Transmissible human proteopathies: an expanding field

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Abstract

Prions are considered the prototype of transmissible proteopathies, and this property has for many decades been considered unique. More recently the transmissibility of other misfolded proteins, notably Aβ, tau and synuclein, has been recognised. Initially, the transmission of these proteins was shown experimentally but the relevance for humans was debated. The co-transmission of Aβ with prions through medical procedures involving preparations derived from cadaveric human tissues, such as human growth hormone treatment, dura mater transplants or the use of neurosurgical instruments carrying traces of Aβ protein has fundamentally changed the opinion in the field. In this article, we will summarise the key features of the most common neurodegenerative diseases involving protein misfolding and their established or potential role in disease transmission.

Glossary:

Amyloid: Aggregation of proteins forming a β-sheet structure. Most, but not all, show Congo red birefringence.

\textit{PrP}\textsuperscript{Sc}: Misfolded (abnormally shaped) normal cellular prion protein (PrP\textsuperscript{C}).

\textit{Prions}: Operational term to designate an infectious particle, containing a protein as an essential component.

Isoforms: Proteins that have a similar, but not an identical amino acid sequence; for tau, all isoforms are produced by the same gene \textit{MAPT}.

Misfolding: Change in the shape of normal cellular proteins.

\textit{Proteopathies}: Diseases characterised by the deposition of misfolded protein. Many neurodegenerative diseases are associated with pathological deposition and aggregation of misfolded proteins.
Introduction

The majority of adult-onset neurodegenerative diseases, such as the most prevalent dementia Alzheimer’s disease (AD) and the most common movement disorder Parkinson’s disease (PD), develop through pathological aggregation of proteins or peptides. They are part of a large spectrum of more than 50 diseases, all caused by misfolding and dysfunction of soluble host proteins [25]. These abnormal proteins have the ability to self-assemble and many of them display amyloid characteristics.

Neurodegenerative diseases are leading causes for disability accompanied by a significant emotional and economic burden to the immediate family, community and society as a whole. The majority of neurodegenerative diseases are sporadic, i.e. are not associated with specific gene mutations. The strongest risk factor for the development of all sporadic neurodegenerative diseases is age. As a result of an increasing life expectancy in the years to come, there will be growing numbers of people with neurodegenerative diseases. Apart from genetic (familial, inherited) forms of neurodegenerative diseases, the underlying biological mechanisms of sporadic neurodegenerative diseases are complex and not fully understood. This complexity contributes to the difficulty to develop effective disease-modifying treatments, and currently, neurodegenerative diseases are incurable and invariably fatal.

After the discovery of the transmissible nature of prion diseases in the 1960’s, it was long thought that such properties are unique to prions, and have led to the establishment of precautionary measures when handling infected tissue [5, 36, 37, 40]. Transmission of other neurodegenerative diseases, although hypothesised years ago, has been much less in the spotlight until recently. Following early studies demonstrating experimental transmission of amyloid-β (Aβ), there was a steady growth of publications which increasingly underpinned the hypothesis of its transmissibility in experimental settings. Only the recent discovery of a possible link between human transmissions of Aβ through medical procedures (human cadaveric pituitary-derived growth hormone (c-hGH), dura mater transplantations and other neurosurgical interventions), suggested that transmission of proteopathic seeds in humans was possible beyond prions.

Here, we will give a succinct overview of neurodegenerative diseases caused by misfolded proteins, review evidence of human transmission of neurodegenerative disease-associated misfolded proteins and discuss how to identify potential at-risk-cases, as well as practical health and safety aspects for pathologists.

Overview of protein misfolding diseases associated with neurodegeneration

Most proteopathies are associated with distinct and variably overlapping clinical phenotypes and neuropathological alterations. A clinical neurodegenerative phenotype caused by misfolded protein accumulation can be broadly grouped into disorders dominated by dementia or by movement impairment. Whilst for some of the diseases additional clinical, imaging or biochemical alterations allow the distinction on clinical grounds, often an accurate diagnosis can only be established by neuropathological examination of the brain. The main proteins or peptides associated with neurodegenerative diseases are Aβ, hyperphosphorylated tau protein (p-tau), α-synuclein and TDP43.

