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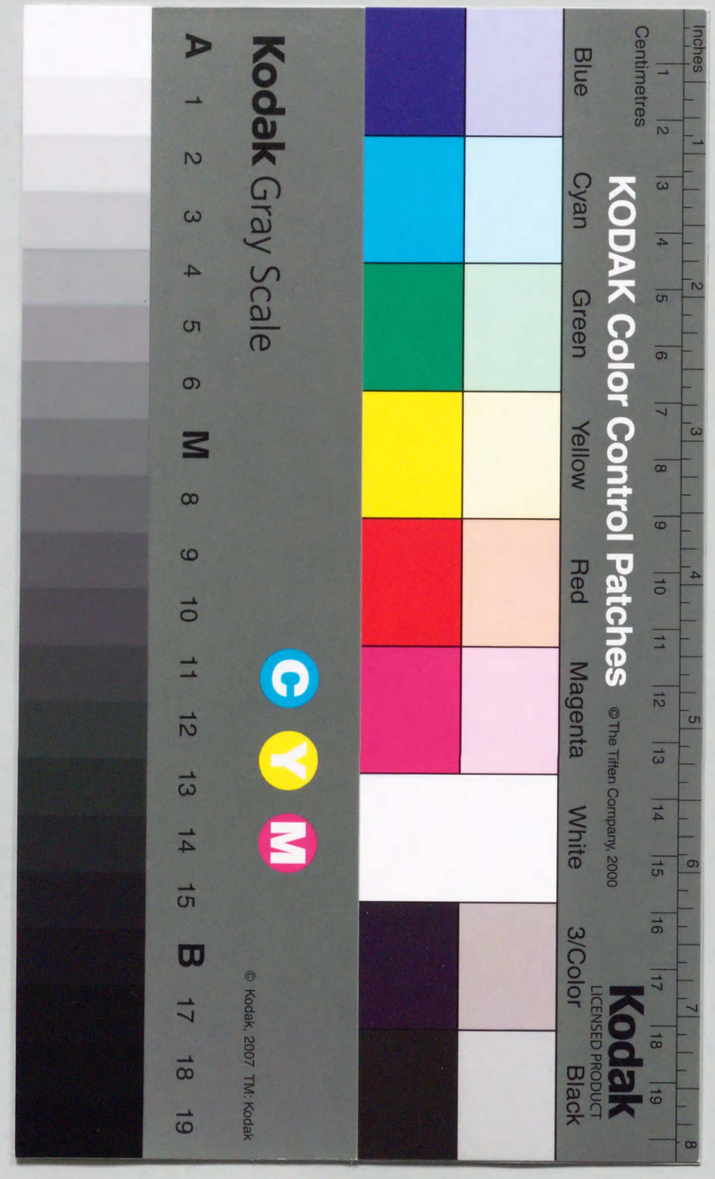

Creutzfeldt-Jakob Disease Patients with Congophilic Kuru Plaques Have the Missense Variant Prion Protein Common to Gerstmann-Sträussler Syndrome

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Congophilic kuru plaques, one of the pathological hallmarks in kuru and Gerstmann-Sträussler syndrome, are sometimes present in patients with Creutzfeldt-Jakob disease (CJD). The congophilic kuru plaques are composed partly of a host-encoded prion protein, and a missense variant prion protein with the codon 102 proline-to-leucine change (Leu^{102}) is commonly present in patients with Gerstmann-Sträussler syndrome. To investigate the relationship between this syndrome and CJD with congophilic kuru plaques, we made a sequence analysis of the prion protein gene from patients with CJD, with or without congophilic kuru plaques. We found no alterations other than the Leu^{102} change, common to Gerstmann-Sträussler syndrome, in one of the prion protein alleles of the patient with congophilic kuru plaques. In the prion protein genotype analysis of other patients with CJD, the Leu^{102} allele was revealed to be carried heterozygously by 6 of 7 patients who had CJD with congophilic kuru plaques, yet no patient with CJD without congophilic kuru plaques had this allele. Interestingly, the Leu^{102} allele was also carried by some unaffected relatives of 3 patients with CJD with congophilic kuru plaques but with no apparent familial occurrence of a similar neurological disorder. Our findings show that CJD with congophilic kuru plaques should be categorized as belonging to Gerstmann-Sträussler syndrome, not CJD, and also suggest that the variant prion protein with Leu^{102} is closely related to the amyloidgenesis seen in subjects with congophilic kuru plaques.

