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Review

The role of host PrP in Transmissible Spongiform Encephalopathies

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Abstract

PrP has a central role in the Transmissible Spongiform Encephalopathies (TSEs), and mutations and polymorphisms in host PrP can profoundly alter the host's susceptibility to a TSE agent. However, precisely how host PrP influences the outcome of disease has not been established. To investigate this we have produced by gene targeting a series of inbred lines of transgenic mice expressing different PrP genes. This allows us to study directly the influence of the host PrP gene in TSEs. We have examined the role of glycosylation, point mutations, polymorphisms and PrP from different species on host susceptibility and the disease process both within the murine species and across species barriers.
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Keywords: PrP; Prions; Gene targeting; Transgenic mice; Point mutations; Infection

1. Introduction

The host-encoded protein PrP^C has been shown to be essential for development of a TSE since PrP knock-out mice are resistant to TSE infection [1,2]. PrP is a glycoprotein containing two N-glycan attachment sequences (N–X–T) at amino acids 180 and 196 in mice. These sites are variably glycosylated *in vivo* such that un-, mono- and di-glycosylated glycotypes are observed [3,4]. Both N-glycosylation sites are conserved in the PrP gene (*Prnp*) from all species suggesting that N-glycans may play an important role in the protein function [5]. A central event in TSEs appears to be a conformational modification of the normal cellular prion protein (PrP^C) from a soluble form with a predominant alpha-helical conformation to the disease associated form (PrP^{Sc}) which is rich in beta sheets and partially resistant to proteinase-K (PK) digestion. Moreover PrP^{Sc} has been proposed to be both the neurotoxic and infectious particle in these diseases, however the precise form of these particles is still under debate [6].

The host PrP is the most important factor determining the susceptibility of the host to an infectious TSE agent. However the mechanism by which susceptibility is determined has not yet been defined. Mutations in the human PrP gene (*PRNP*) are thought to lead directly to disease without the requirement for an exogenous infectious agent [7,8]. Polymorphisms in PrP from a number of species are thought to play a role in both the control of incubation times of disease and host susceptibility [9,10]. The sequence and structure of PrP in the host and the donor of infectivity have been hypothesized to influence the barrier to TSE infection both within and between species with identity leading to high susceptibility and short incubation times whereas differences between the proteins are predicted to lead to longer incubation times and lower susceptibility of the host to infection [11,12]. The glycosylation of host PrP has been proposed to be important in the conversion of PrP^C to PrP^{Sc} and may be also the factor determining the TSE strain characteristics and strain targeting in the CNS and in the periphery [13–15].

To clarify the role of host PrP in the disease process we have developed a number of gene targeted transgenic mouse lines expressing different PrP genes with specific alterations introduced into the endogenous murine PrP gene by gene targeting. We have infected these mice with different TSE strains to establish the influence of different forms of host PrP in host susceptibility, the species barrier, and the infectious process and disease outcome.

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2. Gene targeted transgenic models

Gene targeting allows the generation of transgenic mice that possess either one or two copies of the desired transgene in the correct location in the murine genome regulated by the correct transcriptional controls. Thus the mutated PrP is expressed in the same tissues and at the same level as that of wild type PrP. Transgenic mice generated in this way are therefore ideal models for studying not only the CNS events of disease, but also for peripheral routes of inoculation to study the disease process in the periphery.

To produce these mice embryonic stem cells derived from 129/Ola mice are electroporated with a plasmid carrying the mutated PrP. By homologous recombination the endogenous murine gene is replaced with the mutated one. These stem cells are then injected into C57BL mouse blastocysts to produce chimaeric pups which are then bred with 129/Ola mice to produce inbred heterozygous and homozygous transgenic lines carrying the mutated PrP gene. By maintaining inbred lines of gene targeted mice we have ensured that any alteration in the disease process and host susceptibility can be directly attributed to the alteration in the PrP gene [16]. The additional advantage that this approach gives over the standard production of transgenic mice is that each of the lines can be directly compared not only with wild type mice but also with each other. We have developed transgenic lines to investigate the influence of point mutations and polymorphisms in host PrP, glycosylation of PrP and the species of PrP on the host susceptibility and the TSE disease process.

