propagation of pathological isoforms of wild type PrP may also make a significant contribution to phenotypic variability in inherited prion disease [23,115–117]. These collective data indicate that PrP^{Sc} isoforms may be generated in inherited prion disease with unique physicochemical properties, reflected by sensitivity to proteinase K digestion or PrP^{Sc}/prion infectivity ratios that are very different from the PrP^{Sc} types propagated in sporadic and acquired forms of human prion disease. For example in P102L inherited prion disease, it is now apparent that three isoforms of protease resistant PrP with divergent physicochemical properties can be propagated. Two distinct abnormal conformers derived from PrP 102L generate protease-resistant fragments of either ca. 21–30 kDa or 8 kDa [23,108–110,118], while abnormal conformers of wild type PrP appear to generate proteolytic fragments of only ca. 21–30 kDa [23]. Glycoform ratios of ca. 21–30 kDa proteolytic fragments generated from PrP 102L and wild type PrP are not only distinct from each other, but are also distinct from those generated from wild type PrP in sporadic or acquired CJD [23]. Differences in neuropathological targeting of these distinct disease-related PrP species, together with differences in their abundance and potential neurotoxicity, provides a molecular mechanism for generation of multiple phenotypes in P102L inherited prion disease. Clearly, new experimental methods may be required in order to fully document the spectrum of abnormal PrP isoforms seen in inherited prion disease. In this context it is important to note that not all inherited prion disease may invoke disease through the same mechanism. For example the *PRNP* A117V mutation gives rise to transmembrane forms of PrP (PrP^{ctm}) which it is proposed may invoke a neurological disease without generation of PrP^{Sc} [58]. It is also not presently known whether all inherited prion diseases are transmissible by inoculation.

Efforts to produce an unified international classification and nomenclature of human PrP^{Sc} types has been complicated by the fact that the N-terminal conformation of some PrP^{Sc} subtypes seen in sporadic CJD can be altered *in vitro* via changes in metal-ion occupancy [12,15] or solvent pH [119–121]. Additionally there is now increasing evidence for co-existence of different PrP^{Sc} types within the same brain [13,15,21,23,108–110,122–127]. While it has recently been proposed that pH alone determines the N-terminal structure of PrP^{Sc} in sporadic CJD [120,121], this interpretation has not been supported by other studies [12,17,124], and specific PrP^{Sc} conformations show critical dependence upon the presence of copper or zinc ions under conditions where pH 7.4 is strictly controlled [12]. Although agreement has yet to be reached on methodological differences, nomenclature and the biological importance of relatively subtle biochemical differences in PrP^{Sc}, there is strong agreement between laboratories that phenotypic diversity in human prion disease relates in significant part to the propagation of disease-related PrP isoforms with distinct physicochemical properties [9–18,124,127,128]. Importantly, it is now also becoming apparent that protease-sensitive pathological isoforms of PrP may have a significant role in both animal and human prion disease [18,104,129,130] and therefore development of new diagnostic tests that do not rely on protease digestion are required. In this context, the conformation-dependent immunoassay [104] has recently been reported to have high diagnostic sensitivity in human prion disease [18].

The hypothesis that alternative conformations or assembly states of PrP provide the molecular substrate for clinicopathological heterogeneity seen in human prion diseases (and that this relates to the existence of distinct human prion strains) has been strongly supported by transmission experiments to conventional and transgenic mice. Transgenic mice expressing only human PrP with either valine (V) or methionine (M) at residue 129 have shown that this polymorphism constrains both the propagation of distinct human PrP^{Sc} conformers and the occurrence of associated patterns of neuropathology [10,77,79,131,132]. Biophysical measurements suggest that this powerful effect of residue 129 on prion strain selection is likely to be mediated via its effect on the conformation of PrP^{Sc} or its precursors or on the kinetics of their formation, as it has no measurable effect on the folding, dynamics or stability of PrP^{C} [133]. These data are consistent with a conformational selection model of prion transmission barriers [2,80,131,134] (a model also recently supported by work with yeast prions [135]) and strongly support the "protein only" hypothesis of infectivity by suggesting that prion strain variation is encoded by a combination of PrP conformation and glycosylation [2]. These findings also provide a molecular basis for *PRNP* codon 129 as a major locus influencing both prion disease susceptibility and phenotype in humans.

