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Citation for published version:

Digital Object Identifier (DOI):
10.1128/JVI.02316-15

Link:
Link to publication record in Edinburgh Research Explorer

Document Version:
Peer reviewed version

Published in:
Journal of Virology

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Prion-type dependent deposition of PRNP-allelic products in heterozygous sheep.

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running title: PrP-allotype deposition in BSE/scrapie ARR/VRQ sheep.

keywords: prion, strain, heterozygosity, PrP polymorphism, BSE, scrapie, sheep, genetic resistance, allotype

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word count for the abstract: 205
and the word count for the text: 3041
ABSTRACT

Susceptibility or resistance to prion infection in humans and animals depends on single prion protein (PrP) amino acid substitutions in the host, but the agent’s modulating role has not been well investigated. Compared to disease incubation times in wild type homozygous ARQ/ARQ sheep, scrapie susceptibility is reduced to near resistance in ARR/ARR animals while it is strongly enhanced in VRQ/VRQ carriers. Heterozygous ARR/VRQ animals exhibit delayed incubation periods. In BSE infection the polymorphism effect is quite different, though the ARR allotype remains the least susceptible. In this study, PrP allotype composition in protease resistant prion protein (PrPres) from brain of heterozygous ARR/VRQ scrapie infected sheep was compared with that of BSE infected sheep with similar genotype. The triplex-Western blotting technique was used to estimate the two allotype PrP fractions in PrPres material from BSE infected ARR/VRQ sheep. PrPres in BSE contained equimolar amounts of VRQ- and ARR-PrP which contrasts with the excess (>95%) VRQ-PrP fraction found in scrapie. This is evidence that TSE agent properties alone, perhaps structural aspects of prions (such as PrP amino acid sequence variants and PrP conformational state) determine the polymorphic dependence of the PrPSc accumulation process in prion formation as well as the disease associated phenotypic expressions in the host.
IMPORTANCE

Transmissible spongiform encephalopathies (TSEs) are fatal neurodegenerative and transmissible diseases caused by prions. Amino acid sequence variants of the prion protein (PrP) determine transmissibility in the hosts as known for classical scrapie in sheep. Each individual produces a separate PrP molecule from its two PrP gene copies. Heterozygous scrapie infected sheep that produce two PrP variants associated with opposite scrapie susceptibility (136V-PrP, high; 171R-PrP, very low) contain in their prion material over 95% of the 136V PrP variant. However, when infected with prions from cattle (BSE), both PrP variants occur in equal ratios. This shows that the infecting prion-type determines the accumulating PrP variant ratio in the heterozygous host. While the host’s PrP is considered a determining factor, these results emphasize that prion structure plays a role during host infection and that PrP variant involvement in prions of heterozygous carriers is a critical field for understanding prion formation.
INTRODUCTION

Transmissible spongiform encephalopathies (TSEs) or prion diseases are fatal neurological diseases occurring in some mammalian species including man. The TSE agent or prion is characterised by the pivotal role of the host prion protein (PrP) that in disease appears aggregated and structurally abnormal, and is named PrP<sub>Sc</sub>. Sc refers to scrapie in small ruminants which was recognized in the 18<sup>th</sup> century in Spanish Merino sheep (1). In healthy situations PrP is a cellular membrane protein (PrP<sub>C</sub>) and fully susceptible to proteases, while its PrP<sub>Sc</sub> isoform is partially resistant to digestion with proteinase K (PK) usually leading to an N-terminally shortened protein called PrP<sup>res</sup> and contains infectivity (2-4).

From many studies it is obvious that TSEs occur in distinct phenotypic forms that are recognized as TSE- or prion disease-types such as classical scrapie in sheep and goat, Creutzfeldt-Jakob disease in humans, chronic wasting disease in cervids and bovine spongiform encephalopathy (BSE) encephalopathy cattle (5-15). In the experimental situation these can be considered as strains when sub-passaged to homogeneity in rodent bioassays (16-20). Susceptibility (and resistance) to animal and human prion diseases, either in infectious or spontaneous conditions, is dependent on single amino acid substitutions in the host’s PrP sequence. In most species such substitutions occur as naturally occurring polymorphisms (7, 10, 21-24).

