# Mechanisms of phenotypic heterogeneity in prion, Alzheimer and other conformational diseases

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mutations has either a mixed phenotype which includes features of both CJD and GSS or a pathological phenotype with no distinctive features. The form acquired by infection consists of three phenotypes that are also quite distinct, namely the iatrogenic CJD, kuru, and the new variant CJD reported almost exclusively in the United Kingdom.

A major interest of our group has been the study of the mechanisms that regulate the phenotypic heterogeneity. The understanding of these mechanisms may lead to a classification of prion diseases based on the causes rather than the effects of this heterogeneity. Such classification is likely to be more accurate and may result in a wider detection and more precise diagnosis of these diseases.

## 1. Introduction

One of the challenges faced by clinicians and pathologists is dealing with the variability of the clinical signs and of the pathological changes that are associated with diseases. The truism that two patients with the same disease are never identical poses no serious diagnostic problems under most circumstances. However, there are diseases that present considerable variability in their clinical and pathological phenotype, which not only makes the diagnosis difficult but also raises the issue of the mechanisms that regulate it.

While phenotypically Alzheimer disease, amyotrophic lateral sclerosis and Huntington chorea have been considered fairly homogenous diseases, prion diseases include several clinically and pathologically distinct conditions (Table 1) [33]. Until a few years ago, the sporadic form of prion diseases included only the Creutzfeldt-Jakob disease (CJD) phenotype. The inherited form included familial CJD, fatal familial insomnia (FFI), and Gerstmann-Sträussler-Scheinker disease (GSS). These three phenotypes are quite different and have very unique features. In addition, a fourth subtype of familial prion diseases associated with insertion

## 2. Molecular, cell biology and pathology of the prion protein

The mature normal or cellular prion protein ($PrP^C$) in humans is a glycoprotein that has 209 residues (residues 23 to 231), two sites of N-glycosylation, a disulfide bond and a glycolipid anchor [36,44,47]. The glycosylation is non-obligatory leading to the presence of three PrP glycoforms, which carry two, one or no sugar chains and migrate upon gel electrophoresis at 33–42 kDa, 28–30 kDa and 27 kDa, respectively [9, 12,28]. The full-length $PrP^C$ molecule can arbitrarily be divided into three regions based on the NMR structural studies of recombinant PrP [16,27,37]: a) the N-terminus region, which encompasses approximately the first 90 amino acids, is flexible and unstructured; b) the central region, which is located approximately between amino acid 110 and 125, and is hydrophobic and lacks a well defined secondary structure; c) the C-terminus region, which includes three $\alpha$-helices and two short anti-parallel $\beta$-strands, and forms a well-structured core domain.

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Table 1
Human prion diseases

| Form | Phenotype |
|---|---|
| Sporadic | Creutzfeldt-Jakob disease |
| | Sporadic fatal insomnia |
| Inherited | Creutzfeldt-Jakob disease |
| | Fatal familial insomnia |
| | Gerstmann-Sträussler-Scheinker disease |
| | Mixed or lacking distinctive features |
| Acquired | Iatrogenic CJD (medical procedures) |
| by infection | Kuru (contaminated food) |
| | New Variant CJD (contaminated food?) |

Following the addition of the anchor, the disulfide bond and the sugar chains in the endoplasmic reticulum (ER), $PrP^{C}$ is transported along the secretory pathway to the Golgi apparatus, where the sugar chains are modified, and then to the plasma membrane where the three $PrP^{C}$ glycoforms are anchored to the extracellular space [6,10]. They are then internalized and cleaved at residue 111/112, probably in the endosomal-lysosomal compartment, reinserted into the membrane by the anchor and then finally reinternalized and degraded [12, 20,43].