Notably, our transgenic modeling studies indicate that there is no common preferred PrP^{Sc} conformation for V129 and M129 human PrP that can be generated as a result of exposure to the vCJD/BSE prion strain [131]. Depending on the origin of the inoculum and the PrP codon 129 genotype of the host, primary or secondary transmission of BSE-derived prions can result in four distinct prion disease phenotypes. Transgenic mice homozygous for human PrP M129 propagate either type 2 or 4 PrP^{Sc} with respective neuropathologies consistent with human sporadic CJD or vCJD [79,131], whereas transgenic mice homozygous for human PrP V129 either propagate type 5 PrP^{Sc} and a distinct pattern of neuropathology, or develop clinical prion disease in the absence of detectable PrP^{Sc} [77,131]. Depending on the source of the inoculum, multiple disease phenotypes are also seen in human PrP codon 129MV heterozygous transgenic mice [132]. However in the PrP codon 129MV genotype, none of these phenotypes resemble the neuropathological phenotype of vCJD as the propagation of type 4 PrP^{Sc} is dissociated from the occurrence of abundant florid PrP plaques [132]. While caution must be exercised in extrapolating from animal models, even where faithful recapitulation of molecular and pathological phenotypes is possible [79,131] (Fig. 2), our data, together with closely similar findings of Manson and colleagues [136], argue that primary human BSE prion infection, and secondary infection with vCJD prions by iatrogenic routes, may not be restricted to a single disease phenotype. Stratification of all human prion disease cases by PrP^{Sc} type will enable rapid recognition of any change in relative frequencies of particular PrP^{Sc} sub-types in

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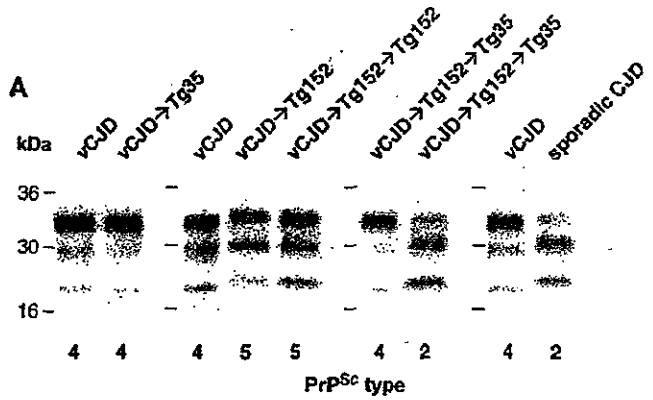

