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Prion Type-Dependent Deposition of *PRNP* Allelic Products in Heterozygous Sheep

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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 (where each triplet represents the amino acids at codons 136, 154, and 171, respectively) 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 bovine spongiform encephalopathy (BSE) infection, the polymorphism effect is quite different although the ARR allotype remains the least susceptible. In this study, PrP allotype composition in protease-resistant prion protein (PrP^{res}) from brain of heterozygous ARR/VRQ scrapie-infected sheep was compared with that of BSE-infected sheep with a similar genotype. A triplex Western blotting technique was used to estimate the two allotype PrP fractions in PrP^{res} material from BSE-infected ARR/VRQ sheep. PrP^{res} in BSE contained equimolar amounts of VRQ- and ARR-PrP, which contrasts with the excess (>95%) VRQ-PrP fraction found in PrP in scrapie. This is evidence that transmissible spongiform encephalopathy (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 PrP^{res} 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 has been shown 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 susceptibilities (136V-PrP variant, high; 171R-PrP variant, very low) contain in their prion material over 95% of the 136V PrP variant. However, when these sheep are infected with prions from cattle (bovine spongiform encephalopathy [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.

Transmissible spongiform encephalopathies (TSEs), or prion diseases, are fatal neurological diseases occurring in some mammalian species, including humans. The TSE agent or prion is characterized by the pivotal role of the host prion protein (PrP) that in disease appears aggregated and structurally abnormal and is named PrP^{Sc}, where "Sc" refers to scrapie in small ruminants, which was recognized in the 18th century in Spanish Merino sheep (1). In healthy situations PrP is a cellular membrane protein (PrP^{C}) and fully susceptible to proteases, while its PrP^{Sc} isoform is partially resistant to digestion with proteinase K (PK), usually leading to an N-terminally shortened protein called PrP^{res} that still retains the associated 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) in cattle (5–15). In the experimental situation, these types can be considered strains when they are subpassaged to homogeneity in rodent bioassays (16–20). Susceptibility (and resistance) to animal and human prion diseases, either under infectious or spontaneous conditions, is dependent on single amino acid substitutions in the host's PrP sequence. In most species such substitutions are naturally occurring polymorphisms (7, 10, 21–24).

In sheep two PrP polymorphisms in the PrP sequence, V_{136} and R_{171} (where V is valine and R is arginine, according to the single-letter code used by the IUPAC-IUB Joint Commission on Biochemical Nomenclature), provide, respectively, high and very low susceptibilities to natural scrapie compared to the homozygous

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wild-type variants with A_{136} and Q_{171} (where A is alanine and Q is glutamine). Other variants also influence susceptibility, for example, H_{154} (where H is histidine) (13, 24–30). Together, this evidence has led to policies for eradication of scrapie in sheep breeds by focusing on codons 136, 154, and 171, in which the different alleles are designated, in respective order, ARQ (the wild type), VRQ, AHQ, and ARR (31, 32). When both codon 136 and 171 variants occur in heterozygous sheep, the genotype code is indicated as 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^{res} was highly overrepresented, with 91 to 100% VRQ-PrP product (33, 34). Yet the expression levels of the PrP^{C} alleles in heterozygous animals are considered equal (34, 35), which means that during PrP^{Sc} formation in ARR/VRQ scrapie-infected animals, there occurs a selective incorporation of the VRQ-PrP allotype. *In vitro* assays confirm the relatively high, but not absolute, resistance to conversion of ARR-PrP when this allotype is subjected to scrapie or BSE prions (12, 15, 26, 36). This special property of the ARR-PrP allotype is confirmed under *in vivo* intracerebral (i.c.) BSE challenge conditions, but the VRQ-PrP allotype, in contrast to its strong link to susceptibility to scrapie, in VRQ/VRQ sheep appeared 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^{res} from ARR/VRQ BSE i.c. infected 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^{res} in scrapie- and BSE-infected ARR/VRQ sheep. A previously developed robust triplex Western blotting (WB) method (38, 39) was used to quantitatively estimate PrP concentrations. In this technique the Q171-PrP fraction (VRQ and ARQ) can be quantitatively estimated using a mixture of two antibodies on the same blot membrane, with one antibody (SAF84) recognizing only the VRQ fraction while the other binds equally well both VRQ-PrP and ARR-PrP. The outcome yielded a clear-cut difference in VRQ contents 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 effects 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 1. The BSE and classical scrapie diagnosis was carried out on brain stem tissue of each animal by immunohistochemistry and by Western blotting (40–42). Tissues used in the different laboratories were obtained from sheep experiments performed under EU convention ET S 123 in accordance with the rules for ethical animal experimentation carried out in the European Community.

