Altered trafficking of abnormal prion protein in atypical scrapie: prion protein accumulation in oligodendroglial inner mesaxons

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Aims: Prion diseases exist in classical and atypical disease forms. Both forms are characterized by disease-associated accumulation of a host membrane sialoglycoprotein known as prion protein (PrP\textsuperscript{\textalpha}). In classical forms of prion diseases, PrP\textsuperscript{\textalpha} can accumulate in the extracellular space as fibrillar amyloid, intracellularly within lysosomes, but mainly on membranes in association with unique and characteristic membrane pathology. These membrane changes are found in all species and strains of classical prion diseases and consist of spiral, branched and clathrin-coated membrane invaginations on dendrites. Atypical prion diseases have been described in ruminants and man and have distinct biological, biochemical and pathological properties when compared to classical disease. The purpose of this study was to determine whether the subcellular pattern of PrP\textsuperscript{\textalpha} accumulation and membrane changes in atypical scrapie were the same as those found in classical prion diseases. Methods: Immunogold electron microscopy was used to examine brains of atypical scrapie-affected sheep and Tg338 mice. Results: Classical prion disease-associated membrane lesions were not found in atypical scrapie-affected sheep, however, white matter PrP\textsuperscript{\textalpha} accumulation was localized mainly to the inner mesaxon and paranodal cytoplasm of oligodendroglia. Similar lesions were found in myelinated axons of atypical scrapie Tg338-infected mice. However, Tg338 mice also showed the unique grey matter membrane changes seen in classical forms of disease. Conclusions: These data show that atypical scrapie infection directs a change in trafficking of abnormal PrP to axons and oligodendroglia and that the resulting pathology is an interaction between the agent strain and host genotype.

Keywords: atypical scrapie, oligodendroglial mesaxons, prion disease, protein misfolding, sheep, transgenic mice

Introduction

Prion diseases or transmissible spongiform encephalopathies are fatal neurodegenerative diseases affecting several ruminant species, mink, cats and man. Prion diseases of sheep and man have been recognized since the 18th and early 20th centuries respectively. More recently, variant forms of prion disease have been described in man, cattle and sheep [1,2]. A variant form of sheep scrapie was originally called Nor98 scrapie [1], but is now more commonly referred to as atypical scrapie and is clinically, epidemiological and biologically distinct from the classical forms of scrapie [3,4]. All naturally occurring classical prion diseases of sheep and man have been recognized since the 18th and early 20th centuries respectively. More recently, variant forms of prion disease have been described in man, cattle and sheep [1,2]. A variant form of sheep scrapie was originally called Nor98 scrapie [1], but is now more commonly referred to as atypical scrapie and is clinically, epidemiological and biologically distinct from the classical forms of scrapie [3,4]. All naturally occurring classical prion diseases of sheep and man have been recognized since the 18th and early 20th centuries respectively. More recently, variant forms of prion disease have been described in man, cattle and sheep [1,2].
PrP<sub>d</sub> is situated on the exterior leaflet of neuronal and highly unusual morphological changes of membranes. When viewed by immunogold electron microscopy, these different morphological types of PrP<sub>d</sub> resolve to three subcellular localizations: (i) small aggregated and fibrillar forms of PrP<sub>d</sub> present in the extracellular spaces where they can accumulate into amyloid fibrils and plaques, (ii) intracellular forms of PrP<sub>d</sub> found in endo-lysosomes and (iii) morphological types associated with membranes [9,10]. Only intralysosomal forms of PrP<sub>d</sub> are truncated in vivo. The membrane accumulations of PrP<sub>d</sub> are associated with highly unusual morphological changes of membranes. PrP<sub>d</sub> is situated on the exterior leaflet of neuronal and dendritic plasma membranes and is cross linked to ubiquitin on the cytoplasmic face of the membrane [9]. This membrane binding appears to be unusually resistant to degradation leading to impaired excision from the membrane during recycling endocytosis and results in bizarre clathrin-coated, spiral or branched membrane invaginations. In contrast, PrP<sub>d</sub> binding on astrocytes is associated with membrane extraversion and microfolding or ruffling. These subcellular localizations are common to each of the classical forms of prion disease irrespective of species or strain involved [10] and even occur in experimental disease generated by inoculation of synthetic forms of PrP [11]. PrP<sub>d</sub> is found in some visceral tissues where it is also associated with plasma membrane changes of follicular dendritic cells and chromaffin cells and endo-lysosomes of tingible body macrophages in sheep and in mice [12,13].