3. Point mutations in PrP alter incubation time and/or susceptibility to disease

Several point mutations in *PRNP*, linked to familial forms of TSE, have been described and these mutations are thought to destabilize PrP structure making it more prone to conversion into the abnormal disease associated isoform, PrP^{Sc}, causing the development of a 'spontaneous' TSE disease in the absence of any exogenous infectious agent [7,8]. Transgenic mice were produced to model one of these mutations, P102L, which has been closely linked to the development of Gerstmann–Straässler–Scheinker (GSS) disease in humans [17]. It was demonstrated that mice over-expressing the equivalent mutation in murine PrP (P101L) by 8–16 fold developed a neurological disease between 150 and 300 days [7]. This spontaneous disease was moreover transmitted to low copy number 101L transgenic mice and hamsters, but not to wild type mice [18], thus suggesting that the P101L mutation in PrP was sufficient to lead to the development of a TSE. However, gene targeted P101L transgenic mice have shown that the presence of this disease-linked mutation alone is not sufficient for the development of a spontaneous disease since aged gene targeted mice homozygous for P101L (101LL) did not show any overt phenotype or clinical signs of TSE. Moreover the brains of these mice were analyzed for TSE pathology but no vacuolation or PrP deposition was detected, and no PrP^{Sc} was detected by immunoblotting. Additionally, homogenates of brain and spleen from 101LL mice over 600 days old have been bio-assayed for the presence of infectivity by inoculation in 101LL and 101PP mice, but no infectivity was detectable in these tissues [19] (Barron, unpublished).

However, despite the absence of a spontaneous disease in these mice we have shown that this amino acid change in host PrP can dramatically modify the host susceptibility to TSE infection [19–21]. Indeed the transgenic mice have different incubation times of disease compared to wild type animals when infected with several murine TSE strains (Table 1) and more dramatically when infected with TSE strains from different species (human, hamster and sheep). These experiments suggested that while this mutation in human PrP may not be sufficient alone to cause disease, it may alter the susceptibility of the host to disease. This study has also highlighted the differences obtained in models with physiological and non-physiological expression levels of PrP in the host. Indeed, a recent report has suggested that the spontaneous disease in the standard P101L transgenic mice was due to the level of over-expression of the P101L PrP, and that the observed transmission was instead an acceleration of the phenotype already present in the low level over-expressing transgenic mice [22].

4. PrP sequence identity between host and donor does not always shorten incubation time

It has been proposed that identity between host PrP and the PrP sequence of the donor of infectivity is important for high susceptibility of the host to infection and short incubation times of disease whereas differences in PrP sequence were proposed to lead to lower susceptibility and longer incubation times. This was demonstrated in transgenic mice over-expressing hamster PrP which were shown to be more

Table 1
Incubation times of mouse adapted scrapie strain ME7, 22A, 139A, 79A and BSE derived mouse strain 301V in mice with different genetic combinations at codons 108/189 and 101LL

Mouse line	PrP genotype	ME7 (i.t.±SEM)	301V (i.t.±SEM)	22A (i.t.±SEM)	139A (i.t.±SEM)	79A (i.t.±SEM)
LT/LT	$Prnp^{a(108L-189T)}$	155±2	240±6	493±6	147±2	139±2
FV/FV	$Prnp^{a(108F-189V)}$	295±7	125±6	227±3	240±2	382±12
LV/LV	$Prnp^{a(108L-189V)}$	261±5	141	NA	NA	NA
FT/FT	$Prnp^{a(108F-189T)}$	168±1	202±1	NA	NA	NA
LT/FV	$Prnp^{a(108L-189T)}/Prnp^{a(108F-189V)}$	223±4	202±1	NA	NA	NA
LV/FT	$Prnp^{a(108L-189V)}/Prnp^{a(108F-189T)}$	265±2	NA	NA	NA	NA
101LL	$Prnp^{a101L}$	338±8	181±1	527±28	306±7	298±3

susceptible to a hamster strain of scrapie than the wild type mice [23]. Moreover transgenic mice over-expressing bovine PrP developed disease rapidly when inoculated with BSE whereas mice over-expressing a chimaeric bovine/human PrP were resistant to BSE [24]. This has also been observed recently in transgenic mice expressing human PrP where human *PRNP* 129 heterozygotes were more susceptible to infection with vCJD than to BSE [25]. Moreover, studies performed using recombinant PrP have also suggested that sequence or structural homology may have a profound effect on TSE susceptibility [26].