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Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler syndrome (GSS), and kuru in humans and scrapie in animals are progressive neurodegenerative disorders characterized by amyloid deposits (kuru plaques) and spongiform changes in the brain and by transmissibility to experimental animals [1–5]. The infectious agent has not been clearly identified, but an isoform of a protein called prion protein (PrP), a species-specific host-encoded protein [6–10], has been proposed to be a component of the agent, or the agent itself [11, 12]. PrP has been shown to aggregate to form amyloid fibrils [13]. Hsiao and associates [14] reported that a missense variant PrP with the codon 102 proline (Pro^{102})-to-leucine (Leu^{102}) change is tightly linked to ataxic GSS in two unrelated Caucasian families. They suggested that this missense variant does not merely represent a genetic marker, but rather might provoke development of the illness. This has been supported by our findings that the Leu^{102} change is the most common mutation found in patients with GSS, irrespective of their ethnic backgrounds, but it is not the sole mutation related to GSS [15]. In amyloid deposits or accumulations immunohistochemically evidenced to be partly composed of PrP, there are deposits detected by congo-red stain and some that are unstainable [16]. The former, congophilic amyloid deposits or kuru plaques (KPs), which are frequently seen in brains affected by kuru or GSS and are regarded as being pathological hallmarks of these diseases, are present in some brains affected by CJD. To clarify the relation-

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Table 1. Patient Data

Case No.	Sex	Age at Onset (yr)	Clinical Duration (Mo)	Diagnosis	Onset with S-C	Myoclonus	PSDs	Congophilic Plaques	Immunostained Deposits[a]	Transmission to Rodents[b]
1	M	58	12	CJD-KP	+	+	+	+	+	+
2	M	58	48	CJD-KP	+	+	?	+	+	Ongoing
3	M	62	54	CJD-KP	+	+	?	+	+	Ongoing
4	F	44	60	CJD-KP	+	−	−	+	+	Ongoing
5	M	53	96	CJD-KP	+	−	−	+	+	NE
6	F	65	96	CJD-KP	+	+	−	+	+	Ongoing
7	F	37	108	CJD-KP	+	+	+	+[c]	+	+
8	M	61	5	CJD	−	+	+	−	−	Ongoing
9	M	77	10	CJD	−	+	+	−	−	+
10	F	70	12	CJD	−	+	+	−	+	Ongoing
11	F	68	13	CJD	−	+	+	−	+	Ongoing
12	F	63	21	CJD	−	+	+	−	+	Ongoing
13	M	69	22	CJD	−	+	+	−	+	Ongoing
14	F	63	24	CJD	−	+	+	−	+	+
15	F	55	30	CJD	−	+	+	−	+	Ongoing

[a]Immunostaining of amyloid deposits was performed with anti–Gerstmann-Sträussler syndrome amyloid plaque core antiserum, as described [16].
[b]The transmission experiment was performed as previously described [3, 5].
[c]Retrospective examination revealed congophilic kuru plaques.

S-C = spinocerebellar signs; NE = not examined; ? = questionable data; PSDs = periodic synchronous discharges; CJD-KP = Creutzfeldt-Jakob disease with congophilic kuru plaques; CJD = Creutzfeldt-Jakob disease without congophilic kuru plaques.

ship between GSS and CJD with congophilic KPs, we investigated the PrP gene from Japanese patients who had CJD with or without congophilic KPs.

Materials and Methods

Subjects

Specimens from 7 patients with sporadic CJD with congophilic KPs (CJD-KP) and 8 patients with sporadic CJD without congophilic KPs were analyzed (Table 1). The patients with CJD-KP had no known affected family members, but the clinicopathological features resembled those of GSS rather than CJD. Generally, the patients with CJD-KP presented with spinocerebellar signs and had an insidious clinical course with amnesia and disorientation for over 1 year before lapsing into a bedridden state. Pathologically, they showed numerous congophilic KPs. On the other hand, patients with CJD without congophilic KPs started with acute or subacute psychiatric signs, behavior abnormality, character change, visual impairment, memory and calculation disturbance, and gait disturbance, and had an acute progression for several months, proceeding to an apallic state with remarkable myoclonus and periodic synchronous discharges on electroencephalogram. There were remarkable brain atrophy and spongiform changes but no congophilic KPs. The diagnoses of CJD and CJD-KP were confirmed histopathologically, immunohistochemically, and by kuru plaque core purification and PrP detection using Western blots and, in some cases, by experimental transmission to small rodents [3, 5, 16, 17]. All of these patients were nonconsanguineous.

