Title: A novel combination of prion strain co-occurrence in patients with sporadic Creutzfeldt-Jakob disease

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Running head: Co-occurrence of prion strains in sCJD

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Abstract

Six subgroups of sporadic Creutzfeldt-Jakob disease have been identified by distinctive clinicopathological features, genotype at polymorphic codon 129 (methionine/valine, M/V) of the *PRNP* gene, and type of abnormal prion proteins (type 1 or 2). In addition to the pure subgroups, mixed neuropathological features and co-existence of two types of abnormal prion proteins in the same patient have also been reported. Here, we found that a portion of the patients previously diagnosed as MM1 had neuropathological characteristics of MM2 thalamic form, *i.e.*, neuronal loss of the inferior olivary nucleus of the medulla. Furthermore, co-existence of biochemical features of MM2 thalamic form was also confirmed in the identified cases. In addition, in transmission experiments using prion protein-humanized mice, the brain material from the identified case showed weak infectivity and generated characteristic abnormal prion proteins in the inoculated mice resembling those after inoculation with a brain material of MM2 thalamic form. Taken together, these results show that the co-occurrence of MM1 and MM2 thalamic form is a novel entity of sporadic Creutzfeldt-Jakob disease prion strain co-occurrence. The present study raises the possibility that the co-occurrence of MM2 thalamic form might have been overlooked so far due to scarcity of abnormal prion protein accumulation and restricted neuropathology.

Introduction

Prion diseases are lethal transmissible neurodegenerative diseases including Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler-Scheinker syndrome, fatal familial insomnia (FFI), and variably protease-sensitive prionopathy in humans, or scrapie, bovine spongiform encephalopathy, and chronic wasting disease in animals. The central event in the pathogenesis of prion diseases is a conformational conversion of the normal cellular isoform of prion protein (PrP^{C}) into an abnormal misfolded isoform (PrP^{Sc}), which is a component of a proteinaceous infectious particle, namely prion (1). The conformational conversion of PrP^{C} can occur due to either one of three causes: [1] spontaneous conversion in sporadic CJD (sCJD) and variably protease-sensitive prionopathy, [2] pathogenic mutations of the *PRNP* gene in genetic CJD (gCJD), Gerstmann-Sträussler-Scheinker syndrome, and FFI, or [3] prion infection in acquired prion diseases such as iatrogenic CJD, kuru, variant CJD (vCJD), scrapie, bovine spongiform encephalopathy, and chronic wasting disease (2).

Patients with sCJD show clinical and neuropathological heterogeneity associated with the genotype (methionine (M) or valine (V)) at polymorphic codon 129 of the *PRNP* gene and the type (1 or 2) of PrP^{Sc} accumulating in the brain (3). The types 1 and 2 PrP^{Sc} can be distinguished by the size of proteinase K-resistant core of the protein (21 and 19 kDa, respectively) (3, 4). Based on these two determinants, sCJD patients are currently classified into six subgroups: MM/MV1, MM2 cortical form (MM2C), MM2 thalamic form (MM2T), VV1, VV2, and MV2 (5). The MM1 and MV1 subgroups are merged into one subgroup as MM/MV1 because they are indistinguishable in clinicopathological and biochemical features (3). On the other hand, the MM2 subgroup is further divided into two subgroups, MM2C and MM2T, because

92 they show distinctive neuropathological features (3). Widespread confluent vacuoles
93 and intense perivacuolar PrP deposition in the cerebral cortices are characteristics of
94 MM2C patients. By contrast, in MM2T patients, neuronal loss and gliosis are restricted
95 within thalamic nuclei and the inferior olivary nucleus of the medulla, and PrP
96 deposition is faint or negative. Transmission properties of the six sCJD subgroups have
97 also been examined systematically, and five prion strains have been recognized based
98 on the distinctive transmission properties, namely M1 (sCJD-MM/MV1), M2C
99 (sCJD-MM2C), M2T (sCJD-MM2T), V1 (sCJD-VV1), or V2 (sCJD-VV2 and -MV2)
100 strain (6, 7).

101 Co-occurrence of prion strains in the same brain is possible and results in the
102 presentation of mixed neuropathological features and more than one PrP^{Sc} type (3, 8-12).
103 The most frequently observed mixed subgroup is co-occurrence of M1 and M2C prion
104 strains, *i.e.*, sCJD-MM/MV1+2C, which accounts for 26% of total sCJD cases (12). The
105 sCJD-MM/MV1+2C patients show neuropathological and biochemical features of
106 sCJD-MM/MV2C, *i.e.*, confluent vacuoles, perivacuolar PrP deposition, and type 2
107 PrP^{Sc} accumulation, besides sCJD-MM/MV1 characteristics. Moreover, co-existing
108 M2C prion strain can affect the clinical features of patients as well as neuropathological
109 and biochemical properties, and the duration of illness becomes longer with increasing
110 M2C prion strain load (11, 12). However, co-existing M2C prion strain does not affect
111 the transmission properties because the infectivity of M2C strain is very low (13).

112 The relatively high incidence of co-occurrence of M1 and M2C strains
113 prompted us to investigate the possibility of other combinations of sCJD prion strain
114 co-occurrence. To date, co-occurrence of V1 and V2 strains, M2C and V2 strains, M1
115 and V2 strains, or M2C and M2T strains has been reported though the incidence rates

are very low (12). In the present study, we have demonstrated that a portion of sCJD cases previously diagnosed as MM1 had neuropathological characteristics of sCJD-MM2T. Furthermore, co-existence of biochemical properties of sCJD-MM2T was also confirmed in the identified cases. The present study suggests that the co-occurrence of M1 and M2T prion strains may also occur with a relatively high incidence rate.