Monoclonal antibodies used were L42, Sha31, and SAF84 (43–45) with respective linear ovine PrP epitope sequences consisting of residues 148 to 153, 148 to 155, and 166 to 172, as determined using Pepscan epitope mapping technology (46), and IgG class numbers a2, 1, and b2.

TABLE 1 Sheep genotypes, TSE type tissues, laboratory origin, and breed[a]

TSE type	Genotype	No. of cases	Lab source[d]	Breed
BSE[b]	ARR/VRQ	4	Roslin-UEDIN[c]	Cheviot
	VRQ/VRQ	5	Roslin-UEDIN[c]	Cheviot
	ARQ/ARQ	3	INRA-Tours^{2nd}	Suffolk
	ARR/ARR	3	INRA-Tours	Poll Dorset
Natural scrapie	ARR/VRQ	7	CVI-Wageningen UR	Texel crossbreed
	VRQ/VRQ	2	CVI-Wageningen UR	Texel crossbreed
	ARQ/ARQ	4	CVI-Wageningen UR	Texel crossbreed
	ARQ/VRQ	4	CVI-Wageningen UR	Texel crossbreed

[a] 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 INRA-Tours^{2nd}, obtained by i.c. passage from bovine BSE-infected ARQ/ARQ sheep to ARQ/ARQ sheep.
[b] Intracerebral infection.
[c] Houston and Hunter, unpublished data.
[d] INRA, Institut National de la Recherche Agronomique; UEDIN, University of Edinburgh; CVI, Central Veterinary Institute.

Though L42 and Sha31 share nearly the same linear epitope, they were raised with very different antigens, with L42 being a linear peptide derived from ovine PrP and Sha31 derived from PK-digested nondenatured scrapie-associated fibrils from Syrian hamsters. Molecular Probes Zenon Alexa Fluor mouse labeling 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 (Institut National de la Recherche Agronomique [INRA], Jouy-en Jozas, France) (47).

PrP^{res} preparation and quantification of allotype expression with mixed-antibody Western blotting. PrP^{res} was prepared from 10% (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 previously (38). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of denatured samples in loading buffer (with lithium-dodecyl sulfate and β-mercaptoethanol) was performed in 17-well gels (33). Detection of PrP^{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 labeled with Zenon Alexa Fluor kits before application on the blot. Immunochemical quantification of PrP^{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 fractions in PrP^{res}, a mixture of two antibodies was applied, one of which (SAF84) binds only if the 171Q polymorphism is present (VRQ-PrP or ARQ-PrP) while the other binds equally well to 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 labeling Alexa 488 kit, and L42 or Sha31 was detected with a Zenon labeling Alexa 647 kit (see above for kit specifications). The VRQ-PrP and ARQ-PrP fractions in PrP^{res} samples were calculated as follows (33, 38, 39). When the SAF84/L42 antibody combination was used, the fraction of the 171Q-PrP product [Fr(171Q-PrP); the VRQ- or ARQ-PrP levels] in scrapie or BSE was obtained by applying the formula $Fr(171Q\text{-}PrP) = ratio_x/ratio_{Q/Q}$, where $ratio_x$ is the SAF84/L42 ratio of an unknown sample, and $ratio_{Q/Q}$ is the SAF84/L42 ratio determined for Q/Q homozygous material, which is the average of measurements of the different scrapie ($n = 10$) or BSE ($n = 8$) Q/Q samples; the fraction of the 171R-PrP product (the ARR-PrP level) could be de-