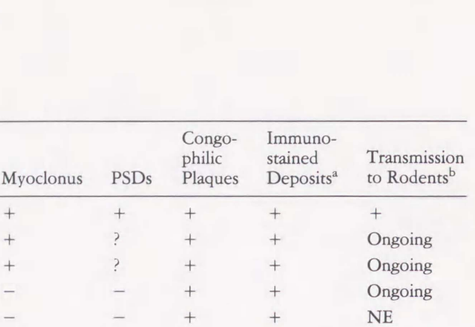


Fig 1. Pedigrees of 3 families affected by Creutzfeldt-Jakob disease with congophilic kuru plaques. Numbers indicate age (years) of the living individuals. I, II, III indicate Pedigrees of families of Cases 1, 2, and 5 with Creutzfeldt-Jakob disease with congophilic kuru plaques, respectively.

The subjects analyzed in the family study of CJD-KP are listed in Figure 1.

Polymerase Chain Reaction

High-molecular-mass DNA was prepared from frozen brain tips (from all CJD and CJD-KP subjects except for Case 5), formalin-fixed brain tissues (Case 5), or peripheral blood cells (all subjects tested in the family study). The PrP coding region was amplified by polymerase chain reaction (PCR) using a pair of primers: 5′-GATGCTGGTTCTCTTTGTGG-3′ is complementary to the antisense strand around the initiation codon and 5′-TCCCACTATCAGGAAGATGA-3′ is complementary to the sense strand at the terminus of the coding region. PCR was performed as described by Saiki and associates [18], with slight modification. One microgram of high-molecular-mass DNA was mixed with 50 pmol of each primer and 200 μM of each deoxyribonucleoside 5′-triphosphate in 100 μl of reaction buffer containing 10 mM Tris hydrochloride (pH 8.3), 50 mM potassium chloride, 1.5 mM magnesium chloride, and 0.01% gelatin. The mixture was heated to 94°C for 7 minutes for strand separation before annealing for 2 minutes at room temperature. Five units of *Taq* polymerase (Perkin-Elmer Cetus, Norwalk, CT) was then added. The cycle of denaturation (94°C, 1 minute), annealing (55°C, 1 minute), and extension (73°C, 1 minute) was carried out on a DNA thermal cycler (Perkin-Elmer Cetus) for 30 cycles.

Cloning and Sequencing of Amplified DNA

PCR-amplified products of Case 2 (CJD-KP) and Case 9 (CJD) (see Table 1) were doubly digested with *Bal* I and *Sau*3AI and then cloned into pUC18. Nucleotide sequence of the cloned fragments was determined by the dideoxy chain termination method [19].

Dot Hybridization Analysis

Dot blot differential hybridization of PCR-amplified DNA was performed with a pair of allele-specific oligonucleotide probes: 5′-ACAAGCCGAGTAAGCCA-3′ for Pro^{102} (normal); 5′-TGGCTTACTCAGCTTGT-3′ for Leu^{102} (variant), which were designed not to form stable G:T mismatches in hybrids [20]. Ten microliters of PCR-amplified DNA was alkaline-denatured with 0.2 N sodium hydroxide at room temperature for 10 minutes and then neutralized to 1 M ammonium acetate before being dot-blotted onto nylon membranes. The membrane was prehybridized for 30 minutes at 42°C in 10 × SSC (1.5 M sodium chloride, 150 mM sodium citrate; pH 7.0), 20 × Denhardt's solution (0.4% bovine serum albumin, 0.4% polyvinylpyrolidone, 0.4% Ficoll), 1% sodium dodecyl sulfate, and 2 mg/ml herring sperm DNA. Each allele-specific oligonucleotide probe, the 5′-end of which was labeled with [γ-^{32}P]ATP, was then added to the same buffer and hybridization was performed at 42°C for 1 hour. The membrane was rinsed twice with 6.67 × SSC (1.0 M sodium chloride, 100 mM sodium citrate; pH 7.0) and 0.1% sodium dodecyl sulfate and washed once with the same solution at 52°C for the normal probe and 50°C for the variant, which is about 2°C below the melting temperature of each of the probes. The membrane was then exposed for several hours to x-ray film at −70°C with intensifying screens.

Results

PrP Gene Analysis

To investigate the nature of PrP from patients who had CJD with or without congophilic KPs, we analyzed the PrP genes of a patient with CJD-KP and of a patient with CJD who was negative for congophilic KPs and immunostained deposits (Cases 2 and 9 in Table 1, respectively). The coding region of PrP gene was amplified by PCR, cloned into plasmid pUC18, and then sequenced. As a result, several clones from the patient with CJD-KP were found to contain a C to T transition at the second letter of codon 102, resulting in a proline-to-leucine change (Leu^{102}) (Fig 2). This substitution is the same change that has been commonly found in patients with GSS regardless of ethnic origins [15]. In contrast, 20 of the clones from the patient with CJD showed no base changes other than artifacts occurring during PCR or cloning experiments, as confirmed by restriction fragment length polymorphism with appropriate restriction enzymes or by dot differential hybridization with appropriate pairs of allele-specific oligonucleotide probes.