In contrast to classical sheep scrapie, the so-called atypical scrapie is a sporadic disease affecting older animals, which may occur in geographical regions where classical scrapie is absent [3]. Because most cases of atypical scrapie are diagnosed at the slaughterhouse few data are available on clinical disease. However, pruritus is a common clinical sign of classical scrapie that is absent from atypical scrapie presentation [1.3,14,15]. Atypical scrapie often occurs in sheep of PRNP genotypes considered relatively resistant to classical scrapie [3,16]. Abnormal prion protein extracted from atypical scrapie brains shows relatively milder resistance to protease digestion [3,17]. Western blots of this weakly protease-resistant prion protein show multiple PrP<sub>res</sub> bands, with a characteristic low molecular weight band of around 12 kDa. This fragment is used to distinguish between atypical and classical sheep scrapie. Atypical scrapie does not transmit readily to wild-type laboratory rodents but does so to the transgenic Tg338 mouse and to sheep [14,18]. Brain immunohistochemistry of atypical scrapie differs from classical scrapie and in particular shows a distinctive white matter PrP<sub>d</sub> deposition [14]. Lesion profiles of Tg338 mice also typically show prominent vacuolation of white matter [17,18].

Classical prion diseases are mainly characterized by abundant accumulation of PrP<sub>d</sub> in grey matter with white matter pathology being only a minor component. In classical ruminant and feline prion diseases, white matter granular PrP<sub>d</sub> accumulation is associated with astrocytes in the absence of significant degenerative changes of myelin or axons [10,19]. Granular PrP<sub>d</sub> accumulation in the absence of significant pathology is found in the white matter of each of the PRNP subtypes of sporadic Creutzfeldt-Jakob disease (sCJD), except for panencephalopathic sCJD, in which there is profound white matter degeneration [20]. This latter feature is considered to be the result of widespread loss of neurons in grey matter. Following experimental murine intracerebral challenge with prions or synthetic abnormal prion protein aggregates, plaques may often be found in white matter [21,22] and white matter plaque-like deposits may also occur in variant CJD [23], in the MM1 sCJD patients [24] and in some inherited prion disease [25]. Filamentous, punctuate and rare myelin sheath-associated inclusions in white matter have been reported in multiple inherited prion diseases, including several octapeptide repeat insertions and...
point mutations [26]. These filamentous white matter inclusions are proportionate to the abundance of cortical PrP\textsuperscript{d} load. Although there is often severe grey matter diffuse punctate labelling of the cerebral and cerebellar cortices as described above, a distinctive feature of atypical scrapie is frequent punctuate or circular PrP\textsuperscript{d} profiles in the white matter [14,17,18].

In this study, we wished to determine the subcellular localization of PrP\textsuperscript{d} in atypical scrapie-infected sheep and Tg338 mice, using immunogold electron microscopy. Primarily we wished to determine whether atypical PrP\textsuperscript{d} caused the same membrane changes that are common to all classical forms of prion diseases so far examined, and to determine the subcellular localization of PrP\textsuperscript{d} in white matter tracts. For the purposes of this report, we will define those prion diseases that have predominantly 19 or 21 kDa PrPres that is robustly resistant to protease digestion as ‘classical’, irrespective of the species in which they occur, and sheep and murine scrapie with 12 kDa PrPres of weak protease susceptibility as ‘atypical’ scrapie.

Materials and methods

Animals and experimental procedure

Two AHQ/AHQ Cheviot sheep, where A, H and Q represent the single letter amino acid codes for alanine, histidine and glutamine at codons 136, 154 and 171, respectively, of the \textit{PRNP} gene, were intracerebrally inoculated with an atypical scrapie isolate. Inoculum SE1847/BP0021 was from a naturally occurring case of atypical scrapie in a sheep of homologous genotype. Sheep were killed following the onset of clinical signs, and immediately subjected to necropsy.

