

## Human Prion Disease and Human Prion Protein Disease

Kitamoto, Tetsuyuki

Department of Neurological Science, Tohoku University School of Medicine

Tateishi, Jun

Department of Neuropathology, Neurological Institute, Kyushu University

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# Human Prion Disease and Human Prion Protein Disease

T. KITAMOTO<sup>1</sup> and J. TATEISHI<sup>2</sup>

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## 1 Introduction

Creutzfeldt-Jakob disease (CJD), kuru, and Gerstmann-Sträussler syndrome (GSS) show clinical and pathological characteristics similar to those of scrapie, a transmissible neurodegenerative disease of sheep and goats. These diseases are caused by slow infectious agents designated as prions (PRUSINER 1982). The major component of prions is prion protein (PrP; MCKINLEY et al. 1983), which is encoded in normal human genomes located on the short arm of chromosome 20 (SPARKES et al. 1986). In 1989 codon 102 or codon 117 point mutations of human PrP were reported to be linked to GSS (HSIAO et al. 1989; DOH-URA et al. 1989). The results in codon 102 transgenic mice also strengthen the idea that this mutation is one of the essential events that cause GSS (HSIAO et al. 1990). The several polymorphisms or mutations were also reported in familial CJD and familial dementia (GOLDGARBER et al. 1989; GOLDFARB et al. 1991; MEDORI et al. 1992; KITAMOTO et al. 1993a,b).

Recently, several studies showed that scrapie form of PrP (PrP<sup>Sc</sup>) is an essential component of prions. These studies included the following results: copurification of PrP<sup>Sc</sup> and scrapie infectivity (MCKINLEY et al. 1983; GABIZON et al. 1988), PrP<sup>Sc</sup> detection only in clones of cultured cell producing prion infectivity (TARABOULOS et al. 1990), PrP amyloid plaque detections in prion diseases (BENDHEIM et al. 1984; KITAMOTO et al. 1986), genetic linkage between human PrP gene mutation and hereditary CJD or GSS (HSIAO et al. 1989, 1992; TRANCHANT et al.

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<sup>1</sup> Department of Neurological Science, Tohoku, University School of Medicine, Sendai 980, Japan

<sup>2</sup> Department of Neuropathology, Neurological Institute, Kyushu University 60, Fukuoka 812, Japan

1992; GOLDFARB et al. 1992), and genetic linkage between mouse PrP gene and scrapie incubation time (WESTAWAY et al. 1987). One of the major confirmations is the result in PrP knockout (PrP<sup>0</sup>) mice (BÜELER et al. 1992). These PrP<sup>0</sup> mice did not develop the scrapie and did not amplify the infectivity (prions titer; BÜELER et al. 1993). Therefore, PrP is an essential component for the infectivity. However, the mechanism of prion multiplication is still unclear. One fascinating hypothesis (the prion dimer hypothesis) was proposed by PRUSINER (1991). This hypothesis can explain the infectious form of prion disease. We report here whether the dimer hypothesis can explain the human prion disease with germ-line mutation. We also report a new variant GSS with codon 102 Leu mutation and codon 219 Lys polymorphism.

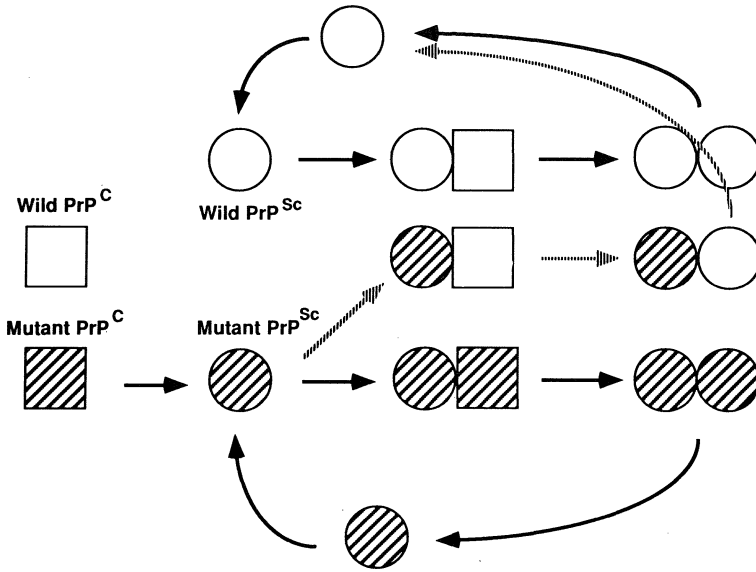
## 2 Prion Dimer Hypothesis and Mutant PrP Molecule

The mechanism by which prions multiply is unknown. The multiplication of prion infectivity is an exponential process in which the posttranslational conversion of PrP<sup>C</sup> (normal cellular form) to PrP<sup>Sc</sup> appears necessary. According to the prion dimer hypothesis, a PrP<sup>Sc</sup> molecule combines with one PrP<sup>C</sup> molecule giving rise to one heterodimer. This heterodimer is subsequently transformed into one homodimer (PrP<sup>Sc</sup>/PrP<sup>Sc</sup>) that dissociates to combine with two PrP<sup>C</sup> molecules creating an exponential process.