In sheep two PrP polymorphisms in the PrP sequence - V<sub>136</sub> and R<sub>171</sub> - provide respectively a high and very low susceptibility to natural scrapie compared to the homozygous wild type variants A<sub>136</sub> and Q<sub>171</sub>. Other variants also influence susceptibility for example H<sub>154</sub> (13, 24-30). Altogether, this has led to policies for

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1 amino acids are indicated by single-letter code as used by the IUPAC-IUB Joint Commission on Biochemical Nomenclature (JCBN); A=alanine, Q=glutamine, R=arginine, V=valine, H=histidine.
eradication of scrapie in sheep breeds focused on codons 136, 154 and 171, in which the different alleles have the respective nomenclature: ARQ (the wild type), VRQ, AHQ, and ARR (31, 32). The codon 136 and 171 variants when both occur in heterozygous sheep are indicated with genotype code ARR/VRQ, while homozygous sheep could have genotype ARQ/ARQ (the wild type), ARR/ARR or VRQ/VRQ (7).

In a previous study we reported that in scrapie infected ARR/VRQ sheep the VRQ-PrP in PrP\textsuperscript{res} was highly overrepresented with 91-100% VRQ-PrP product (33, 34). Yet the expression levels of the PrP\textsuperscript{C} alleles in heterozygous animals are considered equal (34, 35) which means that during PrP\textsuperscript{Sc} formation in ARR/VRQ scrapie infected animals there occurs a selective incorporation of the VRQ-PrP allotype. \textit{In vitro} assays confirm the relatively high - but not absolute - resistance to conversion of ARR-PrP when subjected to scrapie or BSE prions (12, 15, 26, 36). This special property of the ARR-PrP allotype is confirmed in \textit{in vivo} intracerebral BSE challenge (i.c.) conditions, but the VRQ-PrP allotype in contrast to its strong link to susceptibility to scrapie appeared in VRQ/VRQ sheep to confer far more resistance to BSE than that found in ARQ/ARQ sheep (37).

In this paper we investigated whether the level of the VRQ-PrP allotype in PrP\textsuperscript{res} from ARR/VRQ BSE-infected i.c. sheep generated by Houston et al. (37) would be comparably high to that found in the same genotype of sheep with natural scrapie. This was accomplished by comparing brain PrP\textsuperscript{res} in scrapie and BSE infected ARR/VRQ sheep. A previously developed robust triplex Western blot method (38, 39) was used to quantitatively estimate PrP concentrations. In this technique the Q171-PrP fraction (VRQ, ARQ) can be quantitatively estimated using a mixture of two antibodies on the same blot membrane of which one antibody (SAF84) only recognizes the VRQ fraction, while the other binds equally well both VRQ-PrP and
ARR-PrP. The outcome yielded a clear-cut difference in VRQ content deposited in the prions of these two different TSE types. This new information is special since it reports on PrP allotype expression for two separate prion types from a mammalian species (sheep) heterozygous for two non-wild type PrP alleles differing widely in their effect on susceptibility/resistance to prion infection.
MATERIALS AND METHODS

Sheep brain and antibodies

Brain tissues were available from ARR/VRQ, VRQ/VRQ, ARQ/ARQ and ARR/ARR sheep clinically affected following intracerebral challenge with cattle BSE, and from naturally infected scrapie sheep with genotypes ARR/VRQ, VRQ/VRQ, ARQ/ARQ, and ARQ/VRQ detected in active surveillance monitoring. The details of the different groups of sheep are presented in Table I. The BSE and classical scrapie diagnosis was carried out on brain stem tissue of each animal by immunohistochemistry and by Western blotting (40-42).