Materials and methods

Ethics statement

This study was approved by the Institutional Ethics Committee of Hokkaido University Graduate School of Veterinary Medicine (VET27-1). All experiments using human materials were in compliance with the Helsinki Declaration. Animal experiments were performed in strict accordance with the Regulations for Animal Experiments and Related Activities at Hokkaido University and Fundamental Guidelines for Proper Conduct of Animal Experiment and Related Activities in Academic Research Institutions by Ministry of Education, Culture, Sports, Science and Technology in Japan, Notice No. 71. The protocol was approved by the Institutional Animal Care and Use Committees of Hokkaido University (14-0170).

Patients

CJD cases included in this study were patients with clinically, genetically and histopathologically proven sCJD, gCJD, and FFI. Brain tissues were obtained at autopsy from CJD patients after receiving written informed consent for research use. The diagnosis of CJD, histopathological type, and PrP^{Sc} type were confirmed by PrP immunohistochemistry and western blot analysis. The genotype and mutations in the

open reading frame of the *PRNP* gene were determined by sequence analysis as described (14). According to the classification by Parchi *et al* (5), the CJD cases had been classified as follows: sCJD-MM1, 18 cases; sCJD-MM1+2C, 9 cases; sCJD-MM2C, 3 cases; sCJD-MM2T, 4 cases; sCJD-MV2, 3 cases; gCJD-V180I, 9 cases; gCJD-E200K, 2 cases; and FFI, 5 cases. Detailed clinicopathological features of one sCJD-MM1+2C patient (H186) with neuronal loss of the inferior olivary nucleus have been reported elsewhere (15).

Histopathological analysis

Formalin-fixed brain tissues were treated with formic acid (99% for human tissues or 60% for mouse tissues) for 1 hour to inactivate the infectivity and embedded in paraffin. The embedded tissues were sectioned at a thickness of 5 μm. For PrP immunohistochemistry, tissue sections were pretreated by hydrolytic autoclaving (16). The anti-PrP antiserum PrP-N (17) was used as the primary antibody. Goat-anti-rabbit immunoglobulin polyclonal antibody labelled with the peroxidase-conjugated dextran polymer, EnVision^{+} (Dako, Glostrup, Denmark) was used as the secondary antibody. For routine histopathological analysis, the tissue sections were stained with hematoxylin and eosin (H&E). For quantification of neuronal loss in the inferior olivary nucleus, the tissue sections of the medulla were stained by Klüver-Barrera method (18), and the remaining neurons in the right and left inferior olivary nuclei were counted. The number of the remaining neurons was divided by the area of the inferior olivary nucleus that was measured by ImageJ software version 1.52a (http://rsb.info.nih.gov/ij/), and neuronal cell density was calculated.

PrP^{Sc} purification

PrP^{Sc} was purified from human brains or mouse brains as described (19). Briefly, brain tissues were homogenized in 2 ml of lysis buffer (100 mM Tris-HCl pH 8.0, 10 mM NaCl, 10 mM $MgCl_2$, 2% Triton X-100, and 25 units/ml DNase I (Takara Bio)) and digested with collagenase (1 mg/200 mg tissue) (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan) overnight at room temperature. Collagenase digestion disrupts the connective tissue and improves the accessibility of detergents and/or proteinase K to PrP^{Sc} (20). The digested homogenates were ultracentrifuged at 453,000g for 30 min at 4°C, and the pellets were resuspended and sonicated in 870 μl of proteinase K-digestion buffer (100 mM Tris-HCl pH 8.0 and 5% Sarkosyl (Sigma-Aldrich, St. Louis, MO)). The resuspended samples were centrifuged at 1,000g for 3 min to remove the cell debris, and the supernatants (800 μl) were digested with proteinase K (4 μg/200 mg tissue) (FUJIFILM Wako Pure Chemical Corporation) for 1 h at 37°C. It has been reported that these conditions for proteinase K-digestion were sufficient for the complete digestion of normal PrP^{C}, and that higher proteinase K concentrations caused unfavorable degradation of PrP^{Sc} (21). The proteinase K-digested proteins were precipitated by adding 200 μl of 99.5% ethanol and ultracentrifugation at 135,000g for 30 min at 4°C. The pellets were resuspended in 400 μl/200 mg tissue of Laemmli's sample buffer (60 mM Tris-HCl pH 6.8, 5% glycerol, 2% SDS, and 0.01% bromophenol blue) and boiled at 100°C for 10 min.

Western blotting

Protein samples were subjected to SDS-PAGE using 13.5% Bis-Tris long gels of 15 cm length and western blotting as described (22). The anti-PrP monoclonal antibody 3F4

(BioLegend, San Diego, CA) and type 2 PrP^{Sc}-specific anti-PrP polyclonal antibody Tohoku 2 (23) were used as the primary antibodies. The anti-mouse EnVision+ (Dako) and anti-rabbit EnVision+ (Dako) were used as the secondary antibodies. The blots were visualized with Clarity Max Western ECL substrate (Bio-Rad, Hercules, CA), and images were obtained by imaging device ImageQuant LAS 4000 mini (GE Healthcare, Chicago, IL). The signal intensities of the western blots were quantified with ImageQuant TL software version 7.0 (GE Healthcare).

Transmission experiments

The production of knock-in mice expressing human PrP with the 129M/M genotype (Ki-Hu129M/M) has been reported previously (22). Knock-in mice expressing human PrP carrying a causative mutation of FFI (aspartic acid to asparagine at codon 178, Ki-HuD178N) were generated as described (24). The codon 129 genotype of the Ki-HuD178N mice was 129M/M. Intracerebral inoculation was performed as described (25). Briefly, 10% brain homogenates were prepared in sterile phosphate-buffered saline using glass homogenizers, and 20 μl of the homogenates were intracerebrally inoculated into mice. The inoculated mice were sacrificed at a predefined clinical endpoint, or at the time point showing intercurrent illness. One hemisphere of the brain was fixed in 10% buffered formalin for histopathological analysis, and the other hemisphere was immediately frozen for biochemical analysis.

