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Human prion diseases with variant prion protein

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SUMMARY

Recent molecular genetic studies revealed that the human prion protein (PrP) gene has a large repertoire of polymorphisms and mutations. Each variant PrP seems to correspond to a distinct type of prion diseases. We report herein that it is useful to classify prion diseases into plaque type or non-plaque type, based on the distribution of PrP in the central nervous system. The variant PrP including codon 102, codon 105, codon 129, codon 145 and insertional polymorphisms belong to the plaque type prion diseases, whereas the wild-type PrP and the variants including codon 180, codon 200, and codon 232 polymorphisms belong to the non-plaque type. The non-plaque type prion diseases showed a rapidly progressive dementia, myoclonus and periodic synchronous discharges in the electroencephalogram, and in the pathological findings diffuse grey matter PrP accumulations including the synaptic structures. The plaque type prion diseases showed a long clinical course without myoclonus and periodic synchronous discharges, and the major PrP accumulation sites were extracellular PrP plaques. The distribution of PrP deposits in the central nervous system influences the clinical and pathological aspects of prion diseases. Thus, PrP accumulations may play a central role in the pathogenesis of prion diseases.

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 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 1986). In mice, polymorphisms in the open reading frame (ORF) of the mouse PrP gene directly influence the incubation period of scrapie infection (Westaway *et al.* 1987). Therefore, many researchers have concentrated their attention on the polymorphism of the human PrP gene. 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). Several polymorphisms were also reported in familial CJD and familial dementia (Goldgaber *et al.* 1989; Goldfarb *et al.* 1991*a*,*b*; Owen *et al.* 1990; Hsiao *et al.* 1992; Medori *et al.* 1992). The distinct type of PrP seems to correspond to the distinct type of clinical and pathological features.

Recently, we developed hydrolytic autoclaving pretreatment to enhance the immunoreactivity of tissue sections (Kitamoto *et al.* 1991, 1992*a*). This pretreatment revealed diffuse grey matter staining including synaptic structures in the central nervous system of sporadic CJD patients. Using this enhancement, we were able to classify either plaque type or non-plaque type (synaptic type) PrP accumulations in a CJD patient with PrP polymorphism (Kitamoto *et al.* 1992*b*). Combined with these immunohistochemical approaches and genetic studies, we also observed several atypical cases with variant PrP (Kitamoto *et al.* 1993*a*,*c*). We report here on Japanese patients with variant PrP genes.

2. CODON 102 MUTATION (P102L)

(*a*) *Genetic analysis*

Proline-to-leucine substitution at codon 102 was first reported by Hsiao *et al.* (1989), followed by detection in a descendant of the original case reported by Gerstmann, Sträussler and Scheinker (Kretzschmar *et al.* 1991). A CCG-to-CTG transition creates a new cutting site of Dde I and Alu I in the PrP gene. The polymerase chain reaction (PCR) using primers T1 (GATGCTGGTTCTCTTTGTGG) T-2 (CCCAC-TATCAGGAAGATGAG) amplifies the open reading frame (ORF) of the PrP gene. The 738 base pair (b.p.) PCR product was digested with Dde I or Alu I. Alu I cuts the wild-type PCR products into 405 and 333 b.p. fragments, and cuts the mutant-type into 405, 284, and 40 b.p. Dde I cuts the wild-type PCR products into 513, 133, and 92 b.p., and cuts the mutant-type into 361, 152, 133, and 92 b.p. Using Dde I and Alu I restriction fragment length polymorphism (RFLP) (Kitamoto *et al.* 1993*c*) or dot differential hybridization with allele-specific oligonucleotidases (Doh-ura *et al.* 1990), we can easily distinguish a GSS102 patient from demented patients with spinocerebellar degeneration. At present, we have found more than 20 families with the codon 102 mutation in Japan.

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(*b*) *Clinical aspects*

We examined 15 patients with Leu102 variant PrP. Onset of the illness is at 52 ± 10 years. Patients showed mainly cerebellar signs such as ataxic gait, lack of coordination and slurred speech similar to patients with olivo-ponto-cerebellar atrophy. Dementia appeared later. Myoclonus and periodic synchronous discharges (PSD) in the electroencephalogram (EEG) were seen only in limited patients with Leu102 variant PrP. Myoclonus and PSD were detected in almost all CJD patients with the wild-type PrP gene. Akinetic mutism appeared several years later and the mean duration of total clinical course was about 6 years.

(*c*) *Pathological aspects*

A constant pathological feature is PrP plaques which appear throughout the central nervous system. PrP plaques are observed predominantly in the cerebral and cerebellar cortex, but also in the brain stem and the spinal cord. Using hydrolytic autoclave enhancement, diffuse grey matter staining including synaptic structures were also revealed by PrP immunostaining, in addition to PrP plaques.

Spongiform change, neuronal loss and astrocytosis showed a marked variation in degree in each patient. In the same family (Fuj family), one patient showed the destructive changes such as neuronal loss, severe astrocytosis and spongiform changes (Tateishi *et al.* 1979), while another showed well-preserved central nervous system except for PrP plaques (Tateishi *et al.* 1988). Therefore, spongiform changes do not seem to be a pathological hallmark in patients with Leu102 variant PrP.