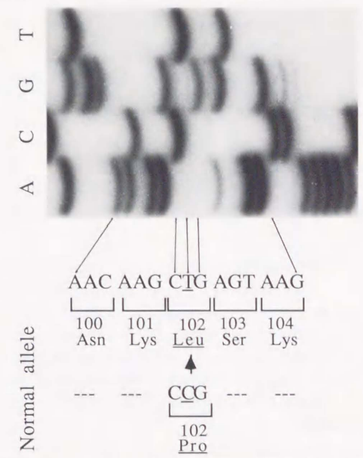


Fig 2. Sequence analysis of one of the prion protein alleles from a patient with Creutzfeldt-Jakob disease with congophilic kuru plaques (Case 2). The second letter of codon 102 is not C but T, resulting in a change from proline to leucine.

PrP Polymorphism Genotype Analysis

We determined genotypes of 7 patients with CJD-KP and 8 patients with CJD for the polymorphism in codon 102. As shown in Figure 3 and summarized in Table 2, 6 of 7 patients with CJD-KP carried a Leu^{102} allele, heterozygously. In contrast, none of the 8 patients without congophilic KPs carried this allele. The only patient (Case 7) with CJD-KP who was negative for the Leu^{102} allele was a 9-year survivor, presenting with an acutely progressive gait disturbance, visual impairment, disorientation, and dementia preceding a 7-year apallic state and pathologically showing severe cerebral atrophy and a few congophilic KPs. Since clinical signs and symptoms progressed acutely at the

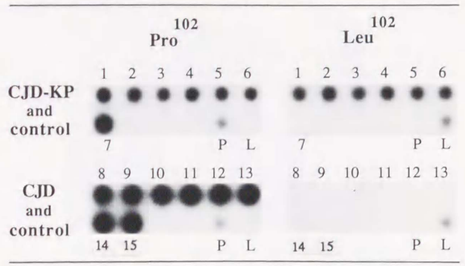


Fig 3. Examples for dot blot analysis for the prion protein polymorphism at codon 102 with a pair of allele-specific probes. Data on all patients with Creutzfeldt-Jakob disease (CJD) or CJD with congophilic kuru plaques (CJD-KP) who were tested are shown. Numbers indicate the case numbers of subjects in Table 1. Case 5 was derived from a formalin-exposed DNA sample. P = cloned prion protein gene with Pro^{102}, L = cloned prion protein gene with Leu^{102}.

beginning of the clinical course and the congophilic KPs were fewer than in other patients with CJD-KP, this case seems to differ from the other cases of CJD-KP.

Family Analysis

Eight individuals, with no signs or symptoms of GSS or CJD, from 3 families of patients with CJD-KP who had a Leu^{102} allele were examined concerning substitution in codon 102, and the results are shown in Figure 1. The Leu^{102} allele was carried by a child (pedigree I), a brother (pedigree II), and three children (pedigree III) of patients with CJD-KP.

Table 2. Results of Prion Protein Polymorphism Genotype Analysis Summarized in Relation to Diagnosis

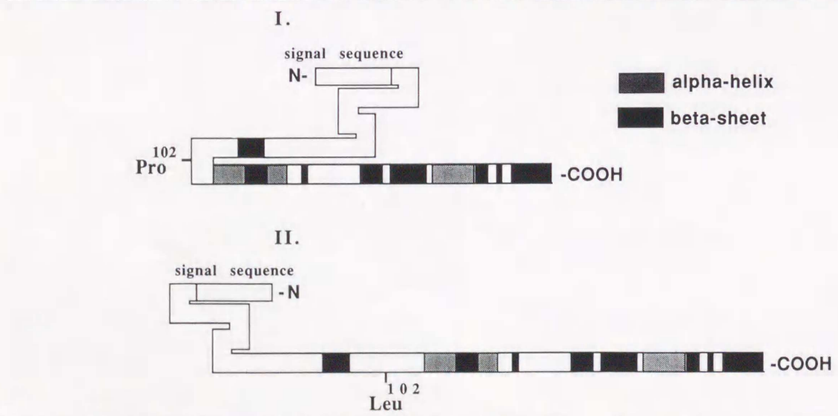
		Pro-Leu^{102}		
Genotype	No.	Pro/Pro	Pro/Leu	Leu/Leu
CJD-KP	7	1	6	0
CJD	8	8	0	0

CJD-KP = Creutzfeldt-Jakob disease with congophilic kuru plaques; CJD = Creutzfeldt-Jakob disease without congophilic kuru plaques.

