Acta neurol. belg., 2001, **101**, 81-87

Invited review

The many faces of human prion diseases in Belgium and the world

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Abstract

Prion diseases are rare neurodegenerative disorders that always lead to death and that can be transmissible under certain conditions. Although sporadic Creutzfeldt-Jakob's disease (CJD) is the best known human variant of these transmissible spongiform encephalopathies with an incidence of about 1 in 106 inhabitants, several other types of human prion disease have been described (e.g. Familial CJD, Gerstmann-Sträussler-Scheinker syndrome, Fatal Familial Insomnia, …). In 1996, a variant of CJD has been linked to the epidemic of bovine spongiform encephalopathy (BSE). Therefore, vigilance concerning prion diseases was increased throughout the whole of Europe. In Belgium, a comprehensive, nation-wide study has been conducted both retrospectively (1960-1997) and prospectively (1998-…) to identify prion disease patients. In 1998, a surveillance system has also been created to monitor the incidence of CJD and other prion diseases. Using data from both studies and the surveillance program, the occurrence and phenotype of all types of prion diseases in Belgium was investigated. The sporadic type of CJD was identified in 116 patients, while 4 suffered from a hereditary form. In our series, we could find no evidence for variant or iatrogenic CJD, neither for the more rare types of prion diseases.

Key words : Transmissible spongiform encephalopathy ; prion protein ; Belgium ; variant ; bovine spongiform encephalopathy ; BSE.

Introduction

This article provides an overview of the different types of human prion diseases or transmissible spongiform encephalopathies (TSE) and their clinical and neuropathological characteristics. In a second part, the occurrence of the different types of human TSE in Belgium are described. Creutzfeldt- Jakob disease (CJD) is the most common human form of the prion diseases. Ten years ago, CJD was an obscure form of dementia unknown to most physicians. The epidemic of bovine spongiform encephalopathy (BSE) and the subsequent recognition of a variant of CJD (vCJD) with the possible link to BSE caused worldwide recognition of the disease. Creutzfeldt-Jakob disease is a neurodegenerative disorder that can be transmissible under certain circumstances (Gajdusek and Gibbs, 1971 ; Gibbs *et al.*, 1968). The disease is characterised by rapidly progressive mental deterioration, always leading to death, often within one year. The causative agent is thought to consist of a post-translationally modified form of a host encoded glycoprotein (the prion protein or PrP^{C}) designated PrP^{Sc}, named after scrapie, the oldest known form of prion disease, which originated in sheep and goat (Fisher *et al.*, 1998). The mechanism that converts PrP^{C} into PrP^{Sc} remains unknown. The TSE or prion diseases also include Kuru, Gerstmann-Sträussler-Scheinker syndrome (GSS) and Fatal Familial Insomnia (FFI) in humans (Parchi and Gambetti, 1995) and Transmissible Mink Encephalopathy (TME), Feline Spongiform Encephalopathy (FSE), Bovine Spongiform Encephalopathy (BSE), and many others in animals (Prusiner and Scott, 1997).

The different human TSE are characterised by a distinct pattern of cause, frequency, symptoms, neuropathological features, clinical tests, incubation period and risk factors. These features will be discussed in detail for each type of human TSE. Depending on the pathogenesis of CJD, 4 different variants can further be differentiated. The most frequent type consists of sporadic CJD (sCJD), which consists of about 80-85% of all cases. Secondly, in about 10% of all cases a familial background can be found (fCJD). The two rarest types are iatrogenic CJD (iCJD), caused by medical interventions, and vCJD.

Sporadic CJD

In the 1920s, two German neuropathologists, Gerhard Creutzfeldt (Creutzfeldt, 1920) and Alfons Jakob (Jakob, 1921a, b), separately described patients suffering from a disorder that had similar neuropathological features : spongiosis, gliosis and neuronal loss. Retrospective analysis of these cases by Masters and Gajdusek revealed that only 2 of the 5 cases described by Jakob and none of the cases described by Creutzfeldt really suffered from CJD (Masters and Gajdusek, 1982). Afterwards, an array of eponyms was applied to similar diseases differing in clinical symptoms or affected brain regions. Only after the experimental transmission of CJD to chimpanzees in 1968 (Gibbs *et al.*, 1968), a rational delineation of the syndrome and acceptance of the designation of CJD became custom.

