**REVIEW**
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# Distinct origins of dura mater graft-associated Creutzfeldt-Jakob disease: past and future problems

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## Abstract

Dura mater graft-associated Creutzfeldt-Jakob disease (dCJD) can be divided into two subgroups that exhibit distinct clinical and neuropathological features, with the majority represented by a non-plaque-type of dCJD (np-dCJD) and the minority by a plaque-type of dCJD (p-dCJD). The two distinct phenotypes of dCJD had been considered to be unrelated to the genotype (methionine, M or valine, V) at polymorphic codon 129 of the *PRNP* gene or type (type 1 or type 2) of abnormal isoform of prion protein ($PrP^{Sc}$) in the brain, while these are major determinants of clinicopathological phenotypes of sporadic CJD (sCJD). The reason for the existence of two distinct subgroups in dCJD had remained elusive. Recent progress in research of the pathogenesis of dCJD has revealed that two distinct subgroups of dCJD are caused by infection with different $PrP^{Sc}$ strains from sCJD, i.e., np-dCJD caused by infection with sCJD-MM1/MV1, and p-dCJD caused by infection with sCJD-VV2 or -MV2. These studies have also revealed previously unrecognized problems as follows: (i) the numbers of p-dCJD patients may increase in the future, (ii) the potential risks of secondary infection from dCJD, particularly from p-dCJD, may be considerable, and (iii) the effectiveness of the current $PrP^{Sc}$ decontamination procedures against the $PrP^{Sc}$ from p-dCJD is uncertain. To prevent secondary infection from p-dCJD, the establishment of effective decontamination procedures is an urgent issue. In this review, we summarize the past and future problems surrounding dCJD.

**Keywords:** Creutzfeldt-Jakob disease, Prion protein, Dura mater grafts, Humanized knock-in mouse

## Introduction

Dura mater grafts used to repair the dural defects at neurosurgery can cause fatal disease years to decades later. The tragedy of dura mater graft-associated Creutzfeldt-Jakob disease (dCJD) was considered to be nearly over. However, recent progress in research of the pathogenesis of dCJD has revealed previously unrecognized problems. In this review, we summarize the past and future problems surrounding dCJD.
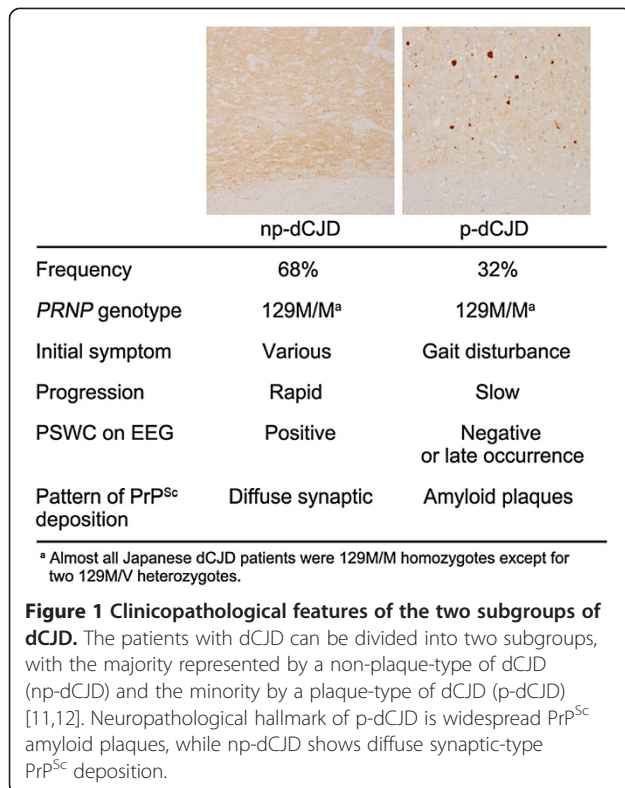
Creutzfeldt-Jakob disease (CJD) is a lethal transmissible neurodegenerative disease. The central event in the pathogenesis of CJD is a conformational change of the normal cellular isoform of prion protein ($PrP^{C}$) into an abnormal infectious isoform of prion protein ($PrP^{Sc}$) [1]. The conformational change of $PrP^{C}$ can occur due to either one of three causes: spontaneous conversion in sporadic CJD (sCJD), mutations in the *PRNP* gene in genetic CJD, or infection with $PrP^{Sc}$ in iatrogenic CJD and variant CJD.

