



SEAC No: 77/3

**BSE PRIONS PROPAGATE AS EITHER VARIANT CJD-LIKE OR SPORADIC CJD-LIKE PRION STRAINS IN TRANSGENIC MICE EXPRESSING HUMAN PRION PROTEIN. ASANTE *et al.* (2002) EMBO 21, (23) 6358-66**

**Issue**

1. A research team at the MRC Prion Unit has recently published the above paper. A copy of the paper is attached at Annex 1.

**Background**

2. SEAC members were alerted via e-mail to the publication of this paper on the Internet on 27<sup>th</sup> November 2002. The paper has now been published in hard copy.
3. The researchers have previously reported (Collinge *et al.*, 1995; Hill *et al.*, 1997) that Tg(HuPrP129V<sup>+/+</sup> Prnp<sup>0/0</sup>)-152 mice, which express only human PrP V129 (129VV Tg152 mice), are highly susceptible to infection with human prions from patients with sporadic and iatrogenic forms of CJD, regardless of patient genotype at polymorphic codon 129. However, these mice are much less susceptible to prions from patients with vCJD. These data were relatively reassuring, in that transmission of BSE to transgenic mice expressing only human PrP was inefficient, with <40% of intracerebrally inoculated mice succumbing to prion disease after prolonged incubation periods, consistent with the presence of a substantial transmission barrier. However, an important caveat with respect to public health considerations was that vCJD was occurring in humans of the PRNP 129MM genotype, while these mice expressed human PrP 129V. Although classical CJD from patients with all three PRNP codon 129 genotypes (MM, VV and MV) transmitted efficiently to these mice, it is possible that part of the transmission barrier to vCJD infection of these mice resided in the mismatch at codon 129 between inoculum and host.
4. Using the same inocula, the researchers have now extended these studies to mice expressing human PrP M129 to further study both the bovine-to-human species barrier and the propagation of human and BSE prion strains.

5. These researchers have previously reported both FVB and C57BL/6 mice, when inoculated with BSE develop a BSE-like pattern with a characteristic PrP<sup>Sc</sup> fragment size and glycoform ratio BSE (Collinge et al., 1996b; Hill et al., 1997). However, these transmissions involve PrP from another mammalian species of different molecular mass, such that the proteins are not directly comparable, as with transmissions of human prion disease to transgenic mice expressing only human PrP. This mouse PrP<sup>Sc</sup> pattern is, therefore, referred to as 'diglycosylated dominant'. The research team had also demonstrated that FIIS and SJL mice inoculated with BSE accumulate a glycoforms of PrP<sup>Sc</sup> in a ratio similar that found in human sporadic CJD and termed monoglycosylated dominant.

### **Summary of paper by Asante *et al* 2002**

6. The paper describes work investigating human susceptibility to TSEs, using transgenic mice models (129MM Tg35 and 129MM Tg45) which express the human form of the PrP gene. Mice were inoculated with vCJD, sporadic CJD and BSE. As judged by clinical disease, both mouse models were more susceptible to inoculation with sporadic CJD, and less susceptible to vCJD and BSE. Inoculation with vCJD and BSE resulted in both clinical and subclinical infection, confirmed by human PrP<sup>Sc</sup> detection in brain tissue by western blot analysis.
7. This group has previously used western blot analysis to investigate fragment size and the glycoform ratio of di-, mono- and nonglycosylated forms of the disease-specific PrP protein, after treatment with protease. The glycoform profiles of a number of TSEs have been ascribed to different categories (or "types") according to their glycoform profile. In vCJD (or "type 4"), diglycosylated PrP<sup>Sc</sup> is the predominant glycoform; a similar molecular "signature" has been demonstrated in BSE. In this study, transmission of vCJD (containing human PrP<sup>Sc</sup>, type 4) to Tg35 and Tg45 mice resulted in "faithful" propagation of human PrP<sup>Sc</sup> closely resembling that of type 4 PrP<sup>Sc</sup> in human brain. However, the most surprising aspect of the study demonstrated that some of the Tg35 mice inoculated with BSE also showed a molecular phenotype indistinguishable from that of a sub type of sporadic CJD ("type 2"). This contrasted with Tg45 mice, inoculated with BSE, all of which showed a phenotype indistinguishable from type 4.
8. The authors conclude that these findings further strengthen the evidence that vCJD is caused by a BSE-like prion strain. They also

suggest that the finding that transgenic mice, inoculated with BSE, can produce a phenotype similar to that of sporadic CJD, could raise the possibility that some humans infected with BSE prions may develop a clinical disease indistinguishable from classical CJD associated with type 2 PrP<sup>Sc</sup>.

