# Could novel genomic alterations clarify phenotypic heterogeneity and aetiology in human prion diseases?

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## Abstract

Prion diseases, or transmissible spongiform encephalopathies, are a group of rare fatal invariably neurodegenerative diseases that occur in humans and animals. Prion diseases are characterized by neurodegeneration that generally have a sponge-like appearance. The infectious prion protein forms aggregates and accumulates in tissue. A unique feature is the manifestation of three prion disease forms; sporadic, acquired and genetic. High levels of heterogeneity are observed in the disease phenotype, age at onset (sporadic and genetic) and incubation period (acquired). In the majority of cases there is an absence of positive family history. For sporadic forms there is no known aetiology, the cause of acquired forms is by transmission and the genetic forms by mutations in the *PRNP* gene. Age at onset is an important factor in the disease and an improved understanding of the reported variance is necessary. Here, an in-depth literature review is presented on the phenotypic heterogeneity in regards to the age at onset and incubation period in human prion diseases. Modifying factors and novel genomic alterations concerting the phenotypic heterogeneity are discussed. In addition, novel genomic alteration concerting the aetiology of sporadic human prion diseases is discussed.

## Introduction

Prion diseases, or transmissible spongiform encephalopathies (TSEs), are a group of rare fatal invariably neurodegenerative diseases that occur in humans and animals. The brain of humans and animals that have been affected by various forms of TSE are characterized by the accumulation of protease-resistant aggregates of the infectious prion protein (Bolton *et al*., 1982). The TSEs cause neurodegeneration in several parts of the brain, in general producing a distinctive sponge-like (spongiform) appearance (Prusiner, 1998). A hallmark of prion diseases is the prolonged, clinically silent, incubation period.

Well-known examples of TSE are bovine spongiform encephalopathy (BSE) a prion disease in bovines, commonly known as 'mad cow disease', and scrapie a prion disease in sheep and goats. In humans, prion diseases can be classified in three forms; sporadic, acquired and genetic (Table 1). A form of Creutzfeldt-Jakob disease (CJD) is present in all three forms and the majority of CJD cases occur sporadically with no evidence of infectious or genetic aetiology. Different transmission routes can result in an acquired form of prion disease. Genetic or inherited forms are caused by autosomal dominantly inherited mutations in the *PRNP*. High levels of heterogeneity are observed in all forms of prion diseases in the clinical presentation, age at onset (sporadic and genetic forms) and incubation time (acquired forms) (Lukic and Mead, 2011). The incubation period is defined as the moment of exposure to the infectious agent until symptoms of disease appear. The age at onset is defined as the age at which an individual develops symptoms of the disease. In a high proportion of cases (50-90%) there is an absence of positive family history in prion diseases (Kovacs *et al.*¸ 2005).

Sporadic Creutzfeldt-Jakob disease (sCJD) was the first recognized form in the early 1920s and accounts for 85% of all human prion diseases, occurring at a rate of one per million per year (Masters *et al.*, 1979). The risk of developing sCJD increases with age and over the past ten years an increase of sCJD incidence is reported (NCJDRSU, 2012). After identification of sCJD two other sporadic cases of human prion diseases have been established; sporadic Fatal Insomnia (sFI) and the Variably Protease-Sensitive Prionopathy (VPSPr) (Parchi *et al.,* 1999; Gambetti *et al.*, 2008). Hypotheses about the sporadic prion disease aetiology include somatic mutation of *PRNP*, undetected horizontal transmission and the spontaneous conversion of the prion protein in the misfolded form (Gajdusek, 1977; Colby and Prusiner, 2011).

**Table 1.** Classification of human prion diseases.

| Form | Human prion disease |
|---|---|
| **Sporadic** | Sporadic Creutzfeldt-Jakob disease |
| | Sporadic Fatal Insomnia |
| | Variably Protease-Sensitive Prionopathy |
| **Acquired** | Kuru |
| | Variant Creutzfeldt-Jakob disease |
| | Iatrogenic Creutzfeldt-Jakob disease |
| **Genetic** | Familial Creutzfeldt-Jakob disease |
| | Fatal Familial Insomnia |
| | Gerstmann-Sträussler-Scheinker disease |
| | Prion protein cerebral amyloid angiopathy |

Acquired prion diseases account for nearly 5% of human prion disease cases (McKintosh *et al.*, 2003). In the 1950s, the first acquired form of prion disease, kuru, was recognized by a Western physician in a native population in Papua New Guinea (Gajdusek and Zigas, 1957). Kuru is acquired through dietary exposure (consumption of infected human tissues) and caused an epidemic outbreak of prion disease in this region. Dietary exposure through the consumption of BSE infected meat results in a different type of CJD called variant CJD (vCJD). Acquired human prion diseases can additionally be obtained by accidental exposure to the infectious prion protein during medical and surgical procedures causing iatrogenic CJD (iCJD).

Genetic prion diseases account for approximately 10-15% of cases in human prion diseases and are caused by mutations in the *PRNP* gene which are inheritable. Four forms currently known are familial Creutzfeldt-Jakob disease (gCJD), Fatal Familial Insomnia (FFI), Gerstmann-Sträussler-Scheinker disease (GSS) and the recently introduced prion protein cerebral amyloids angiopaty (PrP-CAA) form. In the late 80s, gCJD was the original form to linked to mutations in *PRNP*, decades later after the original case was recorded (Kirschbaum, 1924; Goldgaber *et al.,* 1989; Owen *et al.*, 1989). To date, over 40 different mutations in the *PRNP* gene have been identified as the causal role in genetic prion disease. Numerous mutations are extremely rare or are restricted to specific population groups resulting in four mutations accounting for approximately 70% of all the genetic diseases (Kovacs *et al.*, 2002; Kovacs *et al.*, 2005; Mead 2006; Colby and Prusiner, 2011). The mutations induce different phenotypes of disease; E200K as a CJD phenotype, D178N as a CJD or FFI phenotype and P102L consequently a GSS phenotype. The octapeptide repeat insertions (OPRI) can present in a typical sCJD phenotype, a GSS-like phenotype or a blended phenotype of both sCJD and GSS (Jansen, 2011).
The single-nucleotide polymorphism (SNP) located at codon 178 resulting in the D178N point mutation is linked to both gCJD and FFI depending on the amino acid, valine or methionine, positioned at codon 129 (Goldfarb *et al.*, 1992). Therefore, FFI is distinguished from CJD on a molecular level at codon 129 displaying a methionine residue at the mutated allele in contrary with gCJD where a valine residue is displayed (Goldfarb *et al.*, 1992). GSS is a distinctive form characterized by prion amyloid deposits and predominantly caused by the P102L mutation (Ghetti *et al.*, 2003). The recently established PrP-CAA for is phenotypic distinct form GSS and has only been reported in a few patients (Ghetti *et al.*, 1996; Ghetti *et al.*, 2003; Revesz *et al.*, 2009; Jansen *et al.*, 2010). Various mutations in genetic human prion diseases are incompletely penetrant (Kovacs *et al.*¸2005).