One of the most frequent sources of iatrogenic $PrP^{Sc}$ infection is dura mater grafts obtained from human cadavers undiagnosed as CJD. The sum of dCJD (228 cases) and growth hormone-associated CJD (226 cases) accounts for 97% of total iatrogenic CJD cases [2]. A single brand of dura mater graft, Lyodura®, was used for all the dCJD cases in whom the brand name was identified. Although the causative dura mater grafts were manufactured by a German company, 62% (142 cases) of total dCJD cases have been found in Japan [2,3]. Persistent efforts of a Japanese CJD surveillance team have clarified the outline of dCJD outbreaks. The onset of Japanese dCJD patients peaked in the late 1990s, and most of the patients had received the grafts during 1983–1987, while as many as 100,000 persons received the Lyodura® grafts

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**Figure 1 Clinicopathological features of the two subgroups of dCJD.** The patients with dCJD can be divided into two subgroups, with the majority represented by a non-plaque-type of dCJD (np-dCJD) and the minority by a plaque-type of dCJD (p-dCJD) [11,12]. Neuropathological hallmark of p-dCJD is widespread PrP<sup>Sc</sup> amyloid plaques, while np-dCJD shows diffuse synaptic-type PrP<sup>Sc</sup> deposition.

during this period [4,5]. In the process of conducting this elaborate survey, a puzzling mystery about dCJD emerged.
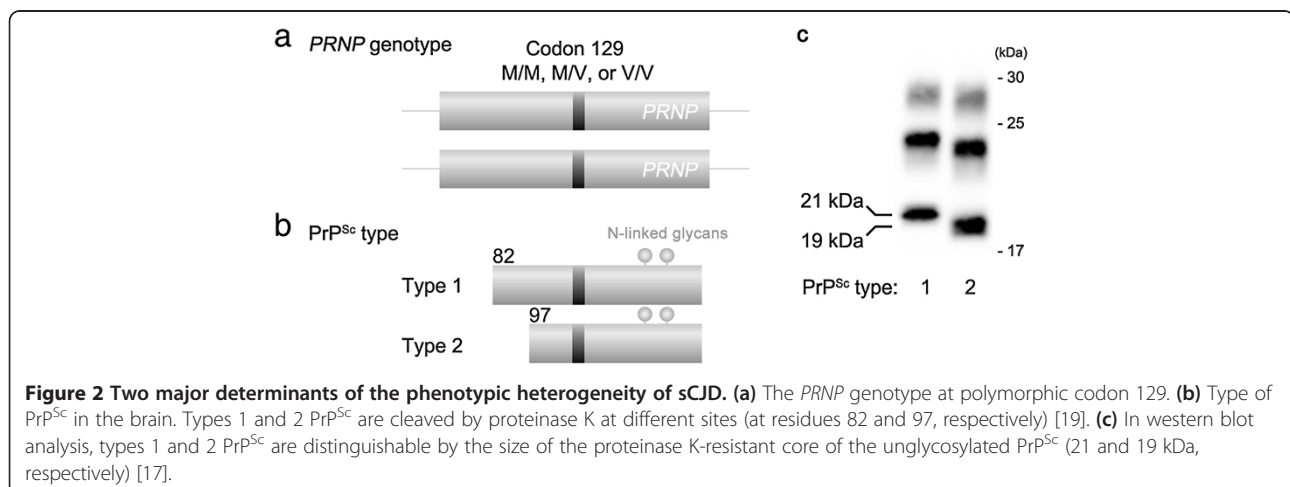
## A mystery about dCJD

There is growing evidence that dCJD can be divided into two subgroups that exhibit distinct clinical and neuropathological phenotypes, with the majority (68%) represented by a non-plaque-type of dCJD (np-dCJD) and the minority (32%) by a plaque-type of dCJD (p-dCJD) (Fig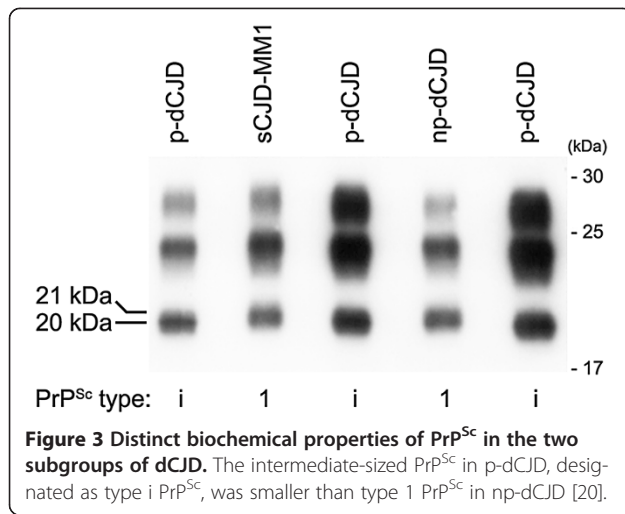ure 1) [6-12]. The clinicopathological features of np-dCJD are identical to those of classical sCJD, whereas p-dCJD is characterized by (i) ataxic gait as an initial symptom, (ii) slow progression of neurological symptoms, (iii) absence or late occurrence of periodic sharp-wave complexes (PSWC) on electroencephalogram (EEG), and (iv) widespread PrP$^{Sc}$ amyloid plaques in the brain [11-16]. There is no significant difference in gender composition, site of graft, age at grafting, and year of grafting between the two subgroups [11,12].