3. CODON 105 MUTATION (P105L)

(*a*) *Genetic analysis*

Proline (CCA) to leucine (CTA) substitution at codon 105 was found in a patient with familial spastic paraparesis and kuru plaques (Kitamoto *et al.* 1993*c*). This mutation was detected on the Val129 allele of PrP gene. The codon 105 mutation creates a new Alu I site, but not a Dde I site. Alu I cuts the mutant-type PCR products into 405, 293, and 40 b.p. fragments. The pattern of Alu I RFLP in codon 105 mutations was similar to that in the codon 102 mutation. Therefore, it is necessary to compare the Dde I cutting and Alu I cutting differentiate of codon 102 and codon 105 mutations (Kitamoto *et al.* 1993*b*). At present, we have identified four Japanese families with the codon 105 mutation (Amano *et al.* 1992; Nakazato *et al.* 1991; Terao *et al.* 1992).

(*b*) *Clinical aspects*

All of the patients with Leu105 variant PrP had a familial occurrence of the disease. The disease started at 44 ± 4 years, presenting with a spastic gait disturbance. Sometimes, patients were diagnosed as familial spastic paraparesis at the onset. One patient showed extrapyramidal signs such as fine finger tremor and rigidity of limbs. However, the most prominent findings were pyramidal tract signs. All patients showed hyper-reflexia, especially in lower limbs and Babinski's signs. During a long clinical course (9 ± 3 years), paraparetic clinical findings progressed to quadriparesis with emotional incontinence. None of the patients with Leu105 variant PrP had myoclonus, PSD in EEG, or showed serious cerebellar signs.

(*c*) *Pathological aspects*

We examined three autopsy patients with Leu105 variant PrP. The most prominent finding was PrP plaques in the cerebral cortex. Numerous PrP plaques were found in the frontal cortex, especially in motor cortex. In the superficial cortical layer, multicore PrP plaques were observed. In the deep cortical layer of the motor cortex, diffuse PrP plaques were confluent and laminar in arrangement, and associated with neuronal loss. These PrP plaques were predominantly seen in the frontal cortex, but rarely seen in the visual cortex. PrP plaques were also observed in basal ganglia and in the thalamus. However, only a few plaques were seen in the molecular layer of the cerebellum. There were no PrP plaques in the spinal cord. Diffuse grey matter PrP staining including synaptic structures was not observed in the cerebral and cerebellar cortices in patients with Leu105 variant PrP.

Histopathological examinations in patients with Leu105 variant PrP revealed no spongiform change in the cerebral cortex in any of the three patients. Neuronal loss and astrocytosis were observed in the deep cortical layer of the frontal cortex where these neurodegenerative changes were associated with numerous diffuse PrP plaques. Neurofibrillary changes were detected in two patients to a mild degree. In the cerebellum, Purkinje cells and granular cells were well-preserved. In the brainstem and spinal cord, a secondary degeneration was observed in the pyramidal tracts. Vacuolation and loss of myelin was seen in the corticospinal tract.

4. CODON 129 POLYMORPHISM (M129V)

(*a*) *Genetic analysis*

Methionine (ATG) to valine (GTG) substitution at codon 129 was first reported by Doh-ura *et al.* (1989). This substitution is a polymorphism seen in the general population. In Japan, we examined the codon 129 polymorphism from 179 individuals from the general population. One hundred and sixty four (82%) had Met/Met alleles and 15 (8%) had Val/Met alleles (Doh-ura *et al.* 1991). We did not find Val/Val in the general population. From August 1991 to August 1993 we examined 61 sporadic CJD cases in our laboratory. Sporadic CJD cases were separated into 50 cases (82%) with Met/Met, 11 (18%) with Val/Met, and 0 with Val/Val. Our data on codon 129 polymorphism were different from those in the United Kingdom (Palmer *et al.* 1991). Codon 129 homozygosity does not seem to be a predisposing factor in Japanese CJD.

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(*b*) *Clinical aspects*

We previously examined six autopsy CJD patients with Val129 variant PrP. One autopsy case was homozygous (Val/Val), and the others were heterozygous (Val/Met). Five patients out of six cases had cerebellar ataxia at the onset. Disease progression was very slow, and the average duration from onset to akinetic mutism was 33 months. However, in CJD patients with homozygosity (Met/Met), the average duration was only 3.6 months. Therefore, clinical manifestations of patients with Val129 variant PrP were similar to those with Leu102 variant PrP (Miyazono *et al.* 1992). Some patients with Val129 variant PrP had myoclonus, but only one patient showed PSD in the EEG. All CJD patients with Val129 were sporadic cases.

(*c*) *Pathological aspects*

A constant pathological finding was PrP plaques, which have a unicentric core. PrP plaques were much less frequent than those seen in patients with Leu102 or Leu105 variant PrP. The major site of PrP plaque deposition was the cerebral cortex. The number of PrP plaques in the cerebellum was less than that in the cerebral cortex. There were no PrP plaques in the brain stem and spinal cord. These results were based on the Japanese sporadic CJD patients with Val129 variant PrP. In addition to Japanese cases, we examined four Caucasian CJD cases due to human growth hormone therapy (Hoque *et al.*, unpublished results). Two Caucasian CJD patients with Met/Met129 showed only synaptic PrP staining, while two patients showed PrP plaques. One of the plaque-type patients had Val/Val129, and the another was not analysed genetically. Therefore, Val129 variant PrP influences the pathologic phenotype and results in plaque formation in both sporadic and infectious CJD.