Monoclonal antibodies used were L42, Sha31 and SAF84 (43-45) with respective linear ovine PrP epitope sequences 148-153, 148-155 and 166-172 as determined using Pepscan epitope mapping technology (46), and IgG class numbers a2, 1 and b2. Though L42 and Sha31 share nearly the same linear epitope, they were raised with very different antigens being respectively a linear peptide derived from ovine PrP and PK digested non-denatured scrapie associated fibrils from Syrian hamsters. Molecular Probes™ Zenon® Alexa Fluor® mouse labelling kits for mouse IgG1 (Alexa 647), IgG2a (Alexa 647) and IgG2b (Alexa 488) were from ThermoFisher. For molecular mass estimation a Pre-Stained SeeBlue Standards kit (LC5625; ThermoFisher) was used. Ovine recombinant ARQ-PrP was a gift from Human Rezaei (INRA, Jouy-en Jozas France) (47).

PrPres preparation and quantification of allotype expression with mixed antibody Western blotting

PrPres was prepared from ten percent (wt/vol) brain stem homogenates prepared in lysis buffer, digested with PK at 37°C, and further partially purified by precipitation...
with 1-propanol as described (38). Sodium dodecyl sulphate poly-acrylamide gel electrophoresis of denatured samples in loading buffer (with lithium-dodecyl sulphate and β-mercaptoethanol) was performed in 17 wells gels (33). Detection of PrP\textsuperscript{res} on blot membranes was carried out in our triplex Western blotting system, but for this study a mixture of only two primary antibodies instead of three was used. The antibodies were labelled with Zenon Alexa Fluor kits before application on the blot. Immunochemical quantification of PrP\textsuperscript{res} was subsequently performed by fluorimetric detection monitored in a three laser beam imager (Typhoon Trio variable-mode imager, Amersham Biosciences) (38). For estimation of the ARR- and VRQ-PrP fraction in PrP\textsuperscript{res}, a mixture of two antibodies was applied of which one (SAF84) will bind only if the 171Q polymorphism is present (VRQ-PrP or ARQ-PrP) while the other is equally well binding to both VRQ-, ARQ- and ARR-PrP (33, 38, 39). Two different mixtures with SAF84 were used: SAF84 with L42 (L42/SAF84 combination) and SAF84 with Sha31 (Sha31/SAF84 combination). SAF84 detection was carried out with a Zenon labelling Alexa 488 kit, and L42 or Sha31 with a Zenon labelling Alexa 647 kit (see above for kit specifications). The VRQ-PrP and ARQ-PrP fractions in PrP\textsuperscript{res} samples were calculated as follows (33, 38, 39). When using the SAF84/L42 antibody combination the fraction of the 171Q-PrP (the VRQ- of ARQ-PrP levels) product in scrapie or BSE was obtained by applying the formula Fr(171Q-PrP) = \(\frac{\text{ratio}_x}{\text{ratio}_{Q/Q}}\) where \(\text{ratio}_x\) is the SAF84/L42 ratio of an unknown sample and \(\text{ratio}_{Q/Q}\) is the SAF84/L42 ratio determined for Q/Q homozygous material, which was an average of measurements of the different scrapie (n=10) or BSE (n=8) Q/Q samples; likewise, the fraction of 171R-PrP product (the ARR-PrP level) could be deduced from the formula \(\frac{\text{ratio}_{Q/Q} – \text{ratio}_x}{\text{ratio}_{Q/Q}}\). For the SAF84/Sha31 combination the same formulas were applied but replacing the L42 values for those of Sha31.
The validity of the approach was confirmed by mixing in loading buffer samples from a VRQ/VRQ and an ARR/ARR sheep both infected with BSE in volume ratios 9/1, 8.5/1.5, 8/2, 7.5/2.5, 7/3, 6/4, 5/5, 4/6, 3/7, 2/8 and 1/9 (for both antibody combinations). To exclude the possibility that the outcomes were influenced by the concentration of the PrP\textsuperscript{res} signal, a further check was performed by calculating the PrP\textsuperscript{res} signal per sample in ng PrP as observed from the L42 and Sha31 detection using the recombinant PrP signal as a reference of which 15 ng was run in a lane of each gel.
RESULTS