Alzheimer’s disease (AD) is by far the most common neurodegenerative disorder and it is characterised by the deposition of misfolded Aβ peptide and p-tau. During disease progression, both Aβ and p-tau show a distinct but stereotypical anatomical progression across different brain regions, defining the pathological disease stages [29]. However, Aβ and tau spread does not equally correlate
with the clinical progression, in that p-tau load much better corresponds to clinical symptoms than Aβ load. In some ways, this is not surprising, firstly, because p-tau pathology early on affects the medial temporal lobe, a region important for memory formation, and secondly, p-tau forms predominantly intracellular aggregates within neurones, whilst Aβ is deposited mainly extracellularly in the surrounding neuropil. Genetic forms of AD develop only due to mutations in genes involved in Aβ peptide generation from transmembrane APP protein. This discovery was followed by the widely accepted amyloid-cascade hypothesis, postulating that Aβ is required for the initiation, and p-tau for the progression of AD pathology. This hypothesis, which dominated the neurodegeneration field over the last two decades, has recently been challenged, proposing that impaired APP metabolism in general (and not specifically its proteolytic fragment Aβ) may have a much wider role in AD development [24].

**Tauopathies.** There are a number of other neurodegenerative diseases in which p-tau is the sole disease-associated protein. These disorders are referred to as tauopathies. They can broadly be categorised into two groups, based on the dominant p-tau isoform. Under normal conditions, adult human neurones express 6 tau isoforms: three of these isoforms comprise three exons 9, 11 and 12, and are referred to as 3 repeat tau (3R). The other three isoforms express an additional exon 10 and are referred to as 4 repeat tau (4R). In Alzheimer’s disease, p-tau is composed of 3R and 4R tau isoforms. Instead, certain tauopathies, such as progressive supranuclear palsy (PSP), corticobasal degeneration (CBD), globular glial tauopathy (GGT) and argyrophilic grain disease (AGD) are characterised by a strong predominance of 4R tau, whilst another tauopathy - Pick’s disease - is characterised by a dominance of pathologically aggregated 3R tau (Figure 1). The clinical phenotypes of these tauopathies range from frontotemporal dementias to parkinsonian-type movement disorders. The majority of these primary tauopathies occur sporadically. Genetic forms of tauopathies are caused by mutations in the tau encoding MAPT gene, but their histopathological appearance is similar or identical to sporadic forms. An acquired, recently described tauopathy is caused by mild, repetitive head trauma (e.g. in boxers) or even by a single severe head trauma [28]. Like other neurodegenerative diseases, chronic traumatic encephalopathy (CTE) can only be definitively diagnosed by post-mortem examination of brain tissue.

**Alpha-synucleinopathies.** Pathological aggregation of α-synuclein is a hallmark feature of three clinically defined neurodegenerative diseases (Figure 1). In two of them, the misshaped protein is deposited in the cytoplasm of neurones as small globules, called Lewy bodies or within neuronal processes, referred to as Lewy neurites. Lewy bodies and neurites are named after the neurologist Friedrich Heinrich Lewy, who first described them in 1912 (at the age of 27) [34]. Lewy bodies and neurites are diagnostic features of Parkinson’s disease (PD) and dementia with Lewy bodies (DLB). PD initially presents with a characteristic movement impairment which may in some patients be followed by cognitive decline several years later. Instead, DLB initially presents with cognitive impairment and may be followed by movement disorder during disease progression. Of note, Lewy bodies in the amygdala region are occasionally also seen in patients with an advanced AD. Whilst the majority of PD and DLB are sporadic forms, a small proportion of Parkinson’s diseases are familial, associated with mutations in a number of different genes. The third α-synucleinopathy is multiple system atrophy (MSA). In contrast to PD and DLB, the dominant pathology in MSA is in oligodendrocytes rather than neurones. MSA is considered sporadic as no pathogenic mutations have been found to date.