sCJD also shows wide phenotypic heterogeneity, and its clinicopathological phenotypes are determined by the genotype at polymorphic codon 129 of the *PRNP* gene and type of PrP$^{Sc}$ in the brain (Figure 2) [17,18]. The codon 129 of the *PRNP* gene shows methionine (M)/valine (V) polymorphism. Two types of PrP$^{Sc}$ (type 1 and type 2) are distinguishable according to the size of the proteinase K-resistant core of unglycosylated PrP$^{Sc}$ (21 and 19 kDa, respectively), reflecting differences in the proteinase K-cleavage site (at residues 82 and 97, respectively) [19]. On the other hand, the two distinct phenotypes of dCJD had been considered to be unrelated to their *PRNP* genotype or type of PrP$^{Sc}$ in the brain [11]. In Japan, almost all dCJD patients had the same genotype, i.e., homozygous for methionine at codon 129 (129 M/M), except two heterozygotes [2], and the type of PrP$^{Sc}$ in their brains had been reported as type 1 [6,10,11]. The reason for the existence of two distinct subgroups in dCJD had remained elusive.

### Solving the mystery

In 2003, an unusual p-dCJD case was reported [9]. This patient showed the accumulation of unusual PrP$^{Sc}$ with intermediate electrophoretic mobility between types 1 and 2 PrP$^{Sc}$. Then, we reevaluated the biochemical properties of PrP$^{Sc}$ in the two subgroups of dCJD and found that the size of PrP$^{Sc}$ from p-dCJD was invariably smaller than that of type 1 PrP$^{Sc}$ from np-dCJD



**Figure 2 Two major determinants of the phenotypic heterogeneity of sCJD. (a)** The *PRNP* genotype at polymorphic codon 129. **(b)** Type of PrP$^{Sc}$ in the brain. Types 1 and 2 PrP$^{Sc}$ are cleaved by proteinase K at different sites (at residues 82 and 97, respectively) [19]. **(c)** In western blot analysis, types 1 and 2 PrP$^{Sc}$ are distinguishable by the size of the proteinase K-resistant core of the unglycosylated PrP$^{Sc}$ (21 and 19 kDa, respectively) [17].

**Figure 3 Distinct biochemical properties of PrP^Sc in the two subgroups of dCJD.** The intermediate-sized PrP$^{Sc}$ in p-dCJD, designated as type i PrP$^{Sc}$, was smaller than type 1 PrP$^{Sc}$ in np-dCJD [20].

(Figure 3) [20]. This intermediate-sized PrP$^{Sc}$ was designated as type i PrP$^{Sc}$.

To resolve the mystery of the existence of two distinct subgroups in dCJD, we hypothesized that they might be caused by infection with different PrP$^{Sc}$ strains from distinct sCJD subgroups. According to the *PRNP* genotype and type of PrP$^{Sc}$ in the brain, sCJD is classified into six subgroups (MM1, MV1, VV1, MM2, MV2, or VV2) [17]. MM1 and MV1, which are the predominant subgroups in sCJD, show the same clinicopathological features. Meanwhile, MM2 can be divided into three subgroups based on histopathological criteria (MM2T, thalamic form showing characteristic atrophy of thalamic and inferior olivary nuclei; MM2C, cortical form showing a predominant cortical pathology; or MM2T + C, mixed form) [17,21]. In addition, MV2 is also divided into three subgroups based on histopathological

criteria (MV2K showing kuru type PrP$^{Sc}$ amyloid plaques, MV2C showing a predominant cortical pathology, or MV2K + C showing mixed histopathology) [17,18]. The clinicopathological features of np-dCJD, such as short duration of illness, PSWC on EEG, or diffuse synaptic-type PrP$^{Sc}$ deposition in the brain, are identical to those of sCJD-MM1/MV1. In contrast, the clinicopathological features of p-dCJD, such as ataxic gait as an initial symptom, slow progression of neurological symptoms, absence or late occurrence of PSWC on EEG, or formation of PrP$^{Sc}$ plaques in the brain, are similar to those of sCJD-VV2, −MV2K, or -MV2K + C. These similarities raised the possibility that np-dCJD might be caused by infection with sCJD-MM1/MV1, whereas p-dCJD might be caused by infection with sCJD-VV2, −MV2K, or -MV2K + C.

