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2 Structure and Assembly

3 **Prion-type dependent deposition of *PRNP*-allelic products in heterozygous**

4 **sheep.**

5

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15

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17

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20

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25 **ABSTRACT**

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

44

45 **IMPORTANCE**

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

59 INTRODUCTION

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

69 From many studies it is obvious that TSEs occur in distinct phenotypic forms that are
70 recognized as TSE- or prion disease-types such as classical scrapie in sheep and
71 goat, Creutzfeldt-Jakob disease in humans, chronic wasting disease in cervids and
72 bovine spongiform encephalopathy (BSE) encephalopathy cattle (5-15). In the
73 experimental situation these can be considered as strains when sub-passaged to
74 homogeneity in rodent bioassays (16-20). Susceptibility (and resistance) to animal
75 and human prion diseases, either in infectious or spontaneous conditions, is
76 dependent on single amino acid substitutions in the host's PrP sequence. In most
77 species such substitutions occur as naturally occurring polymorphisms (7, 10, 21-24).
78 In sheep two PrP polymorphisms in the PrP sequence - V₁₃₆ and R₁₇₁¹ - provide
79 respectively a high and very low susceptibility to natural scrapie compared to the
80 homozygous wild type variants A₁₃₆ and Q₁₇₁. Other variants also influence
81 susceptibility for example H₁₅₄ (13, 24-30). Altogether, this has led to policies for

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.

82 eradication of scrapie in sheep breeds focused on codons 136, 154 and 171, in
83 which the different alleles have the respective nomenclature: ARQ (the wild type),
84 VRQ, AHQ, and ARR (31, 32). The codon 136 and 171 variants when both occur in
85 heterozygous sheep are indicated with genotype code ARR/VRQ, while homozygous
86 sheep could have genotype ARQ/ARQ (the wild type), ARR/ARR or VRQ/VRQ (7).

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

98 In this paper we investigated whether the level of the VRQ-PrP allotype in PrP^{res} from
99 ARR/VRQ BSE-infected i.c. sheep generated by Houston et al. (37) would be
100 comparably high to that found in the same genotype of sheep with natural scrapie.
101 This was accomplished by comparing brain PrP^{res} in scrapie and BSE infected
102 ARR/VRQ sheep. A previously developed robust triplex Western blot method (38, 39)
103 was used to quantitatively estimate PrP concentrations. In this technique the Q171-
104 PrP fraction (VRQ, ARQ) can be quantitatively estimated using a mixture of two
105 antibodies on the same blot membrane of which one antibody (SAF84) only
106 recognizes the VRQ fraction, while the other binds equally well both VRQ-PrP and

107 ARR-PrP. The outcome yielded a clear-cut difference in VRQ content deposited in
108 the prions of these two different TSE types. This new information is special since it
109 reports on PrP allotype expression for two separate prion types from a mammalian
110 species (sheep) heterozygous for two non-wild type PrP alleles differing widely in
111 their effect on susceptibility/resistance to prion infection.

112

113 **MATERIALS AND METHODS**

114 **Sheep brain and antibodies**

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

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

133

134 **PrP^{res} preparation and quantification of allotype expression with mixed** 135 **antibody Western blotting**

136 PrP^{res} was prepared from ten percent (wt/vol) brain stem homogenates prepared in
137 lysis buffer, digested with PK at 37°C, and further partially purified by precipitation

138 with 1-propanol as described (38). Sodium dodecyl sulphate poly-acrylamide gel
139 electrophoresis of denatured samples in loading buffer (with lithium-dodecyl sulphate
140 and β -mercaptoethanol) was performed in 17 wells gels (33). Detection of PrP^{res} on
141 blot membranes was carried out in our triplex Western blotting system, but for this
142 study a mixture of only two primary antibodies instead of three was used. The
143 antibodies were labelled with Zenon Alexa Fluor kits before application on the blot.
144 Immunochemical quantification of PrP^{res} was subsequently performed by fluorimetric
145 detection monitored in a three laser beam imager (Typhoon Trio variable-mode
146 imager, Amersham Biosciences) (38). For estimation of the ARR- and VRQ-PrP
147 fraction in PrP^{res}, a mixture of two antibodies was applied of which one (SAF84) will
148 bind only if the 171Q polymorphism is present (VRQ-PrP or ARQ-PrP) while the other
149 is equally well binding to both VRQ-, ARQ- and ARR-PrP (33, 38, 39). Two different
150 mixtures with SAF84 were used: SAF84 with L42 (L42/SAF84 combination) and
151 SAF84 with Sha31 (Sha31/SAF84 combination). SAF84 detection was carried out
152 with a Zenon labelling Alexa 488 kit, and L42 or Sha31 with a Zenon labelling Alexa
153 647 kit (see above for kit specifications). The VRQ-PrP and ARQ-PrP fractions in
154 PrP^{res} samples were calculated as follows (33, 38, 39). When using the SAF84/L42
155 antibody combination the fraction of the 171Q-PrP (the VRQ- or ARQ-PrP levels)
156 product in scrapie or BSE was obtained by applying the formula $Fr(171Q-PrP) =$
157 $ratio_x/ratio_{Q/Q}$ where $ratio_x$ is the SAF84/L42 ratio of an unknown sample and $ratio_{Q/Q}$
158 is the SAF84/L42 ratio determined for Q/Q homozygous material, which was an
159 average of measurements of the different scrapie (n=10) or BSE (n=8) Q/Q samples;
160 likewise, the fraction of 171R-PrP product (the ARR-PrP level) could be deduced
161 from the formula $(ratio_{Q/Q} - ratio_x)/ratio_{Q/Q}$. For the SAF84/Sha31 combination the
162 same formulas were applied but replacing the L42 values for those of Sha31.