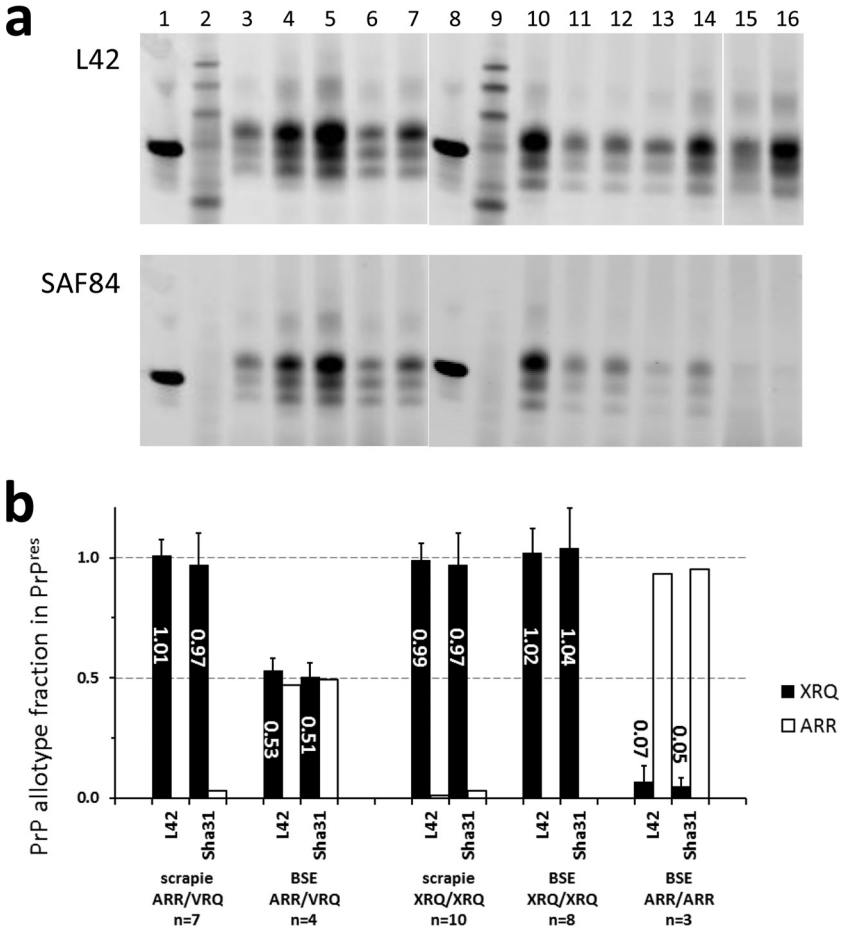

FIG 1 PrP allotype fraction estimates in PrP^{res} from brain of PrP scrapie- and BSE-infected sheep with different *PRNP* genotypes. (a) Western blot of scrapie and BSE PrP^{res} samples of infected sheep with heterozygous and homozygous genotypes as tested with the L42/SAF84 antibody combination. Lanes: 1 and 8, recombinant ovine PrP; 2 and 9, molecular mass standards; 3 to 5, VRQ/VRQ sheep with scrapie; 6 and 7 ARR/VRQ sheep with scrapie; 10 to 12, VRQ/VRQ sheep with BSE; 13 and 14, ARR/VRQ with BSE; 15 and 16 ARR/ARR sheep with BSE. Blotting procedures followed the triplex WB method as described previously (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, and ARQ/VRQ) or two (BSE ARQ/ARQ and VRQ/VRQ) genotypes, respectively. The results shown of the two antibody combinations, SAF84/L42 and SAF84/Sha31, appeared very similar. Black bars, VRQ- and/or ARQ-PrP fraction; open bars, ARR-PrP fraction. The numbers within the bars reflect the average XRQ-PrP fraction values, and vertical lines indicate the standard deviation of the XRQ fraction. *n*, number of individual samples.

duced from the formula $(\text{ratio}_{Q/Q} - \text{ratio}_x)/\text{ratio}_{Q/Q}$. For the SAF84/Sha31 combination, the same formulas were applied but the L42 values were replaced with those for 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, at volume ratios of 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^{res} signal, a further check was performed by calculating the PrP^{res} signal per sample in nanograms of PrP as observed from detection of L42 and Sha31 and using as a reference the recombinant PrP signal, 15 ng of which was run in a lane of each gel.

RESULTS

PrP^{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 to 5 and lanes 10 and 11). As expected, the PrP^{res} from ARR/ARR BSE-infected sheep reacted with antibody L42 but not at all with SAF84 (Fig. 1a, lanes 15 and 16). Scrapie-infected ARR/ARR sheep were not available since these animals remained TSE negative throughout their experimental lifetime, which is indicative of the high scrapie resistance contributed by the 171R codon (>2,000 days) (F. Houston and N. Hunter, unpublished data). The analyses from the heterozygous ARR/VRQ sheep with scrapie and BSE yielded contrasting results in that the staining with SAF84 relative to that with L42 on scrapie-infected sheep samples produced results very similar to each other while that with SAF84 on the BSE samples was reduced. Similar results were observed when the SAF84/Sha31 antibody duplex combination (Fig. 1b) was used. A further calculation of the fraction of VRQ-PrP in the PrP^{res} samples from the heterozygous animals using the SAF84/L42 combination yielded in scrapie-infected ARR/VRQ sheep a VRQ-PrP fraction, Fr(171Q-PrP), of 1.01 ± 0.07 (average ± standard devi-