To test this possibility, we examined the transmission properties of the dCJD and sCJD subgroups using humanized mice carrying human PrP with either the 129 M/M or V/V genotype [20,22,23]. In these transmission experiments, p-dCJD and sCJD-VV2, −MV2K, or -MV2K + C were identical in the transmissibility to the PrP-humanized mice (Table 1, Figure 4a) and in the neuropathological and biochemical features in the inoculated mice (Figure 4b, c). By contrast, np-dCJD showed the same transmission properties as sCJD-MM1. In particular, the 129 M/M mice inoculated with sCJD-VV2, −MV2K, or -MV2K + C material showed widespread PrP$^{Sc}$ plaques and type i PrP$^{Sc}$ accumulation similar to the p-dCJD patients, whereas the 129 M/M mice inoculated with sCJD-MM1 material showed diffuse synaptic-type PrP$^{Sc}$ deposition and type 1 PrP$^{Sc}$ accumulation similar to the np-dCJD patients. Thus, these animal models support the hypothesis that the origin of np-dCJD is sCJD-MM1/MV1 and that of p-dCJD is

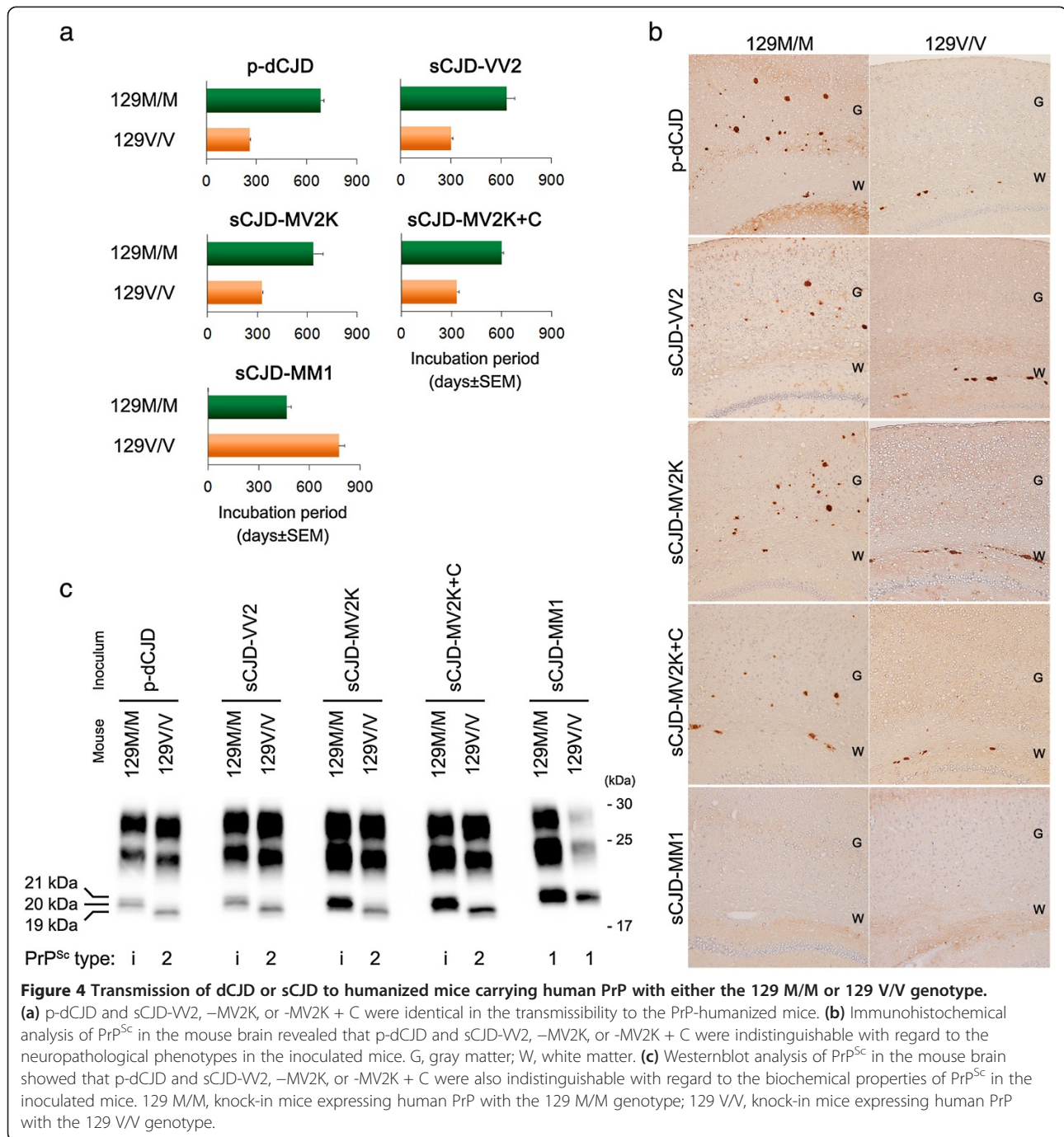**Table 1 Transmission of dCJD or sCJD to PrP-humanized mice**