### *Prion protein*

For several decades the causative agent of prion diseases was inconclusive. The nature of transmission was initially attributed to a 'slow virus' and in 1982 Stanley Prusiner proposed that the disease is caused by a transmissible infectious particle, the prion protein, and suggested the prion hypothesis (Gajdusek, 1966; Prusiner, 1982). The prion hypothesis states that the infectious agent in TSEs is composed of the misfolded form of the prion protein, which replicates in infected individuals conversion of the normal version of the prion protein and is currently completely accepted. The normal non-infectious prion protein is referred to as $PrP^{C}$ and the misfolded prion protein is denoted as $PrP^{Sc}$ (superscript Sc referring to scrapie) (Prusiner, 1998).
The conversion changes the secondary structure of the protein from a predominantly alpha helix to a beta sheet rich form. The misfolded protein and aggregates of misfolded prion protein causes prion disease. Important in the prion replication process is the breakdown of $PrP^{Sc}$ aggregates in

subsequent seeding-competent polymers that amplify the prion replication process resulting in rapid and exponential accumulation of $PrP^{Sc}$ (Saborio *et al.*, 2001). The exact function of the protein remains unknown, although it is located throughout the body with the majority in the central nervous system.

Prions can be identified based on their properties and phenotypic characteristics, producing different prion strains. A prion strain is an infectious isolate that, upon inoculation into genetically identical hosts, causes a prion disease with consistent phenotypic characteristics, for example the incubation period (Fraser *et al.*, 1973; Aguzzi *et al.*, 2007)
In addition to the prion strain diversity, strains occur in different forms of glycosylation. $PrP^{c}$ and $PrP^{Sc}$ exist in three particular glycosylation states, called glycoforms: unglycosylated, monoglycosylated and diglycosylated forms, creating a wide variety of over 400 different forms (Endo *et al*., 1989). It is generally accepted that different glycosylation patterns of $PrP^{Sc}$ construct complexes composed of di-, mono-, or unglycosylated moieties, in ratios that are characteristic to each prion strain. It is poorly understand how the different glycoforms are related to disease characteristics and strain properties (Jansen, 2011).

The homogenization of prion disease affected brains and incubation with proteinase K (a protease) results in the identification of two major human $PrP^{Sc}$ subtypes which initially can be observed in patients with sCJD; type 1 and type 2 (Parchi *et al.*, 1996; Parchi *et al.*, 1997). The ratio of the $PrP^{Sc}$ glycoforms, together with the type of $PrP^{Sc}$ and the genotype at codon 129, are major determinants of the disease phenotype and can serve as surrogate 'strain-typing' markers (Jansen *et al.,* 2012).
The genotype at codon 129 of *PRNP* (MM, VV or MV) and the strain type (1 or 2) have led to the identification of six subtypes of sCJD, with two exceptions: the MM1 and MV1 cases are merged into one category due to phenotypically homogeneity and the MM2 subjects are divided into two subtypes, were one subtype is known as sFI. Additionally, the co-occurrence of type 1 and type 2 in 12-44% of sCJD patients divides the molecular classification into pure subtypes and mixed subtypes (Parchi *et al.*, 2009).

### *Prion protein gene (PRNP)*

The prion protein is encoded by the human gene *PRNP*. The human *PRNP* is a single copy gene located on the short arm of chromosome 20 and contains two exons. The second exon entirely contains the open reading frame (ORF) and encodes the prion protein of 253 amino acids (figure 1) (Puckett *et al.*, 1991).
The N-terminal domain of *PRNP* contains an octapeptide repeat (OR) region between codon 51 and 91, which contains five copies of the OR. The five copies are composed of one nonapeptide followed by a tandem repeat of four copies of an octapeptide (Kretzschmar, 1986). In addition, the N-terminal end contains a signal sequence for endoplasmic reticulum targeting. The protein is posttranslationally processed and sustains glycosylation at two sites, residues 181 and 197, accounting for the three glycoforms of the protein (Haraguchi *et al.*, 1989). At the C-terminal end, a fragment is removed during the addition of the glycosylphosphatidylinositol (GPI) anchor on the serine residue at position 231, attaching the protein to the cell membrane (Stahl *et al.*, 1990). The single-nucleotide polymorphism (SNP), a polymorphic site, at codon 129 containing valine (V) or methionine (M) at codon 129 is a major determinant of susceptibility in human prion diseases. The codon 129 SNP is linked to several other mutations in *PRNP* denoted as 129M or 129V.

MANLGCWMLVLFVATWSDLGLCKKRPKPGGWNTGGSRYPGQGSPGGNRYPPQGGG
GWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQGGGTHSQWNKPSKPKTNMK
HMAGAAAAGAVVGGLGGYVLGSAMSRPIIHFGSDYEDRYYRENMHRYPNQVYYRP
MDEYSNQNNFVHDCVNITIKQHTVTTTTKGENFTETDVKMMERVVEQMCITQYER
ESQAYYQRGSSMVLFSSPPVILLISFLIFLIVG

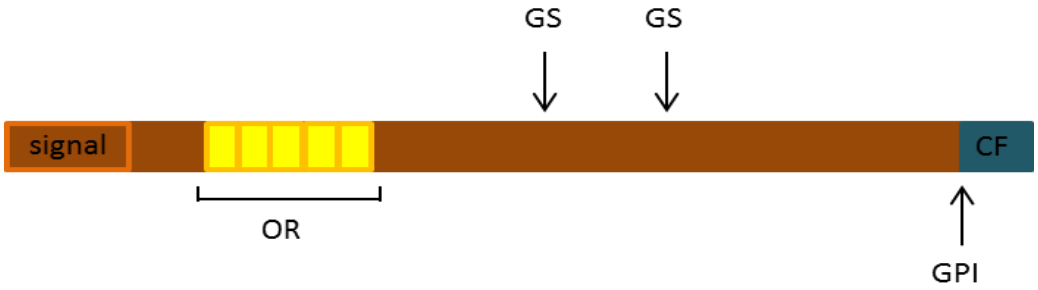


**Figure 1. Amino acid sequence and schematic overview of the prion protein.** In the amino acide sequence (top) the fice octapeptide repeat sequences and the serine residue at which the GPI-anchor is attached are underlined. In the schematic overview (bottom) at the N terminal end is a signal sequence and five octapeptide repeats (OR); the two glycosylation sites (GS) are depicted, the place where the GPI anchor (GPI) is added and the C terminal fragment (CF) that is cleaved off.