Statistical analysis

Signal intensities of PrP^{Sc} bands are expressed as mean±SEM (n = 3). The Kaplan-Meier log-rank test was used to analyze survival data in transmission study. The

statistical tests were carried out using the statistical software EZR version 1.36 (26).

Results

Co-existence of neuropathological characteristics of sCJD-MM2T

Neuronal loss in the thalamus and the inferior olivary nucleus of the medulla is a key neuropathological features of sCJD-MM2T and FFI (3, 27). Since thalamic nuclei are also affected in other CJD subgroups (Supplemental Figure S1), we focused on neuronal loss of the inferior olivary nucleus to identify co-existing M2T prion strain. Systematic neuropathological analysis of archived prion disease tissue samples revealed that 3 out of 18 (17%) sCJD-MM1 patients showed neuronal loss of the inferior olivary nucleus, as with sCJD-MM2T or FFI patients (Figure 1). Other neuropathological changes were typical of sCJD-MM1 such as neuronal loss, spongiform changes, gliosis, and diffuse synaptic-type PrP deposition in the cerebral and cerebellar cortices. Neuropathological features of sCJD-MM2C, *e.g.*, confluent vacuoles or perivacuolar PrP deposition, were not observed in these sCJD-MM1 patients. In addition, 3 out of 9 (33%) sCJD-MM1+2C patients also showed neuronal loss of the inferior olivary nucleus (Figure 1).

Although the sCJD-MM1 patients with the inferior olivary degeneration showed prolonged duration of illness *i.e.*, duration from onset to death (Table 1), long clinical course was not solely responsible for the inferior olivary degeneration because sCJD-MM1 patients with prolonged clinical course did not always show neuronal loss of the inferior olivary nucleus (Figure 2). Thus, a part of sCJD patients previously diagnosed as MM1 or MM1+2C had neuropathological characteristics of sCJD-MM2T.

Co-existence of biochemical features of sCJD-MM2T

To identify co-existing M2T prion strain biochemically, next we performed western blot analysis of proteinase K-resistant PrP^{Sc} in the brain of the sCJD-MM1 patients with the inferior olivary degeneration. Among the three sCJD-MM1 patients with the inferior olivary degeneration, frozen brain tissues from multiple brain regions were available in two patients (H89 and 0303). In both patients, faint type 2 PrP^{Sc} bands (~19 kDa) were visible alongside with type 1 PrP^{Sc} (~21 kDa) at least one brain region in western blot analysis using a conventional anti-PrP antibody 3F4 (Figure 3). By contrast, no type 2 PrP^{Sc} band was detected in a typical sCJD-MM1 case lacking the inferior olivary degeneration (Supplementary Figure S2). Moreover, type 2 PrP^{Sc}-specific antibody, Tohoku 2 (23), revealed that small amounts of type 2 PrP^{Sc} were widely distributed throughout the brain except cerebellum in the two sCJD-MM1 patients with the inferior olivary degeneration. Thus, although the previous examination using only a single brain region had shown only type 1 PrP^{Sc}, re-examination using multiple brain regions identified co-existing type 2 PrP^{Sc} in the sCJD patients previously diagnosed as MM1. Therefore, these patients also had biochemical features of sCJD-MM2T in addition to neuropathological characteristics. The sCJD-MM1+2C patients with the inferior olivary degeneration (H186 and I197) had been previously examined biochemically, and both types 1 and 2 PrP^{Sc} were detected in the brain as shown in Figure 4 (15).

Co-existence of transmission properties of sCJD-MM2T

To identify co-existing M2T prion strain based on transmission properties, we performed a transmission study using brain homogenates of sCJD-MM1 or -MM1+2C patients with the inferior olivary degeneration. The inocula were prepared from brain

regions where both types 1 and 2 PrP^{Sc} co-existed. In the sCJD-MM1+2C patient, more than one brain region was used for inoculum preparation to enhance the chance of detecting concurrent M2T prion strain because M2T and M2C prion strains are indistinguishable by western blot analysis. The brain homogenates were intracerebrally inoculated into knock-in mice expressing human PrP with the 129M/M genotype (Ki-Hu129M/M) or knock-in mice expressing human PrP carrying a causative mutation of FFI (Ki-HuD178N). The transmission patterns of one sCJD-MM1 material (occipital lobe of 0303) and sCJD-MM1+2C materials (thalamus (I197 Th) or a mixture of the hippocampus and parahippocampus of I197 (I197 Hip)) were identical to those of a typical sCJD-MM1 material. Briefly, the mean incubation period (mean±SD) was shorter in Ki-Hu129M/M mice compared with Ki-HuD178N mice (584±3 days vs. 789±82 days for sCJD-MM1 (0303), $P < 0.005$; 554±26 days vs. 653±56 days for sCJD-MM1+2C (I197 Th), $P < 0.005$; 597±4 days vs. 703±55 days for sCJD-MM1+2C (I197 Hip), $P < 0.005$) (Figure 5A-D). In addition, the inoculated Ki-Hu129M/M mice produced a large amount of type 1 PrP^{Sc} whereas Ki-HuD178N mice produced faint type 2 PrP^{Sc} (Figure 6A and B). By contrast, the transmission patterns of the other sCJD-MM1 material (thalamus of H89) was quite different from those of typical sCJD-MM1. Briefly, the infectivity was weak regardless of the mouse genotype (Figure 5E-G), and the inoculated Ki-Hu129M/M mice produced faint type 1 PrP^{Sc} whereas Ki-HuD178N mice produced di- and monoglycosylated form-dominant type 2 PrP^{Sc} (Figure 6A and B). These transmission properties were similar to those of typical sCJD-MM2T materials.