Most described cases of CJD are sporadic which means that no cause for the disease in these patients has been identified until now. Most sCJD patients suffer from a rapidly evolving dementia. The age of onset range is 50 to 72 years (90% CI) with a mean of 63 years. The mean disease duration of sCJD is 6 months and about 80% of patients die within 1 year after onset of the disease (Brown *et al.*, 1985), (Will and Matthews, 1984). The diagnosis is primordially based on the clinical symptoms of the patient. Roughly about one third of patients initially experience fatigue, disordered sleep or decreased appetite. Another third have neurological symptoms such as memory loss, confusion or uncharacteristic behaviour. The final third have focal signs such as ataxia, aphasia, visual loss or amyotrophy. In all patients, a typical rapid deterioration of cognitive abilities from week to week and in some

Table 1

Diagnostic criteria for sporadic CJD (sCJD)

I		Rapidly progressive dementia
II	A	Myoclonus
	B	Visual or cerebellar problems
	C	Pyramidal or extrapyramidal features
	D	Akinetic mutism
III	A	Typical EEG
	B	positive 14-3-3
Definite sCJD :		neuropathologically/immunohistochemically confirmed case
Probable sCJD :		I and 2 of II and III A and/or B
Possible sCJD :		I and 2 of II and duration less than 2 years

patients even from day tot day is present. Other typical symptoms include myoclonus, particularly startling myoclonus, pyramidal or extrapyramidal signs, and akinetic mutism. These symptoms are therefore the basis of the diagnostic criteria for sporadic CJD (table 1).

Clinical laboratory studies show no evidence of inflammation, no consistent abnormalities of the internal organs, and no antibodies to neutralise the disease agent. The cerebrospinal fluid (CSF) does not show pleocytosis or increased immunogobulins and has a normal or only slightly elevated protein level. Over the last ten years, several CSF proteins have been identified that can aid in the clinical diagnosis of CJD. The immunodetection of the 14-3-3 (Hsich *et al.*, 1996) and tau proteins (Otto *et al.*, 1997b) have been reported to be the most sensitive and specific markers. Increases in neuron specific enolase (Zerr *et al.*, 1995) or S-100 protein (Otto *et al.*, 1997a) have been reported but are less sensitive and specific (Beaudry *et al.*, 1999). Determination of the concentration of CSF amyloid-beta 1-42,43 showed a decreased level that approached the levels found in Alzheimer's disease. On the other hand, normal or elevated levels of CSF amyloid-beta 1-42,43 were found in patients suffering from viral encephalitis or other neurological disorders that might clinically resemble CJD and have a positive 14-3-3 or tau result. Therefore, amyloid-beta 1-42,43 determination in CSF can be helpful in distinguishing true and false positive 14-3-3 or tau results (Van Everbroeck *et al.*, 1999 ; Otto *et al.*, 2000).

Another marker for CJD is the electro-encephalogram, which may be normal or diffusely disturbed at disease onset but later in the disease course displays periodic triphasic synchronous sharp wave complexes superimposed on a slow background rhythm. The typical EEG pattern has a sensitivity ranging from 50 to 67 percent and specificity from 70 to 86 percent (Steinhoff *et al.*, 1996), (Pals *et al.*, 1999), (Zerr *et al.*, 2000b). If repeated recordings are obtained, more then 90% patients may show the typical periodic abnormalities (Chiofalo *et al.*, 1980).

Although the sporadic form is the most frequent, it still is very rare with an incidence range of (90% CI) 0.5 to 1.36 patients per million inhabitants per year. Only a few risk factors have been identified in the literature, namely the presence of idiopathic dementia in the family or methionine homozygosity for the PRNP codon 129 polymorphism (Zeidler *et al.*, 1997). Other reported risk-factors like the consumption of raw beef (vanDuijn *et al.*, 1998) or surgical stress (up to 6 month prior to disease onset) must be looked at with caution since "observer/reporter bias" may play an important role (Zerr *et al.*, 2000a).

Genetically determined human prion diseases : fCJD, GSS and FFI

Between 5 and 10 percent of persons with CJD have a family history consistent with an autosomal dominant inheritance of the disease. The fCJD type was already known for a long time (1930s) but it had to wait until the 80s and the discovery of the prion protein and the prion gene (PRNP) to find the cause. Since then numerous point mutations, 1 stop-codon mutation (V145Stop), and several octarepeat insertions/deletions in the coding sequence of the gene for PrP on the short arm of chromosome 20 have been reported in the literature (Fig. 1). Mutations in PRNP are associated with phenotypes mimicking typical sCJD or induce distinctive progressive diseases with spongiform changes in the brain. In general, fCJD has an earlier age of onset and a more protracted course than sporadic disease. The typical electro-encephalographic changes are often missing, and the 14-3-3 protein is only detected in CSF in about half of cases (Zerr *et al.*, 1998). The topography of the neuropathological lesions varies. In brain of some patients plaques are observed, but the essential changes of vacuolisation of neural cells with gliosis and neuronal loss are generally present. The most common mutation leading to the typical clinical and pathological findings of fCJD is linked to codon 200. Clusters of disease among Libyan Jews in Israel, in a region of Slovakia, and in Chile are all explained by this mutation (Goldfarb *et al.*, 1991). Several mutations lead to phenotypes that have been regarded as different diseases.