### *Protein misfolding disorders (PMDs)*

Prion diseases were the first disorders in which a misfolded protein is indicated to be the cause of disease and the acceptance of the prion concept took several decades. A misfolded protein is defined as 'any partially-structured conformational state of a protein or polypeptide that is distinct from both the well-structured native state and the fully unstructured, conformationally heterogeneous unfolded state' (Mulligan and Chakrabartty, 2013). The concept of prions, accumulation and aggregation of misfolded proteins resulting in disease, is presently not restricted to prion diseases; in fact a considerable group of various human diseases, called protein misfolding disorders (PMDs), have been recognized over the past decade. Over twenty PMDs have been identified, including neurodegenerative disorders like Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD) and Amyotrophic lateral sclerosis (ALS).

The transmission of misfolded proteins has only been noticed in prion diseases, however recently the possibility of transmission in these neurodegenerative protein misfolding disorders have been addressed (Soto, 2012). Prion diseases and similar PMDs are generally fatal and incurable, no effective treatment exists and there is been an increase incidence reported in neurodegenerative PMDs in developed countries (Pritchard *et al.*, 2013). Currently, the concept of prions is even thought to be influential in cancer and cardiac diseases (Silva *et al.*¸2010; Willis and Patterson, 2013).

High levels of phenotypic heterogeneity are observed in age at onset (sporadic and genetic) and incubation period (acquired) in human prion disease. For sporadic forms of disease there is no known aetiology. Prion diseases presently are unpreventable, are untreatable and are generally fatal and incurable. Moreover, as life expectancies are rising and different causes of death are successfully treated, susceptibility of prion disease will probably increase and it may become a higher incidence in

causes of death. An improved understanding of these concepts is desirable to potentially develop a treatment for prion diseases and similar neurodegenerative PMDs.

Misfolded proteins are consequently presumed to be involved in cancer and cardiac diseases, leading to new attention to the prion concept and a desirable improved understanding of the 'original' prion misfolding disease. Prion diseases represent the most complicated case of PMDs due to the unique characteristic that they manifest in sporadic, acquired and genetic forms. The age at onset or incubation period are an essential variable in the diseases.

Here, an in-depth literature review is presented on the phenotypic heterogeneity in regards to the age at onset and incubation period in human prion diseases. Modifying factors and novel genomic alterations concerting the phenotypic heterogeneity are discussed. In addition, novel genomic alteration concerting the aetiology of sporadic human prion diseases is discussed. Could novel genomic alterations clarify phenotypic heterogeneity and aetiology in human prion diseases?

## Sporadic human prion diseases

Sporadic CJD cases are the major group of CJD cases without evidence of an infectious or genetic aetiology. Sporadic CJD has an incidence of approximately one per million per year (Masters *et al*., 1979). The peak age at onset is approximately 60 years with a wide range of up to 90 years; a few sCJD cases have been reported in individuals under the age of 20 (Murray *et al.,* 2008). Six subtypes, MM1/MV1, VV2, MV2 2K, MM 2C, MM 2T and VV1, have been identified, for which the age at onset and disease duration differ. The majority of the subtypes have a mean age at onset of approximately 60 years (MM1/MV1: 63.2; VV2, MV2 2K and MM C2: 60.3 years), except the subtype VV1 which may include patients with an early onset of disease and average age at onset of 46 years (Jansen, 2011). The majority of the subtypes have a mean disease duration extended over one year, except for the MM1/MV1 and VV2 were disease duration is respectively 3.8 and 6.6 months (Jansen, 2011).
In sporadic cases homozygosity at codon 129 were predisposed and MM homozygotes resulted in an earlier death than MV heterozygotes in the Caucasian UK population (Palmer *et al.*, 1991). The M allele is subsequently correlated with 'early death' and the V allele is correlated with delayed death (Baker *et al.*, 1991).

For the extremely rare form of sporadic prion diseases subtype MM 2T, named sFI, fourteen cases are described in literature and account for approximately one percent of all sporadic prion disease cases (Mastrianni *et al.*, 1999; Parchi *et al.*, 1999; Scaravilli *et al.*, 2000; Gambetti et al 2003; Piao *et al.*, 2005; Capellari *et al.*, 2008; Priano *et al*., 2009; Moody *et al.*, 2011; Luo *et al.*, 2012).
Identification is based on the absence of a mutation in *PRNP*, since sFI is virtually phenotypic indistinguishable from FFI, a prion disease related to a mutation in *PRNP* (Parchi *et al.*, 1999; Montagna *et al.*¸ 2003). The mean age at onset is approximately 60 years and the mean duration 14 months. Interestingly, one case has been reported by Lou *et al.* (2012) were the patient with sFI was linked to an asparagine to serine substitution at codon 171 (N171S) linked with valine at codon 129 in one allele and a 24 base pair deletion linked with methionine at codon 129 in the other allele.

In addition to these six subtypes of sporadic prion diseases, in 2008 a novel sporadic disease was identified; protease-sensitive Prionopathy (PSPr), subsequently renamed to variably protease-sentitive prionopathy (VPSPr) (Gambetti *et al*., 2008; Zou *et al.*, 2010). In total 33 patients have already been recorded developing the disease (Gambetti *et al.*, 2008; Head *et al.,* 2009; Jansen *et al.*¸2010; Zou *et al.*, 2010; Rodriquez-Martinez *et al.*, 2010; Head *et al.,* 2010; Head *et al.*, 2013).
The first 11 patients reported were homozygous for valine (VV) at codon 129 of *PRNP* (Gambetti *et al*., 2008), however further cases of MV and MM were identified subsequently (Zou *et al.,* 2010). Mean age at onset and disease duration were 67 years (range 48 – 81) and 30 months (range 7 – 72), respectively (Zou *et al*., 2010).
The rarity of 129 MM subject is in contrast to the observation in sCJD, as the majority of cases is reported for VSPSr are 129 VV homozygous (Zou *et al.*, 2010). Additionally, codon 129 significantly influences the mean age at onset and the duration in disease, 129VV and 129MV groups had a mean age at onset of respectively 65 and 72 years and duration of 23 and 45 months (Zou *et al.*, 2010).

## Acquired human prion diseases

The first acquired form of human prion diseases, kuru, primarily affected women and children due to participation in consumption of predominantly the brain of deceased family members. Incubation periods are difficult to determine, as time of infection is generally unknown. Based on predictions, the shortest incubation period is an estimated five years and ranges between five to over 60 years (Collinge *et al.*, 2006). The mean clinical duration of illness is twelve months (range 3 - 23), although in a few atypical cases it may be considerable longer and tends to be concise in children (Alpers, 1964).