Sequence or structural differences between host and donor PrP are therefore considered to be a possible cause of the species barrier effect, where long incubation times and low susceptibility are often observed when a TSE strain enters a new species, followed by a subsequent shortening of incubation time and increased susceptibility on serial transmission in the new species [23,27]. However we have demonstrated that replacement of the murine PrP gene with a bovine PrP gene by gene targeting led surprisingly to longer incubation times for BSE in the transgenic mice than in the wild type mice despite the increase in identity between the host and donor PrP in the transgenic mice. Moreover a similar increase in incubation time was also observed on inoculation of gene targeted transgenic mice expressing human PrP with vCJD when compared with wild type mice, despite again the apparent sequence compatibility [28]. However a number of sCJD strains can transmit more efficiently to the same human PrP transgenic mouse lines than to wild type mice, and in this case sequence identity leads to shorter incubation times and higher susceptibility (Bishop, unpublished data). Thus while PrP sequence of the host is an important factor in determining host susceptibility to a TSE agent it is clearly difficult to predict the transmissibility of a particular TSE strain in a new host based on the sequence of the host and donor PrP. Moreover in standard transgenic mice the expression level of the PrP gene is also likely to determine both the incubation time and susceptibility of the mice to a particular agent thus further complicating any studies of host susceptibility in over-expressing mouse lines. Our experiments have therefore shown that the sequence of PrP has a profound influence over host susceptibility and incubation time of disease. However, increasing the sequence homology between host and donor PrP can increase or decrease incubation time. The mechanism underlying PrP sequence and host susceptibility therefore still remains to be elucidated and the influence of other genes and their interaction with PrP yet to be determined [29–31].

5. Polymorphisms in host PrP are important factors controlling disease incubation time

While overall sequence identity between host and donor PrP does not always appear to be a good indicator of incubation time of disease, are specific mutations or polymorphisms more critical in determining host susceptibility and incubation time? Most human TSE strains are difficult to transmit to wild type mice. However 101LL mice show 100% susceptibility and short incubation time when inoculated with infected brain homogenate from P102L GSS patients [19]. While this may demonstrate that donor and host identity at 102/101 increase the efficiency of transmission, a very surprising effect is seen when these mice are inoculated with experimental sheep scrapie strain SSBP/1 and hamster scrapie strain 263K. These two strains, derived from hosts which lacked the corresponding leucine mutation in PrP, produced shorter incubation times in the transgenic mice (101LL) than in wild type mice (101PP) [20]. This point mutation has therefore altered the susceptibility of the host to TSE agents from three different species and the mechanism by which this is achieved is not dependent on PrP sequence compatibility between host and donor.

In order to understand the basis of host PrP sequence and disease incubation time we have studied the polymorphisms at amino acids 108 and 189 of murine PrP. Only two PrP alleles are commonly found in inbred laboratory strains of mice, and these alleles differ by two polymorphisms: the $Prnp^a$ allele encodes 108L_189T (PrP-A), while $Prnp^b$ PrP encodes 108F_189V (PrP-B). All other positions in the two proteins are identical. It was thought that these polymorphisms were responsible for the differences in incubation time in PrP-A and PrP-B mice inoculated with the same strain of agent. However, due to the different genetic backgrounds of inbred lines expressing these different alleles, it has been difficult to determine by classical genetics whether these polymorphisms do control TSE incubation time in mice [32]. We have produced a gene targeted transgenic model expressing $Prnp^a$ which has been modified by replacing leucine with phenylalanine at codon 108, and threonine with valine at codon 189 ($Prnp^{a(108F_189V)}$). By inoculating these mice with several TSE strains we have demonstrated that the codon 108 and 189 polymorphisms are the major factor controlling TSE incubation time in mice [16]. We have also produced and inoculated gene targeted models in which the codon 108 and 189 polymorphisms have been introduced separately into the endogenous murine $Prnp^a$ gene producing two unique lines of transgenic mice expressing $Prnp^{a(108L_189V)}$ and $Prnp^{a(108F_189T)}$. TSE inoculation of inbred lines of mice expressing all allelic combinations at codons 108 and 189 has revealed a complex relationship between PrP allele and incubation time. It has been established that both codons 108 and 189 control TSE incubation time (Table 1), and that each polymorphism plays a distinct role in the disease process. Comparison of ME7 incubation times in mouse lines that are heterozygous at both codons has also identified a previously unrecognized intramolecular interaction between PrP codons 108 and 189 [33].