According to the prion hypothesis [13,36], the central event in the pathogenesis of prion diseases is a change in conformation of $PrP^{C}$, which is converted into an isoform referred to as protease-resistant PrP ($PrP^{res}$) or scrapie PrP ($PrP^{Sc}$) that is pathogenic, infectious and in part resistant to proteases. It is believed that the predominantly $\alpha$-helical region in $PrP^{C}$ is converted into a predominantly $\beta$-structure [11,29, 41]. The molecular location of the newly formed $\beta$-structure in $PrP^{Sc}$ is not known, but it is thought to involve the first $\alpha$-helix and the two existing $\beta$-sheet structures, approximately between residues 120 and 170 [22,24]. The cellular locale in which the conversion takes place is also not known, but it is believed that the conversion starts at the plasma membrane and is completed in the endosomal-lysosomal compartment following internalization [10]. Finally, the $PrP^{C}$ to $PrP^{Sc}$ conversion is thought to result from the direct physical interaction between $PrP^{Sc}$, either acquired by infection or spontaneously formed, and $PrP^{C}$ (36). This interaction would occur either following the formation of a $PrP^{C}$-$PrP^{Sc}$ heterodimer or between $PrP^{C}$ and PrP aggregate. In these models, $PrP^{Sc}$ would act as a "template" or a "seed" and induce (or catalyze) the $PrP^{C}$ to $PrP^{Sc}$ conversion triggering an exponential conversion cascade [23].

## 3. $PrP^{Sc}$ associated with human prion diseases

The acquisition of the resistance to enzymatic proteolysis has introduced a formidable diagnostic test for prion diseases [3,4]. Following treatment with proteases, such as proteinase K (PK), $PrP^{C}$ is completely degraded. However, only the N-terminus region of $PrP^{Sc}$ is degraded by PK, leaving undigested C-terminal fragments. These PK-resistant fragments (also called $PrP^{Sc}$ 27–30) include three glycoforms and can be easily detected with immunoblotting. After examining brain tissues from a large number of patients affected by various prion diseases, we observed two sets of $PrP^{Sc}$ fragments generated by the PK treatment [28, 32]. The two sets of fragments differ in their electrophoretic mobility on polyacrylamide gels. Following enzymatic deglycosylation of $PrP^{Sc}$ with peptide: N-glycosidase F (PNGase F, which eliminates the heterogeneity of the $PrP^{Sc}$ bands related to the presence of the glycoforms), one type of the $PrP^{Sc}$ fragment, that we called type 1, has a gel mobility of 21 kDa; the other, identified as type 2, has a gel mobility of 19 kDa. N-terminal sequencing by automated Edman degradation and epitope mapping have demonstrated that the type 1 $PrP^{Sc}$ fragment commonly starts at residue 82 while type 2 $PrP^{Sc}$ starts at residue 97 [35]. Both types have an intact C-terminus linked to the lipid anchor [28]. The different gel migration therefore is the result of the differential PK cleavage in the two types of $PrP^{Sc}$, which in turn is an indication of a different conformation or ligand binding of the two $PrP^{Sc}$ types.

## 4. Diagnosis and classification of human prion diseases

### *4.1. Sporadic prion diseases*

Patients who have identical prion protein genotype (*PRNP*) but a different $PrP^{Sc}$ type, either type 1 or 2, often have a distinct disease phenotype. Furthermore, the topography of the brain lesions in the large majority of the subjects with sporadic CJD is different according to whether the subjects have type 1 or 2 $PrP^{Sc}$ (Fig. 1). The histological lesions tend to be rostral in the presence of type 1 $PrP^{Sc}$ affecting preferentially the cerebral neocortex. In contrast, in most subjects with type 2 $PrP^{Sc}$ the lesions preferentially involve more caudal regions including basal ganglia, thalamus and brain stem. Therefore, the $PrP^{Sc}$ type acts as a determinant of the disease phenotype. The other determinant