In kuru, the to date prion disease with the longest known incubation periods, homozygosity at codon 129 was associated with earlier age at onset of the disease and a shorter duration of illness than in heterozygosity patients (Cervenakova *et al.*, 1998). Additionally, the concise incubation period of MM homozygosity in kuru was confirmed by Lee *et al.* (2001) and has resulted in human resistance for prion disease in the native population (Atkins *et al.*, 2013). An additional study on kuru patients having extremely lengthy incubation periods (up to 50 years) revealed that eight out of twelve were heterozygous at codon 129 and presented an extended disease duration compared to homozygous patients (Collinge *et al.*, 2006).

To date, 172 cases of variant CJD have been confirmed in the UK and an additional 47 additional cases have been confirmed in ten different countries (Ironside, 2010). Depending on the period of transmission of BSE to humans, the incubation period can be estimated. The probable period of transmission was around 1984-1989, leading to the incubation period for the early cases of vCJD by transmission of BSE seem to be in the order of ten years (Collee *et al.*, 2006).

A new route of transmission has been revealed in 2004, when transmission occurred after a blood transfusion of an asymptomatic person with vCJD in the United Kingdom as part of the aftermath of the 1980s BSE outbreak (Llewelyn *et al.*, 2004). Currently, four cases have been recorded where iCJD was contracted after blood transfusions (Llewelyn *et al.,* 2004; Peden *et al.*, 2004; Health Protection Agency, 2006; Wroe *et al.*, 2006). Three of the patients died due to the iCJD and had an incubation period between six to eight years (Llewelyn *et al.*, 2004; Health Protection Agency, 2006; Wroe *et al.*¸2006). Concerns have been raised if cases of iCJD will increase in the following decades due to blood transmission of asymptomatic donors revealed to have vCJD (Bishop *et al.*, 2006).

The MM genotype at codon 129 is detected in all patients with probably and definite vCJD, except for one debated case (Kaski *et al.*, 2009; Brandel *et al.*, 2010; Ironside, 2010; Haik and Brandel, 2011). This could indicate a possible protection effect of the other genotypes (VV and MV) (Ironside, 2010; Haik and Brandel, 2011). However, as the vCJD epidemic in the UK occurred in the 90s and incubation period in kuru in 129 heterozygotes have been observed to occur after 50 years, cases of vCJD in VV and MV population could still be identified after several decades. The mean incubation period of vCJD is suggested to be extended to 30 years or lengthier and a second wave of vCJD infections is then plausible (Collinge *et al.*, 2005; Valleron *et al.*, 2006). The extended incubation period might favor secondary transmission (Haik and Brandel, 2011). Options to keep in mind is that the vCJD cases in VV and MV could simple be unrecognized due to occurrence of subclinical forms or unrecognized as the incubation time could exceed a natural life span (Hill *et al.*, 2000; Garske and Ghani, 2010). In mice is has been observed that transgenic mice models expression MV at codon 129 were still susceptible to vCJD infection, although less efficient than MM homozygotes (Saba and Booth, 2013). The suggestion that subclinical forms might conceal MV and VV infected cases in the manifestation of a different phenotype is observed in primates (Krasemann *et al.*, 2013). Infection

with BSE was able to cause prion amyloidosis in the heart of primates, together with a remarkably prolonged duration of the disease (Krasemann *et al.*, 2013).

Two major sources of iCJD, responsible for over 95% of all cases, are respectively dura mater grafts and human-derived growth hormone therapy (Brown *et al.*, 2012). Additional sources of infection, surgical instrument and corneal transplants, have to date led to a few cases in specific countries (Brown *et al.*, 2012).

The largest outbreak of iCJD following dura mater grafts has been in Japan, accounting for 132 cases until 2008 (CDC, 2008), presumably due to the fact that non-sterilized grafts were still used here for an extended time period compared to other countries (Triendl, 1997; Hannah *et al.*, 2001). The source of contamination with prions predominantly originates from Lyodura® grafts which were processed before 1987. The mean incubation period is 11 years (ranges 16 months to 23 years), for which the mean in Japan is 11.8 years (Brown *et al.*, 2012; CDC, 2008). The shortest incubation period is fourteen years and the longest incubation period 25 years (CDC, 2008).

Three countries have had large breakouts of iCJD caused by administration of human cadaver-derived growth hormones, respectively France, United Kingdom and United States (Brown *et al.*, 2012). Approximately half of the cases were reported in France with the final case to date in 2009 (Brandel *et al.*, 2013). Incubation periods appear to be longer in growth hormone cases compared to the dura mater grafts cases, being on average 17 years, ranging between five to 42 years (Brown *et al.*, 2012). Interestingly, the average incubation period in France is seven to nine years shorter in comparison with the United Kingdom and United States (Brown *et al.*, 2012).

In iCJD acquired by growth hormone therapy an extended incubation period is associated with codon 129 heterozygosity in the French cases (Deslys *et al*., 1998; Huillard d'Aignaux *et al.*, 1999).

The association was similarly observed in UK patients, where growth hormone acquired iCJD patients homozygous at codon 129 shortened the incubation period (Brown *et al.*, 2000). Remarkably, in dura mater transplantation iCJD patients no association between codon 129 and the incubation period was observed (Brown *et al.*, 2000).

Two cases of iCJD (one definite and one probable) after corneal transplant surgery have been identified and a striking variability in incubation period of 30 years and 1.5 years is observed (Duffy *et al.*, 1974; Heckmann *et al.*¸ 1997). In the 1970s two patients in Switzerland attracted iCJD through the use of contaminated electrodes during stereotactic electroencephalographic (SEEG) exploration (Bernoulli *et al.*, 1977). The incubation periods of these patients were sixteen and twenty months (Bernoulli *et al.*, 1977).

## Genetic human prion diseases

Incubation periods and phenotypes in genetic prion diseases have a widely range, for example a reported German pedigree with point mutation D178N with the age at disease onset ranging from nineteen to 72 years and included an asymptomatic 73-year-old gene carrier (Synofzik *et al.*¸ 2009). In the four forms the diseases are currently classified according to the haplotype based on the *PRNP* mutation and codon 129 on the mutant allele (Gambetti *et al.*, 2003).

Genetic CJD compared to the sCJD there has an earlier age at onset of the disease in familial cases and an extended duration of illness. The disease typically manifests between 30-50 years, although a few individuals commenced prior the age of 30 years. The disease duration ranged from a few months to several years (Jansen, 2011). In gCJD the mutations E200K and D178N mutations are predominantly present.

In several genetic CJD cases a correlation was established between the age at onset and codon 129. Genetic CJD with VV homozygosity at codon 129 are constituted to have a rapid disease course (Gambetti *et al.*, 1995). Although in Libyan Jews with CJD, no correlation between age at onset of the disease and the codon 129 was established (Gabizon *et al*., 1994).