Spongiform changes, astrocytosis and neuronal loss showed a variation in degree in each patient (Miyazono *et al.* 1992). However, at least slight spongiform changes could be detected in all CJD cases with Val129 variant PrP.

5. CODON 145 MUTATION (Y145ST0P)

(*a*) *Genetic analysis*

Tyrosine (TAT) to amber codon (TAG) changes at codon 145 was recognized in a Japanese autopsy patient with early-onset dementia (Kitamoto *et al.* 1993*a*). This mutation creates a new Mae I site. Mae I cuts 738 b.p. PCR products of the mutant allele into 427 and 311 b.p. fragments, but does not cut PCR products of the wild-type allele. To identify the expression of the mutant allele, we extracted total RNA from the brain of this patient, and performed reverse transcriptase (RT)-PCR. The RT-PCR products showed both mutant and wild-type alleles using Mae I cutting in the same results of genomic PCR products. Therefore, both mutant and wild-type PrP mRNA were expressed in the central nervous system of this patient. We did not examine family members of this patient. We did not find the same mutation using Mae I RFLP from 300 demented patients and 100 individuals from the general population in Japan.

(*b*) *Clinical aspects*

This patient demonstrated memory disturbance at 38 years. Slow progressive dementia was the only clinical finding, and the clinical diagnosis was early-onset Alzheimer's disease. At 43 years, this patient showed marked disorientation, and could not communicate with other people. At 50 years, she became bedridden. This patient did not have PSD in the EEG.

(*c*) *Pathological aspects*

Autopsy examinations revealed many amyloid plaques in the cerebral and cerebellar cortices and diffuse neuropil threads of paired helical filaments in the cerebral cortex. There were no spongiform changes in the cerebral cortex. These amyloid plaques were positively labelled with PrP antibodies, but not labelled with βA4 antibody. To analyse the component of PrP plaques, we prepared N-terminal antibody and C-terminal antibody. N-terminal antibody was raised against the synthetic peptide (Arg25 to Tyr49), and C-terminal antibody was raised against the synthetic peptide (Gln212 to Tyr226). N-terminal antibody positively immunolabelled PrP plaques in this patient and PrP plaques in patients with Leu102 variant PrP. C-terminal antibody positively immunolabelled PrP plaques in patients with Leu102 variant PrP, but not PrP plaques in this patient. This result indicates that only mutant PrP molecules aggregated in the PrP plaques. In the plaque formation of this patient, the prion heterodimer hypothesis (Prusiner 1991) does not seem to fit in this peculiar case.

6. CODON 180 MUTATION (V180I)

(*a*) *Genetic analysis*

Valine (GTC) to isoleucine (ATC) substitution at codon 180 was recognized by using Tth111 I digestion (Kitamoto *et al.* 1993*c*). The Tth111 I digestion has been reported to detect the codon 178 mutation (GAC to AAC) seen in familial CJD and fatal familial insomnia (Goldfarb *et al.* 1991*a*, 1992; Medori *et al.* 1992). However, a Japanese patient with CJD did not have the codon 178 mutation and direct sequencing revealed codon 180 mutation; both mutations lost a Tth111 I cutting site. Previously, we recommended sequencing to differentiate these two mutations (Kitamoto *et al.* 1993*c*); we now report a new approach to differentiate them.

The new approach was based on the mismatched primer which introduces a new restriction enzyme site (figure 1). To detect the codon 180 mutation, we prepared a mismatched sense primer (TE-1: AGAA-CAACTTTGTGCACG-AATGC) and a matched antisense primer (K-6: ACACATCTGCTCAAC-CACGC). We designed a mismatched A (underlined)

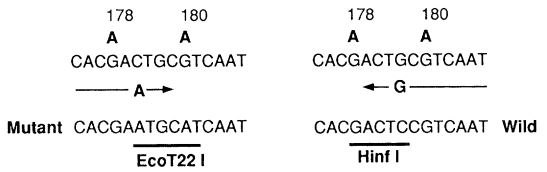

Figure 1. Strategy for the identification of codon 178 and codon 180 mutation. Left panel: to detect the codon 180 mutation, we prepared a mismatched sense primer (TE-1). This primer contains a mismatched adenine instead of cytocine in the 3′ portion. Thus, polymerase chain reaction (PCR) products of the mutant allele have a EcoT22 I site (ATGCAT). PCR products of the wild-type allele do not have a EcoT22 I site. Right panel: to detect the codon 178 mutation, we prepared a mismatched antisense primer (TE-2). This primer induces a Hinf I site in the PCR products of the wild-type allele, but not of the mutant allele.