PrP\textsuperscript{res} samples from sheep homozygous for the 171Q codon allele (genotypes VRQ/VRQ and ARQ/ARQ) exhibited full reactivity with the antibodies L42 and SAF84 in both BSE and scrapie infected animals (Fig. 1a, respectively lanes 3-5 and 10-11). As expected, the PrP\textsuperscript{res} from ARR/ARR BSE infected sheep reacted with antibody L42 but not at all with SAF84 (Fig. 1a, lanes 15-16). Scrapie infected ARR/ARR sheep were not available since these animals remained TSE negative throughout their experimental life time indicative for the high scrapie resistance contributed by the 171R codon (>2000 days, data to be published by Houston and Hunter). The analyses from the heterozygous ARR/VRQ sheep with scrapie and BSE yielded contrasting results in that the staining with SAF84 relative to L42 on scrapie infected sheep samples were very similar to each other while that of SAF84 on the BSE samples was reduced. Similar results were observed when using the SAF84/Sha31 antibody duplex combination (Figure 1b). A further calculation of the fraction of VRQ-PrP in the PrP\textsuperscript{res} samples from the heterozygous animals using the SAF84/L42 combination yielded for scrapie infected ARR/VRQ sheep a VRQ-PrP fraction Fr.(171Q-PrP) of 1.01 ± 0.07 (average ± standard deviation; n=7, Fig.1b). This compared fairly well with previous estimations using 2D gel electrophoresis on isolated PrP\textsuperscript{res} fragments and two different Western blotting techniques (an enzymatically enhanced chemo-luminescence immunodetection method and a triplex-WB based fluorescence immunolabelling method) (33). It further implied that the ARR-PrP fraction varied between different ARR/VRQ sheep derived samples from 0 to only 0.1. In contrast, for BSE infected ARR/VRQ sheep, the VRQ-PrP fraction was 0.53 ± 0.05 (n=4) indicating that PrP\textsuperscript{res} of the BSE infected ARR/VRQ animals contained a nearly equal amounts of both VRQ-PrP and ARR-PrP allotype.
product. Similar values were obtained when tested with the SAF84/Sha31 combination (Figure 1b).

The validity of this approach was confirmed by mixing a VRQ/VRQ with an ARR/ARR BSE sample in loading buffer in different proportions from 9/1 to 1/9. The output versus input curves for VRQ-PrP fraction of PrP\textsuperscript{res} were concave but approached linearity rather well when using either the SAF84/L42 or the SAF84/Sha31 antibody combination (Fig. 2). The final data shown in Figure 1b represent adjusted values based on these concave curves. Finally, an effect of PrP\textsuperscript{res} concentration in the tissue digest on the outcomes was estimated. The regression curves obtained for scrapie and BSE samples were approaching a horizontal line, pointing to negligible effects from the PrP\textsuperscript{res} concentration on the Fr(171Q-PrP) values (Fig. 3). For all individual and overall sample data, the outcomes with the SAF84/L42 and SAF84/Sha31 antibody combinations were very comparable. Also, the current scrapie data confirm our previous results from ARR/VRQ scrapie infected sheep as determined in different ways and prove the quantitative value of the current immunochemical Western blotting methodology used (33).
DISCUSSION