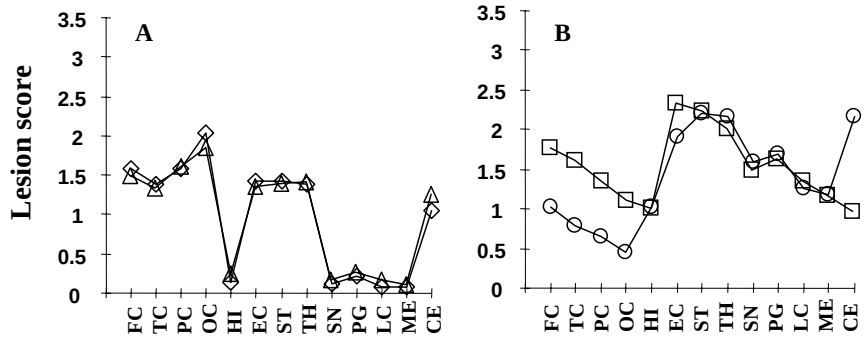


Fig. 1. Brain lesion profile of most common variants of sporadic CJD associated with $PrP^{Sc}$ type 1 (A) or type 2 (B). The MM-1 (◊), MV-1 (△), MV-2 (□) and VV-2 (○) variants were examined. FC: frontal cortex; TC: temporal cortex; PC: parietal cortex; OC: occipital cortex; HI: hippocampus; EC: entorhinal cortex; ST: neostriatum; TH: thalamus; SN: substantia nigra; PG: periventricular gray (midbrain); LC: locus ceruleus ME: medulla; CE: cerebellum. Modified from reference 34.

of the disease phenotype is the codon 129 that is the site of a common methionine/valine polymorphism. The reported frequency of the methionine homozygosity is 37%, the methionine/valine heterozygosity is 51% and the valine homozygosity is 12%, in the normal population [14,18]. Patients who have a different genotype at codon 129 often display a distinct disease phenotype, even if they have the same $PrP^{Sc}$ type. Therefore, the genotype at codon 129 and the $PrP^{Sc}$ type can be used to classify the sporadic prion diseases in groups that differ also in their phenotype. Since the original report by Creutzfeldt and Jakob, several variants of Creutzfeldt-Jakob disease have been reported. They include the Heidenhain or visual variant, the ataxic variant and the thalamic variant [7,21]. These variants are based on subjective findings such as the predominance of some clinical signs or the topography of the lesions. The characterization of prion diseases based on molecular features provides an objective and rapid way to classify human prion diseases [28,30,34]. Using this approach, five variants of sporadic CJD and the sporadic form of FFI or sporadic fatal insomnia (sFI) have been observed (Table 2).

All patients are grouped according to their methionine/valine zygosity at codon 129 and the presence of either type 1 or type 2 $PrP^{Sc}$ (MM-1, MV-1, VV-1, MM-2, MV-2 and VV-2). The MM-1 and MV-1 patients have the phenotype of the classical sporadic CJD. The phenotype of the VV-1 patients is characterized by the early onset (the youngest patient was 24 years old at onset). The MM-2 patients may present with two very different phenotypes. The "cortical" CJD phenotype is characterized by the presence of large, confluent vacuoles throughout the cerebral cortex, and by coarse PrP immunostaining i.e. intense immunostaining at the edges of the vacuoles. In contrast the "thalamic" phenotype is clinically and pathologically indistinguishable from FFI, and corresponds to the sporadic form of FFI or sFI. The MV-2 and VV-2 patients have overlapping but distinguishable phenotypes. Both have non-amyloid PrP deposits. The presence of kuru-type amyloid plaques in the cerebellum associated with little atrophy of the cerebellar cortex is characteristic of the MV-2 phenotype. In contrast the cerebellar cortex is atrophic in the VV-2 subjects.