In gCJD the age at onset was earlier in valine homozygous individuals in comparison to methionine homozygous persons and, to a lesser extent, in MV compared to MM (forms V210I and E200K not included) (Kovacs *et al.*, 2005). In the two kindred in the UK homozygosity, either MM or VV, is correlated with an earlier age of onset in mutation P102L and A117I (Webb *et al.*, 2008).

The GSS form has an age at onset typically in the fifth or sixth decade, however can be 25 years and age at onset is significantly earlier in comparison to gCJD (Kovacs *et al.*, 2005). The average disease duration is around five to six years, ranging from three months to thirteen years (review by Collins *et al.*, 2001). The most common mutation in GSS is the P102L, which has a mean age at onset of around 40 (range 22 - 71) years and disease duration of five and a half (ranges 3 - 12) years (Chi *et al.*, 2010; De Michele *et al.*, 2013). An exceptionally long duration of seventeen years is described in a GSS case with the G131V mutation (Jansen *et al.*, 2011).

In the largest kindred with inherited prion disease P102L, codon 129 modifies the age at onset: the earliest eight clinical onsets were all MM homozygous and the overall age at onset was 7 years earlier for the MM compared to the MV heterozygotes (Webb *et al.*, 2008). Similarly, a different age at onset is noted with the *PRNP* codon 129 homozygosity (VV) in A117V kindred (Webb *et al.*, 2008).

In the Indiana kindred of GSS with mutation F198S homozygosity of VV is correlated with an earlier age at onset of disease (Dlouhy *et al.*, 1992).

The mutation P102L is the only prion disease for which cases in monozygotic twins have been reported. Two cases were reported both showing a considerable variance in age at onset of seven years and eight years (Hamasaki *et al.*, 1998; Webb *et al.*, 2009).

In FFI there is only one known mutation, D178N-129M, causing the disease (Lugaresi *et al.*, 1986). FFI age at onset is generally in the fifth decade, while ranges from twenty to 63 years and as observed in GSS is significantly earlier in age at onset compared to the gCJD cases (Kovacs *et al.*, 2005). The average disease duration is around thirteen to fifteen months (ranges six to 42) (review by Collins *et al.*, 2001). Although an age at onset under 30 years has been reported in fifteen patients worldwide and four patients had an age at onset of under twenty (Harder *et al.*, 2004; Dimitri *et al.*, 2006; Yu *et*

*al.*, 2007; Shi *et al.*, 2012). Seven out of these fifteen patients under 30 years had a Chinese ethnicity and three out of the four patients under twenty years had a Chinese ethnicity.
In FFI there is no influence of codon 129 on the age at onset, however codon 129 influences the disease duration (Gambetti *et al.*, 2003; Harder *et al.*¸2004; Montagna *et al.,* 1998; Parchi *et al.,* 2009*)*. Homozygous patients MM at codon 129 have a shorter disease duration (mean 11 months) than MV heterozygotes (mean 23 months) (Montagna 1998, Gambetti and Russo, 1998, Gambetti *et al.*, 2003).

Octapeptide repeat insertions (OPRI) are inserted repeat regions after codon 51 in the *PRNP* gene.
OPRI are one of the four most common mutations in genetic prion diseases and typically exhibit an earlier age at onset and a prolonged disease course compared to alternative mutations in *PRNP* (Mead *et al.*, 2006). Four-OPRI are the smallest insertional mutations of *PRNP* that are evidently pathogenic (Kaski *et al.*, 2011). Smaller 1-OPRI and 3-OPRI are exclusively pathogenic incidentally and are observed in healthy control populations (Beck *et al.*, 2010). 4-OPRI are not fully penetrant, 5-OPRI and 6-OPRI are fully penetrant by the age 70 and might act as a 'pathological treshold' as in general 5-OPRI and a larger number of OPRI cause disease. 4-OPRI have an older age at onset than several other inherited prion diseases, it is possible that the age at onset of 4-OPRI with 129MV exceeds the human lifespan or the presence of 129V on the wild-type allele prevents stable generation of prion in 4-OPRI (Kaski *et al.*, 2011).
The average age at onset for 4-,5-, 6- and 7-OPRI are respectively 60, 46, 35 and 29 years (Kaski *et al.*, 2011; Mead *et al.*, 2007; Mead *et al.*, 2006; Jansen *et al.*¸2011). The number of repeat regions is related to the age at onset and disease duration, additional inserted repeat regions result in an earlier age at onset and an extended disease duration (Croes *et al.*, 2004; Mead *et al.*, 2007). Recently, a pedigree (including three cases) is identified of harboring a 12-OPRI and having a mean age at onset of 44 years (Kumar *et al.*, 2011). The 12-OPRI would represent an outlier in the relation between the age at onset and the number of octapeptide repeats as calculated by Croes *et al.* (2004).
In 4-OPRI repeats heterozygosity at codon 129 is associated with an earlier age at onset relative to MM and VV genotypes (Sanchez-Valle *et al.*, 2012). In 5-OPRI and 6-OPRI heterozygosity at codon 129 is associated with a delayed age at onset compared to homozygosity (Poulter *et al*., 1992; Mead *et al*., 2007; Webb *et al*., 2008; Webb *et al.*, 2009). A correlation between the age at onset and the age at death was observed between parents and offspring in a kindred with a 6-OPRI (Webb *et al.*, 2008). Croes *et al.* (2004) observed an earlier onset in the MM genotype and a delayed onset in the VV genotype when comparing to the MV genotype in 1-OPRI to 9-OPRI.

## Misfolded proteins and disease

Protein misfolding disorders (PMDs) are a group of diseases characterized by misfolded proteins, aggregation of misfolded proteins and accumulation of aggregates in the tissues. The proteins involved in different PMDs are dissimilar in sequence and structure; however the proteins share the pathological mechanisms as observed in prion diseases, for example resistance to proteolysis and in generally the production of beta-sheet rich oligomers (Soto, 2003; Chiti and Dobson 2006). In the neurodegenerative PMDs, AD, PD, HD and ALS, the prion-like proteins have been named 'proinoids' and the predominant difference with the prion protein is the absence of appearance to cause transmission of disease although the question if transmission is possible has recently been raised (Aguzzi *et al.*, 2007; Soto 2012).

### *Neurodegenerative protein misfolding disorders*

AD, PD and ALS have a sporadic form of the disease, which in all cases represents the majority of the cases with approximately 90% (Martin *et al.*¸ 1999; Chen *et al.*, 2013). Similarly observed in prion diseases, were the majority is sporadic and there is no known aetiology.