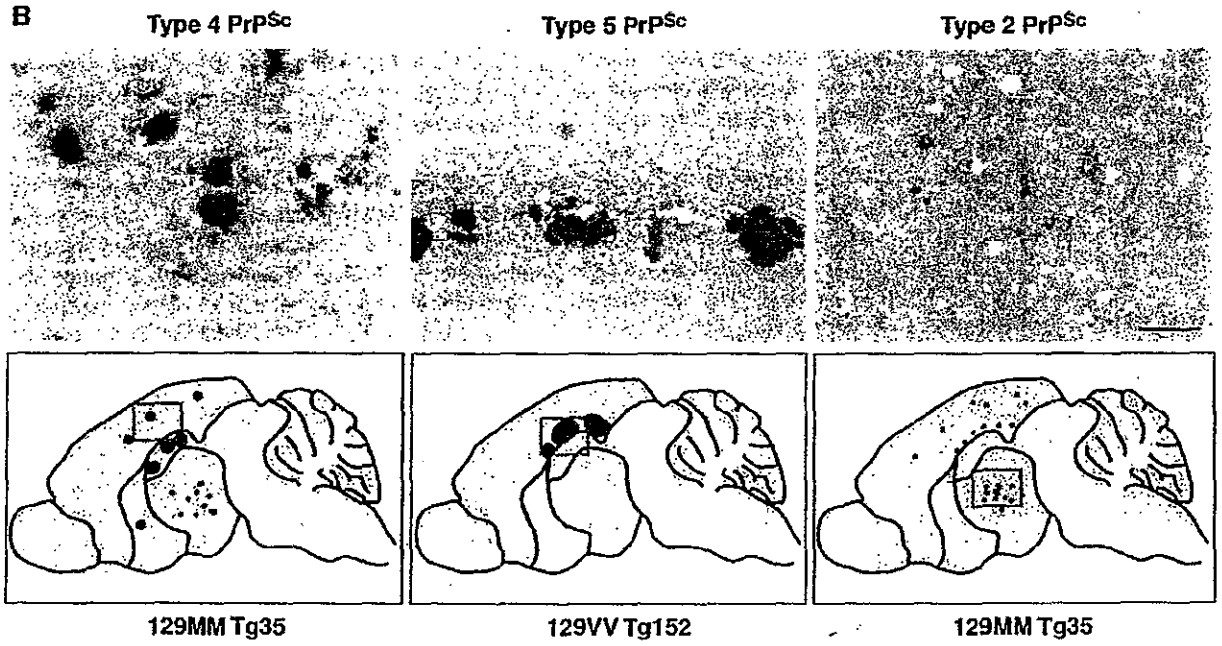

Fig. 2. Recapitulation of vCJD molecular and neuropathological phenotype in transgenic mice. Primary transmission of vCJD prions to transgenic Tg (HuPrP129M$^{+/+}$*Prnp*$^{-/-}$)-35 mice (Tg35) results in faithful propagation of type 4 PrP^{Sc} and the occurrence of abundant florid PrP plaques that are the neuropathological hallmark of vCJD. In contrast, primary transmission of vCJD prions to transgenic Tg(HuPrP129V$^{+/+}$*Prnp*$^{-/-}$)-152 mice (Tg152) produces type 5 PrP^{Sc} and a distinct pattern of neuropathology that is maintained after secondary passage in Tg152 mice but after passage in Tg35 mice induces propagation of either type 4 or type 2 PrP^{Sc} and respective neuropathologies consistent with vCJD or sporadic CJD. (A) Representative immuno-blots of proteinase-K treated brain homogenates from variant and sporadic CJD (*PRNP* 129 MM genotype) and transgenic mice analysed with anti-PrP monoclonal antibody 3F4. The identity of the brain sample is designated above each lane with the type of PrP^{Sc} present in the sample designated below. (B) Representative immunohistochemical analysis of transgenic mouse brain showing abnormal PrP immunoreactivity, including PrP-positive plaques, stained with anti-PrP monoclonal antibody 3F4. Scale bar: 100 μm. Lower panels show the regional distribution of abnormal PrP deposition. Green boxes in the sketches denote the area from which PrP stained sections are derived.

relation to either BSE exposure patterns or iatrogenic sources of vCJD prions [2,79,131,132].

7. Peripheral pathogenesis in human prion disease

Concomitant with studies on PrP^{Sc} from brain has been investigation of PrP^{Sc} in peripheral tissues in human prion diseases. These studies have established that the pathogenesis of vCJD differs significantly from that of other forms of CJD. PrP^{Sc} is readily detectable in lymphoreticular tissues in vCJD and not in classical CJD or inherited prion disease [84,108,137–142]. The fact that tonsillar prion infection has not been detected in iatrogenic CJD associated with use of human cadaveric derived pituitary hormone [138] or kuru (our unpublished data) argues that the distinctive pathogenesis of vCJD relates to the effect of prion strain rather than to a peripheral route of infection.