One millimetre thick slices of cerebellum were taken for paraffin wax embedding and further 1 mm\textsuperscript{3} tissue blocks from cerebellum were immersed in 4% paraformaldehyde for electron microscopy. Western blots of unfixed brain tissue confirmed the presence of typical 12 kDa fragments of PrPres following mild conditions of protease digestion. For use as positive classical scrapie controls, four Tg338 mice terminally ill with the experimental sheep scrapie source CH1641 [27] were perfusion-fixed as above. Tg338 mice infected with CH1641 show unglycosylated PrPres of approximately 19 kDa under stringent digestion conditions. The 12 kDa bands are not found in these brains, using weak concentrations of proteinase K.

Sheep and mouse tissues selected for electron microscopy were further fixed in osmium tetroxide and embedded in araldite.

All inoculations were carried out under general anaesthesia, and in accordance with the United Kingdom (UK) Animal (Scientific Procedures) Act 1986. Sheep inoculations were carried out under license from the UK Government Home Office following approval by the internal Animal and Plant Health Agency ethical review process as mandated by the Home Office, while murine inoculations were reviewed and approved by the Roslin Institute Protocols and Ethics Committee.

Light microscopy procedure for paraffin-wax embedded material The immunohistochemical (IHC) procedure for light microscopy was carried out as described previously [28], using PrP antibodies 1C5, F89 or 2G11 which target amino acid sequence 119–130, 146–159 and 146–182 of the PrP molecule, respectively – [29]: Abcam, Cambridge, UK; Thermo Fisher Scientific, Northumberland, UK. These were applied overnight at 27°C, at dilutions of 1:1500, 1:10000 and 1:500, respectively, in the incubation buffer [28].
Vacuolar lesion profiling on Tg338 mice was carried out on haematoxylin and eosin-stained sections as previously described [30].

Light microscopy procedure for resin embedded material As described previously [31], the avidin-biotin complex IHC staining method was applied to the etched and pretreated sections, using the above antibodies at dilutions of 1:500, 1:750 and 1:150 respectively. Selected 1 mm$^3$ blocks corresponding to areas of sections that had shown appropriate immunolabelling, and positive and normal control blocks (corresponding areas from clinical ovine scrapie and terminal mouse 87 V scrapie and normal ovine and mouse brain) were then taken for subcellular studies.

Ultrastructural immunolabelling procedure Sixty-five nm sections were taken from resin blocks previously found to show PrP$^d$ labelling (or controls containing corresponding areas) and immunolabelled as described previously [31]. Immunolabelling using 2G11 antibody was found to be less intense in resin sections and, therefore, only 1C5 and F89 antibodies were applied to 65 nm sections for ultrastructural analysis. 1C5 was found to give the most intense immunolabelling of sheep tissue, while F89 was the preferred antibody for use on mouse tissue. 1C5 and F89 were used at dilutions of 1:15 and 1:30, respectively, in the incubation buffer, while positive control sheep and mouse tissues were labelled with 523.7 (J. Langeveld, ID–Lelystad, The Netherlands) and 1A8 [32], respectively, both at a 1:500 dilution in incubation buffer. A preimmune serum was used as a control. Multiple blocks from each available brain area were studied from each animal.

Results

Atypical scrapie in sheep cerebellum

Light microscopic lesions consistent with atypical scrapie were present throughout the whole brain, including the cerebrum and cerebellum of both sheep. Small vacuoles were present in the cerebellar molecular layer, particularly in more superficial, subpial regions. Immunocytochemistry of cerebellar sections showed fine diffuse particulate PrP$^d$ present in the molecular layer with irregular foci of particulate deposits in the granular cell layer (Figure 1a). The white matter showed larger particulate immunolabelling associated with scattered white matter fibres (Figure 1b). Abundant myelinated processes lacking axons or showing intramyelinic macrophages were also present. These PrP$^d$ accumulations are in marked contrast to those found in classical scrapie (Figure 1c,d). Electron microscopy showed numerous dilations of endoplasmic reticulum, swollen mitochondria and enlarged processes containing dispersed organelles. These features are recognized artefacts caused by delayed penetration of fixative in immersion fixed material. However, there was no evidence of the membrane changes typically found in classical scrapie. Other subcellular features often associated with sheep scrapie such as tubulovesicular bodies, increased and abnormal multivesicular bodies and autophagy [10,33,34] were not recognized. Post-mortem artefacts made critical analysis of the fine vacuolar changes seen by light microscopy unreliable.

