, Hegyi I, Enari M, Schwarz P, Collinge J, Weissmann C (2001) Transmission of scrapie by steel-surface-bound prions. Mol Med 7:679-684.

89. Hall V, Brookes D, Nacul L, Gill ON, Connor N, Panel CJDI (2014) Managing the risk of iatrogenic transmission of Creutzfeldt-Jakob disease in the UK. J Hosp Infect 88:22-27.
90. Bonda DJ, Manjila S, Mehndiratta P, Khan F, Miller BR, Onwuzulike K, Puoti G, Cohen ML, Schonberger LB, Cali I (2016) Human prion diseases: surgical lessons learned from iatrogenic prion transmission. Neurosurg Focus 41:E10.
91. Brown P, Brandel JP, Preece M, Sato T (2006) Iatrogenic Creutzfeldt-Jakob disease: the waning of an era. Neurology 67:389–393.
92. Maddox RA, Belay ED, Curns AT, Zou WQ, Nowicki S, Lembach RG (2008) Creutzfeldt-Jakob disease in recipients of corneal transplants. Cornea 27:851–854.
93. Heppner FL, Christ AD, Klein MA, Prinz M, Fried M, Kraehenbuhl JP, Aguzzi A (2001) Transepithelial prion transport by M cells. Nat Med 7:976-977.
94. Maignien T, Lasmézas CI, Beringue V, Dormont D, Deslys JP (1999) Pathogenesis of the oral route of infection of mice with scrapie and bovine spongiform encephalopathy agents.
J Gen Virol 80:3035-3042.
95. Beekes M, McBride PA (2000) Early accumulation of pathological PrP in the enteric nervous system and gut-associated lymphoid tissue of hamsters orally infected with scrapie. Neurosci Lett 278:181-184.
96. Beekes M, McBride PA, Baldauf E (1998) Cerebral targeting indicates vagal spread of infection in hamsters fed with scrapie. J Gen Virol 79:601-607.
97. Bencsik A, Lezmi S, Hunsmann G, Baron T (2001) Close vicinity of PrP expressing cells (FDC) with noradrenergic fibers in healthy sheep spleen. Dev Immunol 8:235-241.
98. Banks WA, Robinson SM, Diaz-Espinoza R, Urayama A, Soto C (2009) Transport of prion protein across the blood-brain barrier. Exp Neurol 218:162-167.
99. Taylor DM, McConnell I, Fraser HJ (1996) Scrapie infection can be established readily through skin scarification in immunocompetent but not immunodeficient mice. Gen Virol 77:1595-1599.
100. Thomas JG, Chenoweth CE, Sullivan SE (2013) Iatrogenic Creutzfeldt-Jakob disease via surgical instruments. J Clin Neurosci 20:1207-1212.