| Inoculum (ID) | Incubation period in days ± SEM ($n/n^0$)[a] | | |
|---|---|---|---|
| | **129 M/M** | | **129 V/V** |
| | Tg + Ki-Hu129 M/M[b] | Ki-Hu129M/M[b] | Ki-Hu129V/V[b] |
| | (9.8×)[c] | (1×) | (1×) |
| np-dCJD (GF) | 161 ± 5 (5/5) | N.D. [d] | N.D. |
| np-dCJD (TC) | 208 ± 2 (5/5) | N.D. | N.D. |
| p-dCJD (KR) | 420 ± 10 (5/5) | 685 ± 51 (5/5) | 259 ± 6 (6/6) |
| p-dCJD (KD) | 398 ± 10 (5/5) | 447 ± 51 (6/6) | 317 ± 8 (11/11) |
| sCJD-MM1 | 175 ± 4 (9/9) | 467 ± 24 (8/8) | 774 ± 32 (6/6) |
| sCJD-VV2 | 505 ± 14 (5/5) | 633 ± 49 (6/6) | 302 ± 9 (7/7) |
| sCJD-MV2K | N.D. | 638 ± 57 (4/4) | 329 ± 3 (4/4) |
| sCJD-MV2K+C | N.D. | 600 ± 22 (6/6) | 332 ± 15 (4/4) |
| 129 M/M mouse-passaged sCJD-VV2 | N.D. | 685 ± 17 (6/6) | 309 ± 3 (7/7) |

[a]$n$, number of mice positive for PrP accumulation in the immunohistochemical analysis; $n^0$, number of inoculated mice.
Ki-Hu129M/M, knock-in mice expressing human PrP with the 129 M/M genotype; Ki-Hu129V/V, knock-in mice expressing human PrP with the 129 V/V genotype;
Tg + Ki-Hu129M/M, Ki-Hu129M/M crossed with transgenic mice overexpressing human PrP with the 129 M genotype.
[d]N.D., not done.

**Figure 4 Transmission of dCJD or sCJD to humanized mice carrying human PrP with either the 129 M/M or 129 V/V genotype.**
**(a)** p-dCJD and sCJD-VV2, –MV2K, or -MV2K + C were identical in the transmissibility to the PrP-humanized mice. **(b)** Immunohistochemical analysis of PrP$^{Sc}$ in the mouse brain revealed that p-dCJD and sCJD-VV2, –MV2K, or -MV2K + C were indistinguishable with regard to the neuropathological phenotypes in the inoculated mice. G, gray matter; W, white matter. **(c)** Westernblot analysis of PrP$^{Sc}$ in the mouse brain showed that p-dCJD and sCJD-VV2, –MV2K, or -MV2K + C were also indistinguishable with regard to the biochemical properties of PrP$^{Sc}$ in the inoculated mice. 129 M/M, knock-in mice expressing human PrP with the 129 M/M genotype; 129 V/V, knock-in mice expressing human PrP with the 129 V/V genotype.

sCJD-VV2, –MV2K, or -MV2K + C. Indeed, the incidence rate of p-dCJD (32%) among total dCJD is close to the sum total of the incidence of sCJD-VV2 (15%), –MV2K (8%), and -MV2K + C (3%) [18].