Immunoelectron microscopy showed low levels of PrP$^d$ attached to the membranes of small neurites in the molecular layer (Figures 2a and S1) and in the granular cell layer, or associated with thin strands of microglial or astrocytic processes (Figure S1) coursing between neurites. These changes were not associated with any discernible membrane alterations. In the white matter, there was significant degeneration of myelinated processes. Changes were mainly consistent with Wallerian-type degeneration with occasional dystrophic neurites containing membrane bound, dense cored organelles. These structures were not labelled for PrP$^d$. However, PrP$^d$ immunolabelling was seen in association with occasional myelinated processes, mainly of smaller diameter axons, surrounded by hypertrophic oligodendroglial mesaxons. PrP$^d$ was largely located to irregular electron dense cytoplasm within the inner mesaxon of oligodendroglia (Figures 2b and S2).

Atypical scrapie in Tg338 mice

The light microscopic patterns of vacuolation (Figure S3) and PrP$^d$ accumulation (Figure S4) seen in atypical scrapie-infected Tg338 mice were consistent with those previously described [18,27,35]. Of particular note was the dense punctate PrP$^d$ accumulation in the dorsal thalamus and the laminar punctuate PrP$^d$ accumulation in the mid and deep cerebrocortical layers (Figure S4) with sparing of cerebellum and hindbrain [18].
The atypical scrapie-infected Tg338 mice also showed subcellular lesions of myelinated processes similar to those described above. When compared to immersion fixed sheep tissues, the perfusion fixation of murine tissues allowed greater observational detail of these lesions. Membrane and other changes typical of classical murine scrapie were also present.

Classical scrapie-associated membrane changes were present in the grey matter of the thalamus and cerebral cortex of atypical scrapie-infected Tg 338 mice, where PrP\textsuperscript{d} was most conspicuously and frequently present on membranes of astrocytes showing membrane folding and ruffling (Figure S5c). PrP\textsuperscript{d} also immunolabelled amyloid plaques (Figure 3a) or irregu-
lar arrangements of individual and sparse filaments within the extracellular space (Figure S5b). Infrequently, dendrites and neuronal perikarya showed membrane invaginations including spiral twisted membranes (Figure 3c), branched coated membranes and increased coated pits and vesicles. These all showed PrP\textsuperscript{d} labelling (Figure S5a). Rarely, coated spiral membrane invaginations were located in axon terminals. Sparse PrP\textsuperscript{d} immunolabelling was present in glial and neuronal lysosomes. As described above, these are all features common to experimental classical murine scrapie infection (Figure 3b,d) of various strains and of other naturally occurring prion diseases.

In addition to the classical scrapie-associated membrane changes described above, occasional small or medium diameter myelinated processes showed proliferation of the inner oligodendroglial mesaxon with both increased volume of processes and apparent branching associated with an increased number of separate tongues of cytoplasm (Figure 4a). Processes with abnormal inner mesaxons showed PrP\textsuperscript{d} immunolabelling mainly in association with electron dense mesaxonal cytoplasm often containing electron dense granules, small vesicles, coated pits or coated vesicles (Figure 4b-e). Occasionally, PrP\textsuperscript{d} was associated with axonal membranes (Figure 4c) and, very rarely, axonal membrane invaginations. Some oligodendroglial-associated PrP\textsuperscript{d} was specifically localized to the cytoplasmic loops at some paranodes (Figure 5a-d). PrP\textsuperscript{d} in paranodal loops was associated with paranodal cytoplasmic loop membranes (Figure 5b,c), electron dense granules (Figure 5a,d) and vesicles (Figure 5b,c), and electron dense material between loops (Figure 5d). Occasionally these PrP\textsuperscript{d} labelled paranodes showed irregular organization (Figure 5d).