Although sFI is clinically and pathologically indistinguishable from FFI [31], it shows an invariable difference in the ratio of the three glycoforms (diglycosylated, monoglycosylated and unglycosylated forms) that are commonly present in $PrP^{Sc}$ preparations. $PrP^{Sc}$ present in sFI brains shows the glycoform ratio of $0.6 : 1 : 0.76$, which is characterized by a prominent monoglycosylated form as most sporadic prion diseases. In contrast, $PrP^{Sc}$ associated with FFI has the $1.4 : 1 : 0.13$ glycoform ratio characterized by a marked under representation of the unglycosylated form [28].

### *4.2. Familial prion diseases*

Currently, at least 23 pathogenic mutations have been described in *PRNP*. The mutations obviously play an important role in determining the disease phenotype. The other determinants are the *PRNP* polymorphisms, especially the polymorphism at codon 129, as well as the glutamic acid/lysine polymorphism at codon 219 [2, 42]. The polymorphic codon affects the disease phenotype differently according to whether it is located on the mutant or on the normal *PRNP* allele. A classical example is provided by the D178N *PRNP* mutation. When this mutation is coupled with the methionine codon at

Table 2
Classification of sporadic prion diseases according to the genotype at codon 129 and the $PrP^{Sc}$ type

| Disease | Previous classific. | Cases (%) | Onset (yrs)/ Durat. (mo.) | Clinical features | Neuropathological features |
|---|---|---|---|---|---|
| Creutzfeldt-Jakob disease phenotype | | | | | |
| MM-1 Or MV-1 | Myoclonic, Heidenhain variants | 70 | 66.2/3.9 (42–91)[1] / (1–18) | Rapidly progressive dementia, early and prominent myoclonus, typical EEG. Visual impairment at onset in 40% of cases | "Typical" CJD lesion distribution with marked involvement of occipital cortex; "synaptic" type PrP staining; confluent vacuoles and perivacuolar PrP staining in 1/3 of the cases. |
| VV-1 | Not known | 1 | 41.0/15.3 (24–49)/ (14–16) | Progressive dementia, no typical EEG | Severe pathology in the cerebral cortex and striatum with sparing of brain stem and cerebellum; faint "synaptic" PrP staining |
| MM-2 | Not known | 2 | 67.3/15.7 (49–77)/ (9–36) | Progressive dementia, no typical EEG | Large confluent vacuoles with perivacuolar PrP staining; cerebellum relatively spared |
| MV-2 | Kuru-plaques variant | 9 | 57.7/16.9 (40–81)/ (5–72) | Ataxia in addition to progressive dementia, no typical EEG, duration $>$ 2 years in some cases | Similar to VV2 but with kuru amyloid plaques in the cerebellum and plaque-like PrP deposits. |
| VV-2 | Cerebellar or ataxic variant | 16 | 59.9/6.5 (41–80)/ (3–18) | Ataxia at onset, late dementia, no typical EEG in most cases | Prominent involvement of subcortical regions including brain stem; spongiosis often limited to deep neocortical layers; plaque-like deposits, as well as prominent perineuronal PrP staining. |
| Sporadic fatal insomnia phenotype | | | | | |
| MM-2 | Thalamic Variant or fatal familial insomnia | 2 | 59.5/13.6 (36–71)/ (8–24) | Insomnia and psychomotor hyperactivity, in addition to ataxia and cognitive impairment; no typical EEG | Prominent atrophy of the thalamus and inferior olive (no spongiosis) with little pathology in other areas; spongiosis absent or focal, and $PrP^{Sc}$ is detected in lower amount than in the other variants |

[1] Ranges in parentheses.

position 129, the disease phenotype is FFI with type 2 $PrP^{Sc}$; when the codon 129 coupled to the mutation is valine, the disease phenotype is CJD ($CJD^{D178N}$) and $PrP^{Sc}$ is of type 1 [19,28]. Patients affected by FFI who are homozygous for methionine at codon 129 and those with $CJD^{D178N}$ who are homozygous for valine at that codon, have on average a shorter disease duration and, in the case of $CJD^{D178N}$ an earlier onset. Therefore, on the mutant allele codon 129 determines the basic characteristics of the disease phenotype, on the normal allele it determines the duration and age at onset, i.e. the severity of the disease. Similar findings have been reported for at least two other familial prion diseases (Table 3). Therefore, it is appropriate to identify familial prion diseases with the haplotype, which may also co-distribute with distinct phenotype.