A more detailed summary of the experimental findings is outlined in the following sections.

**Section 1** Summary of results of transgenic mice (129MM TG35) inoculated with sporadic CJD, variant CJD or BSE.

**Section 2** Summary of results of transgenic mice (129MM TG45) inoculated with sporadic CJD, variant CJD or BSE.

**Section 3** Summary of results of 4 inbred mouse strain inoculated with vCJD and BSE.

**Section 1** *Experiments in transgenic mice expressing human PrP (129MM Tg35) inoculated with sporadic CJD, variant CJD or BSE*

9. The researchers challenged transgenic mice homozygous for a human PrP M129 transgene array and murine PrP null (**129MM Tg35**), with inocula of sporadic CJD, variant CJD and BSE. The transgenic mice were derived from an outbred strain. The level of expression of human PrP in the brain of this transgenic line is reported as twice that detected in a sample of (pooled) normal human brain. Members may wish to note that a range of different inocula were used in these experiments (see Table 1, page 6359 of Asante *et al.* 2002<sup>1</sup>) All but one of the inocula used in the transmission experiments were prepared from individual patients or animals (except 1038 which was a BSE brain pooled homogenate). The BSE pooled homogenate gave a titre of 10<sup>3.3</sup> i.c.LD<sub>50</sub> units when titrated in RIII mice. A monoclonal antibody raised against recombinant human PrP was used for immunohistochemistry and a biotinylated form of this antibody was used for Western blotting.

---

<sup>1</sup> Asante, E.A., Linehan, J.M., Desbruslais, M., Joiner, S., Gowland I., Wood, A.L., Welch, J., Hill, A.F., Lloyd, S.E., Wadsworth, J.D.F. and Collinge, J. (2002). BSE prions propagate as either variant CJD-like or sporadic CJD-like prion strains in transgenic mice expressing human prion protein. *The EMBO Journal* **21** (23) 6358-6366.

## Results

10. The results are presented in Table 1 page 6359 of Asante *et al.* and summarised in Figure 1 below.

**Figure 1** Results of vCJD, BSE and sCJD inoculation in transgenic mice expressing human PrP (129MM Tg35 transgenic mice)

Donor \ Recipient	disease and PrP genotype of the inoculum				
	sCJD MM	sCJD MV	sCJD VV	vCJD MM	BSE
<b>Susceptibility to clinical disease</b>	26/26	10/11	4/13	1/14	6/49
<b>Incubation time range (days)</b>	220 - 240	240 - 450	350 – 700	700	300 – 500
<b>Change on second passage</b>	no	NR <sup>1</sup>	NR	NR	NR
<b>Subclinical<sup>2</sup></b>	0	1	3/13	13/14	8/49
<b>Florid plaques</b>	NR	NR	NR	+ <sup>3</sup>	+/-