AD is classified in an early-onset form and a late-onset forms; early-onset AD represents fewer than five percent of all people who have AD and has an age at onset of 30 to 60 years, and the majority are of the familial form. Late-onset AD has an age at onset typically after 60 years and has been linked to different forms of the apolipoprotein E (*APOE*) gene, however the ApoE is not restricted to diseases and is not fully penetrant (Genin *et al.*, 2011).

In AD amyloid plaques are present and their predominant component is the Aβ protein (Glenner and Wong, 1984). In prion diseases a similar form of deposits to the amyloid plaques of AD can be characterized (Ghetti *et al*., 1989;, Rozemuller *et al.*, 2012). Furthermore, aggregates of tau protein play a role in the disease and are observed in degenerating neurons (Grundke *et al.*, 1986). Tau deposits are observed in several prion diseases, including GSS, PrP-CAA, and FFI (Jansen *et al.*, 2011; review by Spillantini and Goedert, 2013). Aβ has been identified as the protein component of the plaques observed in AD. Familial forms of this disease were linked to mutations in the precursor of Aβ, amyloid precursor protein (APP), and the presenilins, which cleave APP to form Aβ (Goate *et al.*, 1991; Sherrington *et al.*, 1995; Levy *et al.*, 1995). In addition duplications and triplication of APP has been described (Rovelet-Lecrux *et al.*¸2006)

In PD the majority of individuals who develop the disease are 60 years of age or older. However, in addition to the late-onset PD, an early-onset form (between 21-40 years) and a juvenile onset form (before age 21) are identified. PD is characterized by aggregates called Lewy bodies, consisting of fragments of a protein named α-synuclein (Forno, 1996; Polymeropoulos *et al.*, 1997; Spillantini *et al.*, 1997). The autosomal dominant forms of PD are caused by missense mutation in α-synuclein, duplication and triplication of the α-synuclein gene (review by Corti *et al.*, 2011). The triplication of α-synuclein gene causes a severe form of PD subject to an early onset (Singleton *et al*., 2003).

HD has a wide range of age at onset, from infancy to seniors, however usually between 35 and 44 years of age. HD is caused by expansions of CAG repeat sequences in the *HTT* gene, resulting in extended glutamine repeat regions in the huntingtin protein. HD is characterized by deposits of this polyglutamine-rich version of the huntingtin protein (DiFiglia *et al.*, 1997). The polyglutamine expansion acts as a pathological threshold and the length of the expansions are correlated with the age at onset (Lee *et al.*, 2012).

In ALS a predisposing mutation results in a disease age at onset ranging between 25-65 years and the sporadic cases typically with a delayed age at onset in the range of 40-80 years. In a familial form of ALS, representing ten percent of all familial cases, patients have aggregates, predominantly composed of superoxide dismutase (SOD1) (Bruijn *et al.*¸1998). The hexanucleotide repeat expansion in the first intron of chromosome 9 open reading frame 72 is the most common genetic cause of familial ALS (Renton *et al.*, 2011).

The familial forms of the neurodegenerative diseases AD, PD, HD and ALS typically have an earlier age at onset, have an increased severity and are associated with a larger load of protein aggregates in comparison with sporadic cases (Hardy *et al.*, 1998).

### *Transmission in prion misfolding disorders*

In acquired human prion diseases the aetiology is the transmission of the infectious $PrP^{Sc}$ to another individual. Currently, transmission of genetic human prion diseases has been unreported. Several studies involving risk assessment of transmission in sCJD and secondary vCJD cases are conducted to analyze if there is a realistic threat of transmission. In addition, the question if other neurodegenerative PMDs could be transmissible as in prion diseases has been raised (Soto *et al.*, 2012).

The findings of transmission of AD by blood transfusion *in vivo* and neuron-to-neuron transmission of Aβ, Tau, SOD1 and α-synuclein aggregates are two major factors in favor of this theory (Desplats *et al.*, 2011; Clavaguera *et al.*, 2009; Nath *et al.*, 2012; Grad *et al.*, 2011; Morales *et al.*, 2012).

A major argument against the transmissibility of other prion misfolding disorders is that there is no known epidemiological evidence. Although, epidemiology in these diseases is complicated by the heterogeneity and extreme long period between exposure and the onset of symptoms, and misfolded aggregates composed of different proteins may interact with each other, causing exposure to a certain misfolded protein to the development of another PMD (Soto *et al.*, 2012).

## Genetics influences

Genetics contribute to the incubation period of human prion disease. In mice models, if experimental conditions are kept constant, genetics are the predominant determinant to the length of the incubation period (Lloyd *et al.*, 2011).

### *Prion diseases in mice*

Mice are used as a model for human prion disease research. Comparative genomics suggests that the mouse and human genome are similar enough that genes discovered in mice studies are likely to be present in humans and to have similarities in biochemical and cellular mechanisms (reviewed by Lloyd *et al*., 2011). Several studies established a large range of inbred lines of mice and indicated that each mouse strain has a characteristic incubation time for a defined prion strain (Lloyd *et al*., 2011; Dickinson *et al.*, 1964; Kingsbury *et al*., 1983; Westaway *et al.*, 1987; Carlson, 1988).

The major genetic determinant of incubation time in mice is the gene *Prnp*, which is located on chromosome 2 and consists of three exons (Oesch *et al*., 1985). Other genes have furthermore been identified has being a significant determinant to variation observed between different inbred lines of mice. Genes suggested to contribute to the incubation time in mice, are *Hectd2, Cpne8, Mmu14, Hspa13, SOD1* (Lloyd *et al.*, 2001; Lloyd *et al.*, 2009; Lloyd *et al.*, 2010; Grizenkova *et al.*, 2010; Grizenkova *et al*., 2012; Akhtar *et al.*, 2013).

### *Human prion diseases*

In humans the *PRNP* gene is linked to prion diseases and is linked to the age at onset and incubation period which are discussed below. The well-studies polymorphism of *PRNP* codon 129 is associated with almost every human prion diseases and influences the age at onset and incubation period as described in previous chapters. Half of the total heterogeneity of age at onset or incubation period is thought to be explained by this SNP (Webb *et al.*, 2008).

The presence of the genotypes (MM, VV and MV) differs between Europe and Asia. In the majority of the European countries and the United Kingdom the genotype distribution of the MM and MV genotype ranges between 40-50% and the VV genotype accounts for approximately ten percent (Collinge *et al.*, 1991; Salvatore *et al.*, 1994; Windl *et al.*, 1999; Lucotte and Mercier, 2005). In several Asian countries, Taiwan, China, Japan and Korea, the MM genotype is the predominant type, observed in 80 to 97% of the population, the MV genotype is observed in three to 18% of the population and the VV genotype is completely absent or up to two percent present in the population (Doh-ura *et al.*, 1991; Tsai *et al.*, 2001; Okhubo *et al.*¸2003; Jeong *et al.*, 2004; Yu *et al.*, 2004).