The analyses of the PrP-allotype composition of prion material in heterozygous ARR/VRQ sheep yielded for BSE infected sheep a VRQ-PrP fraction approaching 0.5. This contrasted to the fraction determined in scrapie infected sheep where the VRQ-PrP fraction approximated 1, thus representing nearly all of the PrP\textsubscript{res} mass. Since in the ARR/VRQ scrapie PrP\textsubscript{res} only one allotype is found while both alleles because of diploidy can and do express PrP (34, 48), it is surprising that the ARR-PrP fraction in the PrP\textsubscript{res} material of the scrapie cases is nearly zero. This is in contrast to the ~50% ARR-PrP fraction in ARR/VRQ BSE PrP\textsubscript{res} mass. This wide difference in VRQ-PrP and ARR-PrP content in the prion material of these sheep with scrapie and BSE infection is unique for three reasons. Firstly, two different acquired (infectious) conditions of prion disease were studied in these animals. Secondly, individual animals carrying two non-wild type PrP alleles with very contrasting TSE-type susceptibilities were investigated - while on the one hand the VRQ-PrP makes them highly susceptible to scrapie, on the other hand the ARR-PrP makes them resistant to both BSE and scrapie. Thirdly, the study was performed on tissues obtained from infected animals, thus the prions studied are products of \textit{in vivo} conditions. These data from heterozygous animals carrying two different TSEs - scrapie or BSE - confirm \textit{in vitro} conversion data that a certain PrP polymorphism of the “host” can be less prone to conversion to PrP\textsubscript{Sc} than another (15, 26). Or as alternative to the species barrier concept, on infection with scrapie, only ARR-PrP forms a polymorphism barrier whereas with primary infection with BSE both ARR- and VRQ-PrP contribute to this barrier. Importantly, these new data also strongly support the concept that type (or strain) of the infecting agent itself has an influence on this conversion event.
The role a certain prion type plays in susceptibility and resistance of the sheep host is strikingly reflected in in vivo situations as will be exemplified with three different TSE types. With BSE infection, ARR/ARR and VRQ/VRQ sheep have long incubation times to clinical disease following intracerebral challenge at respectively >1400 days and >1000 days, compared to that in the wild type ARQ/ARQ sheep (around 600 days) (N. Hunter and F. Houston, personal communication). With classical scrapie infection with the agent derived from VRQ-rich sheep flocks, ARR/ARR sheep are nearly fully resistant to challenge whereas VRQ/VRQ sheep with scrapie have very short incubation times (180-720 days), and the wild type (ARQ/ARQ) sheep have intermediate incubation times (14, 27, 36, 37, 40, 49-51). Interestingly with atypical/Nor98 scrapie, a prion disease that is non-spreading and maybe of spontaneous origin, VRQ/VRQ animals appear highly insensitive based on genotype frequency, while ARR/ARR sheep can be affected but are less frequent than ARQ/ARQ sheep with this scrapie type (Table II) (52). Though the susceptibilities to prion diseases may also be influenced by route of infection, prevailing flock PrP-polymorphism, extent of involvement of the lympho-reticular system and other pathogenic aspects, the above mutual differences in susceptibilities are relatively consistent. A breed effect between the Cheviot and Texel sheep used in this study can not be excluded as another factor for the potential difference in allotype ratio between BSE and scrapie infected ARR/VRQ animals but susceptibilities to TSE within a breed (in casu Romanovs) are expected to be largely independent of polygenic effects and this may also apply to between breed effects (14, 53). Therefore the allotype PrP composition in prion material as found in our results is reflecting the effect of the type of TSE or prion agent rather than variation in the host.
In studies performed on TSE infections other than in sheep, some results have been obtained in bank voles. One polymorphism has been described which if present in 109M/I animals leads to 20-30% differences in incubation times for the heterozygous animals compared to the wild type carriers after intracerebral infection with sheep or goat scrapie, but equal incubation times after infection with mouse scrapie strain 139A (23, 54). In these models deposition of both wild type and non-wild type PrP allotypes were observed in significant amounts pointing to equal allotype levels in the prions. This equal deposition of both allotype PrPs in heterozygous bank voles might indicate that incubation times alone are not sufficiently indicative of a great difference in convertibility of PrP^C to PrP^Sc and therefore leads to 100% attack rates. Thus, the situation in these bank vole experiments is different from that in ARR/VRQ sheep where two non-wild type PrP allotypes have been studied, each of them with a proven influence on susceptibility and PrP^C to PrP^Sc convertibility.

In contrast to infectious conditions, in inherited human TSEs, the patients carry a PrP gene linked predisposition to develop disease by a mutation in the coding region of the PRNP gene. The patients are nearly always heterozygous (55, 56). Depending on the polymorphism the non-wild type variant is frequently the dominant PrP variant present in the PK resistant or detergent insoluble PrP^Sc material, but in some instances wild type and non-wild type PrP are both present in significant amounts (55, 57-63). The PrP allotype prevalence in the deposited prion PrP material is supposed to depend on the position and nature of the amino acid in the PrP sequence. In these spontaneous prion diseases, PrP^C can be considered to be the main host factor determining the PrP allotype ratio of the prion material. However, the
role of non-PrP host factors should also be taken into consideration (64). In infectious conditions such as those studied in animals, the agent itself can have an equally important role to that of host PrP and non-PrP host factors. Probably, binding of PrPSc to PrPC (at least for sheep PrP) does not discriminate between different polymorphic PrP variants, while the PrPC to PrPSc conversion efficiency clearly is related to PrP linked genotype dependent susceptibilities as was shown for sheep prions (12, 15, 27, 36, 65).