Table 3
Effect of codon 129 coupled with the mutation on disease phenotype in familial prion diseases

| Mutation | Codon 129 | $PrP^{Sc}$ | Phenotype |
|---|---|---|---|
| D178N | M | 2 | FFI |
| | V | 1 | $CJD^{D178N}$ |
| E200K | M | 1 | $CJD^{E200K/129M}$ |
| | V | 2 | $CJD^{E200K/129V}$ |
| P102L | M | 1 | $GSS^{P102L/129M}$ |
| | V | 2? | $GSS^{P102L/129V}$ |

## 5. Relationships between the genotype at codon 129 and the $PrP^{Sc}$ type

In sporadic human prion diseases the distribution of the $PrP^{Sc}$ type among the three possible genotypes at codon 129 is markedly uneven (Table 4). Approximately 95% of the patients with type 1 $PrP^{Sc}$ are homozygous for methionine at codon 129. In contrast, type 2 $PrP^{Sc}$ is found in approximately 86% of the patient who carry at least one valine allele. It is obvious that *PRNP* codon 129 is a determinant of the type and, therefore, of the conformation, of $PrP^{Sc}$. The influence of codon 129 on the $PrP^{Sc}$ conformation is even more evident if the fractions containing the PK-resistant fragments are purified and sequenced. This experiment has been recently carried out on a large variety of human prion diseases by our laboratory [35]. In all 36

Table 4
$PrP^{Sc}$ type as a function of codon 129

| $PrP^{Sc}$ | Codon 129 | | |
|---|---|---|---|
| | MM | MV | VV |
| Type 1 (N 86) | 95.3% (N 82) | 3.5% (N 3) | 1.2% (N 1) |
| Type 2 (N 86) | 13.9% (N 12) | 31.4% (N 27) | 54.7% (N 47) |

Table 5
PK cleavage sites in $PrP^{Sc}$ purified from brains of sporadic CJD as determined by N-terminal sequencing

| Codon 129 | $PrP^{Sc}$ type | Primary cleavage | Secondary cleavage |
|---|---|---|---|
| MM | 1 | G82 | G78 |
| MV or VV | 1 | G82 | G78/G86/G90/S97 |
| MM | 2 | S97 | G92/S103 |
| MV or VV | 2 | S97 | G92/S103/G82/G86/G90 |

cases examined, we found two main fragments generated by PK with N-termini beginning at residue 82 and 97, which correspond to $PrP^{Sc}$ type 1 and 2, respectively. A number of other fragments cleaved by PK at different sites were also observed. The location of these secondary cleavages however, is not random but it is clearly influenced by codons 129. For example, the M/M 1 variant of sporadic CJD shows only a secondary cleavage at residue 78, in addition to the main cleavage at residue 82. In the variants M/V 1 and V/V 1 there are several secondary cleavages at residues 86, 90 and 97. Therefore, the presence of one or two valine codons at position 129 in type 1 $PrP^{Sc}$ not only introduces more heterogeneity in the PK resistant fragments but also favors PK secondary cleavages that are closer to the C-terminus of $PrP^{Sc}$ than the cleavage sites occurring in the absence of the valine codon 129. The valine 129 codon in type 2 $PrP^{Sc}$ has a similar effect in causing more heterogeneous cleavages but in opposite directions along the $PrP^{Sc}$ molecule as compared to type 1 $PrP^{Sc}$. While in the M/M 2 variant the secondary cleavages are located at residues 92, 99 and 103, additional secondary cleavages appear at residues 90, 86 and 82 in the M/V 2 and V/V 2 variants. Therefore, the presence of one or two valine 129 codons increases the heterogeneity of the PK-resistant fragments also in type 2 $PrP^{Sc}$. Furthermore, the valine codon favors secondary cleavages that are closer to the N-terminus, rather than the C-terminus as in type 1 $PrP^{Sc}$. In conclusion, *PRNP* codon 129 not only is a determinant of the major PK cleavages which result in the formation of the $PrP^{Sc}$ type 1 and 2 associated with human prion diseases but also affects the secondary cleavages that occur within each of the two $PrP^{Sc}$ types (Table 5). Since the site of protease cleavage in a protein is indicative of its high order structure, it is obvious that codons 129 exercises a fine control on the conformation of the $PrP^{Sc}$ variants in human prion diseases.