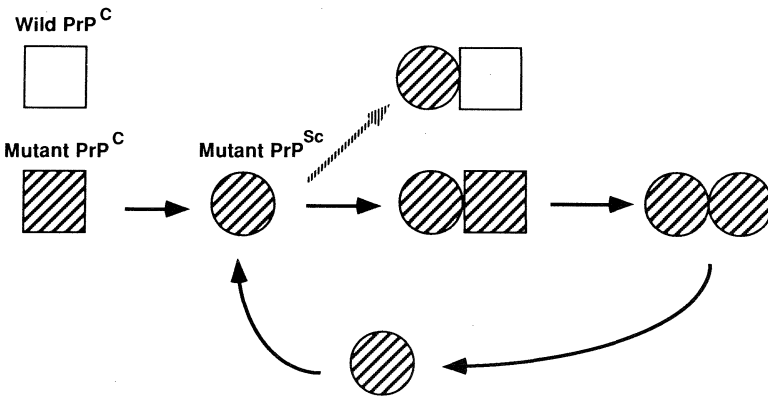
In humans with PrP point mutation, mutant PrP<sup>C</sup> molecules might spontaneously convert into mutant PrP<sup>Sc</sup> (Fig. 1). While the initial stochastic event may be inefficient, once it happens, the process becomes autocatalytic. Whether all GSS and familial CJD patients contain infectious prions is unknown. If the former is found, mutant PrP<sup>Sc</sup> molecules combine with the heterodimer (mutant PrP<sup>Sc</sup>/wild PrP<sup>C</sup>) and are subsequently transformed into mutant PrP<sup>Sc</sup>/wild PrP<sup>Sc</sup>. This wild PrP<sup>Sc</sup> produces the heterodimer (wild PrP<sup>Sc</sup>/wild PrP<sup>C</sup>) in an exponential process (Fig. 1). If the latter is found, presumably, mutant PrP<sup>Sc</sup> molecules alone can produce the central nervous system dysfunction (Fig. 2). To test the dimer hypothesis we examined the following cases with unique point mutation or polymorphism.

## 3 Only Mutant PrP<sup>Sc</sup> Accumulates in the Central Nervous System (Prion Protein Disease)

Most of the point mutations on the PrP gene were heterozygous and missense. Therefore it is difficult to analyze which molecule, wild or mutant, accumulated in the central nervous system because of the identical molecular weights. Previously we and another group identified the mutant PrP molecule (codon 102 Leu



**Fig. 1.** Prion dimer hypothesis in inherited prion disease in humans. Mutant PrP<sup>C</sup> molecule (dashed line in squares) might initiate the conversion of PrP<sup>C</sup> to PrP<sup>Sc</sup> (dashed line in circles). When infectious prions are produced, they stimulate the synthesis of mutant PrP<sup>Sc</sup> (dashed line) and wild PrP<sup>Sc</sup> (open circle)



**Fig. 2.** Inborn error of PrP metabolism in humans. Mutant PrP<sup>Sc</sup> molecule combine with only mutant PrP<sup>C</sup> molecule. Prion infectivity is not generated, but humans develops neurological dysfunction and PrP amyloid plaques

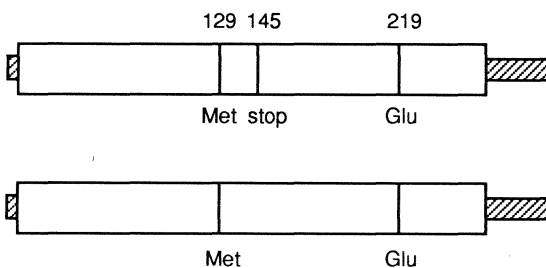
or codon 129 Val/198 Ser) from the kuru plaque core fractions of GSS patients (KITAMOTO et al. 1991; TAGLIAVINI et al. 1991). However, it remains to be established whether mutant PrP are major component of PrP<sup>Sc</sup>. These peptide-sequencing data were based on a purification step which might cause the fragmentation of

PrP or make it difficult to measure the concentration of mutant PrP in the kuru plaques.

Among these limitations of analyzing the mutant PrP molecules, we had a chance to examine a patient with Y145 stop mutation (KITAMOTO et al. 1993a; Fig. 3). The T to G transition at codon 145 was resulted in tyrosine (TAT) to amber codon (TAG). To analyze this stop codon we used the following steps: (a) mRNA expression of mutant PrP in the brain, (b) western blotting, (c) protein expression in *Escherichia coli* and (d) immunohistochemistry using N-terminal and C-terminal antibodies.