In humans polymorphisms at codon 129 have been shown to be important in regulating the susceptibility and phenotype of TSE disease [9]. In the UK population 40% are homozygous for methionine (MM), 11% for valine (VV) and 48% are heterozygous (MV) at this position in *Prnp* [34]. The majority of cases of sCJD occur in patients homozygous for Met or Val at codon 129. Heterozygosity has been reported to lead to lengthened incubation times in iatrogenic CJD cases associated with growth hormone treatment, and also in kuru [35,36]. All instances of clinical vCJD to date have been homozygous for

Table 2
Incubation times obtained after challenge with vCJD in different lines of transgenic mice expressing human PrP

Transgenic mice (vCJD inoc)	HuMM	KiChM	Tg(HuPrP 129M) 35/Prnp$^{0/0}$	HuMV	KiChMV	129MV Tg45/ 152 (Prnp$^{0/0}$)	HuVV	KiChVV	Tg(HuPrP 129V) 152/Prnp$^{0/0}$
Reference	Bishop et al., 2006 [28]	Taguchi et al., 2003 [51]	Asante et al. 2002 [52]	Bishop et al., 2006 [28]	Asano et al., 2006 [53]	Asante et al. 2006 [25]	Bishop et al., 2006 [28]	Asano et al., 2006 [53]	Hill et al., 1997 [54]
Codon 129	Met	Met	Met	Met/Val	Met/Val	Met/Val	Val	Val	Val
Expression level	x1	x1	x2	x1	x1	x4–6	x1	x1	x2
Total Affected*	11/17	13/16	14/14	11/16	13/17	15/15	1/16	0/3	25/56
Total affected (%)	65	81	100	69	76	100	6	0	45

* Positive by clinical and/or pathological analysis.

methionine at codon 129, and it has been proposed that valine homozygosity may be protective for both BSE and vCJD transmission [37]. In order to model human susceptibility to TSE infection we have produced three lines of gene targeted transgenic mice expressing human PrP (HuMM, HuVV or HuMV). Infection of these mice with vCJD was successful in each case with a gradation of transmission efficiency from MM to MV to VV and different pathological characteristics for each genotype (Table 2). The greater transmission efficiency in HuMM mice suggested that homozygosity for methionine at codon 129 leads to earlier onset of TSE-related pathological features and clinical disease than for the other two genotypes. The differences in PrP^{Sc} deposition in the HuMM and HuMV lines suggest that the codon 129 polymorphism in humans is likely to affect the distribution of PrP^{Sc} deposition in the brain (Fig. 1). Importantly these studies suggest that all individuals may be susceptible to vCJD and that subclinical disease may be extensive particularly in 129MV and 129VV individuals. This possibility of extensive subclinical disease has also been highlighted by epidemiological data in humans. A retrospective tonsil and appendix survey identified appendices from two 129VV individuals which stained positive for PrP accumulation [38]. Additionally the recent reports of possible human to human transmission of vCJD by blood transfusion has identified a 129MV transfusion recipient who had not developed clinical disease but showed accumulation of PrP in spleen and lymph nodes at post-mortem [39]. Thus while codon 129 polymorphisms are clearly an important factor controlling disease susceptibility, pathology and incubation time in human TSEs, prediction of the outcome of disease with a particular combination of host genotype and TSE strain is not yet possible. However a series of further experiments using these unique humanized transgenic models aims to unravel the rules governing host susceptibility and risk of disease transmission in humans.

6. Glycosylation of PrP determines cellular location of PrP but no overt phenotype occurs in its absence

The prion hypothesis proposes that the TSE infectious agent is a protease-resistant form of PrP which can self replicate [6]. However the presence of strains of TSE agent with different incubation times, clinical features and neuropathology [40] has proved a challenge to this hypothesis. It has been proposed that