<sup>1</sup> NR - not reported

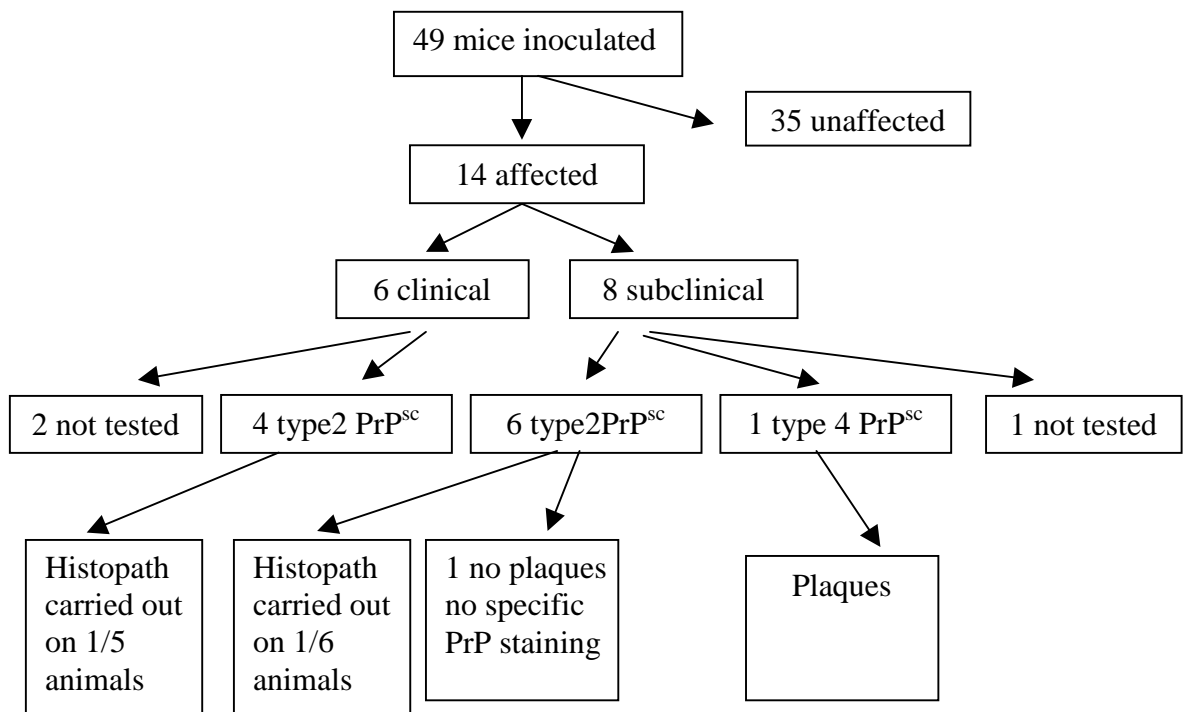
<sup>2</sup> defined as not showing clinical disease but showing pathological and/or biochemical evidence of disease by histology, immunohistochemistry and/or Western blotting

<sup>3</sup> characteristic of vCJD in humans but rarely seen in mice

11. In the mice inoculated with vCJD, the type of PrP<sup>Sc</sup> was non-distinguishable from that seen in the brains of humans with vCJD (with respect to proteinase K fragment size and the ratio of the different glycoforms). The PrP<sup>Sc</sup> type seen, as judged by PrP<sup>Sc</sup> fragment sizes was the same type 4 pattern characteristic of vCJD prions in human brain. The glycoform ratio also closely resembled that of type 4 PrP<sup>Sc</sup> in human brain.

12. The results with the mice inoculated with BSE were, however, unexpected (Table 1 page 6359 Asante *et al.* and summarised in Figure 2 here). Of the mouse brains tested by western blotting (11 in total), 10 showed a molecular phenotype indistinguishable from that of a sub type of sporadic CJD (type 2), while 1 was classified as type 4. However, it is not possible, from the details provided in the paper, to determine whether particular forms of the disease were associated with particular inocula

**Figure 2** Analysis of PrP<sup>sc</sup> type in 129MM Tg35 inoculated with BSE



**Section 2** Results of vCJD, BSE and sCJD inoculation in transgenic mice expressing human PrP (129MM Tg45 transgenic mice)

13. The researchers challenged mice from a second transgenic line (129MM Tg45), which is homozygous for a human PrP M129 transgene array and murine PrP null, with inocula of sporadic CJD, variant CJD or BSE. The brain expression of human PrP in this transgenic line was four times that of pooled normal human brain. The individual inocula used in each of the experiments are not specified in the paper, however a sentence in the discussion

indicates that the same BSE inocula was used in experiments in both the Tg45 and Tg35 mice. The results of the transmission experiments are shown in Figure 3..