The example of possibly different allotype compositions in prion material between two TSE types - scrapie and BSE - as exemplified in the ARR/VRQ sheep of this study is a novel finding for in vivo situations and confirm the in vitro studies that show that different TSE types have a different PrP polymorphism variant preference in the PrPC to PrPSc conversion (13, 14, 36). It also shows that, in disease, the prion type can determine the ability of certain host PrP allotype sequence-variants to be converted from PrPC to PrPSc. The critical issue of how the conversion process works and whether other factors than only PrP amino acid sequence of the host can influence it is still uncertain. The species source from which the infection is derived is one determinant (36), as in our case the BSE material to infect the sheep is from bovine origin. Bovine PrP differs from sheep PrP in having an extra octarepeat in the PrP N-terminus and six further amino acid codon differences (sheep PrP codons 98, 100, 146, 158, 189 and 208) (48, 66). Further structural differences in the folding of the prions of BSE and different scrapie types might well have a role in susceptibility of the host, as has been hypothesized in sheep challenge experiments with BSE, CH1641 scrapie and SSBP1 scrapie (13). Whether a non-PrP factor in the agent could play a role remains to be investigated. However considering the major role of
PrPSc structure in TSEs, our data suggest that further studies on PrP allotype heterozygosity in agent and host are needed in order to understand the factors determining the fate of prion diseases.

ACKNOWLEDGEMENTS

In memory of our colleague Alan Rigter, who died in April 2014 at the stage in his life where he was going to apply his education as molecular biologist as a full time PhD. The Roslin Institute material production was funded by Defra, UK (SE1432). The INRA and CVI material were produced in EU project QLK3-CT-2002-01309. This study was supported by national Dutch funding project WOT-01-002-001.01.
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66. Goldmann W, Hunter N, Martin T, Dawson M, Hope J. 1991. Different forms of the bovine PrP gene have five or six copies of a short, G-C-rich element
within the protein-coding exon. The Journal of general virology 72 ( Pt 1):201-
204.
FIGURE LEGENDS

Figure 1: PrP allotype fraction estimates in PrP\textsuperscript{res} from brain of PrP scrapie and BSE infected sheep with different \textit{PRNP} genotypes. a, Western blot of scrapie and BSE PrP\textsuperscript{res} samples of infected sheep with heterozygous and homozygous genotypes as tested with the L42-SAF84 antibody combination. Lanes: 1 and 8, rec-ovinePrP; 2 and 9, molecular mass standards; 3-5, VRQ/VRQ sheep with scrapie; 6-7 ARR/VRQ sheep with scrapie; 10-12, VRQ/VRQ sheep with BSE; 13-14, ARR/VRQ with BSE; 15-16 ARR/ARR sheep with BSE. Blotting procedures followed the triplex WB method as described (38, 39). Tissue equivalents per each brain sample applied were 0.5 mg per lane. b, VRQ- or ARQ-PrP and ARR-PrP allotype fractions per genotype group of sheep with scrapie or BSE. Genotypes are given for PrP-amino acid residue positions 136, 154 and 171; XRQ means combined data from either three (scrapie: ARQ/ARQ, VRQ/VRQ, ARQ/VRQ) or two genotypes (BSE: ARQ/ARQ, VRQ/VRQ) respectively. The results of the two antibody combinations – SAF84/L42 and SAF84/Sha31 - are presented and appeared very similar. Bar fillings: black represent the VRQ- and/or ARQ-PrP fraction, open the ARR-PrP fraction. The number within the bars reflect the average XRQ-PrP fraction, and vertical lines the standard deviation of the XRQ fraction. Individual sample numbers are given as n=#.