Discussion

Here we identified concurrence of characteristic features of sCJD-MM2T, *e.g.*, neuronal loss of the inferior olivary nucleus of the medulla, type 2 PrP^{Sc} accumulation, and unique transmission properties, in sCJD patients previously diagnosed as MM1. The present study clearly shows that co-occurrence of M1 and M2T sCJD prion strains in the same patient may also occur.

The incidence rate of co-occurrence of M1 and M2T prion strains may be relatively high. In the present study, 3 out of 18 sCJD cases previously diagnosed as MM1 had neuropathological characteristics of sCJD-MM2T. Co-existence of more than one prion strain in the same patient accounts for 35% of total sCJD cases (12). Therefore, the present study, together with previous findings, suggests that co-occurrence of multiple prion strains is more common phenomenon than expected. Indeed, concurrence of M2T prion strain was also suggested in sCJD-MM1+2C patients in the present study. Since faint accumulation of PrP^{Sc} and limited pathological changes are characteristics of M2T prion strain (3), co-existence of M2T prion strain might have been overlooked so far. To estimate the exact prevalence of co-occurrence of M2T prion strain, further analysis will be needed in the future with a larger number of patients. For this aim, the examination of neuronal loss of the inferior olivary nucleus can be one of the sensitive method to identify co-existing M2T prion strain.

The co-existing M2T prion strain may affect transmission properties of sCJD. We previously reported that co-existing M2C prion strain did not affect transmission properties of M1 prion strain in mice inoculated with brain materials from sCJD-MM/MV1+2C patients (13). This is because the infectivity of M2C prion strain is very low (6, 13). Therefore, one can consider that the risk of transmission of M2C prion strain from sCJD-MM/MV1+2C patients is negligible. By contrast, M2T prion strain

has certain infectivity to PrP-humanized mice (7, 28, 29), although its infectivity is lower than that of M1 prion strain. Indeed, in the present study, one out of four inocula prepared from brains in which co-existence of M2T prion strain was neuropathologically suspected showed unique transmission properties resembling those of M2T prion strain. Meanwhile, only transmission properties of M1 prion strain were observed in transmission experiments of the three other inocula, suggesting that the propagation of M2T prion strain might be overwhelmed by the predominant M1 prion strain.

The co-existence of M2T prion strain may also affect duration of illness of sCJD patients. In the present study, all sCJD-MM1 patients with the inferior olivary degeneration showed long duration of illness, *i.e.*, duration from onset to death, compared with the pure form of sCJD-MM1. In sCJD-MM/MV1+2C patients, co-existing M2C prion strain can affect the clinical features of patients, and the duration of illness becomes longer with increasing M2C prion strain load (11, 12). The potential influence of co-existing M2T prion strain on the clinical features, together with those on the neuropathological, biochemical, and transmission properties, suggests that sCJD cases with concurrent M2T prion strain should be considered as distinctive entity such as sCJD-MM/MV1+2T or sCJD-MM/MV1+2C+2T. The clinical features of sCJD-MM/MV1+2T or sCJD-MM/MV1+2C+2T need to be clarified in the future with a larger number of patients.

In conclusion, co-occurrence of M1 and M2T prion strains is a novel subgroup of sCJD prion strain co-occurrence. The co-existing M2T prion strain is easily and reliably detectable by histopathological analysis of the inferior olivary nucleus of the medulla.

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Figure legends

Figure 1 Neuronal loss of the inferior olivary nucleus of the medulla in CJD patients. A portion of sCJD patients previously diagnosed as MM1 (0701, 0303 and H89) or MM1+2C (1301, I197 and H186) showed decreased neuronal cell density, as with sCJD-MM2T patients or FFI patients.

Figure 2 Neuronal loss of the inferior olivary nucleus does not depend on the duration of illness. Data are represented as neuronal cell density of the inferior olivary nucleus of sCJD-MM1 patients (y-axis) plotted against the duration of illness (x-axis).

Figure 3 Type 2 PrP^{Sc} accumulation in the sCJD-MM1 patients with neuronal loss of the inferior olivary nucleus. A and **B**, Multiple brain regions of the sCJD-MM1 patients were examined by western blot using anti-PrP antibody 3F4 or type 2 PrP^{Sc}-specific antibody Tohoku 2. Type 2 PrP^{Sc} was relatively abundant in the occipital lobe (occipital), a mixture of the hippocampus and parahippocampus (hippocampus), and thalamus in one patient (H89) (**A**), while it was prominent only in the occipital lobe in the other patient (0303) (**B**). A brain sample, equivalent to 0.5 mg in wet weight, was loaded in each lane. The mean signal intensities of PrP^{Sc} in type 1 PrP^{Sc} control (sCJD-MM1) and type 2 PrP^{Sc} control (sCJD-MM2T) were assigned as 100/mm^2 in each experiment using the 3F4 antibody (gray bars) and Tohoku 2 antibody (black bars), respectively. The signal intensities of PrP^{Sc} are expressed as mean ± SEM (n = 3).

Figure 4 Type 2 PrP^{Sc} accumulation in the sCJD-MM1+2C patients with neuronal loss of the inferior olivary nucleus. Type 2 PrP^{Sc} was detected in the

occipital lobe, hippocampus, and parahippocampus, though it was indistinguishable whether the detected type 2 PrP^{Sc} was M2T prion strain or M2C prion strain. A brain sample, equivalent to 1 mg in wet weight, was loaded in each lane.