**TDP43 proteopathies.** Displacement of TDP43 from the nucleus to the cytoplasm or into cell processes underlies a majority of motor neurone diseases (MND, also called amyotrophic lateral
sclerosis or ALS) and a proportion of frontotemporal dementias (referred to as FTLD-TDP) (Figure 1). Both MND and FTLD-TDP can be either sporadic or genetic due to mutations in various genes.

Prion diseases are caused by misfolding of the host-encoded prion protein (PrPC) into a pathological isoform (PrPSc). The most common clinical prion disease phenotype, Creutzfeldt-Jakob disease (CJD), is characterised by typically rapidly progressive dementia. The majority of prion diseases are sporadic, with much rarer inherited and transmitted forms. Sporadic CJD (sCJD) develops as a result of a spontaneous alteration of PrPSc, and its conversion into PrPSc. Familial prion diseases are inherited in an autosomal dominant manner, caused by mutations in the PRNP gene. Acquired (transmitted) forms include iatrogenic exposure to prions through medical procedures such as neurosurgical repair with cadaveric dura mater, corneal transplants, use of surgical instruments, treatment of hGH, or dietary exposure. The latter includes ritual endocannibalism in a population in the Eastern Highlands of Papua New Guinea with its peak in the 1950’s. The disease presented as progressive cerebellar ataxia, “kuru” (translating to “trembling” or “shivering”). In the 1980’s the human exposure to BSE (bovine spongiform encephalopathy) prions led to the development of a new strain of prion disease. Clinically, it presents as variant CJD (vCJD) with a distinct neuropathological phenotype and a unique involvement of the lymphoreticular system. vCJD secondary transmission through blood transfusion has been documented in rare instances.

Prion disease transmission – a brief overview

Prion diseases are regarded as the prototype of transmissible proteopathies and were for many decades considered unique. Initially thought to be a slow virus, the unconventional properties of prions have successively been uncovered and characterised over the last five decades. The term “prion” refers to “proteinaceous infectious agent” and was introduced by Nobel Prize winner Stanley Prusiner. The responsible protein, thought to be the sole agent of the infectious particle (the prion), was termed PrPSc (scrapie agent). Following the description and experimental proof of the transmissible nature of prions, the corresponding gene was cloned in 1985 and termed accordingly PRNP, encoding for the gene product PrPC (cellular PrP). In 1966, human prions were transmitted for the first time to non-human primates, by inoculating chimpanzees with preparations from human kuru brains. This was followed in 1968 by experimental transmission of sCJD, again into primates. Since then, experimental transmission of inherited prion diseases has been attempted with varied success. A comprehensive overview of these research studies is given in [6].

The first observation suggesting transmission of prions in humans was documented in 1974 in a patient who had died of prion disease after receiving a cadaver-derived corneal graft less than 2 years earlier. Subsequently, it was found out that the corneal graft came from a patient who had died of autopsy-confirmed CJD [11]. 3 years later transmission through contaminated EEG electrodes was reported [2]. This was followed by further reports of prion disease in patients who received treatments with cadaver-derived tissue (such as extracts of growth hormone or gonadotrophin derived from pooled pituitary glands or dura mater transplants, all obtained during autopsies), or who underwent invasive surgical procedures. These reports triggered the ban of treatment with cadaver-derived hormones in the UK in 1985 and their replacement with genetically engineered peptides. The withdrawal of Lyodura (human cadaver-sourced dural grafts) from the market took place much later, in 1996.

The incubation period of iatrogenic prion diseases varies considerably. Whilst earlier reports [2, 11], documented cases with incubation periods as short as one year, much longer incubation times in the range of four decades have been described more recently in kuru [7] and in iatrogenic CJD (iCJD)
Interestingly, even in patients with long incubation periods once the first symptoms develop the disease typically is of comparably short duration.