Alternative SNPs are linked to age at onset in human prion disease, for example rs1460163 in kuru, vCJD and sCJD (Mead *et al.*, 2009).

In gCJD homozygosity at codon 200 had an earlier age at onset compared to heterozygosity (Simon *et al.*, 2000). A missense mutation at codon 114 causing gCJD identified in a Uruguayan family is associated with a remarkably early age at onset (Rodriguez *et al.*, 2005).

*Hectd2,* first established to influence incubation period in mice, is furthermore associated with human prion diseases and shorter incubation periods (Lloyd *et al.*, 2009). SNPs of *Hectd2* are associated with sporadic and variant CJD and kuru in humans, and an increase in expression is associated with a susceptibility genotype and disease pathogenesis in the British population (Lloyd *et*

*al.,* 2009). In contrary, associations of *Hectd2* and sCJD were undetectable in the Korean population (Jeong *et al.*, 2011).

A SNP located upstream of the *STMN2* gene, was associated with kuru incubation time and resistance to kuru. This risk genotype was associated with vCJD and was in the first instance not linked to sCJD however conferred to an earlier age at onset in a follow-up analysis (Lukic and Mead, 2011).

## Epigenetics and remaining influences

Although, genetic factors already explain a great level of heterogeneity in incubation period and age at onset, additional factors should be considered to contribute in differences. Epigenetics and several remaining factors could additionally influence the incubation period or age at onset in prion diseases.

*Prion diseases in mice*

Sex plays a role in the incubation period in mice and the most current study suggest a shorter incubation period for females however only in particular prion strain and type (Akhtar *et al*., 2011). The age of infected mice at the time of infection and the maternal age at birth of these animals were correlated with incubation periods; an older age leads to a longer incubation period (Manolakou *et al*., 2001).

The place of infection influences the incubation period, injections in the brain generate shorter incubation periods than intraperitonael and shorter in intraspinal compared to intracerebral (Kimberlin *et al.*, 1987; Bartz *et al.*, 2003). Injection in the cerebellum generates shorter incubation periods compared to other sites in the brain (Kim *et al.*¸1990). Injections in the tongue were longer than intracerebral and nerve inoculation, although were shorter in comparison to oral ingestion, intraperitoneal, intravenous and intramuscular inoculations (Bartz *et al*., 2003).

Despite the fact that prion diseases will always lead to disease and 'there is no safe dose of prions', dose-response curves can be created and indicate a relationship between dose and incubation period in mice and in cattle (Fryer and McLean, 2011; Konold *et al.*, 2012)

*Human prion diseases*

In humans there has hardly been research into other factors that could be involved, as it is difficult to determine multiple variables involved and studies performed in mice models are impossible.

Different effect due to the sex of the patients are uncommon, although the age at onset has been indicated to be 2 years lower in female patients with vCJD compared to male patients (Loeuillet *et al.*, 2010). Racial differences were described in sCJD, where non-hispanic whites had a significantly delayed age at onset compared to other racial/ethnic groups (Appleby *et al*., 2012). Probably due to the fact that Japan has increased number of CJD cases, as the MM genotype at codon 129 is located in 97% of the Japanese people (Collins *et al.*, 2006).

Brain weight showed to have a strong correlation with incubation period; a higher brain weight causes a shorter incubation period (Bae *et al.*, 2012).

Environmental factors have a considerable influence in the study of monozygotic twins. Although, historically monozygotic twins were considered to be genetically identical and disconcordance was linked to environmental factors, recently it has been established that they harbor (small) genetic differences (Bruder *et al.*, 2008; Maïti *et al.*, 2011). As mentioned above, two cases of prion disease monozygotic twins have been described in previous studies; both associated with the P102L mutation causing GSS and a large variety in age at onset (Hamasaki *et al.*¸1998; Webb *et al.*, 2009). In addition, in monozygotic twins with PD, HD and ALS novel genomic alterations have been detected, which are discussed in extended detail in the following chapter (Nørremølle *et al.*, 2004; Bruder *et al.*, 2008; Pamphlett and Morahan, 2011).

Despite the fact that monozygotic twins are not genetically similar, they are still an excellent basis in discovering important factors determining phenotype, in addition to the major disease gene, and

shed light on the low penetrance or high age at onset variance in prion diseases (Ketelaar *et al.*, 2011).

## Novel genomic alterations

In genetic human prion diseases genomic alterations are recognized as the cause of the disease. Currently, only point mutations and polynucleotide insertions and deletions restricted to the open reading frame of *PRNP* are recognized as to cause gCJD. Novel forms of genetic variations like copy-number variations (CNVs) and somatic mosaicism may play a role, as they are undetectable by conventional sequencing. Genetic CJD is often clinically indistinguishable from sporadic cases and approximately half of the human genetic prion diseases have an absence of positive family history of human prion disease (Kovacs *et al.*, 2005). Diagnosis of sporadic CJD is partly based on the absence of mutations in the *PRNP* or exclusion of a positive family history of prion disease in first degree relatives.

### *Copy-number variations*

CNVs are genomic regions that have duplications, triplications or deletions of genomic material and are an understudied aspect in genomics. CNVs are relatively common and are nonrandom distributed across the genome and associated with a wide range of diseases. Duplication of *PRNP* is expected to increase PrP expression up to one and a half times (McNaughton *et al.*, 2012). Transgenic mice engineered to overexpress *Prnp* (>8 times) do not develop clinically evident spontaneous prion disease in their normal life span, despite having very short incubation times when inoculated with prions (Fischer *et al.*, 1996).

In AD an extra copy of the APP gene is almost invariably associated with the early development and age at onset of AD (Wisniewski *et al*., 1985; Sleegers *et al.*, 2006; Shaw *et al.*, 2011). APP duplication has been detected in a Finnish family with autosomal dominant early-onset AD and CAA, a phenotype that is observed in prion diseases (Rovelet-Lecrux *et al.*, 2007).
In PD, CNVs has been reported in the *SNCA*, where *SNCA* triplication patients have an approximately ten year earlier onset and shorter disease duration than duplication carriers (Singleton *et al.*, 2003; Kasten and Klein, 2013).

