

Biochemical typing of scrapie strains

Differences in the molecular heterogeneity of the abnormal isoforms of the prion protein, PrP^{Sc}, due to differential glycosylation and partial endogenous or exogenous proteolysis^{1,2} have been found in different scrapie strains³, sources of Creutzfeldt–Jakob disease (CJD) and fatal familial insomnia (FFI)^{4,5}. Recently Collinge *et al.*⁶ extended this analysis to include sporadic CJD and the new variant of Creutzfeldt–Jakob disease (vCJD) and found that the vCJD ‘type 4’ pattern of PrP^{Sc} was common to mice, cats and monkeys infected with bovine spongiform encephalopathy (BSE). We have extended this analysis to include scrapie strains passaged in mice and originally derived from sheep or goats.

Collinge *et al.*⁶ suggested that the prion molecular phenotype ‘type 4’ was distinct and characteristic of BSE, and that its presence in vCJD was further evidence of a direct link between vCJD and BSE. Furthermore, they suggested that this pattern, though also seen in FFI⁷, might be used to identify a BSE origin of the disease — in particular that it would differentiate BSE and natural scrapie in sheep and goats. Unfortunately, no control data of scrapie in mice or the other species were given to support this idea.

We present a glycoform ratio analysis of mouse-passaged scrapie strains from sheep or goats and compare them with a BSE-

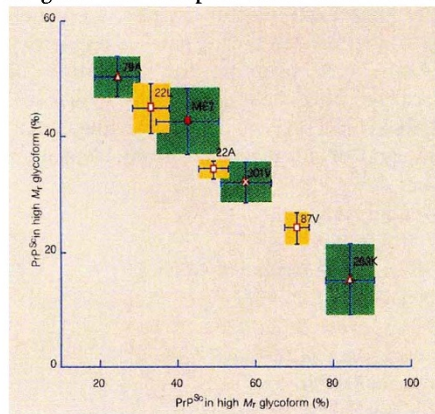


Figure 1 Glycoform profiles were obtained from PrP^{Sc} extracted from whole brains of rodents infected with scrapie or BSE passaged in mice, using an antipeptide rabbit antibody (R30, PrP 90–104)¹⁵ donated by B. Caughey. The models used included 79A and ME7 scrapie strains in the SV mouse strain (*Sinc s7*), 22L in C3H mice (*Sinc s7*), 22A, 87V and 301V in VM mice (*Sinc p7*) and 263K in Syrian hamsters. The glycoform ratio of a particular strain of scrapie is independent of *Sinc* (*Prn-i*) (results not shown). The scattergram shows the percentage of PrP^{Sc} in the high-molecular-mass glycoform plotted against the percentage in the low-molecular-mass glycoform. Error bars, s.d.; *n* = 3–9.

derived strain, 301V. We confirm the original observations of Kasczak and colleagues³ that PrP^{Sc} pattern analysis can be used to differentiate strains of these agents when isolated in inbred mice. However, we have identified strains originally isolated from the same source of scrapie which have radically different patterns of PrP^{Sc}, suggesting that this may compromise its use as a tool to identify the origins of disease.

Over 15 transmissible spongiform encephalopathy strains have been experimentally derived from scrapie-infected sheep and other ruminants infected with a transmissible spongiform encephalopathy^{8,9}. They are characterized biologically by their relative incubation periods in mice of the three *Sinc* (*Prn-i*) genotypes and the distribution of vacuolation in the brain. We analysed five of these strains and a strain passaged in hamsters according to the methods of Collinge *et al.*⁶. The set was compared with 301V, derived from a BSE-infected cow by serial passage in *Sinc p7* mice¹⁰.

Each strain showed a consistent but restricted glycoform profile (Fig. 1). Individual strain glycoform profiles ranged from highly aglycosyl forms (79A) to very heavily glycosylated forms (87V and 263K). The 301V profile is similar to the ‘type 4’ pattern published for other BSE-derived sources, but is also similar to the sheep-derived 22A strain. The ME7 and 87V strains were derived from the same original source of natural scrapie¹¹ and yet they show very different glycoform profiles. Similarly 79A (mouse) and 263K (hamster) derive from the same source¹² but show extreme differences in glycoform profile, albeit in different species.

Our results show that, under controlled experimental conditions, the glycoform ratio can be used to differentiate between strains of transmissible spongiform encephalopathies, but, like other strain properties, it may change at the primary or subsequent passage and not necessarily reflect the characteristics of its progenitor strain^{13,14}. Much more information is needed about the contributions of source of agent and host PrP genotype to the glycoform ratio before it can be used as a valid predictor of the origin of a particular transmissible spongiform encephalopathy.

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Collinge et al. reply — We recently reported that new variant CJD (vCJD) could be distinguished from previously recognized forms of sporadic and acquired CJD by a consistent

pattern on western blots with respect to ratios of PrP^{Sc} glycoforms after proteinase K digestion⁶. This pattern resembled that seen in BSE in cattle and BSE transmitted to mouse, domestic cat and macaque, consistent with the hypothesis that BSE causes vCJD. We did not analyse all prion strains in all species, so the type of pattern that we saw in the ratio of PrP^{Sc} glycoforms may not be unique to BSE and vCJD. We suggested that PrP^{Sc} analysis might be helpful in differentiating BSE and scrapie in sheep to encourage molecular studies in addition to conventional approaches. This approach may be complicated, however, by the multiplicity of PrP alleles in sheep, the many different strains that have been isolated from natural sheep scrapie, and the uncertainty as to whether BSE originated in sheep.

Somerville *et al.* subjected scrapie strains that had been passaged in mice to PrP^{Sc} glycoform analysis. They show that several of these ‘classical’ scrapie strains, previously well characterized by conventional transmission studies and lesion profiling in inbred mice, can also be differentiated by this technique, providing further support for the use of molecular analysis of prion strains.

It is unsurprising that not all of these strains can be completely differentiated from BSE. Given the spread of the clusters on our glycoform scattergrams, it would have been remarkable if none of these scrapie strains showed any overlap with BSE or CJD. This type of analysis remains relatively crude; higher-resolution molecular techniques are needed. But despite the limitations and noise inherent in the current methods, several classical scrapie strains can now be differentiated from BSE in mice, in days rather than years.

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