## Molecular basis of the generation of two distinct subgroups in dCJD

At the molecular level, np-dCJD contains type 1 PrP$^{Sc}$ with the codon 129 M genotype (denoted as M1 PrP$^{Sc}$),

whereas p-dCJD contains type i PrP$^{Sc}$ with the codon 129 M genotype (Mi PrP$^{Sc}$) (Table 2). Meanwhile, sCJD-MM1/MV1 contains M1 PrP$^{Sc}$, and sCJD-VV2 contains type 2 PrP$^{Sc}$ with the codon 129 V genotype (V2 PrP$^{Sc}$). Recently, we found that sCJD-MV2K contains Mi PrP$^{Sc}$ and V2 PrP$^{Sc}$, whereas sCJD-MV2K + C also contains type 2 PrP$^{Sc}$ with the codon 129 M genotype and cortical pathology (M2C PrP$^{Sc}$) in addition to Mi PrP$^{Sc}$ and V2 PrP$^{Sc}$ (Table 2) [23]. M2 PrP$^{Sc}$ can be divided into

**Table 2 Molecular classification of dCJD and sCJD**

| Classification[a] | Codon 129 genotype | PrP$^{Sc}$ type | Transmission type[b] | Original PrP$^{Sc}$ | Existing PrP$^{Sc}$ |
|---|---|---|---|---|---|
| np-dCJD | M/M | 1 | M1 | M1 | M1 |
| p-dCJD | M/M | i [c] | V2 | V2 [d] | Mi |
| sCJD-MM1 | M/M | 1 | M1 | M1 | M1 |
| sCJD-VV2 | V/V | 2 | V2 | V2 | V2 |
| sCJD-MV2K | M/V | i + 2 | V2 | V2 | Mi + V2 |
| sCJD-MV2K+C | M/V | i + 2 | V2 | M2C[e] + V2 | M2C + Mi + V2 |

[a]According to Parchi, 1999 [17]; and Parchi, 2011 [18].
[b]According to Bishop, 2010 [24]; Kobayashi, 2007 [20]; and Kobayashi, 2013 [23].
[c]Intermediate type located between types 1 and 2 PrP$^{Sc}$.
[d]Although not only V2 PrP$^{Sc}$ but also Mi PrP$^{Sc}$ can cause p-dCJD if transmitted to the 129 M/M individuals, the primary origin of Mi PrP$^{Sc}$ is V2 PrP$^{Sc}$ (see text).
[e]M2 PrP$^{Sc}$ can be divided into two subgroups based on histopathological phenotypes. M2C PrP$^{Sc}$ causes a predominant cortical pathology, whereas M2T PrP$^{Sc}$ causes atrophy of thalamic and inferior olivery nuclei.

two subgroups based on histopathological phenotypes. M2C PrP$^{Sc}$ causes a predominant cortical pathology in sCJD-MM2C, –MV2C, or -MV2K + C, whereas M2T PrP$^{Sc}$ causes atrophy of thalamic and inferior olivery nuclei in sCJD-MM2T.

The generation of M1 PrP$^{Sc}$ in np-dCJD is simply due to the infection with M1 PrP$^{Sc}$ from sCJD-MM1/MV1. On the other hand, the generation of Mi PrP$^{Sc}$ in p-dCJD is rather complicated. Transmission of V2 PrP$^{Sc}$, i.e., sCJD-VV2, to the 129 M/M mice generated Mi PrP$^{Sc}$ (Figure 4c) [20]. Similarly, transmission of Mi PrP$^{Sc}$, i.e., p-dCJD, to the 129 M/M mice also generated Mi PrP$^{Sc}$. Therefore, both V2 PrP$^{Sc}$ and Mi PrP$^{Sc}$ can generate Mi PrP$^{Sc}$ if transmitted to individuals with the 129 M/M genotype. Indeed, transmission of sCJD-MV2K containing Mi PrP$^{Sc}$ and V2 PrP$^{Sc}$ to the 129 M/M mice also generated Mi PrP$^{Sc}$ (Figure 4c). Meanwhile, sCJD-MV2K + C contains M2C PrP$^{Sc}$ besides Mi PrP$^{Sc}$ and V2 PrP$^{Sc}$ (Table 2). However, M2C PrP$^{Sc}$ lacks or has very low infectivity and does not affect the transmission properties of the coexisting PrP$^{Sc}$ [23]. Therefore, the transmission of sCJD-MV2K + C to the 129 M/M mice can also result in the generation of Mi PrP$^{Sc}$ (Figure 4c). Taken together, Mi PrP$^{Sc}$ in p-dCJD is generated by infection with Mi PrP$^{Sc}$ and/or V2 PrP$^{Sc}$ from sCJD-VV2, –MV2K, or -MV2K + C. It is noteworthy that Mi PrP$^{Sc}$ can be observed in the 129 M/M mice inoculated with V2 PrP$^{Sc}$ but not in sCJD patients with the 129 M/M genotype, suggesting that Mi PrP$^{Sc}$ in sCJD-MV2K or -MV2K + C is also generated by V2 PrP$^{Sc}$ seed-dependent conversion but not by spontaneous conversion of the 129 M PrP$^{C}$. Therefore, the primary origin of Mi PrP$^{Sc}$ is V2 PrP$^{Sc}$. This can account for the similarities in transmission properties between Mi PrP$^{Sc}$ and V2 PrP$^{Sc}$. Thus, M1 PrP$^{Sc}$ in np-dCJD and Mi PrP$^{Sc}$ in p-dCJD are completely different with regard to the neuropathological phenotypes, biochemical features, and transmission properties, reflecting their distinct PrP$^{Sc}$ origins. In contrast to M1 PrP$^{Sc}$, which is the most common PrP$^{Sc}$ observed in sCJD patients with the 129 M/M genotype, Mi PrP$^{Sc}$ has never been observed in sCJD patients with the 129 M/M genotype. Therefore, the detection of Mi PrP$^{Sc}$ can be sound evidence of iatrogenic infection in individuals with the 129 M/M genotype and would contribute to reliable surveillance of iatrogenic cases such as p-dCJD.