**Disease versus disease-associated misfolded protein transmission**

Increasing evidence from experimental studies supports the concept that misfolded proteins or peptides associated with neurodegenerative diseases can act as a seed or template by inducing conformational changes in native proteins, leading to their continuous corruption and misfolding, trans-cellular propagation throughout the nervous system, and progression of clinical symptoms. Given that ageing is the strongest risk factor for neurodegenerative diseases, it is not surprising that incidental Alzheimer’s type Aβ and tau pathology, Lewy body pathology or TDP43 pathology can be observed within vulnerable (susceptible) brain regions even in clinically healthy individuals. Thus, histopathological identification of these aggregates does not equate to neurodegenerative disease carrier status and it remains speculative that these patients would have progressed to develop a disease phenotype, had they lived longer.

A notable exception is the Aβ cerebral amyloid angiopathy (CAA). CAA is seen in a majority of patients with AD and occurs also sporadically with increasing age with no associated AD. CAA can be found either in leptomeninges overlying the brain, within brain parenchyma or both. The Aβ deposition in the blood vessel walls makes them fragile and prone to rupture, causing intracerebral haemorrhages.

Unlike in prion diseases, there is no epidemiological evidence that advanced AD or PD has been transmitted between humans through blood transfusions and other medical procedures [1]. However, the challenge of epidemiological studies to assess potential transmissibility of neurodegenerative diseases such as AD and PD lies in the comparably high prevalence of these diseases in the general population and in the long incubation periods, overlapping with the onset and the slowly progressive clinical symptoms of the sporadic presentation of AD or PD.

**Transmissible proteopathies - animal models and human disease**

**Aβ (one of the hallmark proteins in AD)**

Experimental transmission of Aβ has been demonstrated through intracerebral injections in primates [32] and rodents and nearly all routes of transmission have been successful in mice [8, 14].

In 2015 we provided the first evidence that Aβ may also be transmissible in humans, through medical procedures [22]. Here, we observed a high load of Aβ in parenchyma and vasculature of a proportion of patients who had died from iCJD decades after receiving cadaver-derived human GH (c-hGH) treatment. Until 1985, when the use of c-hGH preparations was banned in the UK, more than 400,000 pituitary glands had been collected, and pools of glands homogenised for the preparation of GH injections. These GH extracts were repeatedly injected intramuscularly over a variable period of time. The contamination of the pool with a single or several glands derived from carriers of sporadic CJD caused the transmission of prions and the development of GH-related iCJD in the recipients. However, the concomitant contamination with Aβ seeds had thus far been undiscovered, until we analysed a cohort of young adults with gh-iCJD, and noted striking parenchymal and vascular Aβ pathology. After we published this mode of transmission, brains of patients who died of dural transplant related iCJD (d-iCJD) were also confirmed to contain Aβ in parenchyma and blood vessels [17]. Similar findings have been published in further cohorts from different countries (Figure 2 and 3). An important corollary to our study [22] is the finding of Aβ pathology in patients who were treated with c-hGH, but did not develop prion disease [33],
reinforcing our hypothesis that the two pathologies (Aβ and prions) developed independently rather than through a co-dependent seeding process (Figure 2) [9, 12, 18].

None of the patients described in the above studies demonstrated signs of AD and none had any significant p-tau or any other protein aggregate. Therefore, it remains uncertain, if any of these patients would have developed AD had they lived longer. None of the above patients were also documented to have any signs of CAA-related haemorrhages.

An additional mode of transmission of prion diseases is through contaminated neurosurgical instruments. By reviewing past medical histories of young adults who had undergone brain biopsies or had died of intracerebral haemorrhages, we have recently identified four young adults who had developed intracerebral bleeds caused by CAA [23]. All patients had in common one or several neurosurgical interventions in their childhood or teenage years for various reasons, such as head trauma, intracranial tumour or malformations. Through a review of the literature, we identified four more case studies of patients who presented with intracerebral CAA-related haemorrhage and had evidence of childhood neurosurgery. A further recent case report documents a fatal CAA-related intracerebral haemorrhage in a young patient who had received a human cadaver-derived dural graft as a child [19].