## 6. Does the genotype determine the disease phenotype by affecting the conformation of the pathogenic protein in other diseases?

It has been established that while the unbound and water soluble form of the amyloid peptide (A$\beta$) is abundantly present in brains containing amyloid plaque associated with Alzheimer disease, it is undetectable in plaque-free brains [39,45]. In both plaque-containing and plaque-free brains, A$\beta$ is bound to Apolipoprotein E (Apo E) with which it forms soluble complexes of approximately 40 kDa [38]. The Apo E-A$\beta$ complexes from plaque-free brains are much more stable when treated with SDS than the complexes from plaque-containing brains. Therefore, soluble A$\beta$ is likely to be undetectable in plaque-free brains following direct immunoprecipitation with A$\beta$ antibodies because normally it is sequestered in stable complexes with Apo E [38]. In brains from cases with Down syndrome or trisomy 21, in which amyloid precursor protein (A$\beta$PP) is overexpressed due to increased gene dosage and Alzheimer's plaques are almost invariably formed during the second decade of life, soluble A$\beta$ is detectable from birth and often during gestation, long before the appearance of the A$\beta$ plaques [46]. Taken together these findings argue that the failure of the system that normally sequesters A$\beta$, either due to overproduction of A$\beta$ as in Down syndrome or other causes, leads to the abnormal presence of free, soluble A$\beta$ that then aggregates to form Alzheimer's plaques [38,46].

The soluble A$\beta$ present in plaque-containing brains resolves electrophoretically into three bands with ap-