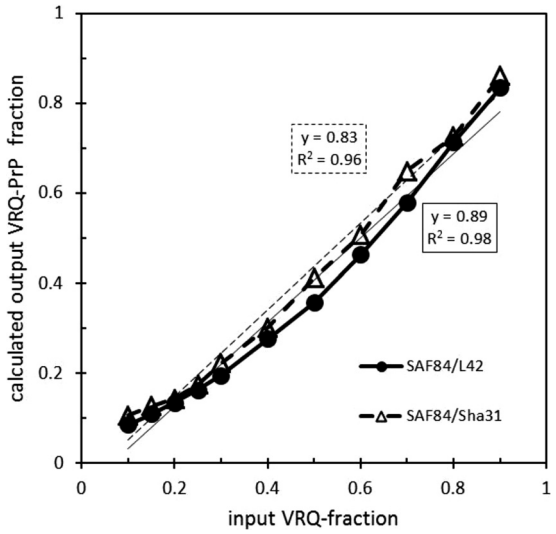

FIG 2 Probing the VRQ-PrP allotype level between input and calculated output levels in PrP^{res} samples in dose-response mixing experiments. See Materials and Methods for the design of the experiment. For both duplex antibody combinations, similar concave curves were obtained. These hollow curves were used for calculation of the final data shown in Fig. 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 combination, 29, 51, 67 and 86% VRQ-PrP. The inset presents the values of the calculated regression lines derived from the data points.

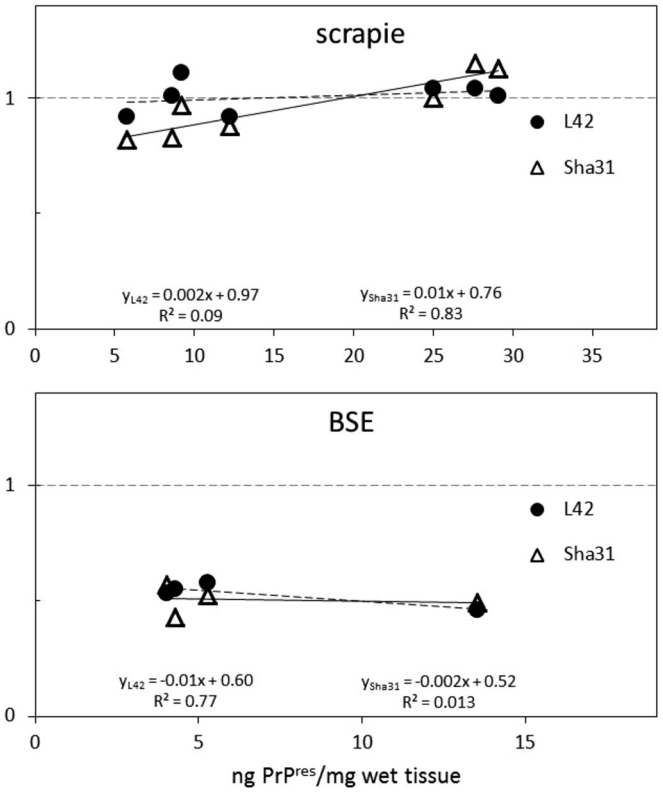

FIG 3 Relation between PrP^{res} concentration and VRQ-PrP level of ARR/VRQ sheep brain. For individual samples from ARR/VRQ sheep, the PrP concentration in the samples was calculated using recombinant PrP as a standard in both blots probed with the SAF84/L42 (filled circles) and SAF84/Sha31 (open triangles) antibody combinations (see Materials and Methods). The VRQ-PrP levels in all individual samples were around 1 in the scrapie samples and 0.5 in the BSE samples. The linear regression formulae for the data from the two antibody combinations point to nearly horizontal curves, indicative of the absence of a concentration effect on the Fr(171Q-VRQ) values in the triplex WB methodology used. Vertical axis, Fr(171-VRQ) fraction.

ation; $n = 7$) (Fig. 1b). This result compared fairly well with previous estimations using two-dimensional (2D) gel electrophoresis on isolated PrP^{res} fragments and two different Western blotting techniques (an enzymatically enhanced chemiluminescence immunodetection method and a triplex WB-based fluorescence immunolabeling method) (33). This result 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^{res} of the BSE-infected ARR/VRQ animals contained nearly equal amounts of both VRQ-PrP and ARR-PrP allotype products. Similar values were obtained when samples were tested with the SAF84/Sha31 combination (Fig. 1b).