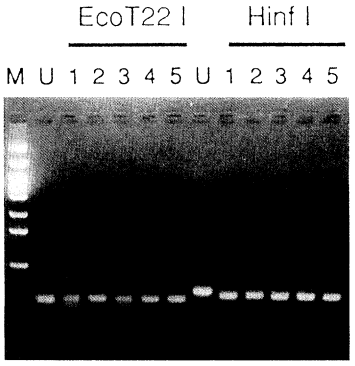

Figure 2. Restriction analysis of the codon 178 or codon 180 mutation. Polymerase chain reactions (PCR) products with TE-1 and K-6 primers were digested with EcoT22 I, and PCR products with TE-2 and K-5 primers were digested with Hinf I. Lane U, undigested PCR products; lanes 1 and 3, PCR products from patients with the codon 180 mutation; lanes 2, 4 and 5, PCR products from healthy control. EcoT22 I cut PCR products (126 b.p.) into 101 b.p. fragments in lanes 1 and 3. Hinf I cut all PCR products (157 b.p.) into 135 b.p. fragments.

instead of a matched C in the TE-1 primer. This mismatched primer induces a EcoT22 I site (ATGCAT) in the mutant allele, but not in the wild-type allele because the wild-type allele PCR products result in ATGCGT at this portion. Using PCR products with T-1 and T-2 primers, we performed the nested PCR with TE-1 and K-6 primer. EcoT22 I cuts the mutant PCR products (126 b.p.) into 101 and 25 b.p., but does not cut the wild-type PCR products (figure 2). For detection of codon 178 mutation, we prepared a matched sense primer (K-5: CATGAGCAGGCCCATCATAC) and a mismatched antisense primer (TE-2: CTTGATTGTGATATTGACGGAGT). This mismatched primer induces a Hinf I site in the wild-type allele, but not in the codon 178 mutant allele (figure 1). Therefore, Hinf I cuts the wild-type PCR products (157 b.p.) into 135 and 22 b.p. fragments, but does not cut the codon 178 mutant PCR products. We examined 300 demented patients and 100 individuals from the general population with Tth111 I, Hinf I and EcoT22 I enzyme cutting. At present, we have found four demented cases with the codon 180 mutation (two CJD cases with only codon 180 mutation, one CJD case with codon 129Val polymorphism and codon 180 mutation on different alleles, and one CJD case with the codon 232 mutation and the codon 180 mutation on different alleles), but no cases with the codon 178 mutation. The codon 180 mutation was located on the Met129 allele in all four patients.

(*b*) *Clinical aspects*

The onset of the disease occurred in old age (one biopsy CJD case with Ile180 variant PrP in a 66 year-old patient, one probable CJD case with Ile180 at 75 years, one autopsy CJD case with Ile180 and Val129 at 81 years old, and one autopsy CJD case with Ile180 and Arg232 at 85 years (Hitoshi *et al.* 1993)). At the onset, patients showed dementia (spatial disorientation, memory and calculation disturbance, etc.). Dementia progressed subacutely. Disease progression from onset to akinetic mutism were slower (6 months to 1 year) than that in wild-type (Met/Met129) CJD patients. Patients showed extrapyramidal signs such as tremor and rigidity, and also showed myoclonus. However, none of the patients with Ile180 variant PrP had PSD in the EEG. Total clinical duration was from 1 to 2 years. Therefore, clinical manifestations in these patients with Ile180 variant PrP are similar to those seen in the wild-type CJD patients except for a lack of PSD. As far as we examined, CJD with the Ile180 variant does not have a familial occurrence. Concerning a lack of familial onset, one possible explanation is that Ile180 variant PrP is a less pathogenic mutation because of the older age at onset compared to the other mutations.

(*c*) *Pathological aspects*

PrP immunostaining sometimes did not reveal PrP accumulation in these patients, even with the hydrolytic autoclaving enhancement. Only weak diffuse grey matter PrP staining was seen. Plaque-type PrP depositions were not documented except for a patient with Ile180 and Val129 variant PrP. Western blot analysis revealed the proteinase-resistant PrP (PrP^{CJD}) in three patients with Ile180 variant PrP. However, the concentration of PrP^{CJD} was much less than that of the wild-type (Met/Met129) CJD patients. We also had negative PrP immunostaining with the brain of fatal familial insomnia (T. Kitamoto & J. Tateishi, unpublished data). Compared with Western blot analysis, PrP immunostaining still has a limitation in sensitivity.

The most prominent histopathologic findings were typical spongiform changes in the cerebral cortex, basal ganglia and thalamus. Neuronal loss and astrocytosis were also observed in the cerebral cortex. However, even in a patient with 2 years clinical duration, spongiform changes were still prominent. The destructive changes, so called 'Status spongiosus' which were often observed in the wild-type CJD patients with 2 years clinical course, were never observed in patients with Ile180. The cerebellum was well preserved in a patient with Ile180 and Val129 variant PrP.

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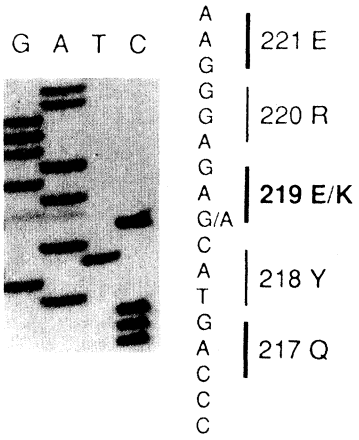

Figure 3. Polymerase chain reaction (PCR) direct sequencing of the codon 219 polymorphism. The G-to-A transversion leads to a Glu to Lys substitution at codon 219 in human prion protein.

7. CODON 200 MUTATION (E200K)

(*a*) *Genetic analysis*

Glutamic acid (GAG)-to-lysine (AAG) substitution at codon 200 was first reported by Goldgaber *et al.* (1989), and was also discovered in families in Slovakia and Libya (Goldfarb *et al.* 1990*a*,*b*). This mutation abolishes a BsmA I site. BsmA I cuts the wild-type PCR products into 578 and 160 b.p. fragments, but does not cut the mutant type. At present, we have found three Japanese families with this mutation. One family has a familial occurrence of CJD (Inoue *et al.* 1994). The other two families have only one CJD patient in each family. In these 2 families, we identified several unaffected family members who have the codon 200 mutation. Therefore, the codon 200 mutation seems to have a low penetrance in Japan. A prospective study will be needed to estimate the penetrance.