parent molecular masses of 4.5 kDa, 4.2 kDa and 3.5 kDa [40]. These bands are indistinguishable from the bands generated by the insoluble A$\beta$ extracted from the plaques indicating that the composition of the insoluble A$\beta$ present in the plaques reflects that of the soluble A$\beta$. Mass spectrometry demonstrated that the 4.5 kDa band predominantly contains full length A$\beta$ ending at residue 42 (A$\beta_{1-42}$), the 4.2 kDa band mostly contains pyroglutamylated A$\beta$ N-terminally truncated at residues 3 (A$\beta_{py3-42}$) and the A$\beta$ truncated at residue 4 (A$\beta_{4-42}$). The 3.5 kDa band is made of pyroglutamylated A$\beta$ N-terminally truncated at residues 11 (A$\beta_{py11-42}$). In sporadic Alzheimer disease, the three bands account for approximately 30%, 50% and 20% of the total soluble A$\beta$, respectively. In brains from affected subjects carrying various presenilin-1 (PS-1) gene mutations, the A$\beta_{py11-42}$ variant was increased by 30% to 40% compared to the relative amount of A$\beta_{py11-42}$ recovered from brains affected by sporadic Alzheimer disease or familial Alzheimer disease associated with the V717I A$\beta$PP gene mutation. The relative increase of the N-terminally truncated A$\beta$ species was especially prominent in brains with the M139L or H163A PS-1 gene mutations. In these brains, A$\beta_{py3/4-42}$ and the A$\beta_{py11-42}$ combined were increased by approximately 70%, while A$\beta_{1-42}$ was decreased 2.5–4 fold. These electrophoretic data were confirmed by the immunohistochemistry of brain sections from carriers of the M139I and H163A mutations which indicated that, on average, eight times more plaques reacted with a specific antibody to the A$\beta$ variant ending at residue 42 than with an antibody against A$\beta$ starting at residue 1. In contrast, the numbers of plaques recognized by the two antibodies in sporadic AD brains were similar. Therefore, the A$\beta$ peptides truncated at the N-terminus are increased over the full length A$\beta$ also in the plaques of brains carrying these two PS-1 mutations. In carriers of PS-1 mutations, Alzheimer disease is clinically more severe. In the cases examined, it has an earlier onset ($43.5 \pm 0.7$ years) and shorter course ($4.5 \pm 0.7$ years) than patients with sporadic Alzheimer disease (onset at $75 \pm 8.7$ years and $8.8 \pm 4.0$ year duration) or with the V717I A$\beta$PP gene mutation (onset at $61 \pm 2.8$ years and $12.5 \pm 2.1$ year duration) [40]. The direct association between presence of A$\beta$ truncated at the N-terminus and a more severe disease phenotype in Alzheimer patients carrying PS-1 mutations raises the possibility that these mutations affect the disease phenotype by favoring the production of the N-terminally truncated A$\beta$ fragments over that of full length A$\beta$. This hypothesis is supported by the finding that N-terminally truncated A$\beta$ fragments are also overproduced in transfected cells carrying an APP or other PS-1 pathogenic mutations that in the human disease are characterized by an early onset or an atypical clinical presentation such as spastic paraparesis [1,15,50]. It has been shown that pathogenic mutations of the PS-1 and A$\beta$PP genes enhance the formation of more toxic A$\beta$ peptides that end at residue 42 compared to those ending at residue 40 [5]. Considerable evidence indicates that PS-1 may indeed be -secretase, the protease that cleaves APP C-terminal fragments to generate the A$\beta_{40}$ and A$\beta_{42}$ variants, or be essential for the enzymatic activity [17,25,50]. The additional increase in N-terminally truncated A$\beta$ variants argues that PS-1 mutations can affect not only the $\gamma$-secretase cleavage but also the A$\beta$PP cleavages produced by the $\beta$-secretase and, possibly, other secretases that determine the A$\beta$ N-terminus. The mechanism by which mutations in the PS-1 gene result in the increased production of N-terminally truncated A$\beta$ variant is unknown. Recent data indicate that presenilins form stable complexes with nascent, full length A$\beta$PP in the ER [51]. While being a component of the PS-A$\beta$PP complexes, APP would be cleaved by the $\beta$-secretase, producing PS-A$\beta$PP C-terminal fragments (PS-C99) complexes. The PS-C99 complexes are suitable substrate of the $\gamma$-secretase that operates in the Golgi and the trans-Golgi network. If PS-1 mediates the secretase activities through its binding with APP and APP derivatives, an attractive hypothesis is that because of the mutations, PS-1 adopts an abnormal conformation and forms aberrant PS-A$\beta$PP complexes. This in turn might favor the cleavage by both $\beta$- and $\gamma$-secretase at sites that are displaced toward the C-terminus of A$\beta$PP resulting in the formation of A$\beta$ variants that are amino terminally truncated and end at residue 42 rather than generating the more physiological A$\beta$ 1–40 form.