Discussion

One of the main findings in this work is that the GSS-common mutation of PrP, the codon 102 proline-to-leucine change, also exists in apparently sporadic cases of CJD-KP. It is difficult to reconcile our findings with those of Hsiao and colleagues [14], who provided strong evidence that the linkage of the codon 102 leucine substitution to GSS indicates that GSS is an inherited illness. However, we found that 3 patients with CJD-KP had one or more unaffected family members with the PrP mutation, despite lack of similar neurological disease in the families. The lack of other affected family members can be variously explained. In pedigree II, one of the parents of the patient (Case 2) must have had a Leu^{102} allele, since both the patient and his brother had this allele. Hence, clinical evaluation of the disease may not have been thorough in the mother, who died at the age of 54 years of undetermined cause, and in the father, who died of gastric cancer at age 85. They may have died before development of the illness, or they were not affected because of absence of other factors necessary for development of the illness. These explanations for pedigree II can also be applied to Case 1 (pedigree I) and Case 5 (pedigree III). If their parents and siblings are free of the Leu^{102} allele, these patients might represent individuals with de novo germline mutations. If this is the case, one would not expect to discover a history of neurodegenerative disease in the previous generation. However, one might well expect that the 4 offspring of Cases 1 and 5, aged 36, 46, 41, and 39 years, are still young enough to be at risk for the disease, and that all who possess the codon 102 leucine substitution will eventually develop neurological disorders. Older individuals, such as Case 2's unaffected brother, who also has the leucine variant, are at risk to develop the illness. Thus, these Leu^{102}-positive patients with CJD-KP, who showed clinicopathological features suggestive of GSS, may well be regarded as cases of GSS with no other apparent familial occurrences.

Fig 4. The predicted secondary structure of prion protein with Pro^{102} (I) and prion protein with Leu^{102} (II).



We do not know the exact role(s) of the variant PrP in the disease process of GSS or CJD-KP, but it might (1) be a constituent of a causative agent or the agent itself, (2) increase susceptibility of the host to the agent, or (3) change the nature of PrP to aggregate to form amyloid. The first and the second possibilities can be clarified by follow-up of Leu^{102}-positive unaffected individuals. The third is now supported by the findings that the leucine variant was found in all congophilic KP-positive patients with GSS [15] or CJD-KP except for one, and is a major component of congophilic KPs (data not shown). Interestingly, the secondary structure of PrP with Leu^{102} predicted by the method of Chou and Fasman [21] significantly differs from that of PrP with the codon 102 proline (Pro^{102}) (Fig 4). The change of proline to leucine in codon 102 predicts abolition of a beta-turn structure at that position. This change might facilitate aggregation of the variant PrP to form amyloid, similarly to variant transthyretins in familial amyloidotic polyneuropathy [22, 23]. However, there is a fundamental distinction between familial amyloidotic polyneuropathy and prion diseases in that the former has systemic deposition of amyloid fibrils and transmission is not horizontal. As for amyloid deposits, immunostaining has facilitated detection of smaller cerebral amyloid accumulations that cannot be detected by congo-red stain [16]. We asked whether the amyloidgenesis seen in congophilic KPs is the same as that in the immunostained deposits undetectable by congo-red stain. We wish to point out that the variant PrP with Leu^{102} was carried by none of the patients having noncongophilic amyloid deposits. This suggests that the amyloidgenesis in congophilic KPs differs from that in noncongophilic amyloid deposits or accumulations.

In conclusion, we propose that CJD-KP be classified in GSS. Analysis of PrP genes in patients and their families with sporadic CJD, especially those with marked spinocerebellar signs or a relatively long clinical course, should be done to rule out CJD-KP, presumably an inherited disorder. It is also necessary to follow up Leu^{102}-positive, unaffected family members of patients with CJD-KP to elucidate and resolve the disease process, in relation to the variant PrP. The variant PrP is involved in the amyloidgenesis seen in congophilic KPs.

Since Hsiao and associates [14] pointed out that the use of DNA extracted from formalin-fixed tissues can alter base composition, the data on Case 5, as derived from formalin-exposed DNA samples, are not absolute. Nevertheless, data on the children (see Fig 1, pedigree III) are interesting and important and support our proposal.

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