As in the cerebellar white matter of the sheep, white matter tracts such as the corpus callosum showed extensive degeneration of myelinated processes. The majority of degenerate processes showed features consistent with Wallerian-type degeneration or infrequent dystrophic processes. No specific co-localization of PrP\textsuperscript{d} was seen with these lesions.

Also present, and common to all prion disease, was vacuolation with scrapie-suggestive subcellular features such as internal membranes and granular debris. These were not associated with PrP\textsuperscript{d} labelling. Degenerate terminal axons are a feature of murine

![Figure 3](image-url)  

**Figure 3.** Immunogold labelling for prion protein (PrP\textsuperscript{d}) with (a): F89 antibody in atypical scrapie-infected Tg338 mice, (b): 1A8 antibody in 87 V-infected C57bl mice. PrP\textsuperscript{d} is associated with amyloid fibrils in plaques (arrows). (c,d) UA/LC staining of (c) Tg338 mouse brains infected with atypical scrapie and (d) C57Bl mice infected with 87 V scrapie showing spiral membrane invaginations (arrowheads). Magnification bars are (a,b) 1 \( \mu \text{m} \) and (c,d) 500 nm.
scrapie [10,36,37], (but not prion disease of ruminants) and were also present. These too were unlabelled for PrP^d.

Tubulovesicular bodies are accumulations of rounded or oval 35 nm diameter membrane bound particles. They are found in all naturally occurring prion diseases and are usually readily found in experimental murine classical scrapie [33]. We were unable to detect tubulovesicular bodies in atypical scrapie-infected Tg338 mouse brain.

**Tg338 infected with CH1641 classical scrapie**

As expected, CH1641-infected Tg338 mice showed patterns of vacuolation (Figure S3) and PrP^d distribution distinct from atypical scrapie-infected Tg338 mice [27]. Electron microscopic examination of CH1641-infected mice confirmed the presence of extracellular amyloid, intra-lysosomal PrP^d (in neurons and glia) and membrane lesions typical of classical scrapie. Tubulovesicular bodies (Figure S6), evidence of autophagy, and abnormal multivesicular bodies which are also consistently found in classical prion diseases, and axonal terminal degeneration typical of murine scrapie [9,37] were also found. Thus, these CH1641-infected Tg338 mice showed an electron microscopic pathological phenotype indistinguishable from that of conventional mice with respect to classical scrapie infection.

**Discussion**

Atypical scrapie-infected sheep show light microscopic lesions [3,14] that are distinct from those seen in classical scrapie [19]. We show here that the subcellular...
lesions in the white matter of atypical scrapie-affected sheep and Tg338-infected mice are also unique with respect to previous descriptions of classical and naturally occurring TSEs [10] and of experimental prion disease generated by recombinant forms of prion protein [11]. We further show that the subcellular PrPd-associated membrane lesions of dendrites, neuronal cytoplasm (lysosomes) and axon terminals typically associated with classical sheep scrapie [9] are absent from atypical scrapie-infected sheep. Membrane changes in classical scrapie and other naturally occurring classical forms of prion disease are thought to occur because of the strong resistance of membrane PrPd to excision and endosomal recycling [10,11]. The absence of such changes in atypical scrapie-affected sheep may suggest that atypical PrPd is more easily endocytosed from membranes than in classical scrapie PrPd.

Proliferation of the oligodendroglial inner mesaxon is an unusual lesion and not one previously described in sheep scrapie or other classical forms of prion disease. Some inherited forms of human prion disease show filamentous abnormal PrP inclusions of white matter that appear to be intramyelinic or para-axonal as shown by double immunofluorescence and electron microscopy [26]. Several of the inherited disorders described by Reiniger et al. [26] showed mutations of the octapeptide repeat sequence. Tg(PG14) transgenic mice also express a nine-octapeptide insertional mutation homologous to that of a familial prion disease of humans [38] and these mice transport a weakly protease resistant form of prion protein in axons [39] which accumulates in linear segments on neurite membranes in the region of synaptic terminals [40]. Tg(PG14) mice also show abnormal proliferation of the inner oligodendroglial mesaxon and segmental demyelination although abnormal PrPd has not

