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Citation	Neuropathology, 36(3), 305-310 https://doi.org/10.1111/neup.12270
Issue Date	2016-06
Doc URL	http://hdl.handle.net/2115/65887
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File Information	Manuscript(HUSCAP).pdf

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Title: Neuropathological and biochemical criteria to identify acquired Creutzfeldt-Jakob disease among presumed sporadic cases

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Running title: Criteria to identify acquired CJD

Abstract

As an experimental model of acquired Creutzfeldt-Jakob disease (CJD), we performed transmission studies of sporadic CJD using knock-in mice expressing human prion protein (PrP). In this model, the inoculation of the sporadic CJD strain V2 into animals homozygous for methionine at polymorphic codon 129 (129M/M) of the *PRNP* gene produced quite distinctive neuropathological and biochemical features, *i.e.*, widespread kuru plaques and intermediate type abnormal PrP (PrP^{Sc}). Interestingly, this distinctive combination of molecular and pathological features has been, to date, observed in acquired CJD but not in sporadic CJD. Assuming that these distinctive phenotypic traits are specific for acquired CJD, we revisited the literature and found two cases showing widespread kuru plaques despite the 129M/M genotype, in a neurosurgeon and in a patient with a medical history of neurosurgery without dura mater grafting. By western blot analysis of brain homogenates, we revealed the intermediate type of PrP^{Sc} in both cases. Furthermore, transmission properties of brain extracts from these two cases were indistinguishable from those of a subgroup of dura mater graft-associated iatrogenic CJD caused by infection with the sporadic CJD strain V2. These data strongly suggest that the two atypical CJD cases, previously thought to represent sporadic CJD, very likely acquired the disease through exposure to prion-contaminated brain tissues. Thus, we propose that the distinctive combination of 129M/M genotype, kuru plaques, and intermediate type PrP^{Sc}, represents a reliable criterion for the identification of acquired CJD cases among presumed sporadic cases.

Key words: Creutzfeldt-Jakob disease, prions, prion diseases, *PRNP*, iatrogenic

INTRODUCTION

Prion diseases are lethal transmissible neurodegenerative diseases caused by an abnormal isoform of prion protein (PrP^{Sc}), a component of a proteinaceous infectious particle named prion, which is converted from the normal cellular isoform (PrP^{C}).[1] The conformational conversion of PrP^{C} is believed to occur: (1) spontaneously in sporadic Creutzfeldt-Jakob disease (sCJD) and variably protease-sensitive prionopathy, (2) as a consequence of a pathogenic *PRNP* mutation in genetic CJD, Gerstmann-Sträussler-Scheinker syndrome, and fatal familial insomnia, (3) through an acquired prion infection in iatrogenic CJD, kuru, and variant CJD. Proven sources of iatrogenic CJD transmission include dura mater grafts, growth and gonadotrophic hormone parenteral administration, neurosurgical instruments, corneal grafts, and stereotactic intracranial electrodes,[2] while kuru, the other acquired form of prion disease related to sCJD, likely originated from the transmission of prions through ritual cannibalism.[3]

As an experimental model of acquired CJD, transmission studies using knock-in mice expressing human PrP^{C} have been extensively pursued over the last decade.[4-15] These animal models have successfully clarified the pathogenesis of acquired CJD, particularly the relationship between *PRNP* polymorphism and disease susceptibility or disease phenotype.[16] One of the most important findings in these animal models is the neuropathological and biochemical characteristics of acquired CJD that would help to distinguish acquired CJD cases from sporadic cases. In this review, we summarize recent advances provided by experimental transmission studies of sCJD prions with implication for the diagnostic approach aimed at identifying acquired CJD cases.