163 The validity of the approach was confirmed by mixing in loading buffer samples from
164 a VRQ/VRQ and an ARR/ARR sheep both infected with BSE in volume ratios 9/1,
165 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
166 combinations). To exclude the possibility that the outcomes were influenced by the
167 concentration of the PrP^{res} signal, a further check was performed by calculating the
168 PrP^{res} signal per sample in ng PrP as observed from the L42 and Sha31 detection
169 using the recombinant PrP signal as a reference of which 15 ng was run in a lane of
170 each gel.

171

172 **RESULTS**

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

197 product. Similar values were obtained when tested with the SAF84/Sha31
198 combination (Figure 1b).

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

213 **DISCUSSION**

214 The analyses of the PrP-allotype composition of prion material in heterozygous
215 ARR/VRQ sheep yielded for BSE infected sheep a VRQ-PrP fraction approaching
216 0.5. This contrasted to the fraction determined in scrapie infected sheep where the
217 VRQ-PrP fraction approximated 1, thus representing nearly all of the PrP^{res} mass.
218 Since in the ARR/VRQ scrapie PrP^{res} only one allotype is found while both alleles
219 because of diploidy can and do express PrP (34, 48), it is surprising that the ARR-
220 PrP fraction in the PrP^{res} material of the scrapie cases is nearly zero. This is in
221 contrast to the ~50% ARR-PrP fraction in ARR/VRQ BSE PrP^{res} mass. This wide
222 difference in VRQ-PrP and ARR-PrP content in the prion material of these sheep with
223 scrapie and BSE infection is unique for three reasons. Firstly, two different acquired
224 (infectious) conditions of prion disease were studied in these animals. Secondly,
225 individual animals carrying two non-wild type PrP alleles with very contrasting
226 TSE-type susceptibilities were investigated - while on the one hand the VRQ-PrP
227 makes them highly susceptible to scrapie, on the other hand the ARR-PrP makes
228 them resistant to both BSE and scrapie., Thirdly, the study was performed on tissues
229 obtained from infected animals, thus the prions studied are products of *in vivo*
230 conditions. These data from heterozygous animals carrying two different
231 TSEs - scrapie or BSE - confirm *in vitro* conversion data that a certain PrP
232 polymorphism of the "host" can be less prone to conversion to PrP^{Sc} than another
233 (15, 26). Or as alternative to the species barrier concept, on infection with scrapie,
234 only ARR-PrP forms a polymorphism barrier whereas with primary infection with BSE
235 both ARR- and VRQ-PrP contribute to this barrier. Importantly, these new data also
236 strongly support the concept that type (or strain) of the infecting agent itself has an
237 influence on this conversion event.