To verify experimentally that Mi PrP$^{Sc}$ originates from V2 PrP$^{Sc}$ and its transmission properties are identical to those of the parental V2 PrP$^{Sc}$, we performed a modeling study using PrP-humanized mice (Figure 5a) [25]. As described above, the 129 M/M mice inoculated with V2 PrP$^{Sc}$ showed widespread PrP$^{Sc}$ plaques and Mi PrP$^{Sc}$ accumulation in the brain as an experimental model of p-dCJD. We then inoculated the Mi PrP$^{Sc}$ from these mice into other PrP-humanized mice with either the 129 M/M or V/V genotype. This secondary passage revealed that the transmission properties of the Mi PrP$^{Sc}$, i.e., 129 M/M mouse-passaged sCJD-VV2, are identical to those of the parental V2 PrP$^{Sc}$. In particular, although the incompatibility of the codon 129 genotypes between host and inoculum usually results in a prolonged incubation period [20], the 129 V/V mice inoculated with the Mi PrP$^{Sc}$ showed a shorter incubation period compared with the 129 M/M mice (Table 1). Moreover, the altered neuropathological phenotype and biochemical properties at the primary passage in the 129 M/M mice reverted to the original ones in the secondary passage in the 129 V/V mice (Figure 5b, c). Thus, this modeling study shows that (i) V2 PrP$^{Sc}$ infection in a host with the incompatible codon 129 M/M genotype generates an unusual PrP$^{Sc}$ with altered conformational properties, i.e., Mi PrP$^{Sc}$, (ii) the emerging Mi PrP$^{Sc}$ retains the memory of the parental V2 PrP$^{Sc}$ within its conformational properties, and (iii) the parental V2 PrP$^{Sc}$ re-emerges and proliferates rapidly if the Mi PrP$^{Sc}$ is transmitted to the original host with the codon 129 V/V genotype. This phenomenon, designated as traceback, can be a useful tool to identify the origin of PrP$^{Sc}$ infection if atypical PrP$^{Sc}$ emerges in the future [20,22,26].

**Figure 5 Modeling of p-dCJD and traceback phenomenon.**
**(a)** Schematic diagram of the modeling study. The 129 V/V mice were highly susceptible to the 129 M/M mouse-passaged sCJD-VV2, i.e., Mi PrP$^{Sc}$, despite their incompatible codon 129 genotypes. Moreover, the altered neuropathological and biochemical phenotypes in the primary passage in the 129 M/M mice reverted to the original ones in the secondary passage in the 129 V/V mice. This is because the origin of Mi PrP$^{Sc}$ is V2 PrP$^{Sc}$. This phenomenon has been designated as traceback [20,26]. **(b)** Immunohistochemical analysis of PrP$^{Sc}$ in the brains from the PrP-humanized mice infected with the 129 M/M mouse-passaged sCJD-VV2. G, gray matter; W, white matter. **(c)** Westernblot analysis of PrP$^{Sc}$ in the brains from the PrP-humanized mice infected with sCJD-VV2 or the 129 M/M mouse-passaged sCJD-VV2. 129 M/M, knock-in mice expressing human PrP with the 129 M/M genotype; 129 V/V, knock-in mice expressing human PrP with the 129 V/V genotype.