Figure 3 Results of vCJD, BSE and sCJD inoculation in transgenic mice expressing human PrP (129MM Tg45 transgenic mice)

Donor \ Recipient	Disease and PrP genotype of the inoculum		
	sCJD not given	vCJD MM	BSE
Susceptibility to clinical Disease	7/7	1/4	0/12
Incubation time (days)	150	580	>700
Change on second passage	No	NR <sup>1</sup>	NR
Subclinical	0	3/4	9/12
Florid plaques	NR	+ <sup>2</sup>	+

<sup>1</sup> NR - not reported

<sup>2</sup> characteristic of vCJD in humans but rarely seen in mice

In all the Tg45 mice affected following vCJD or BSE inoculation, the PrP<sup>Sc</sup> type found was type 4, as expected.

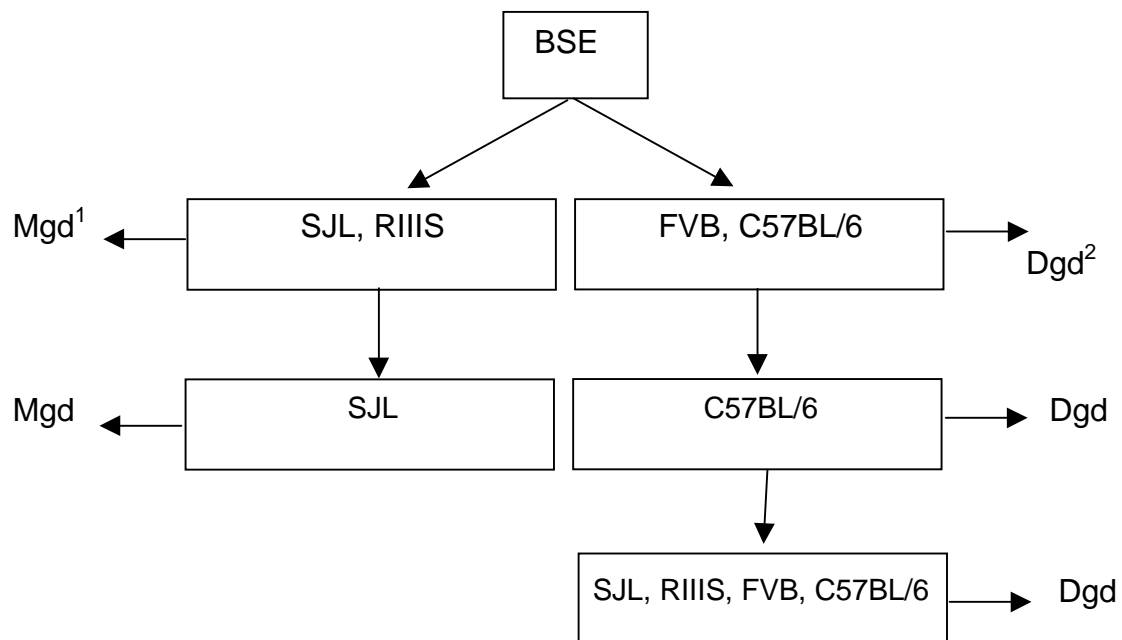
**Section 3** Results of vCJD and BSE inoculation to inbred lines of non-transgenic mice

- The researchers also studied the transmission of BSE and vCJD in four inbred mouse lines (SLJ, RIIS, FVB, C57BL/6). These four inbred lines all have the same *Prnp* coding sequence (*Prnp-a*) and are homozygous for methionine at codon 128, the corresponding murine codon to PRNP codon 129. In the SJL and RIIS lines, BSE transmission is associated with the production of a distinctive PrP<sup>Sc</sup> type, with PrP<sup>Sc</sup> glycoform ratios closely similar

to that of human sporadic CJD and referred to as a monoglycosylated dominant' PrP<sup>Sc</sup> pattern. These lines are also associated with unusually short incubation periods for BSE (Table III page 6363 Ashante *et al.*) Both FVB and C57BL/6 mice when inoculated with BSE develop a BSE-like pattern with a characteristic PrP<sup>Sc</sup> fragment size and glycoform ratio, referred to as diglycosylated dominant'.