Figure 5. Paranodal immunogold prion protein (PrPd) labelling of Tg338 mice infected with atypical scrapie using F89 antibody. Immunogold labelling is present in oligodendroglial cytoplasmic loops (L) of paranodes. The PrPd labelling is associated with loops of cytoplasm containing excess small diameter vesicles (a–d); some PrPd is located on loop membranes (b,c; arrowheads) and some in association with coated vesicles (b; arrows); the regular cytoplasmic loop organization in d is disturbed; ax, axon; my, myelin. Magnification bars are (a,c,d) 500 nm and (b) 1 μm.
been specifically detected at these sites [40]. Oligodendroglial mesaxonal proliferation is detected in some toxic leukoencephalopathies. Such mesaxonal proliferations are thought to be a response to the presence of intra-axonal aggregates or toxins [41]. It has been suggested that the Tg(PG14) mesaxonal proliferation occurs in response to intra-axonal PrPd. We suggest therefore that the mesaxonal proliferation and PrPd association in atypical scrapie-infected sheep and Tg338 mice, and possibly also in some inherited human prion disorders [26], may also be a response to the presence of axonal PrPd aggregates. This suggestion is supported by the occasional presence of PrP d on axonal plasma membranes and, very rarely, within axons in atypical scrapie-infected Tg 338 mice. Although PrP d was most frequently detected in mesaxons, the ease of detection of PrP d in axons is proportionate to its abundance and rate of transport. We suggest that mesaxons are removing and accumulating axonal membrane PrP d, which therefore results in its more frequent detection in these sites.

The predominant cellular localizations of PrP d in classical and atypical scrapie differ. In atypical scrapie PrP d accumulates on membranes of neurites, reactive glial processes between such neurites, the oligodendroglial inner mesaxon and on axons of myelinated processes. Intralysosomal PrP d accumulations are minimal in atypical scrapie [42]. In contrast, most PrP d in classical scrapie and other naturally occurring and experimental classical prion diseases [10] is associated with dendritic and perikaryonal membranes with variable accumulations, depending on strain, on glial processes. Although detailed co-localization studies are not available for man, sCJD and variant CJD both show similar morphological structures [43] suggesting these relate to common pathogenetic processes for classical forms of prion disease. Membrane invaginations are occasionally found on unmyelinated axons but only occur in the presence of other membrane and extracellular PrP d accumulations suggesting that unmyelinated axons acquire their PrP d in classical forms of disease by glycolipidosis anchor transfer from dendrites [10,11]. Intralysosomal PrP d accumulations are often conspicuous in classical TSEs. These data show that the cellular targeting and cellular trafficking of atypical PrP d differs markedly from that of classical scrapie. With respect to neurons, classical prion diseases traffic PrP d aggregates predominantly into dendrites and neuronal cell bodies [10] while a significant fraction of atypical scrapie PrP d appears to be trafficked to axons. Wallerian-type degeneration is not uncommon in classical prion disease especially near terminal disease states where there is extensive autophagy and neuronal loss. However, the extent of Wallerian-type degeneration is particularly conspicuous in the atypical scrapie-affected sheep and mice. We suggest that the enhanced pathology of white matter is related to the putative altered trafficking of atypical PrP d. Altered trafficking of PrP d to axons may also occur in some inherited human prion diseases and in their transgenic mouse homologues.

The nature of membrane lesions found in classical scrapie-affected sheep at the ultrastructural level is the same irrespective of strain and PRNP genotype [9], suggesting that classical sheep scrapie strains each convey common pathological effects. However, the proportions of different components of membrane, intra-lysosomal and extracellular PrP d may differ according to source and PRNP genotypes [44]. As described above, the nature of the subcellular lesions present in these atypical scrapie-affected sheep differs from those found in classical scrapie of sheep and all other classical prion disease sources so far examined. This supports the observation that atypical scrapie infection represents a prion source that is of a different type or strain from that which occurs in classical scrapie. Such conclusions are supported by the distinct epidemiology, unique biochemical properties of PrP res and different genetic susceptibility of sheep to atypical scrapie when compared with classical scrapie sources [3].