238

239 The role a certain prion type plays in susceptibility and resistance of the sheep host is
240 strikingly reflected in *in vivo* situations as will be exemplified with three different TSE
241 types. With BSE infection, ARR/ARR and VRQ/VRQ sheep have long incubation
242 times to clinical disease following intracerebral challenge at respectively >1400 days
243 and >1000 days, compared to that in the wild type ARQ/ARQ sheep (around 600
244 days) (N. Hunter and F. Houston, personal communication). With classical scrapie
245 infection with the agent derived from VRQ-rich sheep flocks, ARR/ARR sheep are
246 nearly fully resistant to challenge whereas VRQ/VRQ sheep with scrapie have very
247 short incubation times (180-720 days), and the wild type (ARQ/ARQ) sheep have
248 intermediate incubation times (14, 27, 36, 37, 40, 49-51). Interestingly with
249 atypical/Nor98 scrapie, a prion disease that is non-spreading and maybe of
250 spontaneous origin, VRQ/VRQ animals appear highly insensitive based on genotype
251 frequency, while ARR/ARR sheep can be affected but are less frequent than
252 ARQ/ARQ sheep with this scrapie type (Table II) (52). Though the susceptibilities to
253 prion diseases may also be influenced by route of infection, prevailing flock
254 PrP-polymorphism, extent of involvement of the lympho-reticular system and other
255 pathogenic aspects, the above mutual differences in susceptibilities are relatively
256 consistent. A breed effect between the Cheviot and Texel sheep used in this study
257 can not be excluded as another factor for the potential difference in allotype ratio
258 between BSE and scrapie infected ARR/VRQ animals but susceptibilities to TSE
259 within a breed (*in casu* Romanovs) are expected to be largely independent of
260 polygenic effects and this may also apply to between breed effects (14, 53).
261 Therefore the allotype PrP composition in prion material as found in our results is
262 reflecting the effect of the type of TSE or prion agent rather than variation in the host.

263

264 In studies performed on TSE infections other than in sheep, some results have been
265 obtained in bank voles. One polymorphism has been described which if present in
266 109M/I animals leads to 20-30% differences in incubation times for the heterozygous
267 animals compared to the wild type carriers after intracerebral infection with sheep or
268 goat scrapie, but equal incubation times after infection with mouse scrapie strain
269 139A (23, 54). In these models deposition of both wild type and non-wild type PrP
270 allotypes were observed in significant amounts pointing to equal allotype levels in the
271 prions. This equal deposition of both allotype PrPs in heterozygous bank voles might
272 indicate that incubation times alone are not sufficiently indicative of a great difference
273 in convertibility of PrP^C to PrP^{Sc} and therefore leads to 100% attack rates. Thus, the
274 situation in these bank vole experiments is different from that in ARR/VRQ sheep
275 where two non-wild type PrP allotypes have been studied, each of them with a
276 proven influence on susceptibility and PrP^C to PrP^{Sc} convertibility.

277

278 In contrast to infectious conditions, in inherited human TSEs, the patients carry a PrP
279 gene linked predisposition to develop disease by a mutation in the coding region of
280 the *PRNP* gene. The patients are nearly always heterozygous (55, 56). Depending
281 on the polymorphism the non-wild type variant is frequently the dominant PrP variant
282 present in the PK resistant or detergent insoluble PrP^{Sc} material, but in some
283 instances wild type and non-wild type PrP are both present in significant amounts
284 (55, 57-63). The PrP allotype prevalence in the deposited prion PrP material is
285 supposed to depend on the position and nature of the amino acid in the PrP
286 sequence. In these spontaneous prion diseases, PrP^C can be considered to be the
287 main host factor determining the PrP allotype ratio of the prion material. However, the

288 role of non-PrP host factors should also be taken into consideration (64). In infectious
289 conditions such as those studied in animals, the agent itself can have an equally
290 important role to that of host PrP and non-PrP host factors. Probably, binding of
291 PrP^{Sc} to PrP^C (at least for sheep PrP) does not discriminate between different
292 polymorphic PrP variants, while the PrP^C to PrP^{Sc} conversion efficiency clearly is
293 related to PrP linked genotype dependent susceptibilities as was shown for sheep
294 prions (12, 15, 27, 36, 65).

295

296 The example of possibly different allotype compositions in prion material between two
297 TSE types - scrapie and BSE - as exemplified in the ARR/VRQ sheep of this study is
298 a novel finding for *in vivo* situations and confirm the *in vitro* studies that show that
299 different TSE types have a different PrP polymorphism variant preference in the PrP^C
300 to PrP^{Sc} conversion (13, 14, 36). It also shows that, in disease, the prion type can
301 determine the ability of certain host PrP allotype sequence-variants to be converted
302 from PrP^C to PrP^{Sc}. The critical issue of how the conversion process works and
303 whether other factors than only PrP amino acid sequence of the host can influence it
304 is still uncertain. The species source from which the infection is derived is one
305 determinant (36), as in our case the BSE material to infect the sheep is from bovine
306 origin. Bovine PrP differs from sheep PrP in having an extra octarepeat in the PrP N-
307 terminus and six further amino acid codon differences (sheep PrP codons 98, 100,
308 146, 158, 189 and 208) (48, 66). Further structural differences in the folding of the
309 prions of BSE and different scrapie types might well have a role in susceptibility of
310 the host, as has been hypothesized in sheep challenge experiments with BSE,
311 CH1641 scrapie and SSBP1 scrapie (13). Whether a non-PrP factor in the agent
312 could play a role remains to be investigated. However considering the major role of

313 PrP^{Sc} structure in TSEs, our data suggest that further studies on PrP allotype
314 heterozygosity in agent and host are needed in order to understand the factors
315 determining the fate of prion diseases.