Gerstmann-Sträussler-Scheinker disease is an autosomal dominant illness characterised by severe cerebellar ataxia and often paralysis. In some families, myoclonus is not prominent, and dementia may develop late in the course of illness. The disease has a prolonged course of 8 to 11 years, yet the average age at death is only 48 years. The neuropathological findings are distinct, with numerous PrP amyloid plaques throughout the brain (Masters *et al.*, 1981). In two different mutations in kindreds from Indiana (Ghetti *et al.*, 1989) and Sweden, neurofibrillary tangles were found in the cerebel-

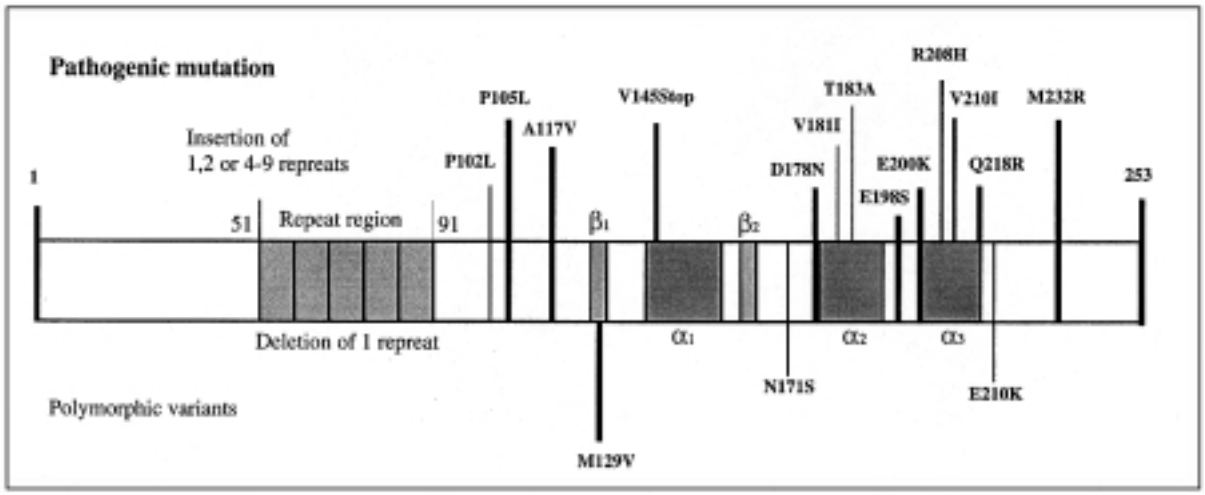

FIG. 1. — Known pathogenic mutations and susceptibility markers in the prion protein gen. Most mutations are located in the regions that are important for its function or the 3D-structure.

lum and neocortex. The most frequent mutation associated with GSS is at codon 102, but the syndrome has also been associated with mutations at other sites. Fatal familial insomnia has an even stranger phenotype. The illness is characterised by progressive insomnia, dysautonomia, and dementia, leading to death in 7 to 15 months after clinical onset. At autopsy, selective atrophy of the ventral and mediodorsal thalamic nuclei is evident (Lugaresi *et al.*, 1998). In some patients, spongiform changes are found in the thalamic nuclei, and immunocytochemical staining for PrP is positive. These findings led to sequence analysis of the gene for PrP in which a mutation at codon 178 was found. However, this mutation had already been found in kindreds with typical fCJD. The association of two distinct phenotypes with the same mutation is explained by the polymorphism at codon 129. In the mutations at codon 178, the methionine allele segregates with fatal familial insomnia and the valine with fCJD. Presumably, this site is important in determining the tertiary structure of PrP^{SC}, and different amino acids at this site can alter the conformation of the prion present in these two diseases (Monari *et al.*, 1994). This hypothesis has been further confirmed by a NMR study of PrP^{c} confirming that both codons are close together in the native structure of PrP^{c} (Riek *et al.*, 1998).