Figure 5 Kaplan-Meier survival curves after intracerebral inoculation into Ki-Hu129M/M mice (129M/M) or Ki-HuD178N mice (D178N). Transmission patterns of brain materials from the occipital lobe of sCJD-MM1 (0303) (**A**), thalamus of sCJD-MM1+2C (I197 Th) (**B**), and a mixture of hippocampus and parahippocampus of sCJD-MM1+2C (I197 Hip) (**C**) were similar to those of typical sCJD-MM1 (**D**). By contrast, transmission patterns of brain material from the thalamus of sCJD-MM1 (H89) (**E**) were similar to those of typical sCJD-MM2T (**F** and **G**). Data are represented as % of surviving animals (y-axis) plotted against the number of days post inoculation (x-axis). Incubation time in Ki-Hu129M/M mice inoculated with typical sCJD-MM1 material has been reported elsewhere (23).

Figure 6 Biochemical properties of PrP^{Sc} in the brain of the inoculated Ki-Hu129M/M mice (129M/M) or Ki-HuD178N mice (D178N). A and **B**, Western blot analysis of proteinase K-resistant PrP^{Sc} using the anti-PrP antibody 3F4 (short and long exposures) or type 2 PrP^{Sc}-specific antibody Tohoku 2. For sCJD-MM1 (0303), sCJD-MM1+2C (I197 Th) and sCJD-MM1+2C (I197 Hip), the inoculated Ki-Hu129M/M mice produced large amounts of type 1 PrP^{Sc} similar to those of the Ki-Hu129M/M mice inoculated with a typical sCJD-MM1 material (23), and the inoculated Ki-HuD178N mice produced faint type 2 PrP^{Sc} similar to those of the Ki-HuD178N mice inoculated with the typical sCJD-MM1 material. By contrast, for

sCJD-MM1 (H89), the inoculated Ki-Hu129M/M mice produced faint type 1 PrP^{Sc}, and the inoculated Ki-HuD178N mice produced di- and monoglycosylated form-dominant type 2 PrP^{Sc}. The glycosylation patterns of PrP^{Sc} in the Ki-HuD178N mice inoculated with the sCJD-MM1 (H89) material (**A**) were identical to those in the Ki-HuD178N mice inoculated with a typical sCJD-MM2T material (**B**). The amount of brain tissue loaded in each lane is indicated beneath the lane.

494 **Table 1. Summary of the clinical features**

	sCJD-MM1 (H89)	sCJD-MM1 (0303)	sCJD-MM1 (0701)
Sex	Male	Male	Female
Age at onset (years)	61	63	71
Initial symptoms	Progressive dementia	Progressive dementia	Progressive dementia
Myoclonus (months)*	15	8	3
Akinetic mutism (months)*	29	12 †	6
PSWC on EEG (months)*	15	- ‡	2
Duration (months)	36	24	24

* The duration until the appearance of myoclonus, akinetic mutism, or PSWC from onset.

† The patient became bedridden 7 months after the initial symptoms.

‡ Only a single EEG examination was performed 2 months after the initial symptoms. EEG revealed a short burst of delta waves and slowing of background activities.

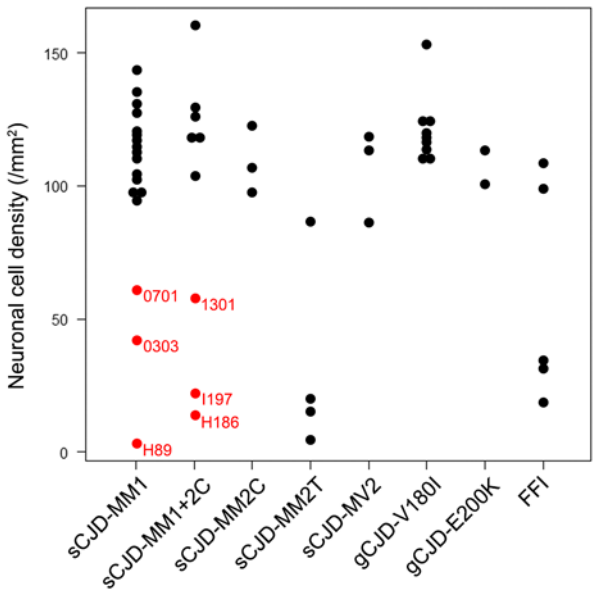

Neuronal cell density (/mm²)
150
100
50
0
0701
1301
0303
I197
H186
H89
sCJD-MM1
sCJD-MM1+2C
sCJD-MM2C
sCJD-MM2T
sCJD-MV2
gCJD-V180I
gCJD-E200K
FFI

Figure 1

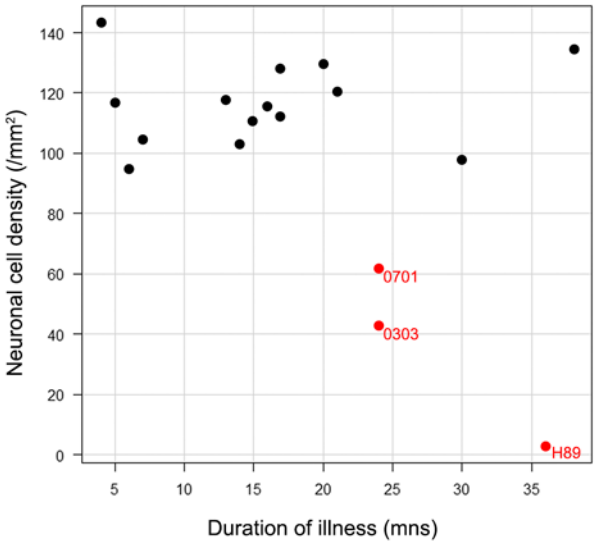

Neuronal cell density (/mm²)
Duration of illness (mns)
0701
0303
H89
0
20
40
60
80
100
120
140
5
10
15
20
25
30
35