First, we checked the mRNA expression with reverse-transcription polymerase chain reaction (RT-PCR) amplification. The RT-PCR product was digested with *MaeI* endonuclease. The mutant PrP gene has a cutting site of *MaeI*. *MaeI* restriction fragment length polymorphism analysis revealed the expression of both wild and mutant PrP mRNA in the brain. Next, we performed western blotting using a proteinase-resistant prion rod fraction in the brain tissue to check for the low molecular weight mutant PrP. The western blot showed a diffuse smear immunoreactivity from the top of the gel to the dye front. The smear immunoreactivity, which may be due to the highly aggregated PrP of kuru plaques, makes it difficult to reveal small molecular weight mutant PrP. Thus we prepared a fusion protein expression plasmid in *E.coli* to check the specificity of the N-terminal and C-terminal PrP antibodies. N-terminal antibody recognized both the wild and mutant PrP fusion protein, and C-terminal antibody recognized only the wild PrP fusion protein. Finally, we examined the mutant PrP molecules in the tissue sections with these PrP antibodies. The N-terminal antibody immunostained positively with kuru plaques in the brain from this patient with Y145 stop, but the C-terminal antibody did not immunolabel the kuru plaque. To confirm the immunoreactivity of the C-terminal antibody, we also immunostained the kuru plaques in the GSS patients with P102L mutation. This C-terminal antibody positively recognized kuru plaques in GSS102. Therefore, in this peculiar case, only mutant PrP molecules aggregated to form kuru plaques.

In this case, mutant PrP<sup>C</sup> molecules might spontaneously convert into PrP<sup>Sc</sup>. While the initial stochastic event may be inefficient, once it happens, the process becomes autocatalytic. Mutant PrP molecules alone produced the amyloid plaques in the central nervous system. Therefore this point mutation represents inborn errors of PrP metabolism (prion protein disease). Transmission study from this case to mouse also showed a negative result (0/10 mice).

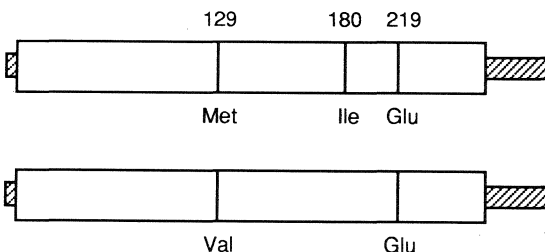


**Fig. 3.** PrP genotype of a patient with codon 145 mutation. Codon 129 and codon 219 represent normal polymorphism seen in Japanese

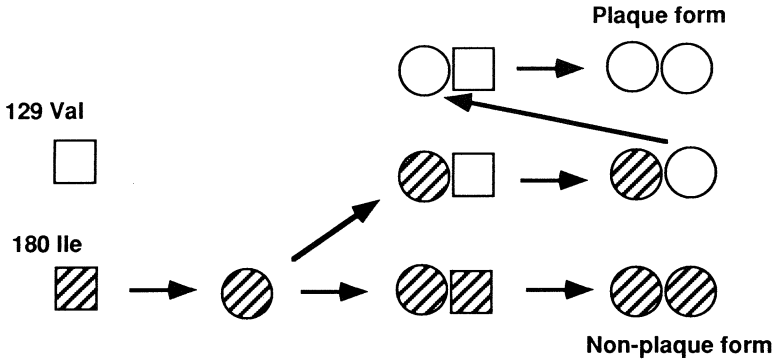
## 4 Mutant PrP<sup>Sc</sup> Influences the Wild PrP<sup>Sc</sup> Conversion (Prion Disease)