The validity of this approach was confirmed by mixing a VRQ/VRQ sample with an ARR/ARR BSE sample in loading buffer in different proportions from 9/1 to 1/9. The output-versus-input curves for the VRQ-PrP fraction of PrP^{res} were concave but approached linearity rather well when either the SAF84/L42 or the SAF84/Sha31 antibody combination was used (Fig. 2). The final data shown in Fig. 1b represent adjusted values based on these concave curves. Finally, an effect on the outcomes of the PrP^{res} concentration in the tissue digest was estimated. The regression curves obtained for scrapie and BSE samples were approaching a horizontal line, pointing to negligible effects from the PrP^{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, 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 with the fraction determined in scrapie-infected sheep, where the VRQ-PrP fraction approximated 1, thus representing nearly all of the PrP^{res} mass. Since in the ARR/VRQ scrapie PrP^{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^{res} material of the scrapie cases is nearly zero. This is in contrast to the ~50% ARR-PrP fraction in ARR/VRQ BSE PrP^{res} mass. This wide difference in VRQ-PrP and ARR-PrP contents in the prion material of these sheep with scrapie and BSE infection is unique for three reasons. First, two different acquired (infectious) conditions of prion disease were studied in these animals. Second, individual animals carrying two non-wild-type PrP alleles with very contrasting TSE-type susceptibilities were investigated; on the one hand, the VRQ-PrP makes them highly susceptible to scrapie, on the

TABLE 2 Susceptibility dependence on TSE/prion type and host PrP polymorphism

Disease type	PrP allotype susceptibility[a]		
	Most	Medium	Least
BSE	Wild type	V_{136}	R_{171}
Classical scrapie	V_{136}	Wild type	R_{171}
Atypical/Nor98 scrapie	Wild type	R_{171}	V_{136}

[a] Susceptibility is presented in a qualitative way for the single amino acid allotype. Wild-type represents the $A_{136}R_{154}Q_{171}$ allele. Data about BSE are from experimental infections, classical scrapie data are from natural and experimental infections, and atypical/Nor98 scrapie data are from active monitoring in a number of European countries.

other hand the ARR-PrP makes them resistant to both BSE and scrapie. Third, the study was performed on tissues obtained from infected animals; thus, the prions studied are products of *in vivo* conditions. These data from heterozygous animals carrying two different TSEs, scrapie and BSE, confirm *in vitro* conversion data that a certain PrP polymorphism of the host can be less prone to conversion to PrP^{Sc} than another (15, 26). Or, as an 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 the 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 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, >1,400 days and >1,000 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 to 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 nonspreading and may be of spontaneous origin, VRQ/VRQ animals appear highly insensitive based on genotype frequency, while ARR/ARR sheep can be affected but are less frequently so than ARQ/ARQ sheep with this scrapie type (Table 2) (52). Though the susceptibilities to prion diseases may also be influenced by route of infection, prevailing PrP polymorphism of the flock, 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 cannot be excluded as another factor for the potential difference in allotype ratios 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 view may also apply to between-breed effects (14, 53). Therefore, the allotype PrP composition in prion material found in our results reflects the effect of the type of TSE or prion agent rather than variation in the host.

With respect to animal species other than sheep, some results have been obtained with TSE infections in heterozygous TSE-infected bank voles. One polymorphism has been described which, if present in 109M/I animals, leads to 20 to 30% differences in incubation times for the heterozygous animals compared to that in the wild-type carriers after intracerebral infection with sheep or goat scrapie but to 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 was observed in significant amounts, pointing to equal allotype levels in the prions. This equal deposition of both PrP allotypes 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 this therefore may lead 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 which has 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 both wild-type and non-wild-type PrPs are 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). Under infectious conditions, such as those studied in animals, the agent itself can have an equally important role as that of host PrP and non-PrP host factors. Probably, binding of PrP^{Sc} to PrP^{C} (at least for sheep PrP) does not discriminate between different polymorphic PrP variants, while the PrP^{C}-to-PrP^{Sc} 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 confirms the *in vitro* studies that show that different TSE types have different PrP polymorphism variant preferences in the PrP^{C}-to-PrP^{Sc} 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 PrP^{C} to PrP^{Sc}. The critical issue of how the conversion process works and of whether other factors than the 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 used to infect the sheep is of 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 re-

mains to be investigated. However, considering the major role of PrP^{Sc} 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.

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