Depending upon the density of lymphoid follicles, PrP^{Sc} concentrations in vCJD peripheral tissues can vary enormously, with levels relative to brain as high as 10% in tonsil [84,138] or as low as 0.002% in rectum [84]. A distinctive PrP^{Sc} type, designated type 4t, is seen in both ante-mortem and post-

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mortem tonsil from patients with vCJD [84,138] (Fig. 1) including secondary vCJD infection resulting from blood transfusion [87]. Type 4t PrP^{Sc} in tonsil differs in the proportions of the PrP glycoforms from type 4 PrP^{Sc} seen in vCJD brain [84,138] implying the superimposition of tissue and strain specific effects on PrP glycosylation [80,138]. As infection of lymphoreticular tissues is thought to precede neuroinvasion, and indeed has been detected in archived surgical samples removed prior to development of vCJD [81,143], tonsil biopsy is likely to allow firm diagnosis at the early clinical stage or indeed pre-clinically [4]. Early diagnosis obviates the need for further investigation, which may include brain biopsy, necessary to exclude alternative potentially treatable conditions and will be increasingly important with the advent of putative treatments and clinical trials where the aim must be to intervene early before extensive CNS damage has occurred [4]. To date tonsil biopsy has shown has shown 100% sensitivity and specificity for diagnosis of clinical vCJD [84,94,137,138] (and our unpublished data).

The distinctive peripheral pathogenesis of vCJD has provided the basis for prevalence screening of the general population for vCJD infection. Two anonymous screens of lymphoreticular tissues, removed during routine surgery, have been performed [81,82]. Tonsil appears a more sensitive reporter of vCJD prion infection than appendix [144] and it is of concern that three positives appendix specimens were reported from a retrospective screen of around 12 000 largely appendix samples [81]. A pilot scale prospective study of 2000 tonsils found no positives, despite use of high sensitivity methods [82]. However, this negative result cannot provide reassurance that significant community infection is unlikely because of the relatively small sample size, demographic and age-related factors and unknown test sensitivity during the prolonged incubation period [82]. Accordingly, a national large scale study is now being organized by the UK Department of Health which aims to screen 100,000 tonsils for disease-associated PrP to estimate prevalence of vCJD prion infection in the UK population.

8. Secondary transmission of vCJD prions

The unknown but potentially high prevalence of clinically silent infection with BSE prions allied with the distinctive pathogenesis of vCJD has led to significant concerns that there may be substantial risks of iatrogenic transmission of vCJD prions via blood products and contaminated surgical and medical instruments [80,84,142,145].

Indeed three cases of transfusion-associated vCJD prion infection have already been reported from amongst a very small cohort of known exposed individuals [85–87]. Prions resist many conventional sterilisation procedures and surgical stainless steel-bound prions transmit disease with remarkable efficiency when implanted into mice [146,147].

Precautionary risk reduction measures have already been taken with respect to blood and blood products [85,148] however major concerns remain relating to possible iatrogenic transmission of vCJD prions via contaminated surgical instruments. Transmission of vCJD to conventional mice involves a transmission barrier that severely limits the ability to detect low titre infectivity [77–79,149]. Pending development of a transmission barrier-free model for bioassay of vCJD prions, high sensitivity immunodetection of PrP^{Sc} has been used to provide an upper limit on PrP^{Sc} levels in peripheral tissues, including blood, to inform risk assessment models [84,141,142]. High concentrations of PrP^{Sc} appear to be largely confined to the central nervous system and lymphoreticular tissues in vCJD. While a range of surgically important tissues have levels of PrP^{Sc} 10^4–10^5 fold lower than found in brain [84], the demonstration of prion infectivity in vCJD rectum despite barely detectable levels of PrP^{Sc} is consistent with PrP^{Sc}/prion infectivity ratios in vCJD being closely similar to experimental rodent prion strains [145]. These findings strongly endorse the value of PrP^{Sc} analysis in vCJD for defining risk reduction strategies to limit secondary transmission of vCJD prions and reinforce concerns that iatrogenic transmission of vCJD prions might occur through contaminated endoscopes, biopsy forceps or surgical instruments [142,145,147,150,151].