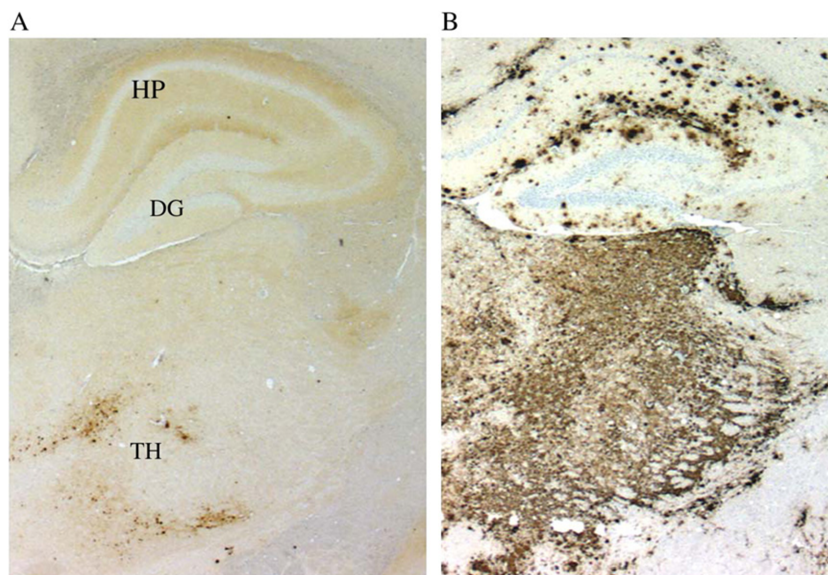

Fig. 1. Different PrP^{Sc} deposition in brains of gene targeted mice expressing human PrP with 129MV (A) or 129MM (B) after inoculation with vCJD. HP: hippocampus; DG: dentate gyrus; TH: thalamus.

Table 3
Biochemical characteristics of glycosylation deficient PrP compared with wild type PrP^C (Wt) and with PrP^{Sc} from a brain infected with the scrapie mouse adapted strain ME7

	Wt	G1	G2	G3	ME7
PK resistance	no	no	no	no	yes
PIPLC sensitivity	yes	yes	yes	yes	no
Solubility in 1 M guanidine	yes	yes	yes	yes	no
Membrane localization in neurons	yes	yes	yes	no	n.a.

each TSE strain represents a different stable conformation of abnormal PrP and glycosylation may have an important role in influencing PrP conformation and determining the strain characteristics [13,41].

Previous studies performed *in vitro* indicated that preventing endogenous PrP glycosylation can alter the structure of PrP favouring a misfolding process that leads PrP^C to acquire scrapie-like properties [42,43]. This spontaneous conversion has been also observed in cell cultures treated with tunicamycin, preventing the attachment of mature sugars at the Golgi apparatus level [44]. Additionally, accumulation of N-terminally truncated degradation products has been observed in cell cultures expressing glycosylation-deficient PrP, supporting the hypothesis that N-glycans function as protein stabilizers [45]. Thus lack of sugars on PrP may facilitate TSE onset by inducing PrP to misfold. However, recent data in transgenic mice over-expressing partially glycosylated PrP have shown that altered glycosylated PrP has some of the