PHENOTYPIC HETEROGENEITY OF ANIMAL MODELS OF ACQUIRED CJD

To model acquired CJD, a range of sCJD isolates have been inoculated into knock-in mice expressing human PrP^{C} with each one of the three *PRNP* codon 129 genotypes (methionine homozygosity, M/M; valine homozygosity, V/V; or methionine/valine heterozygosity, M/V). Current classification of sCJD is largely based on the *PRNP* genotype at polymorphic codon 129 and the type of PrP^{Sc} (type 1 or type 2) accumulating in the brain, *e.g.*, MM1, MM2, MV1,

MV2, VV1, or VV2.[17] Types 1 and 2 are two isoforms of PrP^{Sc} which are distinguishable according to the size of the protease-resistant core (21 and 19 kDa, respectively). However, the molecular-phenotypic correlation based on these two molecular features is not complete. For example, the MM2 group comprises two clinicopathologically distinct subgroups, which include a cortical (MM2C) and a thalamic histotype (MM2T).[17] Similarly, the MV2 group has also been divided into two distinct subtypes based on pathological criteria, which distinguish a cortical type (MV2C) and a kuru plaque type (MV2K).[18] Finally, since both MM1 and MV1 and, MM2C and MV2C share the same clinicopathological features, they have been merged into single entities, *i.e.* the MM/MV1 subtype and the MM/MV2C subtype, respectively. In summary, six phenotypic subtypes are recognized in the current classification of sCJD, *i.e.*, MM/MV1, MM/MV2C, MM2T, MV2K, VV1, or VV2.[19] Interestingly, the transmission of each of these six sCJD subtypes to PrP-humanized knock-in mice, has led to the isolation and characterization of five sCJD prion strains with different transmission properties, namely M1 (sCJD-MM/MV1), M2C (sCJD-MM/MV2C), M2T (sCJD-MM2T), V1 (sCJD-VV1), or V2 (sCJD-MV2K and -VV2).[11,12] Thus, five out of the six subtypes recognized by current sCJD classification seem to be associated with a specific strain of prions, the only exception being the MV 2K, which is indistinguishable from the VV2 subtype after transmission, and therefore also linked to the V2 strain. Disease phenotypes of the PrP-humanized knock-in mice inoculated with each sCJD prion strain are summarized in Table 1. Among the various combinations of neuropathological and biochemical features observed in the inoculated mice, the most intriguing ones were those observed in 129M/M mice inoculated with the V2 strain. These animals showed widespread PrP amyloid plaques (kuru plaques) and a distinctive type of PrP^{Sc} with intermediate electrophoretic mobility (~20 kDa) between types 1 and 2, which has been designated as intermediate type (type i), accordingly.[8,10] Since the combination of the 129M/M genotype, type i PrP^{Sc}, and widespread kuru plaques has not been observed in any subtypes of sCJD, these characteristic features likely result from the conformational adaptation of type 2 PrP^{Sc} with the 129V genotype (V2 PrP^{Sc}) to PrP^{C} with the 129M genotype.[8] Indeed, type i PrP^{Sc} and kuru plaques can also be observed in the 129M/V mice inoculated with the V2 PrP^{Sc} or in patients with sCJD-MV2K, which is considered to have originated from the spontaneous generation of V2 PrP^{Sc}.[14,20]

PHENOTYPIC HETEROGENEITY OF ACQUIRED CJD PATIENTS

Among human CJD cases, the combination of 129M/M genotype, type i PrP^{Sc}, and kuru plaques has only been found in patients with the acquired form of the disease. Dura mater graft-associated CJD (dCJD), one of the two most prevalent subgroups of iatrogenic CJD,[2] can be divided into two subtypes based on clinicopathological features, with the majority represented by a non-plaque-type caused by the M1 sCJD strain and the minority by a plaque-type caused by the V2 sCJD strain.[21-23] While non-plaque-type dCJD patients show clinico-pathological features identical to those affected by sCJD-MM/MV1, plaque-type dCJD patients show the combination of the 129M/M genotype, type i PrP^{Sc}, and kuru plaques.[8,24] Kuru plaque formation combined with the 129M/M genotype has also been found in human growth hormone-associated CJD,[25-28] the other most prevalent subgroup of iatrogenic CJD. Similar to dCJD, two neuropathologically distinct subtypes are also recognized in growth hormone-associated CJD subjects carrying the 129M/M genotype, based on the presence or absence of kuru plaques.[27,28] Following Parchi's classification, the size of protease-resistant PrP^{Sc}, among the relatively low number of cases examined to date, has been reported as type 1 in subjects carrying the 129M/M genotype, regardless of the presence of kuru plaques, and as type 2 in those carrying the 129M/V genotype.[27-29] Since growth hormone-associated CJD cases and dCJD cases show similar divergent neuropathological phenotype, similar etiology has been suspected, *i.e.*, contamination with different sCJD prion strain.[27] Finally, widespread kuru plaques are also the neuropathological hallmark of kuru patients, especially in patients with the 129M/M or 129M/V genotype.[30] Unfortunately, PrP^{Sc} typing of kuru brains has also been performed in a limited number of cases. Among them, the size of protease-resistant PrP^{Sc} has been reported as type 1 in a single 129M/M patient and as type 2 in a single 129M/V and three 129 V/V patients, respectively.[31,32] Of note, transmission study using nonhuman primates indicated that kuru might have originated from the sCJD strain V2.[33] Taken together, the data obtained from the phenotypic analyses of human cases and from experimental transmission studies, strongly suggest that the combination of 129M/M genotype, type i PrP^{Sc}, and widespread kuru plaques is a distinctive feature of acquired cases caused by an infection with