## Remaining problems

Our transmission studies resolved the complicated pathogenesis of dCJD. However, they have also revealed several issues surrounding dCJD that need to be addressed in the future.

First, the numbers of p-dCJD patients may increase in the future. The experimental p-dCJD model, i.e., the 129 M/M mice inoculated with Mi PrP$^{Sc}$ and/or V2 PrP$^{Sc}$ from sCJD-VV2, –MV2K, or -MV2K + C, showed a longer incubation period compared with the np-dCJD model, i.e., the 129 M/M mice inoculated with M1 PrP$^{Sc}$ from sCJD-MM1 (Table 1). This raises the concern that additional p-dCJD patients, who are presenting still at the subclinical stage, may emerge after a longer incubation period in the future. Although the numbers of patients with newly developed dCJD have dropped off, continuous surveillance will be required to find remaining p-dCJD cases.

Second, the potential risks of secondary infection from dCJD, particularly from p-dCJD, may be considerable. As described above, the transmission studies raise a concern about the existence of subclinical p-dCJD patients. dCJD patients may undergo more than one neurosurgical operation due to their underlying diseases (the primary disease for which the neurosurgery was performed) [4]. In addition, p-dCJD patients may be more frequently autopsied because the clinical features of p-dCJD are atypical compared with those of classical sCJD [11]. These facts suggest that there may be considerable risk of secondary infection from p-dCJD patients. Individuals with the 129 V/V genotype may be more vulnerable to the infection with Mi PrP$^{Sc}$ from p-dCJD, as suggested by the fact that the 129 V/V mice were highly susceptible to Mi PrP$^{Sc}$ in the transmission study (Table 1). Additionally, 129 M/M individuals may be also affected after a prolonged incubation period, as suggested by the high attack rate (100%) of the 129 M/M mice inoculated with Mi PrP$^{Sc}$. Therefore, secondary infection from p-dCJD can occur regardless of the codon 129 genotype. Comprehensive analysis of the distribution of PrP$^{Sc}$ in the peripheral tissues of p-dCJD patients will be also required to assess the potential risks of secondary infection.

Finally, the efficacy of the current PrP$^{Sc}$ decontamination procedures against Mi PrP$^{Sc}$ needs to be tested in the future. Mi PrP$^{Sc}$ in p-dCJD and M1 PrP$^{Sc}$ in np-dCJD differ in the sizes of the proteinase K-resistant core, suggesting their conformational differences. Moreover, their parental PrP$^{Sc}$ strains are also different. Different PrP$^{Sc}$ strains can show different thermostability [27,28] and different susceptibility to the decontamination procedures [29]. To prevent the spread of secondary infection from dCJD patients to medical workers or other patients, adequate decontamination and disinfection of

the instruments used for neurosurgery or autopsy are essential. However, the current PrP$^{Sc}$ decontamination procedures were developed using scrapie isolates and tested using CJD isolates other than p-dCJD [30-32]. Therefore, further studies using Mi PrP$^{Sc}$ will be needed to assess the effectiveness of the current procedures. For this purpose, sensitive detection systems for Mi PrP$^{Sc}$ are also prerequisite to evaluating quantitatively the reduction of infectivity after the decontamination procedures. Real-time quaking-induced conversion [33,34], protein misfolding cyclic amplification [35-39], or transgenic mice overexpressing human PrP with the 129 V genotype [20] might be useful to detect the reduced infectivity of Mi PrP$^{Sc}$ at high sensitivity. Using such sensitive detection systems, effective decontamination procedures for Mi PrP$^{Sc}$ can be established in the future.

## Concluding remarks

Recent progress in the study of the pathogenesis of dCJD has revealed that the two distinct subgroups of dCJD are caused by infection with different PrP$^{Sc}$ strains of sCJD, i.e., np-dCJD caused by M1 PrP$^{Sc}$ from sCJD-MM1/MV1 and p-dCJD caused by Mi PrP$^{Sc}$ and/or V2 PrP$^{Sc}$ from sCJD-VV2, −MV2K, or -MV2K + C. Studies have also revealed previously unrecognized problems such as the considerable risks of secondary infection from dCJD, particularly from p-dCJD. To prevent secondary infection from p-dCJD, the effectiveness of the current decontamination procedures should be tested urgently using sensitive Mi PrP$^{Sc}$ detection systems.

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