Figure 2

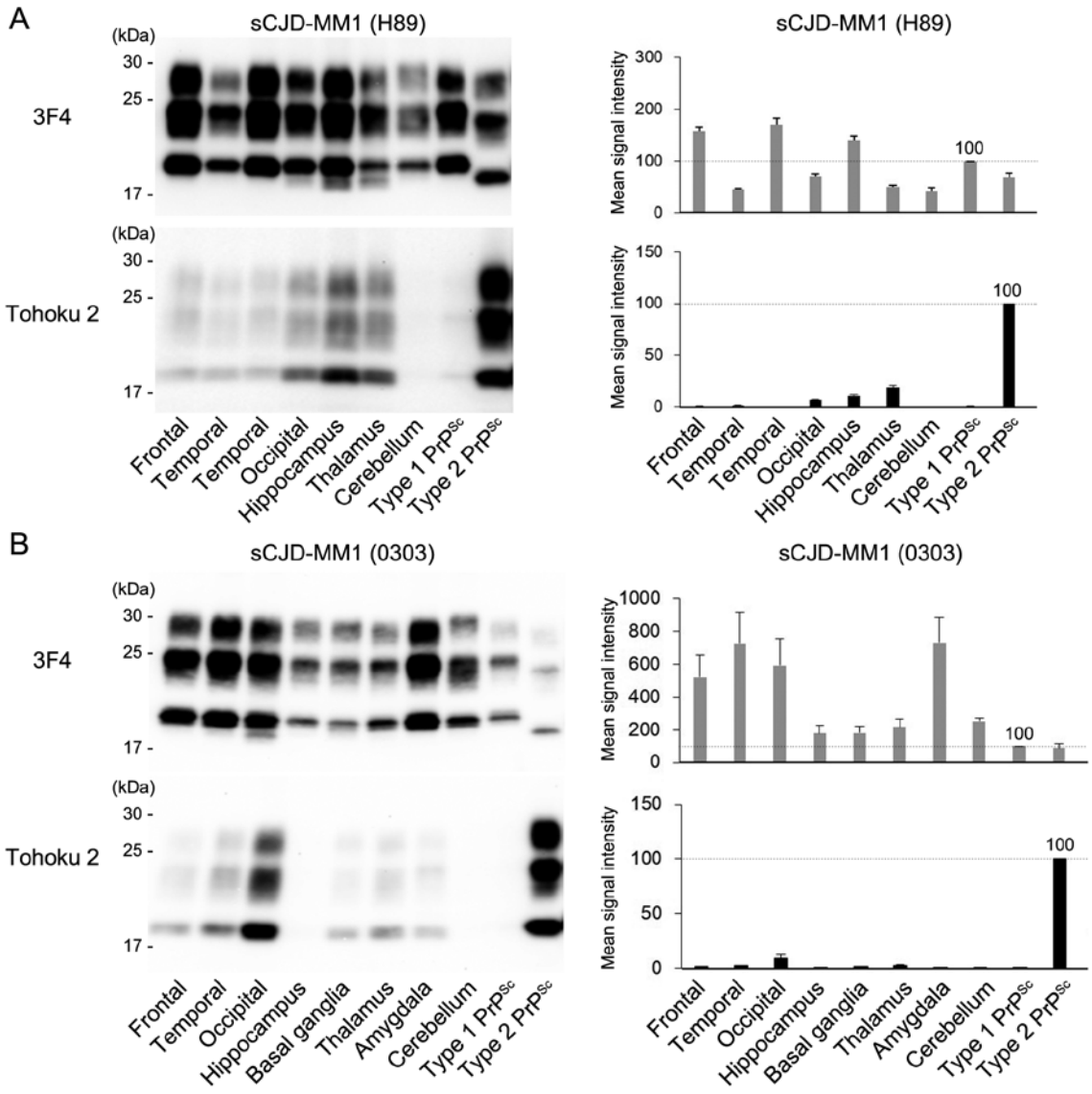

A
sCJD-MM1 (H89)
(kDa)
30 -
25 -
17 -
3F4
Tohoku 2
Frontal
Temporal
Temporal
Occipital
Hippocampus
Thalamus
Cerebellum
Type 1 PrPSc
Type 2 PrPSc
Mean signal intensity
300
200
100
0
150
50
100
B
sCJD-MM1 (0303)
1000
800
600
400
200
Basal ganglia
Amygdala

Figure 3

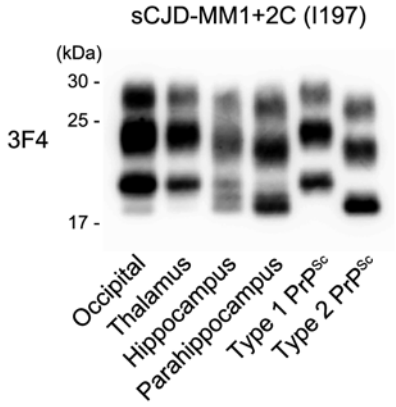

sCJD-MM1+2C (I197)
(kDa)
30 -
25 -
17 -
3F4
Occipital
Thalamus
Hippocampus
Parahippocampus
Type 1 PrPSc
Type 2 PrPSc