Predictions of the eventual size of a vCJD epidemic have varied widely, although some recent estimates, based on current cases of vCJD, suggest that the total epidemic may be relatively small [152]. However, key uncertainties, notably with respect to major genetic effects on the incubation period [54,105], suggest the need for caution. Importantly, such models cannot estimate the number of infected individuals, which remains unknown. The mean incubation period for kuru has been estimated to be around 12 years [75] with a similar estimate in iatrogenic CJD associated with the use of human-cadaver-derived pituitary growth hormone [70]. However, the maximum incubation periods in kuru can exceed 50 years [75]. The transmission barrier of BSE between cattle and human beings is unknown and cannot be directly measured. However, the cattle-to-mouse barrier for BSE has been well characterised experimentally by comparative endpoint titration. BSE prions transmit readily to laboratory mice, including after oral dosing [153]. The murine LD_{50} (lethal dose causing 50% mortality) in C57Bl/6 mice is about 500-fold higher than that in cattle [154]; this barrier also results in a three-fold to four-fold increase in mean incubation period. Mean incubation periods of human BSE infection of 30 years or more should therefore be regarded as possible, if not probable, with the longest incubation periods approaching (and perhaps exceeding) the typical human lifespan [75]. The mean incubation periods of secondary vCJD (involving human-to-human transmission) would however be expected to have considerably shorter mean incubation periods than in primary vCJD resulting from exposure to BSE prions. The incubation periods seen in the first clinical cases of secondary vCJD are 6 and 6.5 years [85,87] suggesting the shortest incubation periods of primary vCJD will be much longer, particularly as the oral route of transmission is also typically associated with longer incubation periods than parenteral routes. A preliminary estimate of the shortest incubation periods of primary vCJD, based on the youngest cases seen, is around 12 years which would be consistent with such extrapolation from the shortest incubation periods in secondary vCJD [75].

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It remains unclear if individuals with the other *PRNP* codon 129 genotypes, VV and MV, will also succumb to clinical disease with longer mean incubation periods after primary infection with BSE prions or secondary transmission of vCJD prions. The *PRNP* 129MV blood transfusion recipient had evidence of prion infection of lymphoreticular tissues at autopsy but died from a ruptured abdominal aortic aneurysm with no reported evidence of a neurological disorder; the brain showed no pathological features of vCJD [86]. It is unclear if this individual would have gone on to develop clinical prion disease had they lived longer and if so would have had the phenotype of vCJD. Subclinical or carrier states of prion infection are recognised in animal models [134,155], including BSE or vCJD prion-inoculated transgenic mice expressing human prion proteins [79,131,132,136]. Modelling susceptibility in such "humanised" transgenic mice suggests such genotypes will be susceptible but may develop disease phenotypes distinct from vCJD because of selection and propagation of different prion strain types [131,132]. The absence of detectable brain PrP^{Sc} in the 129 MV blood transfusion patient precluded molecular strain typing of prions. Although splenic PrP^{Sc} from this case resembled that seen in vCJD, this required use of phosphotungstic acid precipitation of PrP^{Sc} which interferes with molecular strain typing [84]. In addition, type 5 PrP^{Sc}, seen in vCJD-inoculated transgenic mice expressing human PrP 129V, but not yet documented in humans, has a similar glycoform profile [77,131,132]. It is possible that this patient was propagating a prion strain distinct from that causing vCJD. Importantly, Peden et al. noted that they were unable to detect PrP^{Sc} in lymphoid follicles in tonsil, appendix or large intestine from this patient, although abnormal PrP was detected in spleen and in a cervical lymph node. As tonsillar involvement has to date been invariably seen in clinically affected cases of vCJD, Peden et al. speculated that this case may represent a distinctive pathogenesis related to route of exposure (intravenous rather than the oral route of exposure to BSE prions presumed in primary vCJD). It is also possible that the absence of a species barrier in secondary (human to human) infection may affect phenotype. However the detection of type 4t PrP^{Sc} in the tonsil of the most recent codon 129MM transfusion case [87] argues against these interpretations, and suggests that absence of tonsillar PrP^{Sc} in the MV blood recipient may be due to the effect of *PRNP* genotype and perhaps selection of a distinctive prion strain. These findings highlight the importance of the need for continued surveillance and investigation of all forms of human prion disease within the UK and other populations with extensive dietary exposure to BSE prions.

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