(*b*) *Clinical aspects*

Five patients with Lys200 variant PrP were identified in three different Japanese families. The clinical course of these patients was almost the same as that of the wild-type (Met/Met129) CJD patients. Age at the onset was 58 ± 9 years. These patients showed rapidly progressed dementia, extrapyramidal signs, pyramidal tract signs and myoclonus. Disease progression from onset to akinetic mutism was about 4 months. All patients had PSD in the EEG.

(*c*) *Pathological aspects*

We examined two autopsy cases in the same family (Inoue 1994). The pathological changes were almost the same as seen in the wild-type (Met/Met129) CJD patients. There were no plaque-type PrP depositions throughout the central nervous system. Synaptic-type PrP depositions were found in the grey matter of the central nervous system (Kitamoto *et al.* 1992*b*).

Spongiform changes, neuronal loss and severe astrocytosis were observed in the cerebral cortex, basal ganglia, thalamus and the cerebellum. We cannot differentiate CJD patients with Lys200 from the wild-type CJD patients by pathological examination.

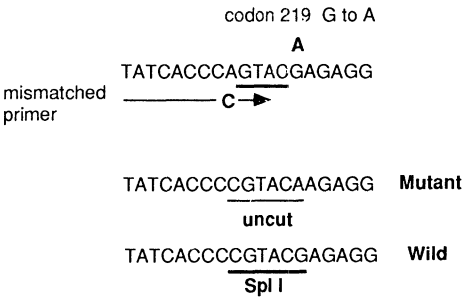

Figure 4. Strategy for the detection of the codon 219 polymorphism (Glu to Lys). We prepared a mismatched sense primer (Sc-7) to induce a Spl I site in the Glu219 allele, but not in the Lys219 allele.

8. CODON 219 POLYMORPHISM (E219K)

(*a*) *Genetic analysis*

We recently found a glutamic acid (GAG)-to-lysine (AAG) substitution at codon 219 in a family with familial schizophrenia (figure 3). One healthy person in this family also has this variant PrP. We examined 100 individuals from the general population of Japan to determine whether this variant is a mutation or a normal polymorphism. Unfortunately, this substitution could not be detected by RFLP. Thus, we prepared a mismatched primer to induce a restriction enzyme site. We designed a mismatched sense primer (Sc-7: AGCAGATGTGTATCACCCCGTA) and a matched antisense primer (Sc-4: AGGAAGATGAGGAAAGAGATC) (figure 4). The nested PCR products with Sc-7 and Sc-4 primers show a Spl I cutting site (CGTACG) in the Glu allele at codon 219, but not in the Lys allele because the Lys allele PCR products result in CGTACA at this portion. Spl I cuts the Glu allele PCR products into 98 and 20 b.p., but does not cut the Lys allele PCR products (118 b.p.) (figure 5).

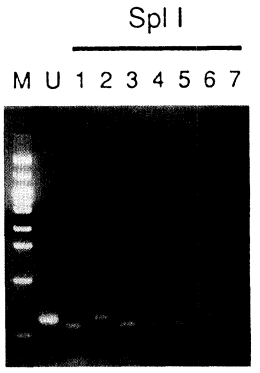

Figure 5. Restriction analysis of the codon 219 polymorphism. Polymerase chain reaction (PCR) products with Sc-4 and Sc-7 primers were digested with Spl I. Lane U, undigested PCR product; lanes 2 and 6, PCR products from DNA with Glu/Lys219 alleles; lane 1, 3, 4, 5 and 7, PCR products from DNA with Glu/Glu219 alleles. In lanes 2 and 6, the undigested 118 b.p. PCR products were detected in addition to the digested 98 b.p. fragment.

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In the sample from the Japanese general population, 88 individuals had Glu/Glu at codon 219, and 12 had Glu/Lys. Allele frequency shows that Glu-to-Lys substitution at codon 219 is 0.94–0.06. We examined five different families with the Lys allele at codon 219 at least in two generations. Therefore, we concluded that Lys variant PrP at codon 219 is a normal polymorphism seen in the Japanese. All examined Lys variant PrP at codon 219 were on the Met129 allele. Interestingly, Lys219 variant PrP was detected in patients with codon 102 mutation or with codon 232 mutation. In almost all these patients, Lys219 variant PrP was located on the wild-type allele, but not on the mutant allele. However, we found one Japanese family with a Leu102 mutation and Lys219 variant on the same allele. Four family members in this family are now affected and have an allele with Leu102 and Lys219. These patients show only slight cerebellar signs compared with GSS patients with Leu102 and Glu219.

At present, we do not have any autopsy cases with Lys219 variant PrP. Therefore, it is not conclusive that this variant PrP influences the phenotype of prion disease.