Figure 4

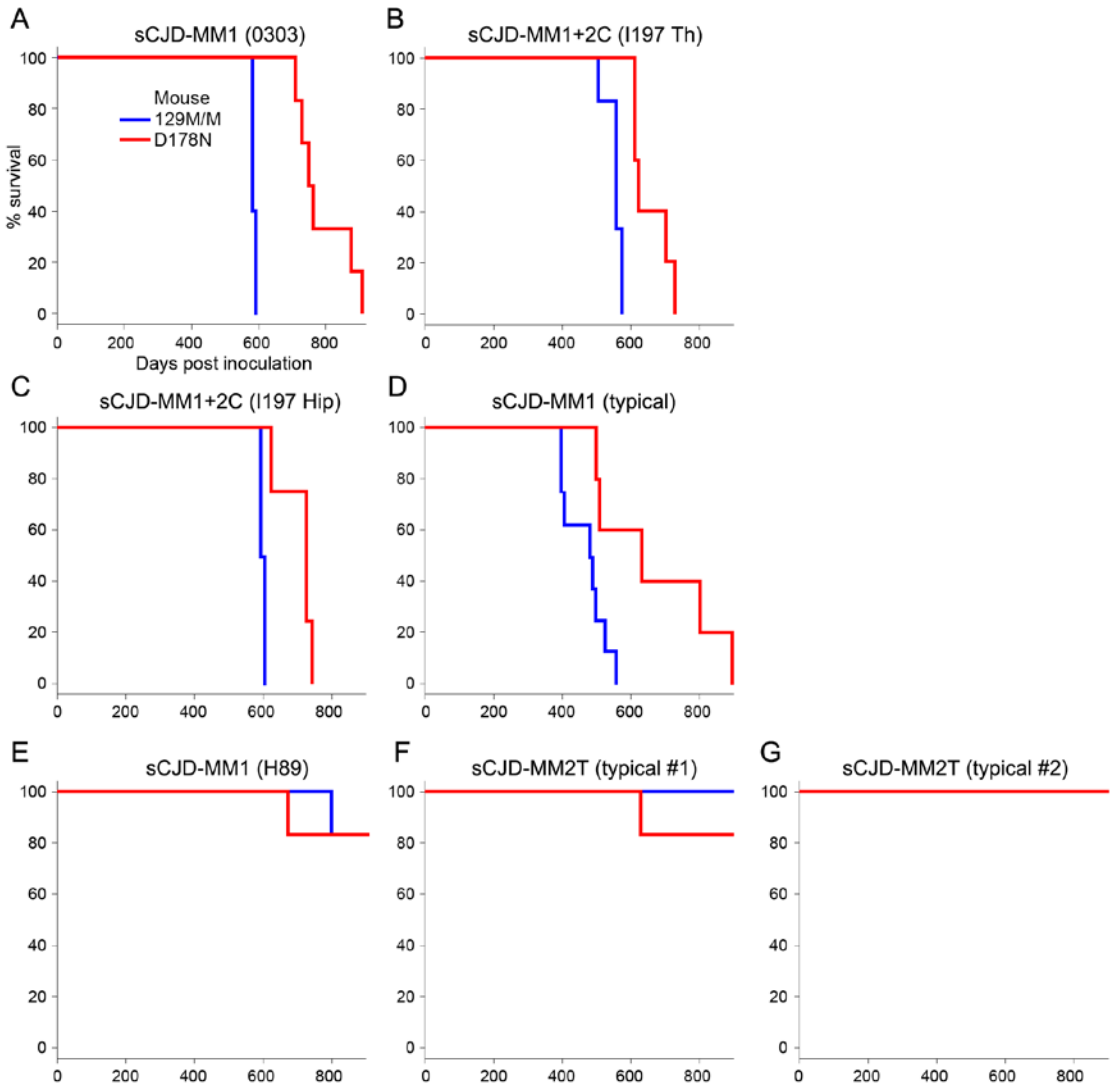

A
sCJD-MM1 (0303)
Mouse
129M/M
D178N
% survival
Days post inoculation
B
sCJD-MM1+2C (I197 Th)
C
sCJD-MM1+2C (I197 Hip)
D
sCJD-MM1 (typical)
E
sCJD-MM1 (H89)
F
sCJD-MM2T (typical #1)
G
sCJD-MM2T (typical #2)