the sCJD strain V2.

IDENTIFICATION OF ACQUIRED CJD AMONG SPORADIC CASES

Assuming that the 129M/M genotype, type i PrP^{Sc}, and kuru plaques represent reliable markers of acquired CJD, we searched the literature for CJD cases with these distinctive features and found two of them, previously reported as sCJD, in a neurosurgeon and in a patient with a medical history of neurosurgery without dural grafting.[34] In transmission experiments, these two atypical CJD cases showed the same properties as those of plaque-type dCJD (Table 2). Most significantly, at variance with most sCJD prions in which the codon 129 genotypic homology between inoculated animals and the inoculum strongly favors disease susceptibility,[16] these two 129M/M cases were transmitted most efficiently into 129 V/V mice, despite the mismatched genotype. Moreover, like the sCJD strain V2 they induced the formation of PrP^{Sc} type 2 (V2 PrP^{Sc}) in the brain of these mice. Based on these findings, we concluded that the two atypical CJD cases, likewise plaque-type dCJD, actually represent acquired cases of CJD caused by the sCJD strain V2.[34] In summary, the two atypical CJD cases and plaque-type dCJD cases showed the same transmission properties as those of the parental sCJD strain V2, with the highest susceptibility in 129 V/V mice and generation of V2 PrP^{Sc}, thus representing an example of the so-called "traceback phenomenon".[6,35]

CRITERIA TO IDENTIFY ACQUIRED CJD

Based on the findings described above, we have proposed neuropathological and biochemical criteria to identify acquired CJD cases caused by transmission of the sCJD strain V2 to the 129M/M individuals, denoted as acquired CJD-MMiK (129M/M genotype, type i PrP^{Sc}, and kuru plaques) (Fig. 1A).[34]

The distinction between type i PrP^{Sc} and type 1 PrP^{Sc} is rather difficult with conventional western blot analysis due to subtle differences in the electrophoretic mobility of the two fragments. This is presumably the reason why PrP^{Sc} type i has not been reported in acquired prion disease patients other than plaque-type dCJD. For their precise distinction, stringent conditions for protease treatment and high resolution gel electrophoresis systems such

as Bis-tris long gels or 10-20% gradient Tris-glycine long gels are needed.[20] Alternatively, an analysis of the electrophoretic patterns of the carboxyl terminal PrP^{Sc} fragments using carboxyl terminus-directing antibodies is also quite useful to distinguish PrP^{Sc} type i from PrP^{Sc} type 1.[34]

Furthermore, the characteristic transmission properties also provide a sound basis for the differential diagnosis of the acquired CJD-MMiK as described above (Fig. 1B). To determine the transmission properties of a given CJD inoculum, the follicular dendritic cell assay following intraperitoneal inoculation into PrP-humanized knock-in mice[4] can be a time-saving alternative to the standard intracerebral transmission that requires long incubation period until disease onset. In addition, *in vitro* conversion assays using PrP^{C} carrying each of the codon 129 genotype as substrate, *e.g.*, protein misfolding cyclic amplification,[36,37] may also be useful to determine the seeded conversion activity and resulting PrP^{Sc} type.