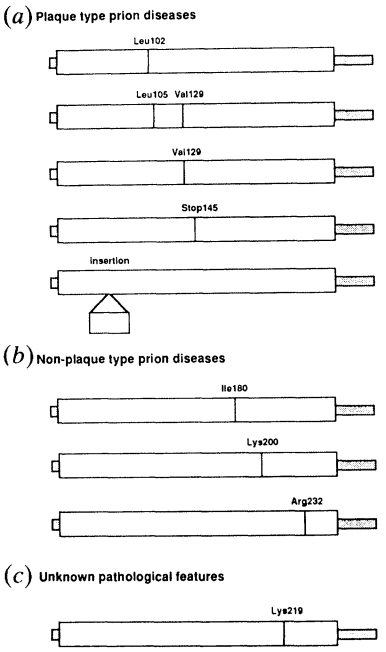

Figure 6. Schematic presentation of prion protein polymorphisms detected in Japanese patients. Interestingly, plaque-type prion diseases have a substitution or insertion at the N-terminal side of prion protein, while non-plaque-type prion disease have a substitution at the C-terminal side.

9. CODON 232 MUTATION (M232R)

(*a*) *Genetic analysis*

Methionine-(ATG)-to arginine (AGG) substitution was found in sporadic CJD patients (Kitamoto *et al.* 1993*c*). To detect this Arg232 variant, we prepared primers K-7 (GTCACC-ACAACCACCAAGGG) and K-8 (CAGGAAGACCTTCCTCATCC). The mutation abolishes a Nla III cutting site. PCR products (738 b.p.) with T-1 and T-2 primers have 12 Nla III cutting sites. Therefore, we designed K-7 and K-8 to make a PCR product (212 b.p.) containing only one Nla III site at codon 232. Nla III cuts the wild-type PCR products into 129 and 83 b.p. fragments, but does not cut the mutant type. Nla III cutting revealed no Arg232 allele in 100 individuals of the general population, and eight CJD cases with the Arg232 allele. Codon 232 mutation was detected on the Met129 allele. In some cases, the codon 232 mutation was combined with another polymorphism. One autopsy patient had both codon 180 and codon 232 mutations each on a different allele. One living patient has the codon 232 mutation and Lys219 variant each on a different allele. Two autopsy cases had only the codon 232 mutation. Almost all cases have no familial occurrence. Recently, we found one family with the codon 232 mutation and two probable CJD cases. Therefore, further study should clarify whether this Arg232 PrP variant is a mutation with low penetrance rate or a rare polymorphism.

(*b*) *Clinical aspects*

The patients with Arg232 variant PrP showed older onset (65 ± 7 years old) than patients with Lys200. All CJD patients with Arg232 had progressive dementia, myoclonus and PSD and EEG. Disease progression from onset to akinetic mutism was about 4 months. These clinical manifestations were almost the same seen in CJD patients with the wild-type (Met/Met129) PrP or Lys200 variant PrP.

(*c*) *Pathological aspects*

We examined two autopsy patients with Arg232 variant PrP. PrP immunostaining revealed diffuse grey matter staining including synaptic structures. However, no plaque-type PrP staining was observed. Histopathologic examination showed typical spongiform change, neuronal loss and severe astrocytosis. Therefore, pathologic changes seen in the patients with Arg232 could not be differentiated from those seen in CJD patients with the wild-type (Met/Met129) PrP or with Lys200 variant PrP.

10. PLAQUE TYPE OR NON-PLAQUE TYPE PRION DISEASES

We summarize variant PrP seen in Japanese patients in figure 6. As described above, prion diseases can be classified into the plaque type and the non-plaque type by the pathological findings. This classification is also useful for clinical applications. The non-plaque-type prion diseases show a short clinical course, rapidly progressive dementia and myoclonus, whereas the plaque-type prion diseases show a long clinical course and slowly developing dementia with a lack of myoclonus and PSD in the EEG. Other clinical manifestations in the plaque-type prion diseases may be influenced by the distribution of the PrP plaques. PrP plaques in patients with Leu105 variant are deposited mainly in the motor cortex, and these patients showed

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the pyramidal tract signs. PrP plaques in patients with Leu102 variant are heavily deposited in the cerebellum, and the patients showed features of spinocerebellar degeneration.

The synaptic PrP accumulations seen in the non-plaque-type prion diseases may directly affect neuronal function, and result in rapidly progressive dysfunction of the central nervous system. In plaque-type prion diseases, extracellular PrP plaques may slowly affect neuronal function in the same fashion as βA4 plaques in Alzheimer's disease, and result in slow deterioration. Therefore, the distribution of PrP accumulations is the key for understanding the pathological mechanism in prion diseases. We claim here that it is important to classify prion diseases into the plaque type or the non-plaque type.

Concerning transmission, brain tissue from the non-plaque-type prion diseases were easily transmitted to small rodents. We have confirmed the successful transmission from CJD patients with the wild-type (Met/Met129) PrP, Lys200 variant or Arg232 variant (Muramoto 1993). However, brain tissue from about 50% of GSS patients with Leu102, or a GSS patient with Val117 did not transmit to small rodents (Tateishi *et al.* 1990). Transmission experiments from patients with Stop145 variant or with Leu105 are now in progress. The transmissibility of plaque-type prion disease may be less than that of non-plaque-type prion disease. At present, we use the term 'prion diseases' for convenience. Among the plaque-type prion diseases, there are some diseases which would be better called 'prion protein diseases'. Further transmission experiments should answer this question.