Acquired human TSE : iCJD and kuru

That human prion diseases were transmissible between humans was proven by the knowledge that was provided by kuru. This TSE was identified in a native tribe in Papua New Guinea and was found to be transmitted between humans by ritualistic cannibalism. Iatrogenic CJD is even rarer than sCJD or fCJD with about 150 known cases worldwide. In 1985, CJD developed in four patients who had received human growth hormone, all of them under 40 years of age. At that time, human growth hormone was derived from pooled cadaveric human pituitary glands (Brown *et al.*, 1985). Since then, other causes of iCJD have been identified such as cornea or dura mater transplantation. All of the known transmissions have occurred when the used source was directly associated with the central nervous system.

More than 100 iCJD patients worldwide have been related to growth hormone and gonadotropic hormones. Most cases (about 50%) have been observed in France, where the disease has developed in about 2.5 percent of recipients, with a mean incubation period of 8 years. In the United Kingdom, about 1 percent of recipients have been affected, with a mean incubation period of 12 years. In the United States, the disease has developed in 0.2 percent of recipients (16 cases out of the 8000 patients) (Ridley and Baker, 1996). The differences in the frequency of transmission and the length of incubation probably reflect variable contamination resulting from different protocols for hormone extraction. Nevertheless, in all affected countries, growth hormone-related disease begins with cerebellar ataxia and movement disorders, with dementia developing late. Pathological changes in the cerebellum and basal ganglia are prominent on autopsy. These findings are reminiscent of kuru, suggesting that age at exposure or the route of inoculation might influence the clinical and neuropathological features. The average incubation period of iCJD is variable, depending on the mode of transmission : from 54 months after dura implantation to 12 years after injection with growth hormones. The age of onset is also strongly dependent on the kind of treatment with a range from 11 years (growth-hormone treatment) to 75 years

(dura mater transplantation) (Collinge and Palmer, 1994 ; Parchi and Gambetti, 1995 ; Prusiner and Hsiao, 1994).

Variant CJD

Because of concern about cross species transmission in the United Kingdom, a national surveillance unit for Creutzfeldt-Jakob disease was established in 1990. No unusual cases were noted during the first four years of monitoring, but a new type of CJD was first described in 10 patients in 1996 and was called (new) variant CJD (Will *et al.*, 1996). Between 1994 and 2000, 94 cases of vCJD disease were reported in the UK, three in France and one in Ireland. Most patients are younger than those with sCJD (average 29 years, range 14-74 years). The symptoms of vCJD involve prominent early psychiatric and behavioural manifestations and persistent pain without an obvious cause. Cerebellar ataxia uniformly develops and the course of the disease is prolonged to an average of 18 months. The electroencephalogram fails to show typical periodic complexes. All these characteristics are represented in a set of diagnostic criteria, which differ strongly from those of sCJD (Table 2) (Will *et al.*, 2000). The pathological examination shows prominent and flower- like PrP plaques, reminiscent of kuru. None of the patients with vCJD disease have had mutations in PRNP, but all have been homozygous for methionine at the codon 129 polymorphism (Ironside *et al.*, 1996 ; Collinge *et al.*, 1996). All patients had eaten meat, although one had become a strict vegetarian in 1991. None had knowingly eaten brains, but before the specified offal ban, brain and spinal cord were regularly included in sausages, hamburger, and processed meats. The oral intake of meat products contaminated with BSE before the ban on the use of ruminant-derived feed in cattle farming and the ban on specified offal, has been proposed as the origin of these cases (Cousens *et al.*, 1997).

Table 2

Diagnostic criteria for variant CJD (vCJD)

I	A	progressive neuropsychiatric disorder
	B	duration of illness >6 months
	C	routine investigations do not suggest other diagnosis
	D	no history of potential iatrogenic exposure
II	A	early psychiatric symptoms
	B	persistent painful sensory symptoms
	C	ataxia
	D	myoclonus or chorea or dystonia
	E	dementia
III	A	EEG does not show the (typical) appearance of sCJD
	B	posterior thalamic high signal on MRI scans
IV		positive tonsil biopsy
Definite vCJD		1 (a) and neuropathologically confirmed case of vCJD
Probable vCJD		I and 4/5 of II and III A and III B (and IV)
Possible vCJD		I and 4/5 of II

Recent laboratory studies provide strong evidence that the causative agents of vCJD and BSE have a common origin. Glycosylation patterns of PrP^{Sc} and susceptibility studies in mice showed that the patterns in brain tissue of vCJD patients and animals with BSE are similar and are distinct from the patterns associated with sCJD and iCJD (Hill *et al.*, 1997). In inbred mouse strains, different strains of scrapie have distinctive incubation periods and a different topographical distribution of lesions. Transmission studies to mice of the agents of BSE, vCJD and the spongiform encephalopathy of exotic ruminants and cats show similarities in incubation periods and in the distribution of lesions, which are distinct from those of sCJD (Bruce *et al.*, 1997).