Besides the neuropathological, biochemical, and transmission properties, clinical features of the acquired CJD-MMiK can also be distinctive as revealed by comparative studies of plaque-type and non-plaque-type dCJD patients.[21,22] The distinctive clinical features of the acquired CJD-MMiK include gait disturbance as an initial symptom, slow progression of disease, and absence or late occurrence of periodic sharp-wave complexes on electroencephalogram.

CONCLUDING REMARKS

Animal models of acquired CJD have contributed greatly to the evaluation of the *PRNP* genotypic effects on disease susceptibility or disease phenotype. The data obtained from animal experiments, combined with the phenotypic characterization of CJD patients have prompted the proposal of diagnostic criteria to identify acquired CJD among presumed sporadic cases as summarized in this review. However, this diagnostic approach is applicable only to a small portion of acquired CJD cases, *i.e.*, the 129M/M individuals infected with the sCJD strain V2. Unfortunately, the other acquired CJD cases, *i.e.*, the other combinations between host *PRNP* genotype and prion strain, cannot be distinguished from sCJD by neuropathological and biochemical analyses, as revealed by the animal models. Nevertheless, further continuous surveillance of acquired CJD cases using the proposed diagnostic approach and the

identification of transmission routes in such cases may help to reduce the risk of iatrogenic CJD transmission in the future.

ACKNOWLEDGMENTS

We thank members of the Creutzfeldt-Jakob Disease Surveillance Committee in Japan, Creutzfeldt-Jakob disease specialists in the prefectures, and Creutzfeldt-Jakob disease patients and families for providing important clinical information. We thank Y. Ishikawa, H. Kudo, M. Yamamoto, and A. Yamazaki for their excellent technical assistance. This study was supported by Grants-in-Aid from the Ministry of Health, Labor and Welfare of Japan (A.K., S.M. and T.K.), Grants-in-Aid for Scientific Research from JSPS (A.K. and T.K.), a grant from MEXT for the Joint Research Program of the Research Center for Zoonosis Control, Hokkaido University (T.K.), and a Grant-in-Aid for Scientific Research on Innovative Areas from MEXT (T.K.).

REFERENCES

1. Prusiner SB, Scott MR, DeArmond JP, Cohen FE. Prion protein biology. *Cell* 1998; **93:** 337-348.
2. Brown P, Brandel JP, Sato T *et al.* Iatrogenic Creutzfeldt-Jakob disease, final assessment. *Emerg Infect Dis* 2012; **18:** 901-907.
3. Wadsworth JD, Joiner S, Linehan JM, Asante EA, Brandner S, Collinge J. Review. The origin of the prion agent of kuru: molecular and biological strain typing. *Philos Trans R Soc Lond B Biol Sci* 2008; **363:** 3747-3753.
4. Kitamoto T, Mohri S, Ironside JW *et al.* Follicular dendritic cell of the knock-in mouse provides a new bioassay for human prions. *Biochem Biophys Res Commun* 2002; **294:** 280-286.
5. Taguchi Y, Mohri S, Ironside JW, Muramoto T, Kitamoto T. Humanized knock-in mice expressing chimeric prion protein showed varied susceptibility to different human prions. *Am J Pathol* 2003; **163:** 2585-2593.
6. Asano M, Mohri S, Ironside JW, Ito M, Tamaoki N, Kitamoto T. vCJD prion acquires altered virulence through trans-species infection. *Biochem Biophys Res Commun* 2006;

342: 293-299.

7. Bishop MT, Hart P, Aitchison L *et al.* Predicting susceptibility and incubation time of human-to-human transmission of vCJD. *Lancet Neurol* 2006; **5:** 393-398.
8. Kobayashi A, Asano M, Mohri S, Kitamoto T. Cross-sequence transmission of sporadic Creutzfeldt-Jakob disease creates a new prion strain. *J Biol Chem* 2007; **282:** 30022-30028.
9. Hizume M, Kobayashi A, Teruya K *et al.* Human prion protein (PrP) 219K is converted to PrP^{Sc} but shows heterozygous inhibition in variant Creutzfeldt-Jakob disease infection. *J Biol Chem* 2009; **284:** 3603-3609.
10. Kobayashi A, Sakuma N, Matsuura Y, Mohri S, Aguzzi A, Kitamoto T. Experimental verification of a traceback phenomenon in prion infection. *J Virol* 2010; **84:** 3230-3238.
11. Bishop MT, Will RG, Manson JC. Defining sporadic Creutzfeldt-Jakob disease strains and their transmission properties. *Proc Natl Acad Sci USA* 2010; **107:** 12005-12010.
12. Moda F, Suardi S, Di Fede G *et al.* MM2-thalamic Creutzfeldt-Jakob disease: neuropathological, biochemical and transmission studies identify a distinctive prion strain. *Brain Pathol* 2012; **22:** 662-669.
13. Takeuchi A, Kobayashi A, Ironside JW, Mohri S, Kitamoto T. Characterization of variant Creutzfeldt-Jakob disease prions in prion protein-humanized mice carrying distinct codon 129 genotypes. *J Biol Chem* 2013 **288:** 21659-21666.
14. Kobayashi A, Iwasaki Y, Otsuka H *et al.* Deciphering the Pathogenesis of Sporadic Creutzfeldt-Jakob Disease with Codon 129 M/V and Type 2 Abnormal Prion Protein. *Acta Neuropathol Commun* 2013; **1:** 74.
15. Kobayashi A, Matsuura Y, Iwaki T *et al.* Sporadic Creutzfeldt-Jakob disease MM1+2C and MM1 are identical in transmission properties. *Brain Pathol* 2015 doi: 10.1111/bpa.12264. [Epub ahead of print]
16. Kobayashi A, Teruya K, Matsuura Y *et al.* The influence of *PRNP* polymorphisms on human prion disease susceptibility: an update. *Acta Neuropathol* 2015 doi: 10.1007/s00401-015-1447-7. [Epub ahead of print]
17. Parchi P, Giese A, Capellari S *et al.* Classification of sporadic Creutzfeldt-Jakob disease based on molecular and phenotypic analysis of 300 subjects. *Ann Neurol* 1999; **46:**

224-233.

18. Parchi P, Strammiello R, Notari S, Giese A, Langeveld JP, Ladogana A. Incidence and spectrum of sporadic Creutzfeldt-Jakob disease variants with mixed phenotype and co-occurrence of PrPSc types: an updated classification. *Acta Neuropathol* 2009; **118:** 659–671.
19. Parchi P, Strammiello R, Giese A, Kretzschmar H. Phenotypic variability of sporadic human prion disease and its molecular basis: past, present, and future. *Acta Neuropathol* 2011; **121:** 91-112.
20. Notari S, Capellari S, Giese A *et al.* Effects of different experimental conditions on the PrP^{Sc} core generated by protease digestion: implications for strain typing and molecular classification of CJD. *J Biol Chem* 2004; **279:** 16797-16804.
21. Noguchi-Shinohara M, Hamaguchi T, Kitamoto T *et al.* Clinical features and diagnosis of dura mater graft associated Creutzfeldt-Jakob disease. *Neurology* 2007; **69:** 360-367.
22. Yamada M, Noguchi-Shinohara M, Hamaguchi T, Nozaki I, Kitamoto T, Sato T. Dura mater graft-associated Creutzfeldt-Jakob disease in Japan: Clinicopathological and molecular characterization of the two distinct subtypes. *Neuropathology* 2009; **29:** 609-618.
23. Kobayashi A, Matsuura Y, Mohri S, Kitamoto T. Distinct origins of dura mater graft-associated Creutzfeldt-Jakob disease: past and future problems. *Acta Neuropathol Commun* 2014; **2:** 32.
24. Kretzschmar HA, Sethi S, Földvári Z *et al.* Iatrogenic Creutzfeldt-Jakob disease with florid plaques. *Brain Pathol* 2003; **13:** 245-249.
25. Delisle MB, Fabre N, Rochiccioli P, Doerr-Schott J, Rumeau JL, Bes A. Creutzfeldt-Jakob disease after treatment with human extracted growth hormone. A clinicopathological study. *Rev Neurol (Paris)* 1993; **149:** 524-527.
26. Billette de Villemeur T, Gelot A, Deslys JP *et al.* Iatrogenic Creutzfeldt-Jakob disease in three growth hormone recipients: a neuropathological study. Neuropathol Appl Neurobiol 1994; **20:** 111-117.
27. Cali I, Miller CJ, Parisi JE, Geschwind MD, Gambetti P, Schonberger LB. Distinct

pathological phenotypes of Creutzfeldt-Jakob disease in recipients of prion-contaminated growth hormone. *Acta Neuropathol Commun.* 2015; **3**: 37.

28. Rudge P, Jaumuktane Z, Adlard P *et al*. Iatrogenic CJD due to pituitary-derived growth hormone with genetically determined incubation times of up to 40 years. Brain. 2015 Aug 11. pii: awv235. [Epub ahead of print]
29. Jansen C, Parchi P, Capellari S *et al*. Human prion diseases in the Netherlands (1998-2009): clinical, genetic and molecular aspects. PLoS One. 2012; **7**: e36333.
30. Cervenáková L, Goldfarb LG, Garruto R, Lee HS, Gajdusek DC, Brown P. Phenotype-genotype studies in kuru: implications for new variant Creutzfeldt-Jakob disease. *Proc Natl Acad Sci U S A* 1998; **95:** 13239-13241.
31. Parchi P, Capellari S, Chen SG *et al.* Typing prion isoforms. *Nature* 1997; **386:** 232-234.
32. Wadsworth JD, Joiner S, Linehan JM *et al.* Kuru prions and sporadic Creutzfeldt-Jakob disease prions have equivalent transmission properties in transgenic and wild-type mice. *Proc Natl Acad Sci U S A* 2008; **105:** 3885-3890.
33. Parchi P, Cescatti M, Notari S *et al.* Agent strain variation in human prion disease: insights from a molecular and pathological review of the National Institutes of Health series of experimentally transmitted disease. Brain 2010; **133:** 3030-3042.
34. Kobayashi A, Parchi P, Yamada M *et al.* Transmission properties of atypical creutzfeldt-jakob disease: a clue to disease etiology? *J Virol* 2015; **89:** 3939-3946.
35. Kobayashi A, Asano M, Mohri S, Kitamoto T. A traceback phenomenon can reveal the origin of prion infection. *Neuropathology* 2009; **29:** 619-624.
36. Saborio GP, Permanne B, Soto C. Sensitive detection of pathological prion protein by cyclic amplification of protein misfolding. *Nature* 2001; **411:** 810-813.
37. Yokoyama T, Takeuchi A, Yamamoto M, Kitamoto T, Ironside JW, Morita M. Heparin enhances the cell-protein misfolding cyclic amplification efficiency of variant Creutzfeldt-Jakob disease. *Neurosci Lett* 2011; **498:** 119-123.

FIGURE LEGENDS

Fig. 1 Diagnostic criteria to identify acquired CJD. (A) The combination of the 129M/M genotype at the polymorphic codon 129 of the *PRNP* gene, intermediate type (type i) PrP^{Sc} with the intermediate electrophoretic mobility between PrP^{Sc} types 1 and 2, and widespread kuru plaques is a reliable criterion for identification of acquired CJD cases caused by infection with the sCJD strain V2, designated as acquired CJD-MMiK. (B) Transmission properties of acquired CJD-MMiK in PrP-humanized knock-in mice are also distinctive. Since the causative origin of acquired CJD-MMiK is the sCJD strain V2, acquired CJD-MMiK shows the same transmission properties as those of the sCJD strain V2. In particular, 129V/V mice show the highest susceptibility despite the mismatched codon 129 genotype, with PrP^{Sc} type 2 accumulation and plaque-like PrP deposition in their brain.

Table 1 Transmission properties of sCJD prions in PrP-humanized knock-in mice

Inoculum		Mouse line	PrP^{Sc} type	PrP deposition†	References
Prion strain	sCJD subgroup				
M1	MM/MV1	129M/M	1	S	10,11,14,15
		129M/V	1	S	
		129V/V	1	S	
M2C	MM/MV2C	129M/M	—‡	—	11,14
		129M/V	—	—	
		129V/V	—	—	
M2T	MM2T	129M/M	2	S	12
		129M/V	N.D.§	N.D.	
		129V/V	N.D.	N.D.	
V1	VV1	129M/M	1	S	11
		129M/V	1	S	
		129V/V	1	S	
V2	MV2K or VV2	129M/M	i¶	K	8,11,14
		129M/V	i+2#	K	
		129V/V	2	P	

† The patterns of PrP deposition in the mouse brain are classified into diffuse synaptic deposition (S), kuru plaques (K), or plaque-like deposition mainly observed in the white matter (P).

‡ The M2C sCJD strain was not transmissible to the PrP-humanized knock-in mice.

§ N.D., not done.

¶ Intermediate type that shows intermediate electrophoretic mobility (~20 kDa) between type 1 (21 kDa) and type 2 (19 kDa) PrP^{Sc}.

A mixture of type i and type 2 PrP^{Sc}.

Table 2 Transmission properties of the atypical CJD cases or plaque-type dCJD cases in PrP-humanized knock-in mice

Inoculum	Mouse line	PrP^{Sc} type	PrP deposition†	References
Atypical CJD	129M/M	i‡	K	34
	129M/V	i+2§	K	
	129V/V	2	P	
Plaque-type dCJD	129M/M	i	K	8,22,34
	129M/V	i+2	K	
	129V/V	2	P	

† The patterns of PrP deposition in the mouse brain are classified into kuru plaques (K) or plaque-like deposition mainly observed in the white matter (P).

‡ Intermediate type that shows intermediate electrophoretic mobility (~20 kDa) between type 1 (21 kDa) and type 2 (19 kDa) PrP^{Sc}.

§ A mixture of type i and type 2 PrP^{Sc}.

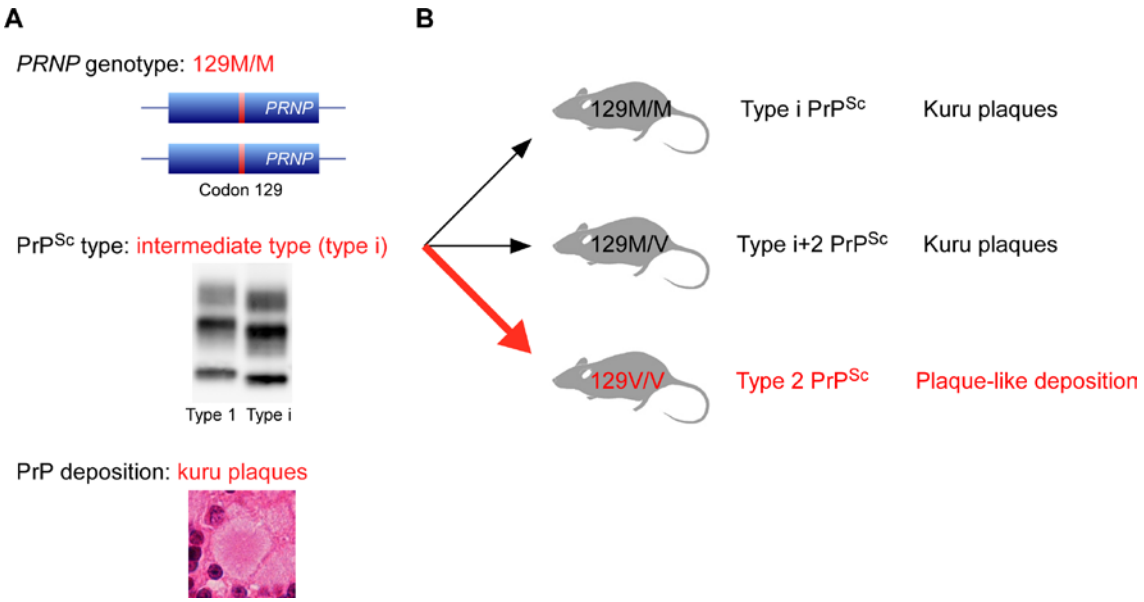
A
PRNP genotype: 129M/M
PRNP
PRNP
Codon 129
PrP^Sc type: intermediate type (type i)
Type 1 Type i
PrP deposition: kuru plaques
B
129M/M
Type i PrP^Sc
Kuru plaques
129M/V
Type i+2 PrP^Sc
Kuru plaques
129V/V
Type 2 PrP^Sc
Plaque-like deposition

Fig. 1