Atypical scrapie occurs most frequently in sheep of PRNP genotypes less common in classical scrapie such as the AHQ/AHQ sheep in the present study [3]. As the nature of light microscopic types of PrP d accumulation, including those in myelinated fibres, is common to sheep of all genotypes naturally affected with atypical scrapie [42] it is likely that sheep of other genotypes affected with atypical scrapie would show the same subcellular changes. AHQ/AHQ sheep also support classical scrapie and we have previously shown that classical scrapie-affected sheep expressing the AHQ allele show the distinctive membrane changes found in all classical forms of prion disease [9,44]. Thus, the nature of membrane lesions found in oligodendroglia and axons in the present sheep can be ascribed to the strain of agent rather than PRNP genotype. Although it is not clear why the VRQ sheep allele expressed in Tg338 mice renders them so susceptible to atypical scrapie.
the white matter lesions in Tg338 mice were similar to those found in atypical scrapie-affected sheep and thus are probably also causally related to the atypical agent strain. However, atypical scrapie-affected Tg338 mice also showed PrP<sub>d</sub> membrane lesions found in classical forms of prion disease. These data suggest that the atypical pathology phenotype is strongly influenced by the genetics of the host. Thus, as previously shown in sheep [8,45], and mice [46], the disease phenotype is an interaction between the PRNP genotype of the host and the strain or isolate of infectious prion.

The distinctive PrP<sub>d</sub> co-localizing membrane and intra-lysosomal lesions of classical prion disease occur in cattle [47] and in murine CH1641 (the present study) both of which accumulate 19 kDa PrP<sub>res</sub> fragments as well as in other sources accumulating 21 kDa fragments [10]. Thus, the fragment size of PrP<sub>res</sub> in classical forms of prion disease has no qualitative effect on the nature of the subcellular lesions co-localizing with PrP<sub>d</sub>. The coated and spiral membrane invaginations of atypical scrapie-infected Tg338 mice accumulating 12 kDa PrP<sub>res</sub> fragments occurred in the absence of detectable 19 or 21 kDa fragments of strongly protease resistant PrP<sub>res</sub>. Thus, the present data show that the unique membrane changes of classical prion disease do not have a simple correspondence with either the fragment size of the unglycosylated band or the degree of protease resistance of PrP<sub>res</sub>.

Tubulovesicular bodies, found in most classical TSE-affected species were not detected in atypical scrapie-affected sheep or Tg338 mice. However, Tg338 mice are capable of generating tubulovesicular bodies as shown by their conspicuous presence in CH1641-affected mice. The molecular structure and pathogenic significance of tubulovesicular bodies is unknown, however their absence in atypical scrapie-affected sheep and Tg338-infected mice may be of discriminatory relevance.

In conclusion, this study shows that the altered biological behaviour of atypical sheep and murine scrapie when compared with that of classical scrapie and other naturally occurring and experimental classical forms of prion disease is accompanied by an altered pathological phenotype most conspicuously evident in white matter. This is associated with different trafficking of abnormal PrP<sub>d</sub> and a modified cellular response to its presence. Some inherited human forms of prion disease also show white matter pathology with abnormal PrP co-localization to myelin that potentially might also be related to different trafficking pathways of abnormal PrP. The studies also show that neither the fragment size of PrP<sub>res</sub> nor its susceptibility to protease digestion correlates with the distinctive membrane lesions seen in classical or Tg338 atypical forms of prion disease.

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Conflict of interest

All authors declare no conflict of interest.

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Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher’s web-site:

Figure S1. Immunogold PrP<sup>d</sup> labelling in atypical scrapie-infected sheep using 1C5 antibody.

Figure S2. Immunogold labelling for PrP<sup>d</sup> with F89 antibody in atypical scrapie-infected Tg338 mice.

Figure S3. Lesion profiles showing nine grey matter sites (G1–9) and 3 white matter sites (W1–3) for atypical scrapie- or CH1641-affected or atypical scrapie challenged Tg338 mice.

Figure S4. Immunohistochemistry for PrP<sup>d</sup> using 1C5 antibody in the cerebral cortex of a Tg338 mouse with atypical scrapie.

Figure S5. Immunogold labelling for PrP<sup>d</sup> with F89 antibody in atypical scrapie-infected Tg338 mice.

Figure S6. UA/LC staining of 35 nm diameter, the so-called tubulovesicular bodies in dendrites of CH1641-infected Tg338 mice (circled).

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