15. A BSE inoculum (1783) from a single bovine brain was used in this experiment. This inoculum was different from that used for the experiments in transgenic mice.
16. Following inoculation with either vCJD or BSE prions, both FVB and C57BL/6 mice showed the expected diglycosylated dominant PrP<sup>Sc</sup> pattern in the brain (Figures 2G) and a prolonged and variable incubation period (Table III page 6363). However, in SJL and RIIS mice, the monoglycosylated form was seen following inoculation with either BSE or vCJD. Although stable on further passage in the same mouse line, the glycosylation pattern in SJL and RIIS mice was the diglycosylated dominant form when the BSE had been passaged twice in C57/BL/6 mice. The results for BSE are shown schematically in Figure 4.

**Figure 4** Inbred lines - Summary of PrP<sup>Sc</sup> glycotype following BSE inoculation



<sup>1</sup> Mgd - Monoglycosylated dominant pattern; <sup>2</sup> Dgd - Diglycosylated dominant pattern

17. The authors state that the neuropathology observed in SJL and RIIIS mice inoculated with either BSE or vCJD showed only diffuse staining for PrP without florid or other PrP immunoreactive plaques. These data are not shown in the paper.

### Key Conclusions

18. i) The species barrier is absent for transmission of sporadic CJD from patients with a codon 129 MM genotype to transgenic mice expressing a human 129 MM form of PrP.
- ii) In this model, the species barrier for transmission from human or cattle to mice was lower if pathological changes, rather than clinical disease are the criteria used to indicate transmission.
- iii) Some mice when inoculated with either BSE or vCJD prions, developed the neuropathological and molecular phenotype of vCJD, consistent with these diseases being caused by the same prion strain.

- iv) Inoculation of BSE prions can induce two distinct phenotypes in one of the transgenic lines examined (129MM Tg 45). 1/14 affected animals showed a molecular phenotype that was indistinguishable from that of vCJD (type 4 PrP<sup>Sc</sup>). 10/14 affected animals showed a molecular phenotype indistinguishable from that of sporadic CJD (type 2 PrP<sup>Sc</sup>). The three remaining affected animals were not tested.
- v) The sCJD like phenotype (type 2 PrP<sup>Sc</sup>) was not detected in the second transgenic line (129MM Tg45) examined after BSE-inoculation and 9/9 affected animals had the vCJD like phenotype (4 PrP<sup>Sc</sup>).

*The authors suggest*

- 19. i) The current definitions of the species barrier (quantified either by comparative titration in the two respective hosts or by a fall in the incubation period between primary and secondary passage) should be reassessed because both depend on the onset of clinical symptoms.
- ii) The findings strengthen the evidence that vCJD is caused by a BSE-like agent, and suggest that more than one BSE derived prion stain may infect humans.
- iii) Some humans infected with BSE may develop a clinical disease that is indistinguishable from sporadic CJD associated with type 2 PrP<sup>Sc</sup>.
- iv) Some of the increase in sporadic CJD in the UK may be related to exposure to BSE.
- v) Other species exposed to BSE may develop prion disease that may not be recognised (as been caused by BSE) by current strain typing methods

### **Advice Sought from the Committee**

- 20. Members are asked to advise on the scientific significance and implications of this research in particular on, .
  - i) The experimental design (in particular on the influence of the transgenic lines and different inocula on the interpretation of the findings)

- ii) The conclusions and speculative interpretation of these data (summarised in the covering paper)
- iii) What are Members views on the implications of this work for differential diagnosis in human and animal TSE's ?
- iv) Does this research indicate a need to include molecular PrP sub-typing in epidemiological studies of CJD?

*List of material attached*

- The scientific paper Asante *et al.*, (2002) *EMBO* **21** (23) 6358-6366
- Comments on the Asante paper 'Mouse model sheds new light on human prion disease' (Published on the MRC Website)

**References:**

Collinge *et al.* (1995) Unaltered susceptibility to BSE in transgenic mice expressing human prion protein. *Nature* 378, 779-83.

Hill *et al.*, (1997) The same prion strain causes vCJD and BSE. *Nature* 389, 448-50.

Lloyd *et al* (2001) Identification of multiple quantitative trait loci linked to prion disease incubation period in mice. *Proc Natl. Acad. Sci USA* 98, 6279-83.