Figure 2: Probing the VRQ-PrP allotype level between input and calculated output level in PrP\textsuperscript{res} samples in dose response mixing experiments. See Methods section for design of experiment. For both duplex antibody combinations similar concave curves were obtained. These hollow curves were used for calculation of the final data in Figure 1b. Thus a sample with an output value of 20, 40, 60 or 80% VRQ-PrP allotype, yielded in case of the SAF84/L42 combination respectively 30, 55, 72, and
87% and for the SAF84/Sha31 29, 51, 67 and 86% VRQ-PrP. The inset presents the values of the calculated regression lines derived from the data points.

Fig. 3: Relation between PrP\textsuperscript{res} concentration and VRQ-PrP level of ARR/VRQ sheep brain. For individual samples from ARR/VRQ sheep the PrP concentration in the samples was calculated using recPrP as standard in both blots probed with the SAF84/L42 (closed circles) and SAF84/Sha31 (open triangles) antibody combination (see Methods section). The VRQ-PrP levels were in all individual samples around 1 in the scrapie samples and 0.5 in the BSE samples. The linear regression formulae for the two antibody combinations data point to near horizontal curves, indicative for absence of a concentration effect on the Fr(171Q-VRQ) values in the triplex-WB methodology used.
Table I: Sheep genotypes, TSE type tissues, laboratory origin and breed

<table>
<thead>
<tr>
<th>TSE</th>
<th>genotype</th>
<th># of cases</th>
<th>lab source</th>
<th>breed</th>
</tr>
</thead>
<tbody>
<tr>
<td>i.c. BSE</td>
<td>ARR/VRQ</td>
<td>4</td>
<td>Roslin-UEDIN&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Cheviot</td>
</tr>
<tr>
<td></td>
<td>VRQ/VRQ</td>
<td>5</td>
<td>Roslin-UEDIN&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Cheviot</td>
</tr>
<tr>
<td></td>
<td>ARQ/ARQ</td>
<td>3</td>
<td>INRA-Tours&lt;sup&gt;2nd&lt;/sup&gt;</td>
<td>Suffolk</td>
</tr>
<tr>
<td></td>
<td>ARR/ARR</td>
<td>3</td>
<td>INRA-Tours</td>
<td>Poll Dorset</td>
</tr>
<tr>
<td>natural scrapie</td>
<td>ARR/VRQ</td>
<td>7</td>
<td>CVI-WageningenUR</td>
<td>Texel-cross breed</td>
</tr>
<tr>
<td></td>
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<td>CVI-WageningenUR</td>
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<tr>
<td></td>
<td>ARQ/ARQ</td>
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<tr>
<td></td>
<td>ARQ/VRQ</td>
<td>4</td>
<td>CVI-WageningenUR</td>
<td>Texel-cross breed</td>
</tr>
</tbody>
</table>

<sup>a</sup> Scrapie brain stem tissues were from natural field cases, BSE brain stem or midbrain tissues were either from intracerebral infections with bovine BSE in VRQ/VRQ, ARR/VRQ and ARR/ARR sheep, or in the case of superscript 2<sup>nd</sup> by i.c. passage from bovine BSE infected ARQ/ARQ sheep to ARQ/ARQ sheep.

<sup>b</sup> i.c., intracerebral infection.

<sup>c</sup> Publication of detailed study in preparation (Houston and Hunter).
Table II. Susceptibility dependence on TSE/prion type and host PrP polymorphism<sup>a</sup>.

<table>
<thead>
<tr>
<th>Disease Type</th>
<th>PrP Allotype Susceptible to Acquire</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>most</td>
</tr>
<tr>
<td>BSE</td>
<td>wt</td>
</tr>
<tr>
<td>Classical scrapie</td>
<td>V&lt;sub&gt;136&lt;/sub&gt;</td>
</tr>
<tr>
<td>Atypical/Nor98 scrapie</td>
<td>wt</td>
</tr>
</tbody>
</table>

<sup>a</sup> Susceptibility is presented in a qualitative way for the single amino acid allotype. Wild type represents the A<sub>136</sub>R<sub>154</sub>Q<sub>171</sub> allele. Data about BSE are from experimental infections, classical scrapie from natural and experimental infections, atypical/Nor98 scrapie from active monitoring in a number of European countries.