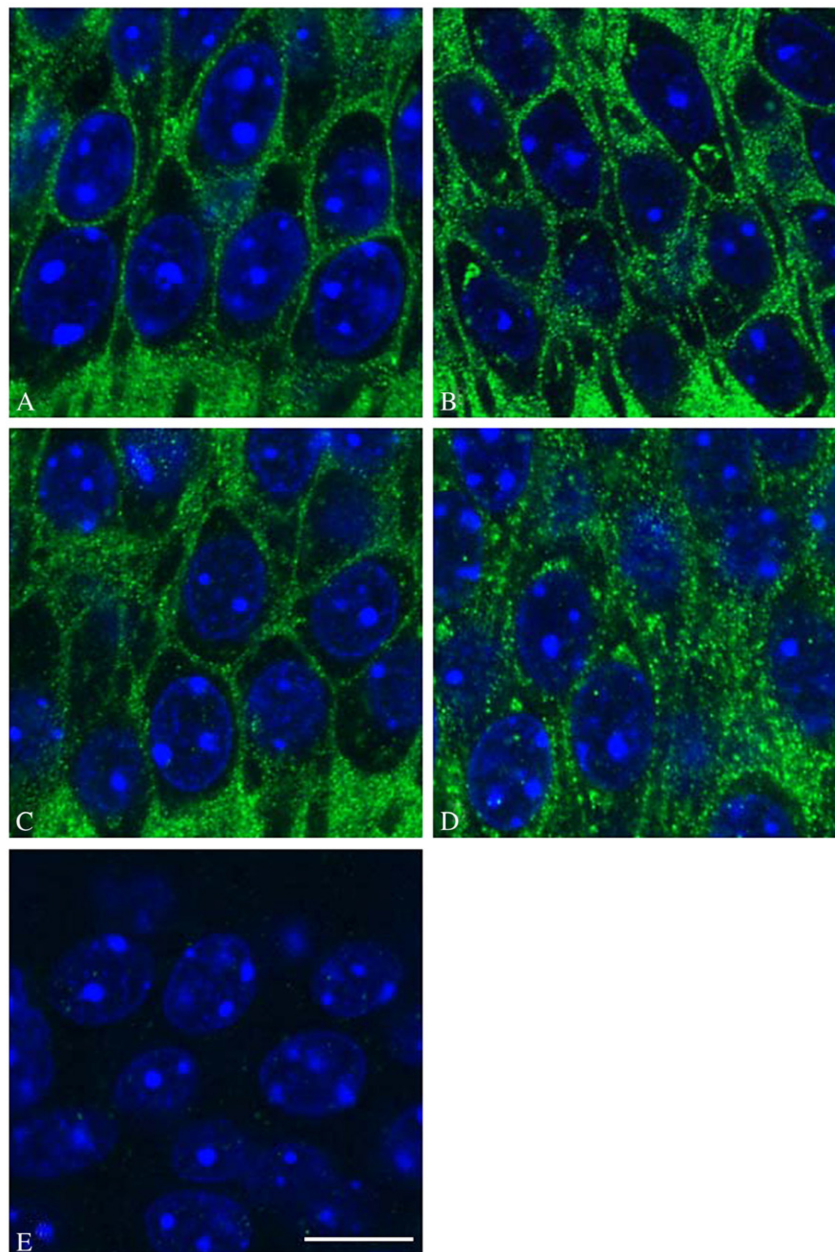

Fig. 2. Localization of Wt PrP (A); G1 PrP (B); G2 PrP (C) and G3 PrP (D) in slides derived from mouse brains. Panel E staining in slides derived from PrP knock out mice.

disease associated PrP characteristics such as detergent insolubility but these proteins maintain the PK sensitivity similar to wild type PrP [46].

In contrast, biochemical analyses carried out on brains of our gene targeted transgenic mice expressing mono-glycosylated or un-glycosylated PrP excluded the possibility of structural changes in PrP when sugars were partially present or completely absent (Table 3). These transgenic mice have been generated by substitution of threonine for asparagine^{180} (G1) or threonine for asparagine^{196} (G2) or both mutations combined (G3), which eliminate the first, second and both glycosylation sites respectively. The total amount of PrP in the G1, G2 and G3 lines was similar to that observed in wild type animals. Un-glycosylated- or mono-glycosylated PrP *in vivo* did not display any of the PrP^{Sc} characteristics such as PK partial resistance or insolubility in detergents. Moreover an ageing experiment carried out in homozygous G3 transgenic mice further supported these findings as no PK resistant PrP, glyosis or vacuolation was observed in the brains of G3 mice over 800 days old [47]. However, sugars may be important in determining the trafficking of PrP^{C} in neurons. While transgenic mice expressing mono-glycosylated PrP revealed similar PrP localization to the wild type protein, with mainly cell membrane localization and some presence in the cytoplasm, G3 transgenic mice expressing unglycosylated PrP showed mainly intracellular localization (Fig. 2).

It has been proposed that accumulation of intracellular PrP may have a toxic effect causing neurodegeneration, and transgenic mice expressing cytoplasmatic PrP lacking a GPI anchor were shown to develop severe ataxia, with cerebellar degeneration and gliosis [48,49]. However other reports have shown that accumulation of PrP in the cytoplasm is not toxic when the cytoplasmatic PrP is expressed under the control of different promoters [50]. Our mice expressing un-glycosylated PrP did not develop any type of neurodegeneration during lifespan suggesting that in this case intracellular accumulation of a GPI anchored PrP is not toxic.

7. Conclusions

Our studies using gene targeted murine models have allowed the effect of specific mutations in PrP on host susceptibility to be examined directly. These studies have clearly identified that the rules underlying host susceptibility are considerably more complex than previously proposed. The amino acid sequence of PrP has a powerful influence on host susceptibility but although overall identity between host PrP and PrP from the donor of infectivity often leads to short incubation times and high host susceptibility the converse can also be true. Specific mutations and polymorphisms in PrP clearly have a profound influence on disease incubation time, host susceptibility and pathogenesis of disease. It has been proposed that these mutations influence host susceptibility through their effect on PrP structure but a greater understanding of the structural effects of the mutations is required to establish if this is indeed the case. Our studies with the 108 and 189 polymorphisms suggest interaction between different parts of the PrP protein appear important in determining host susceptibility and that different strains of agent interact with different regions of PrP. Glycosylation of host PrP also appears from our recent studies to have an important influence on the outcome of disease (data not shown). However before we can predict the susceptibility of a host to new TSE strains we have clearly some way to go in unravelling the mechanism underlying host susceptibility. We hope, with the use of our gene targeted models and both *in vivo* and *in vitro* studies derived from these lines of mice, that we will gradually define these mechanisms and predict host susceptibility to new TSE strains.

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