We and others have shown that Aβ may be contained in pituitary glands [20, 22] or in dura mater [27] and this implies a possible source for transmission. Moreover, Aβ and tau have been found in the c-hGH batches used for GH injection preparations [12]. These studies demonstrate that the cadaver-derived material used for medical procedures and contaminated surgical instruments were indeed the source of Aβ seeds and that iatrogenic CAA may lead to devastating intracerebral haemorrhages.

**Tau, α-synuclein and TDP43**

*In vitro* and/or *in vivo* transmission of tau, α-synuclein and TDP43 aggregates derived from brains from patients with associated neurodegenerative diseases has been demonstrated [38], but at present, human transmission of these proteins has not been described.

**How to identify potential iatrogenic transmission of Aβ**

As discussed above, there is a growing number of *in vitro* studies and animal experiments demonstrating the transmissibility of misfolded proteins implicated in neurodegenerative diseases. There is now strong support for Aβ seed transmission in humans through contaminated human cadaver-derived tissues, and this should prompt consideration of iatrogenic Aβ transmission in all patients with a history of treatment with human cadaver-derived tissues, such as GH or gonadotrophin injections, implantation or administration of any product containing dura mater, or corneal transplants. In analogy to prion diseases, Aβ seed transmission may also occur through contaminated neurosurgical instruments. Hence, potentially in all patients the past medical history of invasive surgical, and notably neurosurgical manipulations may be of relevance in terms of possible proteopathic seed transmission [23].

However, there are two important caveats. First, neurodegenerative proteopathies are highly prevalent in the ageing population, and such high prevalence confounds discrimination from transmitted seeds through prior invasive surgical procedures or administration of human-derived treatments and sporadic cases. Second, all previous observational studies indicate long latencies (more than 2 decades) between the causative intervention and Aβ pathology development. This
implies that an absence of pathology in patients with shorter incubation times does not equate to the absence of transmission.

CAA is a characteristic feature of human Aβ transmission, and CAA is highly unusual in patients under 55 years of age. These two circumstances should prompt further investigation into the cause of CAA in younger people. Once pathogenic mutations in APP, PSEN1 and PSEN2 (genes associated with early-onset Aβ pathology) have been excluded, a critical review and scrutiny of the past medical history, in particular for any evidence of surgical interventions or past medical treatments, is recommended. So far no association of blood transfusions and AD have been observed [3, 10, 26, 30]. Nevertheless, as discussed above, transmission of the misfolded protein or peptide does not necessarily equate to transmission of the associated disease. The limited number of epidemiological studies have looked only for evidence of an advanced neurodegenerative disease based on clinical criteria and these studies have only reviewed medical records but did not correlate with the neuropathological post-mortem findings. Therefore, further studies would be necessary to assess risks of transmission of misfolded proteins associated with neurodegenerative disease.

The risks of transmission - health and safety aspects

The experimental transmission and the circumstantial evidence of human transmission of proteopathic seeds through medical procedures raise questions about safety in relation to handling human tissue and research with transgenic animals.

The safety and transmission aspects of prion diseases have been extensively covered elsewhere and guidelines of best practice have been widely published by official bodies, such as the UK Department of Health and Public Health England (https://www.gov.uk/government/collections/creutzfeldt-jakob-disease-cjd-guidance-data-and-analysis, accessed in September 2018) and this resource is continuously updated. Model systems of the transmission of prions have recently been published in a comprehensive review [6].

For all other transmissible proteopathies, very limited or no guidance is currently available. The human transmission of Aβ occurred more than three decades ago, in the context of administration of c-hGH, and the use of such cadaver-derived GH ceased in 1985 once identified as a source of CJD transmission. Dura mater, another established source of CJD transmission and recently identified source for Aβ transmission, was withdrawn from the market in the 1990’s, but it cannot be excluded that batches of Lyodura stocked in hospitals may have been used for many more years after without adequate documentation, making identification of at-risk patients difficult.