Human prion diseases in Belgium

Patient population

After the description of vCJD and subsequent experiments establishing the link with bovine spongiform encephalopathy in 1998, a surveillance network for CJD was formed in Belgium. Our laboratory has performed a retrospective study from 1960 to 1997 (Pals *et al.*, 1999 ; Van Everbroeck *et al.*, 2000). We also started a prospective study in which we test cerebrospinal fluid samples as aid to differential clinical diagnosis and perform neuropathological investigation of patients suspected of a prion disease (mostly sCJD). Of all referred samples, a clinical history is available. The clinical, demographic, CSF, and neuropathological data were stored in a database (Filemaker Pro) together with the results of the CSF investigation. Additional follow-up information has been integrated into this database. The reported numbers below are extracted out of our database completed with information provided by the surveillance network. All patients described before the first of January 2001 were analysed. The data presented in this article is under constant remodelling as new information and new patients will be added in the future.

Clinical and neuropathological characteristics

On a total of 144 patients suspected of a human prion disease, from 120 cases clinical and neuropathological data was available and the definite diagnosis confirmed in the period form 1960 tot 2000 in Belgium. Neuropathological investigation was performed for the presence of gliosis, neuronal loss, spongiosis, and prion immunohistochemistry (Van Everbroeck *et al.*, 2000). From 75 patients the codon 129 polymorphism was investigated and from 30 patients also the entire sequence of PRNP was examined. Two families with each 2 affected family members were identified that showed dom-

inant inheritance of the disease and in one of the families an insertion of seven octarepeats was found in the PRNP (Dermaut *et al.*, 2000). All other cases (n = 116) could be identified as sCJD patients, based on clinical and neuropathological examination. The 14-3-3 test resulted in a sensitivity of 100% and 87% specificity. The average disease duration of the sCJD patients was 9.6 months for women and 6.7 for men. This apparent shorter disease duration for men was found to be statistically significant (p = 0.0254 ; Mann-Whitney U-test). Of 114 sCJD patients the sex was known, 72 patients were female and 42 were male resulting in a sex ratio of 1.67 (female/male). This shows a preponderance of women who develop the disease. The average age at death was 63 ± 10 (mean ± SD) years. The average age at death was 63 for women and 62 for men, indicating no difference in age of death between the sexes. In order of frequency the following initial symptoms were found : disturbed equilibrium (36%), psychiatric problems (32%), cognitive disturbances (32%), and motor abnormalities (16%).

Incidence of human prion diseases in Belgium

We investigated the incidence of human prion diseases in Belgium. No patients were found with a clinical history and a disease course suggestive of iCJD or vCJD. Furthermore, no patients with a clinical history or a genetic mutation in PRNP linked to GSS or FFI were identified. We did identify 4 fCJD patients (3% of all cases). In all other cases, a diagnosis of sCJD could be made. We calculated the incidence of sCJD patients from 1997 to 2000 in number of afflicted cases/10^6/year (1997 : 0.78 ; 1998 : 0.98 ; 1999 : 1.27 and 2000 : 0.69) in Belgium. Due to the rare nature of CJD, large differences might occur between sequential years ; however the average incidence of the four last years (0.98) represents a good overview of the Belgian situation which is compatible to the average worldwide incidence of 1 case/10^6/year.

Conclusions

Different pathways ranging from a genetic mutation over the consumption of infectious tissue to an accidental transmission of the agent in a medical procedure can cause human prion diseases. These diseases give rise to a large variety of symptoms ranging from insomnia, depression and psychiatric disorders to dementia, ataxia, and akinetic mutism. They can occur in persons in almost all age categories from early teenagers to the elderly (> 80 years).

The clinical and neuropathological characteristics of the Belgian TSE patients are very similar to those found in the rest of the world, although only a low number of genetic TSE cases (3%) were identified compared to the rest of the world (10% to 15%). No patient with clinical or pathological features of iCJD or vCJD has ever been identified in Belgium. The average incidence of CJD in Belgium over the last four years is 0.98 patients per million inhabitants, corresponding to the worldwide average.

Despite all the clues that have been provided by iatrogenic and familial CJD and by the likely transmission of BSE to humans, the cause and progression of sCJD that makes up to 85 to 90 percent remains a mystery. Past exposures, dietary habits, occupational risks, recreational activities, pets, surgical stress, and a myriad of other factors that were suggested at one time to be linked with CJD, provide no aid in establishing the diagnosis and direction in which the cause can be sought.

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