Two studies have been published concerning CNVs in human prion diseases; both were unable to establish the relationship of CNVs and prion disease in cases of sCJD (Collins *et al.*, 2010; McNaughton *et al.*, 2012). The first study, Collins *et al.* (2010) screened Australian and Dutch patients with sCJD, specifically patients with an earlier age at onset (mean 65.4 years) and the second study, McNaughton *et al.* (2012) tested British cases of sCJD with an average age of 55 years. No further studies have been recorded in respect to CNVs in other forms of human prion diseases. The observation that *PRNP* duplications have been undetected to date could indicate that it causes an extremely rare cause of the disease (McNaughton *et al.*, 2012). A possibility could be that CNV may be the underlying aetiology of sCJD solely in the presented cases with a very early age at onset before 20 years of age. These cases are extremely rare and only seven cases have been identified worldwide (Brown *et al.*, 1985; Berman *et al.*, 1988; Kulczycki *et al.*, 1991; Petzold *et al.*, 2004; Murray *et al.*, 2008;). Absence of CNVs could indicate that *PRNP* duplications are not a cause of human prion disease or that the severity of the phenotype prevents development of prion disease (McNaughton *et al.*, 2012).

### *Somatic mutations and mosaicism*

Somatic mutations occur in somatic cells postzygotically and are called *de novo* mutations, due to the fact that the mutations are undetectable in the parents of the affected individual. If a somatic mutation occurs in utero, all of the cells descended from the somatic cell will carry the mutation, resulting in somatic mosaicism. Somatic mosaicism is the presence of increased number of genetically distinct cells in an individual and only a subset of their cells harboring the mutations. Mutations can therefore be undetectable with common DNA analysis techniques as not all cells will harbor the mutations. Somatic mutations can result in a variety of conditions and are specifically associated with cancers (Bamford *et al.*, 2004).

Somatic mutations may be widespread in the brain, in the form of aneuploidy or retrotransposon insertions, perhaps as part of its 'normal' development (Rehen *et al.*, 2005; Muotri *et al.*, 2006; Baillie *et al.*, 2011). Somatic mutation has previously been suggested as a cause of sporadic neurodegenerative disorders (Pamphlett, 2004; van Broeckhoven, 2010). In addition, somatic mutations can occur postdevelopmentally and are referred to as 'age-related somatic mutation', which are known to play a role in cancer and have been suggested to play a role in normal aging processes as well as in neurodegeneration (Jacobs *et al.*, 2012; Kennedy *et al.*, 2012).

Aneuploidy may contribute to disease progression in neurological diseases. Increased aneuploidy has recently been associated with AD, as AD is observed in nearly all Down syndrome individuals by the age of 40, and AD patients (without Down syndrome) have an increase of aneuploidy cells in the brain. Siegel and Amon (2012) speculated that aneuploidy might be involved in plaque formation by neurons, as the brain is considered a naturally aneuploidy organ.

First case of somatic and germline mosaicism in sporadic early-onset AD was reported in 2004, the patient harbored *de novo* mutation P436Q of 8% in peripheral blood cells and 14% in brain cells (Beck *et al.*, 2004). Beck *et al.* (2004) additionally observed a clear gene dosage effect on age at onset. The age at onset of the individual harboring the mosaicism P436Q mutation is 42 years compared to the age at onset in individuals harboring the P436Q is in their late twenties (Beck *et al.*, 2004; Taddei *et al.*¸1998).

Proukakis *et al.* (2013) suggests that in PD, inherited mutations may lead to the highest risk and earliest onset; whereas somatic mutations very early in neuroectodermal development could lead to a similar situation, subsequently originating affecting fewer neurons might result in late-onset disease, and those with the last neuronal development could result in clinically silent incidental Lewy bodies disease. In PD this is consistent with the observation that, in general, the age at onset is earlier and the severity increases in those who have inherited a *SNCA* mutation than in sporadic cases and would lead us to expect a higher prevalence of somatic mutations and higher mutation load in younger-onset cases (Proukakis *et al.*, 2013).

In HD, the CAG repeat length is associated with the age at onset of disease (ref) and somatic instability of the CAG repeat was determined to be a significant determinant of the age at onset, with larger repeat length associated with an earlier disease onset a phenomenon called anticipation (Swami *et al.*, 2009).

The octapeptide repeat region of PRNP has been discovered to be instable and supports the first evidence of somatic octarepeat mutation-based model for human sCJD aetiology (Li et al., 2011).

In ALS it has been proposed by Frank (2011) that the similarity between sporadic and inherited cases suggests that the sporadic cases may possibly commence from an initiating focus of cells with genetic mutations. If most cases derive from either inherited or developmental mutations, then the risk of ALS and perhaps other neurodegenerative diseases may be set very early in life. If sporadic cases generally derive from developmental mutations, then somatic genomics will reveal an association between age at onset and the fraction of cells that carry a predisposing somatic mutation. The inherited cases would simply be the extreme of the risk continuum, in which all cells carry the predisposing mutation.
In general, the age at onset in prion diseases is earlier in genetic cases often between 30 and 50 years and in sporadic cases around 60 years with a wide range of up to 90 years (Jansen, 2011). Although, severe disease duration is typical in sCJD a significant minority of cases have atypical features and disease durations of several years (Collins *et al.*, 2004; Jansen, 2011).

Recently, one case of somatic mosaicism of a mutation in *PRNP* has been reported for the cause of sporadic CJD and was caused by a post-zygotic mutation and was identified by the presence of three alleles for *PRNP*. *De novo* mutation identified is D178N, occurring in 97% of the peripheral blood and brain cells suggesting the mutation occurred at an early stage of embryogenesis (Alzualde *et al.,* 2010).

## Conclusion and discussion

Human prion diseases comprise of at least ten different types of diseases divided into three categories; sporadic, acquired and genetic. High levels of heterogeneity are observed in the age at onset and incubation period. Additionally, mutations in gCJD are not fully penetrant and there is no known aetiology for sporadic prion diseases. Prion diseases and alternative PMDs are generally fatal and incurable, no effective treatment exists and there is been an increase reported in prion diseases and alternative neurodegenerative PMDs in developed countries. An improved understanding of the age at onset, incubation period and aetiology of prion diseases will hopefully lead to the development of a treatment.

A major determinant of the age at onset and incubation period in sporadic, acquired and genetic prion diseases is in general the genotype (MM, VV or MV) present at codon 129 of the *PRNP* gene. In Asian countries the percentage of the V allele is extremely limited and no associations have been observed.
A few mutations in alternative genes have been reported to influence the age at onset in prion diseases and alternate factors are associated with the incubation period, for example place of infection and brain weight.
Somatic mosaicism has been recorded in one case of sCJD and associated with age at onset in alternative neurodegenerative PMDs. CNVs have been observed and associated with age at onset and aetiology in alternative neurodegenerative PMDs.

The investigation of the potential contribution of novel genomic alterations to neurodegenerative disorders is in its infancy and large-scale analysis of brain DNA is needed to test the hypothesis to establish novel genomic alterations indeed contribute to phenotypic heterogeneity in all prion diseases and are underlying aetiology in (a proportion of) sporadic and genetic CJD. Novel genomic alterations could clarify the phenotypic heterogeneity and aetiology in human prion diseases.

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