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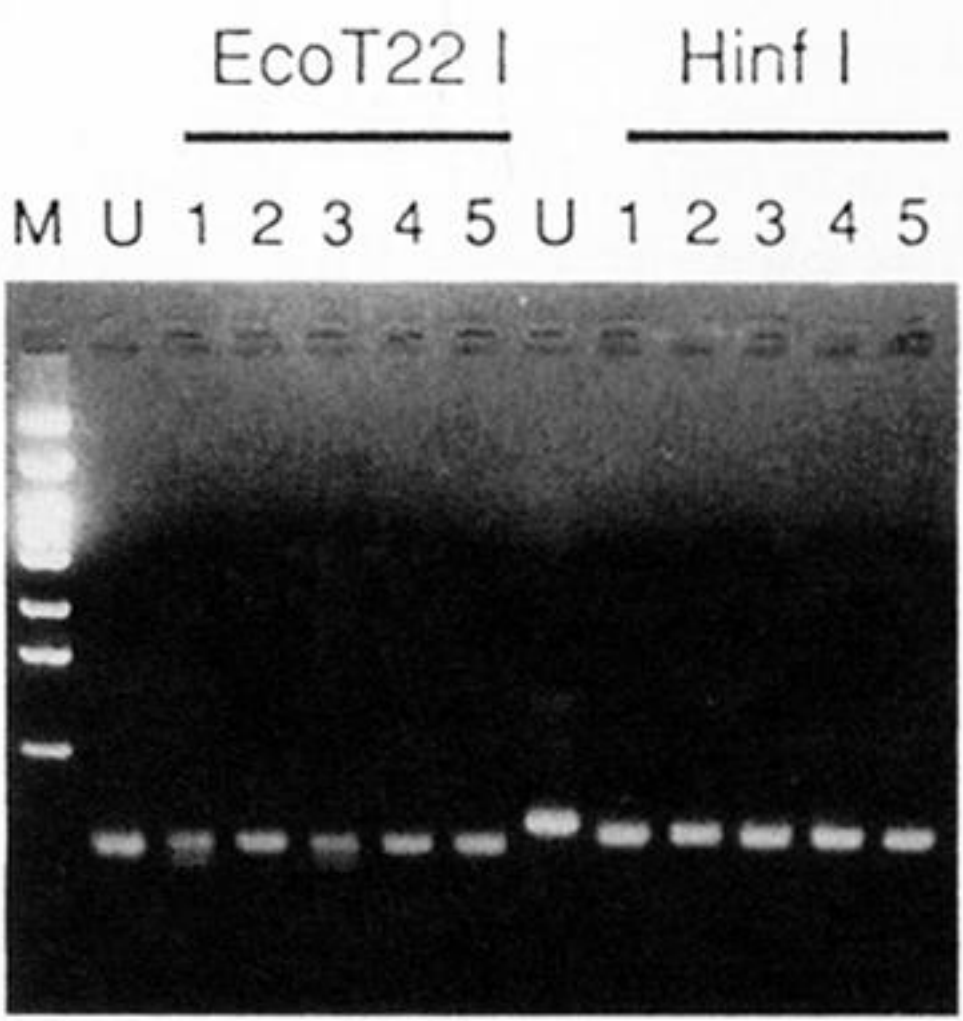
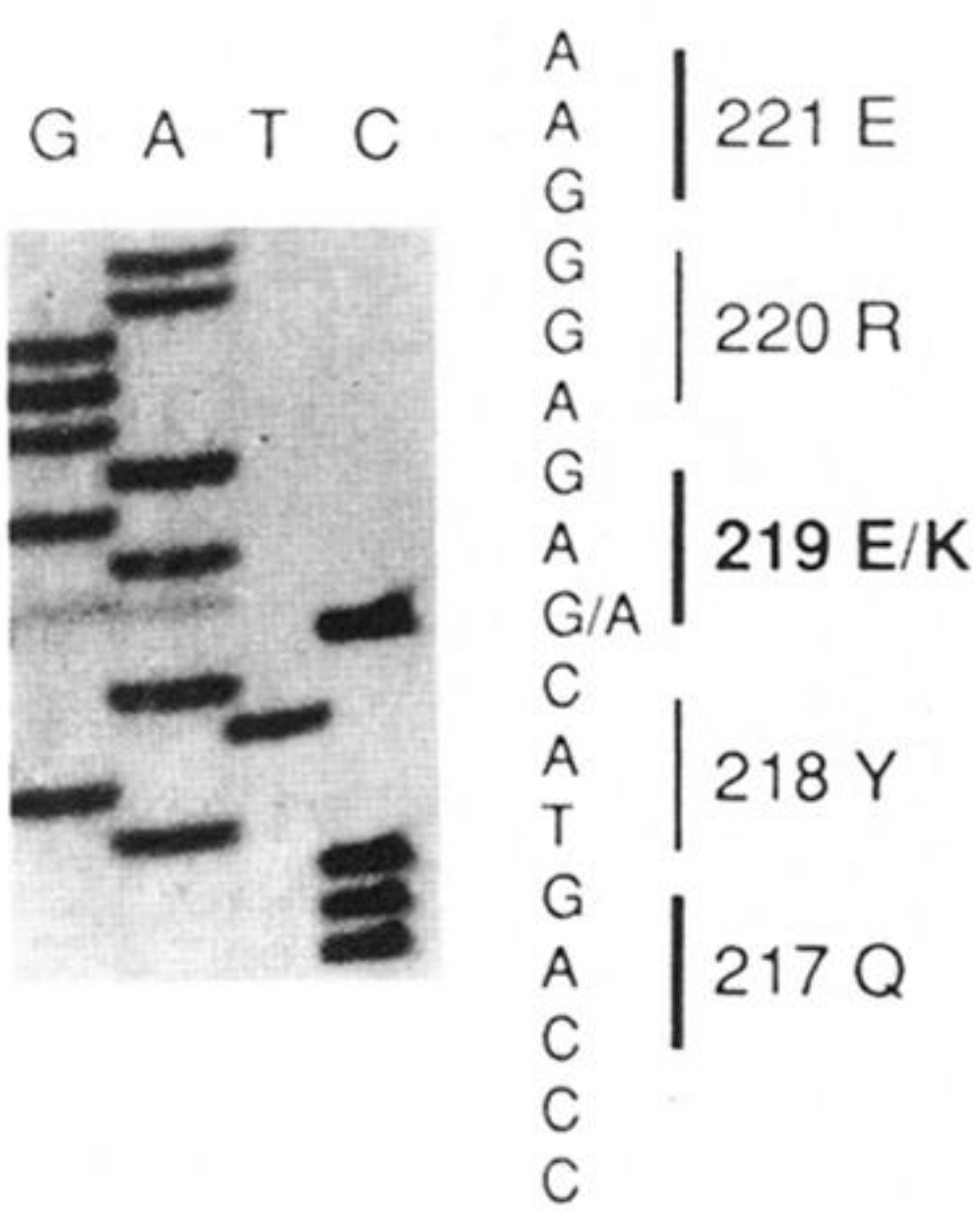
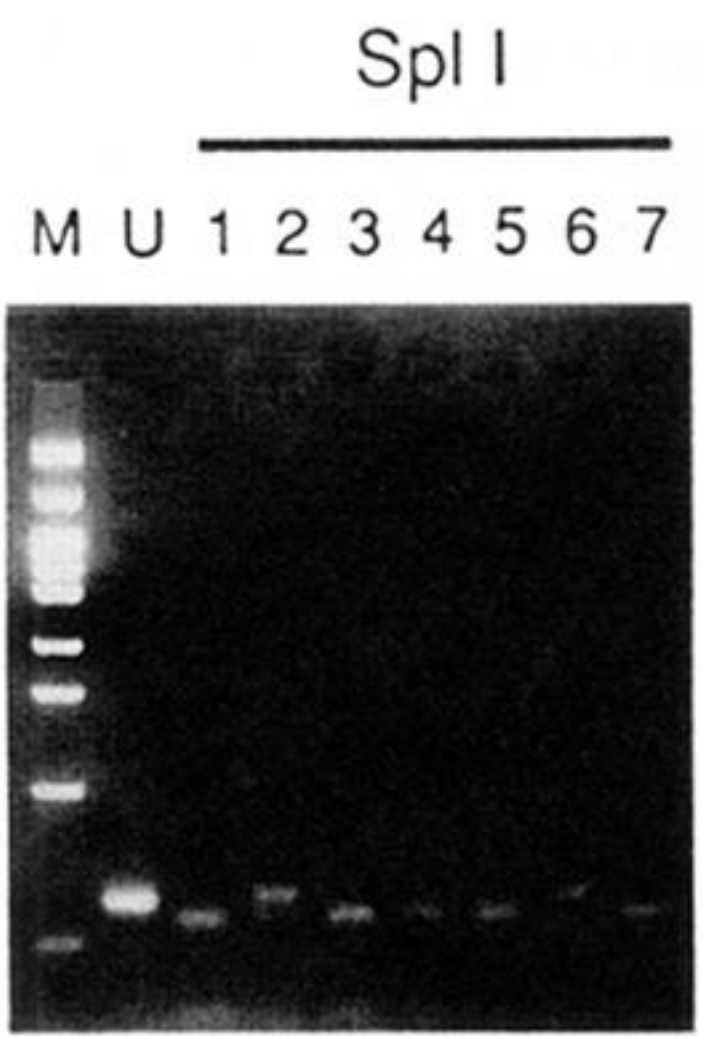


gure 2. Restriction analysis of the codon 178 or codon 180 utation. Polymerase chain reactions (PCR) products with E-1 and K-6 primers were digested with EcoT22 I, and R products with TE-2 and K-5 primers were digested with inf I. Lane U, undigested PCR products; lanes 1 and 3, PCR oducts from patients with the codon 180 mutation; lanes 4 and 5, PCR products from healthy control. EcoT22 I cut R products (126 b.p.) into 101 b.p. fragments in lanes 1 d 3. Hinf I cut all PCR products (157 b.p.) into 135 b.p. agments.



gure 3. Polymerase chain reaction (PCR) direct sequencing the codon 219 polymorphism. The G-to-A transversion ads to a Glu to Lys substitution at codon 219 in human ion protein.



gure 5. Restriction analysis of the codon 219 polymor-
ism. Polymerase chain reaction (PCR) products with Sc-4
d Sc-7 primers were digested with Spl I. Lane U,
digested PCR product; lanes 2 and 6, PCR products from
NA with Glu/Lys219 alleles; lane 1, 3, 4, 5 and 7, PCR
oducts from DNA with Glu/Glu219 alleles. In lanes 2 and
the undigested 118 b.p. PCR products were detected in
ldition to the digested 98 b.p. fragment.