Figure 5

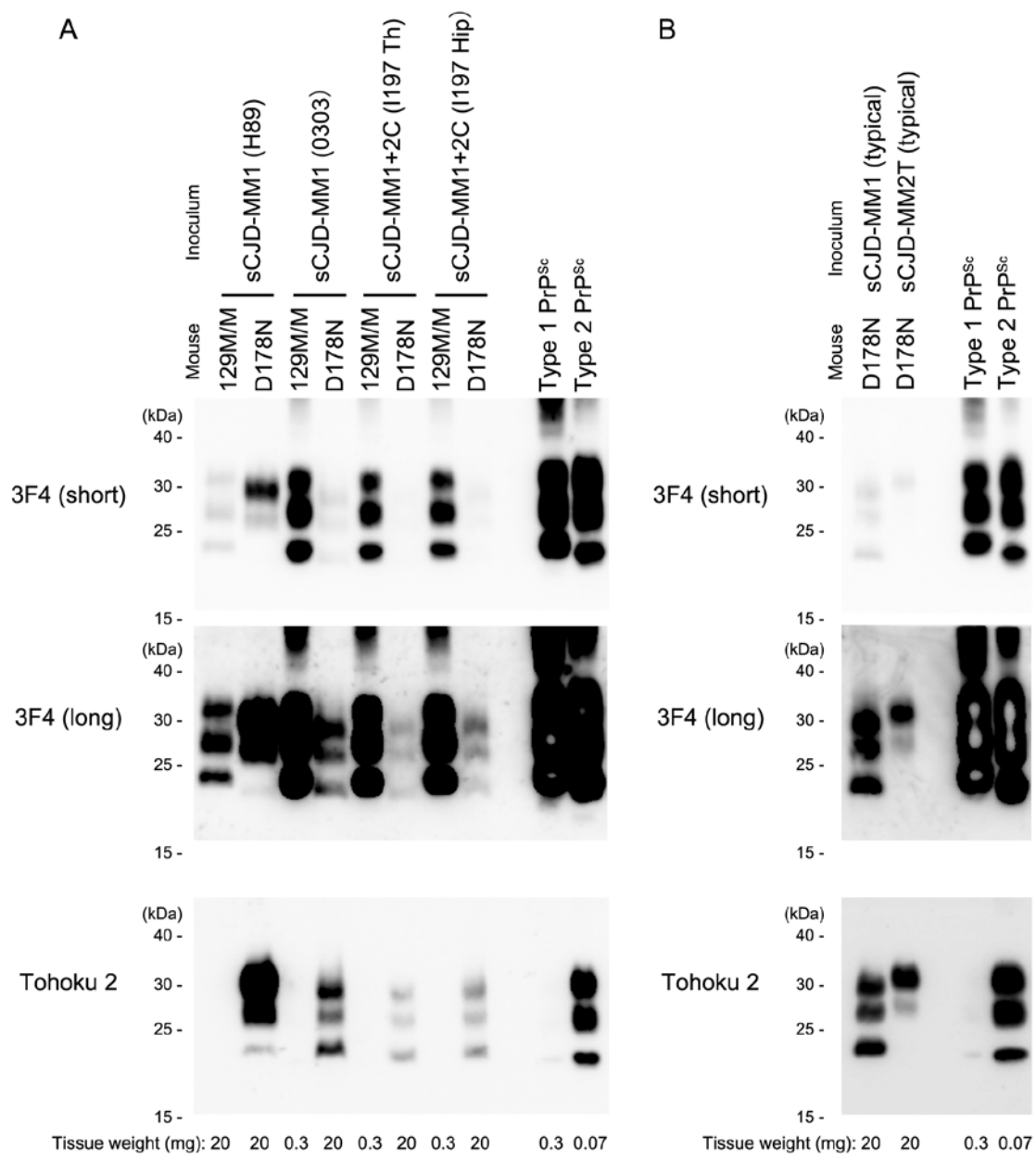
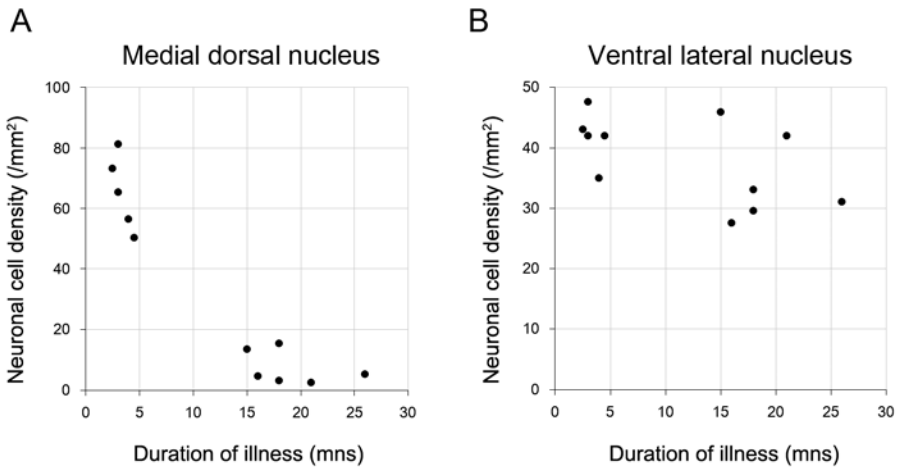
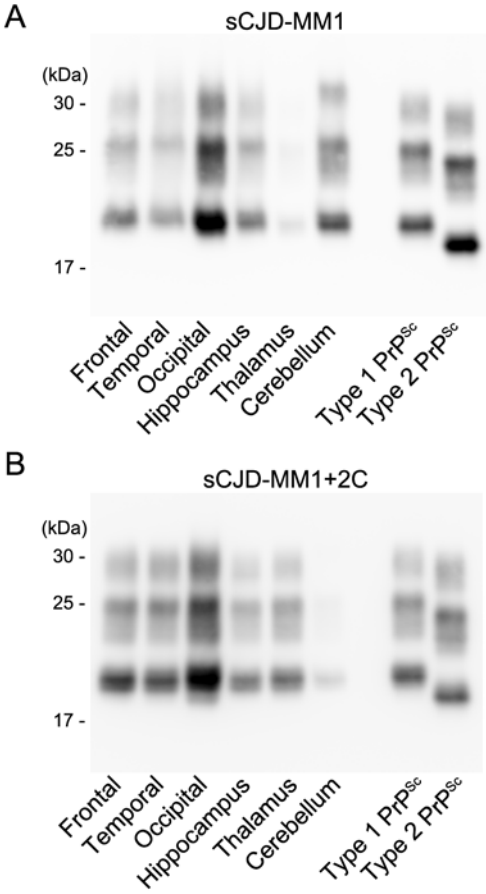

A
Inoculum
Mouse
129M/M
D178N
sCJD-MM1 (H89)
sCJD-MM1 (0303)
sCJD-MM1+2C (I197 Th)
sCJD-MM1+2C (I197 Hip)
Type 1 PrPSc
Type 2 PrPSc
3F4 (short)
3F4 (long)
Tohoku 2
(kDa)
40 -
30 -
25 -
15 -
Tissue weight (mg): 20 20 0.3 20 0.3 20 0.3 20 0.3 0.07
B
Inoculum
Mouse
D178N
D178N
sCJD-MM1 (typical)
sCJD-MM2T (typical)
Type 1 PrPSc
Type 2 PrPSc
3F4 (short)
3F4 (long)
Tohoku 2
(kDa)
40 -
30 -
25 -
15 -
Tissue weight (mg): 20 20 0.3 0.07

Figure 6



Supplemental figure 1



Supplemental figure 2

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