We have another case with codon 180 Ile mutation and codon 129 Val polymorphism each on the different allele (Fig. 4). In our experience with codon 180 Ile mutation, patients had only Met/Met type polymorphism at codon 129 and Glu/Glu type polymorphism at codon 219. Neuropathological examinations showed typical spongiform changes and moderate neuronal loss in the cerebral cortices. Western blot analysis revealed PrP<sup>Sc</sup>, but the concentration of PrP<sup>Sc</sup> was much less than that of the wild-type CJD patients. The wild-type CJD patients have three major PrP<sup>Sc</sup> bands, but CJD patients with codon 180 Ile have two major bands corresponding to nonglycosylated and one glycosylated PrP<sup>Sc</sup> (Hitoshi et al. 1993). PrP immunostainings showed diffuse gray matter stainings but not amyloid plaques. In CJD cases with Val/Met or Val/Val polymorphism at codon 129 we determined the amyloid plaque formation in the central nervous system (Kitamoto et al. 1992; Miyazono et al. 1992). Therefore this patient is a suitable case to examine the both mutant (codon 180 Ile) and wild (codon 129 Val) PrP molecules. Figure 5 shows the working hypothesis for detection of mutant and wild PrP molecules. Mutant PrP<sup>C</sup> (180 Ile) might convert to mutant PrP<sup>Sc</sup>. If the conversion occurs, the mutant PrP<sup>C</sup>/mutant PrP<sup>Sc</sup> heterodimer formation may result in the two mutant PrP<sup>Sc</sup> molecules. The interesting point is whether the wild PrP<sup>C</sup>/mutant PrP<sup>Sc</sup> heterodimer formation occurs. If the wild PrP<sup>C</sup> is converted to wild PrP<sup>Sc</sup>, the process of wild PrP<sup>Sc</sup> formation becomes autocatalytic. These wild PrP<sup>Sc</sup> (codon 129 Val) accumulations could be observed in the amyloid plaque formation in the central nervous system.

To examine this working hypothesis we at first analyzed the neuropathological findings of this case. Routine histopathological findings were severe spongiform changes and neuronal loss in the cerebral cortices, but no congophilic amyloid plaques. Western blot showed two lower PrP<sup>Sc</sup> bands. The results of these histopathological and western blot analyses of this case are compatible with the findings seen in other CJD patients with codon 180 Ile. Finally, we examined PrP immunostainings using the hydrolytic autoclaving pretreatment. PrP immunostainings revealed weakly positive diffuse gray matter stainings and a few amyloid plaques. These amyloid plaques were not observed in other CJD



**Fig. 4.** PrP genotype of a patient with codon 180 mutation and codon 129 Val polymorphism



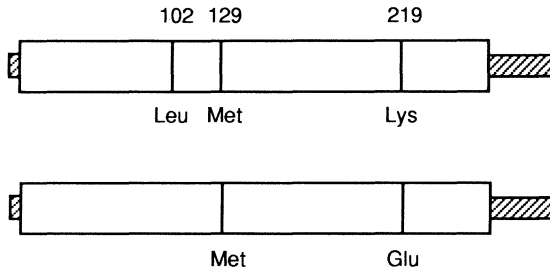
**Fig. 5.** Prion dimer hypothesis in a patient with codon 180 mutation and codon 129 Val polymorphism each on the different PrP allele

patients with codon 180 Ile mutation and codon 129 Met/Met polymorphism. Therefore in this case wild PrP<sup>Sc</sup> molecules also accumulated in the central nervous system. Transmission experiment is now continuing.

## 5 A New Variant PrP Molecule in Gerstmann-Sträussler Syndrome

We determined the new polymorphism (codon 219 Glu or Lys) in a normal Japanese population (KITAMOTO and TATEISHI 1994). The allele frequency of codon 219 Lys is about 6% in normal Japanese. During the search for this polymorphism codon 219 Lys was detected in four patients with Japanese Gerstmann-Sträussler syndrome (P102L). In three patients belonged to the different families, codon 102 Leu mutation was on the codon 219 Glu allele, but not on the codon 219 Lys allele. These three patients have a typical clinical course showing spinocerebellar degeneration. However, in one patient codon 102 Leu mutation was detected on the codon 219 Lys allele (Fig. 6). Family study revealed that four patients in this family have codon 102 Leu and codon 219 Lys on the same allele. Two patients showed only dementia in the absence of cerebellar signs, and two others showed weak cerebellar signs and dementia.

Recently we examined one autopsy case with a clinical course of dementia in the absence of cerebellar signs. Histopathological examinations showed no spongiform changes in the cerebral cortices. Mild gliosis and mild neuronal loss were observed in the deep layer of the cerebral cortices. There were no congophilic amyloid plaques in the cerebral and cerebellar cortices. The absence of amyloid plaques is quite different from GSS patients with only codon 102 Leu. Previously we examined more than 20 GSS patients with codon 102 Leu. These GSS patients have many congophilic plaques in the cerebral and cerebellar



**Fig. 6.** PrP genotype of a new GSS family in Japan. The codon 102 Leu mutation is located on the codon 219 Lys allele

cortices, but this patient does not have congophilic plaques in routine histopathological examinations. PrP immunostainings revealed a few amyloid plaques in the molecular layer of the cerebellar cortices, and diffuse amyloid plaques, so-called moth-eaten PrP plaques, in the deep cortical layers of the cerebral cortices and basal ganglia. There were neither tau-positive neurofibrillary tangles nor senile plaques in the cerebral cortices.

These clinical and neuropathological findings support the hypothesis that codon 219 Lys polymorphism influences the phenotype of the codon 102 Leu mutation. Therefore it is better to classify as a new type GSS with codon 102 Leu/codon 219 Lys.

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