The risk of Aβ transmission through surgical procedures needs to be assessed appropriately. Again, the methods of instrument decontamination and sterilisation have changed over time. It is not possible to reconstruct the procedures used for neurosurgical instrument decontamination 2-3 decades ago, and, therefore, it is currently not possible to draw any conclusions about the safety of current procedures.

For experimental studies involving human tissue in laboratory settings, adherence to good laboratory practice, and observing general health and safety guidelines relevant to handling of human tissue has been considered as appropriate in a recent workshop and expert consultation at the National Institute of Health, (Bethesda, MD USA) May 2, 2018 (Neurodegenerative Disease Transmissibility: Current Science and Recommendations for Future Research, SB personal communication).
It is well established that formaldehyde and standard decontamination and sterilization procedures are insufficient to completely remove infectivity from prion-contaminated instruments and protocols for appropriate decontamination are widely published [5, 13, 21, 36, 40]. There is evidence that Aβ (similar to prions) is resistant to heat and formaldehyde treatment [16] and adheres to stainless steel surfaces [14]. The effectiveness of different commercially available reagents to disassemble Aβ, tau or α-synuclein seeds, adsorbed on non-disposable materials, such as plastic, glass, aluminium or stainless steel surfaces have been recently published [4, 15, 31, 39]. A recent study [15] describes cleaning procedures with commercially available detergents, SDS, TFD4 and Hellmanex (1% in MilliQ water) that are effective in removing α-synuclein, tau and Aβ from non-disposable laboratory tools. However, the detergents TFD4 and Hellmanex corrode aluminium surfaces highlighting the need of taking into account the compatibility between the detergent and the surface when choosing the most appropriate decontamination method. Details of the compatibility and efficacy between different cleaning procedures and laboratory tools in relation to the removal of α-synuclein, tau and Aβ, are published in [15].

Conclusion

In conclusion, there is significant experimental evidence that misfolded proteins implicated in neurodegenerative diseases are potentially transmissible. Apart from the established human transmission of prions, there is also growing evidence that Aβ can be transmitted in humans.

Currently, there is no epidemiological evidence that advanced neurodegenerative diseases could have been iatrogenically transmitted. However, there is strong evidence from observational studies that intracerebral, often fatal, haemorrhages develop as a consequence of iatrogenic transmission of vascular Aβ [19, 23]. Further detailed studies are now required to ascertain the transmission risk of all proteins implicated in neurodegenerative diseases, so that appropriate risk assessments for procedures involving human tissues are carried out and correct, standardised decontamination approaches are used for equipment in clinical and research practice.

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Figure 1: Overview of neurodegenerative diseases associated with misfolded protein aggregation.

Figure 2: **Timeline of published studies on Aβ transmission in humans.** The left column indicates the publication year. The subsequent columns to the right show publications of case series of transmitted proteopathies. Column 2 (dark brown), patients with human cadaver-derived growth hormone (c-hGH) related iatrogenic Creutzfeldt-Jakob disease (iCJD) and Aβ pathology; Column 3 (light brown), patients treated with c-hGH who died of causes other than prion disease but developed Aβ pathology. Column 4 (green), patients who died of iCJD due to dural grafting and had additional Aβ pathology (first two studies from 1996 and 2006 documented Aβ pathology but did not suggest the possibility of iatrogenic Aβ transmission); Column 5 (cyan), patients who received human cadaver-derived dura mater grafts and developed Aβ pathology but did not develop prion disease. Column 6 (blue), patients with past medical history of neurosurgery, who had parenchymal and vascular Aβ but not CJD.
Figure 3: Treatments associated with prion and Aβ transmission in humans. Medical treatments and surgical procedures associated with Aβ and/or prion transmission are shown on the left. The lines in blue demonstrate the treatments or surgical procedures which have resulted in prion transmission and the orange lines denote treatments and procedures resulting in Aβ transmission. Human cadaver-derived corneal grafting, shown on the top left, was the first documented procedure resulting in prion disease transmission, whilst a group of patients who had received human cadaver-derived GH treatment, shown on the bottom left, was the first cohort in which evidence of Aβ transmission was demonstrated.
References:


