

# Defining sporadic Creutzfeldt-Jakob disease strains and their transmission properties

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**The biological determinants of the phenotypic variation in sporadic Creutzfeldt-Jakob disease (sCJD) are unknown. To categorize sCJD cases, the prion protein (PrP) codon 129 genotype and the biochemical characteristics of the disease-associated form of PrP (PrP<sup>Sc</sup>) can be combined to form six subgroups (MM1, MM2, MV1, MV2, VV1, and VV2). This classification largely correlates with the known variation in the clinical and pathological features of sCJD, with the MM1 and MV1 cases representing the “classic” phenotype of sCJD. To address how this classification relates to different strains of sCJD we have inoculated each subgroup of sCJD to a panel of mice expressing different forms of the human *PRNP* gene (129MM, 129VV, and 129MV). We have established that all subtypes are transmissible to at least one genotype of mouse, and both agent and host factors determine transmission efficiency and the form of PrP<sup>Sc</sup> deposited in the brain. Moreover, we have identified four distinct strains of sCJD using our in vivo strain typing panel.**

codon 129 genotype | mouse model | prion disease | transmission strains

**T**ransmissible spongiform encephalopathies (TSE) or prion diseases are a group of fatal neurodegenerative diseases that include Creutzfeldt-Jakob disease (CJD) in humans, scrapie in sheep and goats, bovine spongiform encephalopathy (BSE) in cattle, and chronic wasting disease in deer and elk. These diseases can be sporadic, familial, or acquired by infection, and the common hallmark is a distinct pathology in the central nervous system characterized by neuronal loss, spongiform degeneration, and gliosis (1). Sporadic CJD (sCJD) is the idiopathic form of CJD. There is much that remains to be understood about how and why this disease occurs and the specific biochemical and cellular pathogenesis that leads to the neurodegenerative phenotype. Most frequently, sCJD is a rapidly progressive dementia that occurs between 50 and 75 y of age and has a short clinical duration of weeks to a few months (2).

Expression of the host-encoded cellular prion protein (PrP<sup>C</sup>) is essential for an individual to be susceptible to disease, because PrP null mice are refractory to TSE infection (3). PrP<sup>C</sup> is a glycoprotein with two consensus sites for attachment of N-linked glycans, which are variably occupied and produce di-, mono-, and unglycosylated PrP. A central event associated with TSEs is the conformational conversion of PrP<sup>C</sup> into an abnormal, protease-resistant form, PrP<sup>Sc</sup> (4). During disease, PrP<sup>Sc</sup> is deposited in the brain in the form of plaques and fibrils (5).

An extensively studied polymorphism of the human prion gene is that which results in a methionine-to-valine change at codon 129. The normal frequencies for the three genotypes show wide variability across populations in different geographical areas (6). It has been shown that codon 129 genotype may have effects on susceptibility to disease (7, 8), disease duration (9, 10), phenotype of familial forms of CJD (11), neuropathology of sCJD (2), protease cleavage of PrP<sup>Sc</sup> (12), oligomerization of PrP<sup>Sc</sup> (13), and PrP<sup>Sc</sup> amyloid formation (14).

Sporadic CJD was originally classified as a single disease; however, transmission of 234 sCJD cases to nonhuman primates demonstrated variability in disease characteristics such as incubation times, duration of illness, and the pathological outcome (15). More recently sCJD has been classified into different subgroups according to the codon 129 genotype of the host and profile of PrP<sup>Sc</sup> determined by means of Western blotting (2, 16).

Electrophoresis of the PrP<sup>Sc</sup> protease-resistant core can distinguish between two isoforms of the protein commonly referred to as type 1 (21 kDa) and type 2 (19 kDa), from the mobility of the unglycosylated component (17). The prion protein codon 129 genotype and the biochemical characteristics of the disease-associated form (PrP<sup>Sc</sup>) can be combined to form six subgroups (MM1, MM2, MV1, MV2, VV1, and VV2). Further studies have been carried out to assess whether disease characteristics, according to PrP<sup>Sc</sup> type, are maintained after passage to hosts with different *PRNP* genotypes (18, 19).

In vivo transmission studies have been undertaken to identify sCJD strains. Transmission of sCJD to wild-type mice does not often result in clinical disease (20); however, transmissions to bank voles have proved more productive in terms of a clinical outcome, with sCJD isolates classified as MM1 and MV1 behaving as a single strain, but VV2 and MV2 failed to cause disease after inoculation (21). A number of different lines of transgenic mice have been produced that express full-length or chimeric human and mouse PrP genes to facilitate transmission of CJD (22–24). When challenged with some, but not all, CJD isolates, transgenic mice have shorter incubation times than wild-type mice, and the data demonstrate that identity between host and agent codon 129 genotype often, but not always, facilitates transmission.

Previously we reported the use of gene targeting methodology to produce mice expressing physiological levels of the human prion protein gene (25). The inserted human *PRNP* gene is under the direct control of the normal expression modifiers for the equivalent mouse *Pmp* gene and, after inoculation with human prions, there will be homologous human PrP<sup>Sc</sup>-PrP<sup>C</sup> interaction. These lines have been inbred on a 129Ola background, thus the only genetic variation (between the different mouse lines) is that of the codon 129 genotype in the inserted human prion gene. Thus, the direct effect of an M-to-V substitution in the mature prion protein can be studied in both the homozygous (HuMM and HuVV) and heterozygous (HuMV) lines. Six sCJD cases were selected for transmission to the transgenic mice, each of which showed the typical characteristics of that subgroup: MM1, MM2, MV1, MV2, VV1, and VV2. Our aim was to define the diversity of sCJD strains and the influence of codon 129 genotype on the transmission properties of sCJD.

## Results

**Incubation Times on First Passage Indicate Four Strains of sCJD.** Incubation time data for mice showing clinical TSE symptoms are shown in Table 1. The presence/absence and time of onset of clinical manifestation of TSE disease were dependent on both the *PRNP* genotype of the host and the inoculum. These data suggest that there exist four discrete sCJD strains. The first strain comprises the subgroups sCJD(MM1) and sCJD(MV1) that produced similar incubation times in each of the lines of mice,

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with the shortest in the HuMM and HuMV lines (446–475 d), whereas incubation times in HuVV mice were more than 100 d longer. The second strain comprises sCJD(MV2) and sCJD(VV2) inocula that produced clinical disease with relatively short incubation times ( $\approx 280$  d) in the HuVV mice but much longer incubation times in HuMM and HuMV mice (450–582 d). For these inoculations, only a few mice in the HuMM and HuMV lines displayed clinical signs [sCJD(MV2): 3 of 13 HuMM and 2 of 16 HuMV; sCJD(VV2): 4 of 18 HuMM and 1 of 15 HuMV] compared with the high numbers of HuVV mice displaying clinical signs [sCJD(MV2): 16 of 17; sCJD(VV2): 13 of 16]. Although the two groups of HuMV mice produced different incubation periods after inoculation of sCJD(MV2) and sCJD(VV2), these data were limited to too few mice to compare statistically. The third and fourth strains comprise sCJD(VV1) and sCJD(MM2), which had transmission characteristics that were different from each other and from the other agents. No clinical disease was observed in the HuMM mice inoculated with sCJD(VV1), and only two cases were observed in each of the HuMV and HuVV lines between 546 and 568 d. Sporadic CJD (MM2) inoculation showed no clinical disease in any of the three lines of mice.

**Vacuolation Profiles Demonstrate Strain Diversity of sCJD.** Examination of the vacuolar pathology of sCJD-infected mice allowed further assessment of the similarities and differences in transmission properties of each inoculum and provided further evidence for four discrete sCJD strains (Table 1). Inoculation with sCJD(MM1) and sCJD(MV1) produced similar levels of vacuolation, with 80–100% of HuMM and HuMV mice and  $\approx 80\%$  of HuVV mice scored positive for vacuolation. Inoculation with sCJD(MV2) and sCJD(VV2) produced 100% positive HuVV mice and  $\approx 33\%$  positive HuMV mice, but there was some variation in the HuMM mice, with 69% positive with sCJD(MV2) inoculum compared with 33% with sCJD(VV2). The sCJD(VV1) inoculum showed no evidence of vacuolation in the HuMM mice, and  $\approx 50\%$  of mice were positive in the other two transgenic lines. The sCJD(MM2) inoculum showed no evidence of TSE vacuolation in any of the three mouse lines. Fig. 1 shows the lesion profiles generated from assessment of TSE vacuolation distribution in the brain. Data for sCJD(MM2) inoculation (all genotypes) and HuMM mice inoculated with sCJD(VV1) are absent because no mice were positive for vacuolation. There were similarities in lesion profiles for all genotype mice infected with both sCJD(MM1) and sCJD(MV1). The distribution of lesions is characterized by three peaks in the gray matter scores relating to the superior colliculus (GM3), hippocampus (GM6), and cingulate and adjacent motor cortex (GM9). Similarities were also seen between sCJD(MV2) and sCJD(VV2) inocula; however, unlike sCJD(MM1) and sCJD(MV1), these profiles differed between the mouse lines. The HuMM mice profiles had peak scores for the hippocampus (GM6) and the cingulate and adjacent motor cortex (GM9) and appeared similar but not identical to those of sCJD(MM1) and sCJD(MV1). The HuVV mice lesion profiles for these two inocula had peaks in the pro-

files again at GM3, GM6, and GM9; however, region GM9 had a much higher mean score, and additionally a high score was also recorded for the cortex region GM8 (retrosplenial and adjacent motor cortex). The heterozygous HuMV mice inoculated with sCJD(MV2) and sCJD(VV2) produced lesion profiles that were dissimilar to both homozygous lines of mice and also to the sCJD(MM1) and sCJD(MV1) inocula. Lesion profiles for HuMV and HuVV mice inoculated with sCJD(VV1) were different from each other and from all other profiles generated.

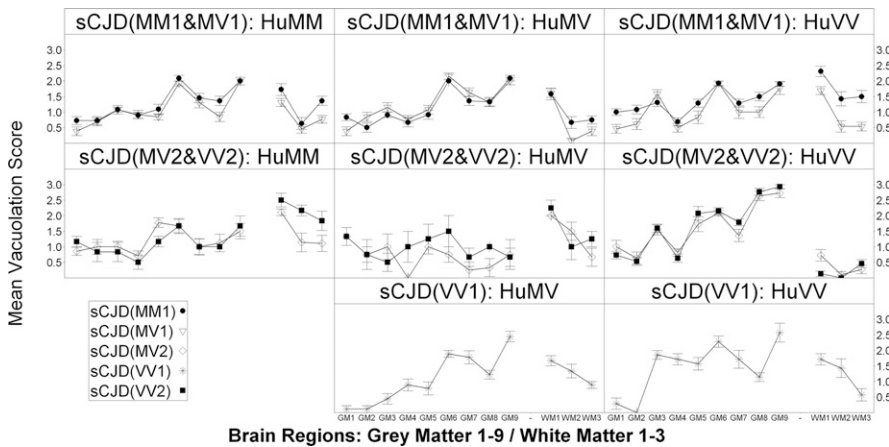
**Detection of PrP<sup>Sc</sup> by Immunocytochemistry.** Analysis of PrP<sup>Sc</sup> deposition was carried out to determine whether distinctive patterns exist for the sCJD subgroups [Fig. 2 (hippocampal/thalamic targeting), Table 1, and Fig. S1 (cerebellar targeting)]. Brain sections from all mice in each inoculated group were examined by immunohistochemistry to assess the specific targeting of PrP<sup>Sc</sup> deposition. The degree of within-group variation seemed to depend on the incubation time of the mice, with an increase in deposition over time. The regions targeted did not change over this time period.

Both sCJD(MM1)- and sCJD(MV1)-inoculated mice of each genotype showed similar overall patterns of staining: in the thalamic region with discrete, punctate, deposits; hippocampal targeting of the stratum lacunosum moleculare; and occasional larger deposits in deep layers of the cerebral cortex. The cerebellum was spared with only very little evidence of PrP<sup>Sc</sup> deposition. The sCJD(MV2)- and sCJD(VV2)-inoculated mice showed very similar patterns of PrP<sup>Sc</sup> deposition, which were different from that found with sCJD(MM1) and sCJD(MV1). The same thalamic regions were targeted as for sCJD(MM1), but as well as the punctate deposits, there was an increase in more diffuse staining. The HuMM and HuMV lines showed a similar degree of staining concentrated in the ventral posteromedial and posterolateral thalamic nuclei. For the HuVV line this area extended to include the lateral dorsal nucleus, the posterior thalamic nuclear group, and the medial dorsal and ventromedial thalamic nuclei. Outside the thalamic region the HuVV mice again showed differences from the other lines. The cerebral cortex was heavily stained with distinctive banding associated with the cell type layers present. Band 6 showed the densest deposits. Intensely stained aggregates were seen in the white matter of the corpus callosum in both HuMM and HuVV mice and not in the HuMV mice. The cerebellum showed similar staining patterns, targeted to the granular layer, for all mouse lines and both inocula. Deposition in the brains of all three genotype mice inoculated with sCJD(VV1) showed similar overall patterns of staining, and this was different from that found for the four sCJD subgroups discussed above. The deposition was less intense, with PrP<sup>Sc</sup> detected only in the stratum lacunosum moleculare of the hippocampus and occasional deposits in the cerebral cortex. The cerebellum showed no evidence of PrP<sup>Sc</sup> deposition. There was no evidence of PrP<sup>Sc</sup> deposition in the brains of HuMM mice inoculated with sCJD(MM2), which distinguishes this sCJD subgroup from all of the others. HuMV and HuVV mice showed a similar pattern of staining, with only very rare small punctate

**Table 1. Primary inoculation of sCJD into the three transgenic mouse lines**

sCJD inoculum	HuMM				HuMV				HuVV			
	IP	Clinical signs	Vacuolation present	PrP <sup>Sc</sup> detected by ICC	IP	Clinical signs	Vacuolation present	PrP <sup>Sc</sup> detected by ICC	IP	Clinical signs	Vacuolation present	PrP <sup>Sc</sup> detected by ICC
MM1	446 ± 3.1	10/13	13/13	13/13	457 ± 10.5	9/14	14/14	14/14	588 ± 20.9	8/16	13/16	14/16
MV1	446 ± 4.9	15/16	16/16	16/16	475 ± 9.6	15/18	16/18	17/18	603 ± 34.2	7/18	15/18	13/18
MV2	563 ± 2.2	3/13	9/13	10/11	575 ± 37.0	2/16	6/17	15/17	288 ± 3.0	16/17	16/16	15/15
VV2	582 ± 47.8	4/18	6/18	15/17	450	1/15	5/15	12/14	274 ± 3.6	13/16	16/16	15/15
VV1	—	0/16	0/16	2/16	557 ± 11.0	2/14	9/14	1/14	568 ± 0.0	2/14	7/14	7/14
MM2	—	0/16	0/16	0/15	—	0/18	0/18	2/18	—	0/17	0/17	3/17

Primary passage data for incubation period (IP), number of clinical-positive, TSE vacuolation-positive, and immunocytochemistry (ICC)-positive mice, shown out of group tested. Values are mean ± SEM or number. —, no clinical signs.

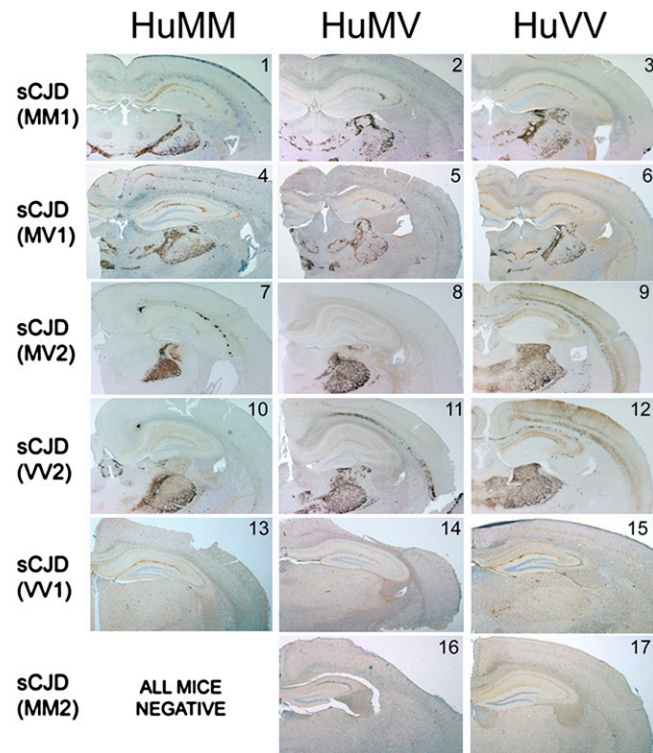


**Fig. 1.** Lesion profile charts of primary passage vacuolation scoring data. The distribution and intensity of TSE-associated vacuolation was assessed for each mouse in the primary inoculation experiments. Mean vacuolation scores for nine gray matter (GM1–GM9) and three white matter (WM1–WM3) brain regions were plotted. Lesion profile data have been grouped horizontally according to the predicted sCJD strain groupings and vertically according to host codon 129 genotype. No data are shown for sCJD(VV1) in HuMM mice and for sCJD(MM2) because no TSE vacuolation was observed. Error bars,  $\pm$ SEM; mean values based on at least five individual mouse scores; brain regions defined in *Materials and Methods*.

deposits in the thalamic region. The cerebellum showed no evidence of PrP<sup>Sc</sup> deposition. These differences observed with each subtype confirm evidence of four major sCJD strains: sCJD(MM1 and MV1); sCJD(MV2 and VV2); sCJD(VV1); and sCJD(MM2).

**Detection of PrP<sup>Sc</sup> by Western Blot.** Western blot detection of PrP<sup>Sc</sup> was performed on brain material recovered during the postmortem of each mouse (Fig. S2). The appearance of type 2 PrP<sup>Sc</sup> was restricted to the HuVV mice inoculated with either sCJD(MV2) or sCJD(VV2) on primary and secondary passage. These mice also

showed a dominance of the diglycosylated form, a Western blot profile similar to variant CJD (see type 2 standard lanes in Fig. S2). There was no evidence for the appearance of type 2 PrP<sup>Sc</sup> in mice homozygous or heterozygous for methionine at codon 129. All of the other mice/inoculum combinations in which PrP<sup>Sc</sup> has been detected showed the typical sCJD type 1, monoglycosylated dominant profile even if the inoculum contained type 2 PrP<sup>Sc</sup>. Very low levels of PrP<sup>Sc</sup> were seen in the sCJD(VV1) experiment. With regards to understanding the strain nature of the inocula, the Western blot data have only limited capacity to confirm the previous findings. It is clear that sCJD(MV2) and sCJD(VV2) share similar properties that are different from the other inocula. Because all other inoculum/host combinations produce type 1 monoglycosylation dominant PrP<sup>Sc</sup> (where this is present), there can be no further distinction between those sCJD subgroups.



**Fig. 2.** Comparison of immunocytochemistry data from transgenic mice inoculated with six sCJD subgroups. PrP<sup>Sc</sup> deposition within the brain was assessed for variation in brain region targeted and morphology of deposits, in the understanding that this may vary according to the sCJD agent strain inoculated and the host codon 129 genotype. Immunocytochemistry with anti-PrP antibody (6H4, brown staining) was performed on histological sections of the mouse brains. Representative sections are shown for coronal sections through the hippocampus/thalamus from primary passage mice. All HuMM mice inoculated with sCJD(MM2) were negative for PrP<sup>Sc</sup> deposition. (Magnification: 2.5 $\times$ .)

**Secondary Passage in Transgenic Mice.** Secondary passage of brain material from each of the three transgenic mouse lines was set up for the three commonest subgroups of sCJD (MM1, MV2, and VV2) to examine the influence of host PrP in modifying the strain of agent. Table 2 lists the incubation periods found for the appearance of clinical TSE signs in the second passage, together with the scores for mice found with TSE vacuolation. Sporadic CJD(MM1) showed remarkably close similarities in onset of clinical TSE signs and number of affected mice between first and second passage regardless of the incubation period or genotype of the mice that donated the inoculum brain material. For example, primary inoculation of HuMM mice produced clinical disease at a mean time of 446 d, and secondary passage from HuMM-, HuMV-, and HuVV-sourced inocula gave mean incubation periods of 451, 446, and 451 d, respectively. Additionally, the extended incubation periods in HuVV mice ( $\approx$ 550 d) were similar in both primary and all secondary passage experiments. These data indicate that sCJD(MM1) strain properties are being propagated efficiently and that this can occur independently of the genotype of the host, even if brain material is sourced from an HuVV mouse.

Sporadic CJD(MV2) and sCJD(VV2) showed similar primary passage incubation times, but differences were observed with these inocula after secondary passage. Incubation times in HuVV mice were not affected by primary passage-sourced inocula from either HuMM or HuMV mice and gave incubation periods of 262–288 d. Second passage from HuVV-sourced brains resulted in reduction of incubation periods to 235–239 d, suggesting that codon 129 methionine may hinder propagation of this otherwise efficient genotype/agent combination. Further differences between primary and secondary passage were restricted to HuMV and HuMM mice with inocula originating from primary sCJD(MV2)-infected HuMV mice and sCJD(VV2)-infected HuMM mice. Reduction in incubation periods (by  $\approx$ 100 d) and an increase in clinically positive mice compared with primary passage suggest that the agent may be adapting toward having properties more similar to sCJD(MM1) and sCJD(MV1).

**Table 2. Secondary passage of sCJD(MM1), sCJD(MV2), and sCJD(VV2) into the three transgenic mouse lines**

Inoculum	HuMM			HuMV			HuVV		
	IP	Clinical signs	Vacuolation present	IP	Clinical signs	Vacuolation present	IP	Clinical signs	Vacuolation present
MM1 via HuMM	451 ± 9.8	16/18	18/18	433 ± 15.0	10/18	17/17	539 ± 20.7	6/18	16/18
MM1 via HuMV	446 ± 9.2	16/18	18/18	439 ± 9.9	13/18	18/18	563 ± 25.2	6/18	16/18
MM1 via HuVV	451 ± 6.6	16/18	18/18	484 ± 10.8	11/18	15/18	564 ± 24.7	7/18	15/17
MV2 via HuMM	617 ± 0.5	2/18	15/18	>700	0/17	12/17	277 ± 5.1	15/18	18/18
MV2 via HuMV	475 ± 9.9*	15/18*	18/18*	491 ± 3.4*	5/18*	17/18*	288 ± 3.2	16/17	16/17
MV2 via HuVV	648 ± 10.0	2/11	9/11	>700	0/12	4/12	235 ± 3.0	11/12	12/12
(duplicate)	637 ± 98.0	2/12	7/12	553	1/11	6/11	239 ± 2.5	11/11	11/11
VV2 via HuMM	474 ± 12.2*	16/18*	18/18*	531 ± 46.6*	3/18*	17/18*	264 ± 6.7	14/18	18/18
VV2 via HuMV	392	1/18	12/18	587	1/18	7/18	262 ± 2.5	17/18	18/18
VV2 via HuVV	649 ± 22.5	2/17	15/17	>700	0/18	16/18	237 ± 2.9	13/18	17/18

Secondary passage data for incubation period (IP), clinical scoring, and TSE vacuolation scoring, with number of positive mice shown out of group tested. Values are mean ± SEM or number. >, No clinical signs up to that time point.

\*Difference seen compared with primary passage.

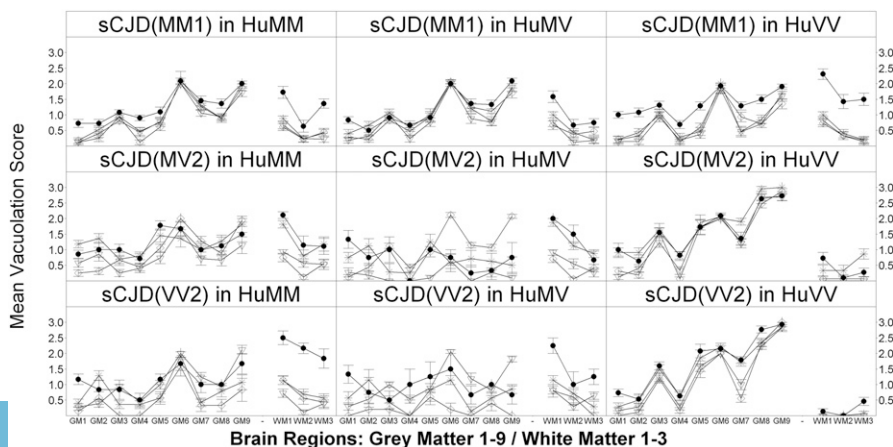
The number of mice positive for the presence of TSE-associated vacuolation closely followed the similarities and differences that were seen for clinical signs. In addition, the HuMM and HuMV mice (discussed above) that showed reductions in incubation periods were >90% positive, unlike 33–50% for primary transmission (see asterisked data in Table 1 and Table 2). In contrast to the primary transmission data, these results for incubation period and the appearance of TSE vacuolation may indicate a divergence of the strain properties of sCJD(MV2) and sCJD(VV2). Visual comparison of the lesion profile data (Fig. 3) showed that with each genotype host there were many instances in which the second passage profile was similar to the first passage. This was seen even when passage occurred from an alternative genotype host. For sCJD(MM1) the lesion profiles showed clear uniformity across all mice lines and with passage from the three genotype primary inoculations. Second passage data showed a general lowering of the scores in regions other than those peak scores seen in primary passage [superior colliculus (GM3), hippocampus (GM6), and cingulate and adjacent motor cortex (GM9)], suggesting adaptation to a more focused pathological response. This effect was not seen in the sCJD(MV2) and sCJD(VV2) passage data. Overall, passage of sCJD(MV2) and sCJD(VV2) gave similar data sets. The HuVV hosts with passage from each different genotype mice had profiles that most closely mirrored the primary data, whereas although the HuMM vacuolation scores gave a similar overall pattern there was more variation between the passage experiments. The HuMV response to the second passage inocula was similar to primary passage in that the profiles were variable and had wide SE ranges. Scores for the hippocampus (GM6) region in HuMM and HuMV mice may highlight a potential adaptation of the sCJD

(MV2) and sCJD(VV2) agents, as shown by a more pronounced peak in the lesion profile at this point.

Sporadic CJD(MM1) seems to have dominance over the genotype of the host, with near identical lesion profile patterns for all primary and secondary passage mice. The patterns seen for both sCJD(MV2) and sCJD(VV2) are similar only within the HuMM and HuVV host mice groups. The heterozygous HuMV host shows the most variation between primary and secondary passage, with no specific pattern apparent. These data confirm the primary transmission findings that sCJD(MM1) has strain properties different from sCJD(MV2) and sCJD(VV2) and that the latter pair could be grouped together as one strain. Second-passage Western blot analysis was undertaken for mice inoculated with the sCJD(MV2) agent from an HuVV genotype host and showed that the same PrP<sup>Sc</sup> types occurred in second passage as was seen in primary passage (Fig. S2); type 1 was seen in the HuMM and HuMV mice brains and type 2 in the HuVV mice.

### Discussion

The similarities and differences that have emerged during this study indicate that six subgroups of sCJD, defined here by PrP<sup>Sc</sup> type and PRNP codon 129 genotype, behave as four different strains of agent. Sporadic CJD(MM1) and sCJD(MV1) isolates have identical transmission properties for all three genotypes of mice. The sCJD(MV2) and sCJD(VV2) isolates have very similar transmission properties, and both the sCJD(MM2) and sCJD(VV1) strains behave differently from each other and from the other isolates. To facilitate discussion of this grouping and for future reference we propose to name these major strains “M1<sup>CJD</sup>,” “V2<sup>CJD</sup>,” “M2<sup>CJD</sup>,” and “V1<sup>CJD</sup>,” respectively. Our



**Fig. 3.** Secondary passage lesion profiles. For secondary passage experiments of sCJD(MM1), sCJD(MV2), and sCJD(VV2), mean vacuolation scores were plotted. Data shown (mean ± SEM) compare profiles between sCJD subgroups (horizontal panels) against the three host genotypes (vertical panels). Each panel includes the primary passage lesion profile for that data set. Filled circles, primary passage; open diamonds, second passage from HuMM inocula; open triangles, second passage from HuMV inocula; asterisks, second passage from HuVV inocula.

studies, based on incubation time, lesion profile, and PrP<sup>Sc</sup> Western blot profile and deposition in the brain using a panel of mice, are based on the “gold standard” strain typing of isolates originally designed using wild-type mice (26). The difference here is that our transgenic mice carrying the human *PRNP* gene have been substituted for the original wild-type panel, but the inbred nature and gene targeting of these mice ensure that they are suitable for such a panel, because there is only a single PrP amino acid difference between each of the lines. Previous sCJD transmission studies have provided additional evidence for sCJD (MM1) and sCJD(MV1) having similar transmission properties and some evidence for similarities between sCJD(MV2) and sCJD(VV2), but insufficient evidence to make a comparison between sCJD(VV1) and sCJD(MM2) (21, 24, 27). This study is a complete analysis of all major sCJD subgroups in inbred mouse models and is thus capable of a true comparison for distinguishing the codon 129 homozygote and heterozygote response. However, the present study examines only single cases of sCJD, with the assumption that transmission characteristics of a single case will be representative of the particular subgroup. Although typical cases of each subgroup were carefully selected for this study, there is phenotypic variation within sCJD subgroups, and it is therefore essential that the findings in this study are replicated in additional cases. Through a grant from the European Union (NeuroPrion: HUMTRANS) the panel of mice used in this study have been provided to a number of other groups, and transmission studies including a significant number of additional sCJD cases are underway. This is of particular importance for the sCJD(MM2) subgroup because our evidence for a unique strain derives partly from the inefficiency in transmission. Inoculum prepared from this specific patient has been used in parallel wild-type and transgenic studies (28) and shows clear evidence of successful transmission to those mice, confirming that infectivity titer was unlikely to be implicated in our findings.

Evidence in support of these four major strains of sCJD has recently been reported through a very different approach. Using *in vitro* assays, Uro-Coste et al. (29) examined the protease sensitivity and conformational stability of PrP<sup>Sc</sup> found in 41 patients with sCJD and found groupings identical to those outlined in this study (i.e., MM1 and MV1; MV2 and VV2; MM2; VV1). Because this study was based entirely on *in vitro* analysis of PrP<sup>Sc</sup>, this suggests that the four strains of agent identified in our study have different conformations of PrP<sup>Sc</sup>. What is perhaps surprising is that only four discrete strains of sCJD have been identified. If the prion protein can exist in many different pathogenic isoforms in a single host, why then do only four different strains of sCJD result in humans? Because the assumption is that each sCJD case arises spontaneously, this would require strong selection factors to be operating for these four strains and against others that may be produced.

There are diverse suggestions as to the origin of sCJD, including proposals that somatic mutations lead to protein misfolding and disease (30) or that sCJD has arisen through infection from an animal source, such as atypical BSE (18, 31). Clearly host *PRNP* sequence is not the major criteria for separating the four strains, although it does seem to have some influence. If differences in protein misfolding are the basis of the origin of these strains then it remains to be established what influences this characteristic. The quasi-species hypothesis put forward by Collinge et al. (32) suggests that a wide range of mammalian PrP<sup>Sc</sup> conformations are possible but that only a subset are compatible with each individual PrP primary structure. This theory may explain both the influence of the host genotype and the limited range of sCJD strains, but such a hypothesis predicts a wider range of strains emerging from heterozygote individuals, which is not apparent in this study. If the strain of sCJD was directed not by the exact somatic mutation but by the cell type in which it arose, then this may lead to differential misfolding of the protein under control of different misfolding cofactors present in that cell (33), which could explain the limited strains of agent associated with each genotype of *PRNP*. Alternatively, there may be only a limited number of somatic mutations that give rise to sCJD disease within the lifetime of the individual, and the number of strains may therefore be restricted by those

somatic mutations that are capable of causing rapid-onset disease. It may be that *in vitro* analysis has not revealed the extent of sCJD strains, although some analyses have suggested a greater diversity of strains (16) than predicted by others (17). Further *in vivo* strain typing will establish the range of sCJD strains.

Our study evaluated the precise effect host *PRNP* codon 129 genotype has on defining transmission and propagation of sCJD strains in the three genotypes 129MM, 129MV, and 129VV. There were some specific combinations of host and inoculum within the dataset that showed similar characteristics across the experiments, such as the observation that the HuVV genotype line developed clinical TSE features with most inocula. HuVV mice also had the shortest incubation periods by more than 100 d, seen for sCJD(MV2) and sCJD(VV2) inocula. These data predict that for human iatrogenic spread of sCJD as a whole, this genotype may be the most susceptible or may show shorter incubation periods. It is of note that there is an increased prevalence of young (<50 y) VV genotype sCJD cases across European countries (United Kingdom, Germany, Italy, and France) (10). The second common characteristic among the data was that HuMM and HuMV mice had similar levels of clinical disease, and mean incubation periods, for four of the six inocula [excluding sCJD(MM2) and sCJD(VV1)]. This suggests that the methionine allele of PrP<sup>C</sup> in the heterozygous HuMV mice may have had a dominant effect over the valine allele PrP<sup>C</sup> with regard to the transmission properties. This study identifies two areas of risk in terms of developing sCJD. The first is that the highest risk of developing CJD after exposure to infection is from strain M1<sup>CJD</sup> [sCJD(MM1) or sCJD(MV1)] and that the VV genotype confers the highest risk of acquiring infection. The epidemiological findings in sCJD demonstrate that approximately 80% of patients are diagnosed with “classic CJD” types MM1 and MV1, which might intriguingly suggest an infectious rather than genetic origin for the majority of sCJD cases.

PrP<sup>Sc</sup> type has typically been used as a diagnostic indicator of the strain of agent infecting an individual. This study, however, clearly demonstrates that the PrP<sup>Sc</sup> type is a result of the interaction between strain and host. Type 2 PrP<sup>Sc</sup> was seen only in HuVV mice that had short incubation periods, after inoculation with primary or secondary sources of strain V2<sup>CJD</sup> [sCJD(MV2) and sCJD(VV2)], indicating a specific pathological response from the valine homozygous mouse host and inocula with valine allele type 2 PrP<sup>Sc</sup>. All other mice genotype/inoculum combinations produced type 1 PrP<sup>Sc</sup>.

Second passage of sCJD(MM1), sCJD(MV2), and sCJD(VV2) in the transgenic mice has allowed us to examine the strain-genotype correlation and to assess the potential risk of transmission of these agents within the human population. The sCJD(MM1) strain transmission properties were unaffected by secondary passage; the strain could propagate within non-MM hosts and could transmit from non-MM hosts to all genotypes with the same properties as primary passage. Because of these facts, there may be difficulty in distinguishing sCJD from iatrogenic CJD caused by infection from sCJD(MM1) or the M1<sup>CJD</sup> strain. However, these data also indicate that passage of the commonest form of sCJD does not cause adaptation to a more highly transmissible form of human TSE. Conversely, the other two sCJD subgroups analyzed by secondary passage seemed to undergo modification of properties dependent on the codon 129 genotype of the source and recipient mouse. The sCJD(MV2) strain transmission properties were unaffected by secondary passage from an HuMM mouse. Inoculation of HuVV mice with sCJD(MV2) from an HuVV mouse produced a reduction in incubation period, suggesting adaptation to a strain with more efficient transmission properties. An overabundance of iCJD cases in VV genotype recipients of contaminated human growth hormone in the United Kingdom supports these data (34), although this is not the case in France (8). A form of adaptation was also observed for HuMM and HuMV mice that received sCJD(MV2) inoculum from an HuMV mouse. Reduction in incubation periods and an increased number of mice positive for vacuolation suggest that a more efficient transmitting strain has been formed. The sCJD(VV2) strain transmission properties showed a similar response as for sCJD(MV2). Trans-

mission properties were unaffected by secondary passage from an HuMV mouse, and inoculation of HuVV mice with sCJD(VV2) from an HuVV mouse produced a reduction in incubation period. Strain adaptation was observed for HuMM and HuMV mice that received inoculum from sCJD(VV2) passaged through an HuMM mouse. Analysis of incubation periods and lesion profile data suggests that the adaptation seen may be producing a host response more similar to that seen for sCJD(MM1) transmission. Therefore if sCJD(MV2) and sCJD(VV2) were to become iatrogenic sources of human infection, the host response may be indistinguishable from sCJD(MM1) and more transmissible with respect to further infection.

The results from this study can be used as a standard against which atypical or novel forms of human TSE can be compared. We have identified four discrete transmission strains, including M1<sup>CJD</sup>: sCJD(MM1 and MV1); V2<sup>CJD</sup>: sCJD(MV2 and VV2); V1<sup>CJD</sup>: sCJD(VV1); and M2<sup>CJD</sup>: sCJD(MM2). We hypothesize that iatrogenic spread of sCJD will depend on sCJD subgroup source and host codon 129 genotype and could produce more transmissible adapted forms of human TSE, highlighting the continued need for CJD surveillance and iatrogenic transmission risk evaluation. The sporadic CJD transmission strain characterization described in this study will now allow the full range of sCJD strains to be examined and atypical strains of human TSE to

be readily identified so that the public health threats from potential new forms of infectious prion disease can be investigated.

## Materials and Methods

Groups of 18 mice were inoculated intracerebrally with brain homogenate prepared from sCJD patients, and from primary inoculation mice for serial passage. Standard methodologies were used throughout for scoring of clinical TSE signs, for assessing the distribution of TSE-associated vacuolation (35), and for detection (immunocytochemistry) and characterization [Western blot (36)] of the disease-associated form of the prion protein. (See *SI Materials and Methods*.)

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1. Ironside JW, Head MW, McCardle L, Knight R (2002) Neuropathology of variant Creutzfeldt-Jakob disease. *Acta Neurol Exp (Warsz)* 62:175–182.
2. Parchi P, et al. (1999) Classification of sporadic Creutzfeldt-Jakob disease based on molecular and phenotypic analysis of 300 subjects. *Ann Neurol* 46:224–233.
3. Büeler H, et al. (1993) Mice devoid of PrP are resistant to scrapie. *Cell* 73:1339–1347.
4. Prusiner SB (1982) Novel proteinaceous infectious particles cause scrapie. *Science* 216:136–144.
5. Bell JE, Ironside JW (1993) Neuropathology of spongiform encephalopathies in humans. *Br Med Bull* 49:738–777.
6. Soldevila M, et al. (2003) Prion susceptibility and protective alleles exhibit marked geographic differences. *Hum Mutat* 22:104–105.
7. Deslys JP, et al. (1998) Genotype at codon 129 and susceptibility to Creutzfeldt-Jakob disease. *Lancet* 351:1251.
8. Brandel JP, et al. (2003) Distribution of codon 129 genotype in human growth hormone-treated CJD patients in France and the UK. *Lancet* 362:128–130.
9. Pocchiari M, et al. (2004) Predictors of survival in sporadic Creutzfeldt-Jakob disease and other human transmissible spongiform encephalopathies. *Brain* 127:2348–2359.
10. Alperovitch A, et al. (1999) Codon 129 prion protein genotype and sporadic Creutzfeldt-Jakob disease. *Lancet* 353:1673–1674.
11. Goldfarb LG, et al. (1992) Fatal familial insomnia and familial Creutzfeldt-Jakob disease: Disease phenotype determined by a DNA polymorphism. *Science* 258:806–808.
12. Parchi P, et al. (2000) Genetic influence on the structural variations of the abnormal prion protein. *Proc Natl Acad Sci USA* 97:10168–10172.
13. Tahiri-Alaoui A, Gill AC, Disterer P, James W (2004) Methionine 129 variant of human prion protein oligomerizes more rapidly than the valine 129 variant: Implications for disease susceptibility to Creutzfeldt-Jakob disease. *J Biol Chem* 279:31390–31397.
14. Baskakov I, et al. (2005) The presence of valine at residue 129 in human prion protein accelerates amyloid formation. *FEBS Lett* 579:2589–2596.
15. Brown P, et al. (1994) Human spongiform encephalopathy: The National Institutes of Health series of 300 cases of experimentally transmitted disease. *Ann Neurol* 35:513–529.
16. Hill AF, et al. (2003) Molecular classification of sporadic Creutzfeldt-Jakob disease. *Brain* 126:1333–1346.
17. Parchi P, et al. (1996) Molecular basis of phenotypic variability in sporadic Creutzfeldt-Jakob disease. *Ann Neurol* 39:767–778.
18. Asante EA, et al. (2002) BSE prions propagate as either variant CJD-like or sporadic CJD-like prion strains in transgenic mice expressing human prion protein. *EMBO J* 21:6358–6366.
19. Wadsworth JD, et al. (2004) Human prion protein with valine 129 prevents expression of variant CJD phenotype. *Science* 306:1793–1796.
20. Bruce ME, et al. (1997) Transmissions to mice indicate that 'new variant' CJD is caused by the BSE agent. *Nature* 389:498–501.
21. Nonno R, et al. (2006) Efficient transmission and characterization of Creutzfeldt-Jakob disease strains in bank voles. *PLoS Pathog* 2:e12.
22. Hill AF, et al. (1997) The same prion strain causes vCJD and BSE. *Nature* 389:448–450, 526.
23. Kitamoto T, et al. (2002) Follicular dendritic cells of the knock-in mouse provides a new bioassay for human prions. *Biochem Biophys Res Commun* 294:280–286.
24. Korth C, et al. (2003) Abbreviated incubation times for human prions in mice expressing a chimeric mouse-human prion protein transgene. *Proc Natl Acad Sci USA* 100:4784–4789.
25. Bishop MT, et al. (2006) Predicting susceptibility and incubation time of human-to-human transmission of vCJD. *Lancet Neurol* 5:393–398.
26. Ritchie DL, et al. (2009) Transmissions of variant Creutzfeldt-Jakob disease from brain and lymphoreticular tissue show uniform and conserved bovine spongiform encephalopathy-related phenotypic properties on primary and secondary passage in wild-type mice. *J Gen Virol* 90:3075–3082.
27. Taguchi Y, Mohri S, Ironside JW, Muramoto T, Kitamoto T (2003) Humanized knock-in mice expressing chimeric prion protein showed varied susceptibility to different human prions. *Am J Pathol* 163:2585–2593.
28. Wiseman F (2006) Glycosylation of PrP and the transmissible spongiform encephalopathies species barrier. PhD thesis (University of Edinburgh, Edinburgh).
29. Uro-Coste E, et al. (2008) Beyond PrP<sup>res</sup> type 1/type 2 dichotomy in Creutzfeldt-Jakob disease. *PLoS Pathog* 4:e1000029.
30. Prusiner SB (1998) Prions. *Proc Natl Acad Sci USA* 95:13363–13383.
31. Capobianco R, et al. (2007) Conversion of the BASE prion strain into the BSE strain: The origin of BSE? *PLoS Pathog* 3:e31.
32. Collinge J, Clarke AR (2007) A general model of prion strains and their pathogenicity. *Science* 318:930–936.
33. Graham JF, et al. (2010) Low density subcellular fractions enhance disease-specific prion protein misfolding. *J Biol Chem* 285:9868–9880.
34. Brown P, et al. (2000) Iatrogenic Creutzfeldt-Jakob disease at the millennium. *Neurology* 55:1075–1081.
35. Fraser H, Dickinson AG (1968) The sequential development of the brain lesion of scrapie in three strains of mice. *J Comp Pathol* 78:301–311.
36. Head MW, et al. (2004) Prion protein heterogeneity in sporadic but not variant Creutzfeldt-Jakob disease: UK cases 1991–2002. *Ann Neurol* 55:851–859.