## 7. Conclusions

In human prion diseases, the PrP genotype and the $PrP^{Sc}$ type have a major influence on the disease phenotype. In the sporadic form, the genotypic influence is related to the codon 129 of the PrP gene, the site of a common methionine/valine polymorphism. In familial prion diseases, major phenotypic features are determined by the haplotype i.e. the interaction between the pathogenic mutation and the polymorphic codon on the mutant allele, most often codon 129 but also

codon 219. Furthermore, codon 129 on the normal allele may regulate the severity, rather then the basic characteristics, of the phenotype. The mechanisms by which the PrP genotype and the $PrP^{Sc}$ type affect the disease phenotype are unclear. However, three lines of evidence argue that codon 129 is a determinant of the $PrP^{Sc}$ type. First, in sporadic prion diseases codon 129 co-segregates largely, although not completely, with the $PrP^{Sc}$ type. Second, in at least three familial prion diseases the $PrP^{Sc}$ type changes according to whether the codon 129 methionine or valine is coupled with the mutation. Third, codon 129 affects not only the site of primary PK cleavage that determines the $PrP^{Sc}$ type but also the secondary cleavages in both types of $PrP^{Sc}$. Therefore, the available evidence is consistent with the scenario that the polymorphic codon alone or in coupling with a pathogenic mutation influences the $PrP^{C}$ to $PrP^{Sc}$ conversion leading to the formation of a specific type of $PrP^{Sc}$. The $PrP^{Sc}$ type in turn would contribute to the distinct disease phenotypes. Since the primary and secondary cleavage sites of $PrP^{Sc}$ reflect the extent of the PK resistant region, codon 129 obviously has an influence on the conversion process that generates $PrP^{Sc}$. The most likely mechanism is that the presence of either methionine or valine at position 129 of $PrP^{C}$ or mutant PrP affects the stability or structure of these PrP variants and, consequently, the structure of the $PrP^{Sc}$ that forms from these variants. Therefore, in human prion diseases the genotype may modulate the disease phenotype by affecting the conformation of $PrP^{Sc}$. Although this scenario is applicable to many prion phenotypes, some phenotypes must be affected by other mechanisms. For example, the co-segregation of codon 129 and the $PrP^{Sc}$ type is not complete. The $PrP^{Sc}$ type fails to co-distribute with the expected 129 codons in almost 20% of the cases and in rare instances both types of $PrP^{Sc}$ coexist in the same patient. Furthermore, distinct phenotypes of sporadic prion diseases are associated with the same codons 129 and protein type. Therefore, the genotype at codon 129 and the $PrP^{Sc}$ type must not be the only factors playing a role in determining the phenotype in these cases.

Although Alzheimer disease has phenotypes that are much less heterogeneous than those associated with prion disease, atypical forms with clinical presentations, age at onset, disease duration and topography of the lesions have been reported in some familial forms [1,15,26]. The increase of A$\beta$N-terminally truncated variants in Alzheimer's subjects carrying different PS-1 gene mutations associated with a more malignant disease phenotype, as compared to subjects with a typical phenotype might be explained by mechanisms similar to, although less direct than, those outlined for the prion diseases. Because of an altered conformation, mutated PS would form aberrant complexes with A$\beta$PP favoring the production of A$\beta$ variants with different pathogenicity. Therefore, the mechanism by which the genotype determines the disease phenotype through a conformational alteration of proteins involved in the disease pathogenesis may be operational in forms of familial Alzheimer disease. The phenotypic homogeneity of sporadic Alzheimer disease might be explained at least in part by the lack of a powerful genetic determinant of the disease phenotype in the A$\beta$PP gene such as the 129 polymorphic codon in the PrP gene.

Protein misfolding with formation of aggregates is the hallmark of many conditions also called conformational diseases [8,48,49]. Conformational diseases causing neurodegeneration include, in addition to prion diseases and Alzheimer disease, the tauopathies, triplet repeat disorders like Huntington's disease and spinocerebellar ataxias, and Parkinson disease. The pathogenetic mechanism of all these diseases, as in prion diseases, is the conversion of a protein that is soluble and functional but conformationally unstable, into a $\beta$-sheet rich molecule that has the tendency to aggregate and is pathogenic. Genetic mutations may further destabilize these proteins causing the diseases almost invariably at middle or advanced age. Therefore, it may be expected that genotype have the capability of affecting phenotype by controlling the conformation of the pathogenic protein in all conformational diseases.

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