316

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323

324 **REFERENCES**

- 325 1. **Fast C, Groschup MH.** 2013. Classical and Atypical Scrapie in Sheep and
326 Goats. *In* Zou W-Q, Gambetti P (ed.), Prions and Diseases: Animals, Humans
327 and the Environment, vol. 2.
- 328 2. **Oesch B, Westaway D, Walchli M, McKinley MP, Kent SB, Aebersold R,**
329 **Barry RA, Tempst P, Teplow DB, Hood LE, et al.** 1985. A cellular gene
330 encodes scrapie PrP 27-30 protein. *Cell* **40**:735-746.
- 331 3. **Prusiner SB.** 1982. Novel proteinaceous infectious particles cause scrapie.
332 *Science* **216**:136-144.
- 333 4. **Prusiner SB, McKinley MP, Bowman KA, Bolton DC, Bendheim PE, Groth**
334 **DF, Glenner GG.** 1983. Scrapie prions aggregate to form amyloid-like
335 birefringent rods. *Cell* **35**:349-358.
- 336 5. **Di Bari MA, Chianini F, Vaccari G, Esposito E, Conte M, Eaton SL,**
337 **Hamilton S, Finlayson J, Steele PJ, Dagleish MP, Reid HW, Bruce M,**
338 **Jeffrey M, Agrimi U, Nonno R.** 2008. The bank vole (*Myodes glareolus*) as a
339 sensitive bioassay for sheep scrapie. *The Journal of general virology* **89**:2975-
340 2985.
- 341 6. **Gambetti P, Kong Q, Zou W, Parchi P, Chen SG.** 2003. Sporadic and
342 familial CJD: classification and characterisation. *British medical bulletin*
343 **66**:213-239.
- 344 7. **Hunter N, Bossers A.** 2006. The PrP genotype as a marker for scrapie
345 susceptibility in sheep., p. 640–647. *In* Hörnlimann B, Riesner D, Kretzschmar
346 H (ed.), Prions in humans and animals. de Gruyter, Berlin, Germany.
- 347 8. **Mead S, Whitfield J, Poulter M, Shah P, Uphill J, Campbell T, Al-Dujaily H,**
348 **Hummerich H, Beck J, Mein CA, Verzilli C, Whittaker J, Alpers MP,**

- 349 **Collinge J.** 2009. A novel protective prion protein variant that colocalizes with
350 kuru exposure. *The New England journal of medicine* **361**:2056-2065.
- 351 9. **Meade-White KD, Barbian KD, Race B, Favara C, Gardner D, Taubner L,**
352 **Porcella S, Race R.** 2009. Characteristics of 263K scrapie agent in multiple
353 hamster species. *Emerging infectious diseases* **15**:207-215.
- 354 10. **Vaccari G, Panagiotidis CH, Acin C, Peletto S, Barillet F, Acutis P,**
355 **Bossers A, Langeveld J, van Keulen L, Sklaviadis T, Badiola JJ,**
356 **Andreeoletti O, Groschup MH, Agrimi U, Foster J, Goldmann W.** 2009.
357 State-of-the-art review of goat TSE in the European Union, with special
358 emphasis on PRNP genetics and epidemiology. *Veterinary research* **40**:48.
- 359 11. **Williams ES, Young S.** 1980. Chronic wasting disease of captive mule deer:
360 a spongiform encephalopathy. *Journal of wildlife diseases* **16**:89-98.
- 361 12. **Bossers A, Belt P, Raymond GJ, Caughey B, de Vries R, Smits MA.** 1997.
362 Scrapie susceptibility-linked polymorphisms modulate the in vitro conversion of
363 sheep prion protein to protease-resistant forms. *Proceedings of the National*
364 *Academy of Sciences of the United States of America* **94**:4931-4936.
- 365 13. **Goldmann W, Hunter N, Smith G, Foster J, Hope J.** 1994. PrP genotype
366 and agent effects in scrapie: change in allelic interaction with different isolates
367 of agent in sheep, a natural host of scrapie. *The Journal of general virology* **75**
368 **(Pt 5):**989-995.
- 369 14. **Gonzalez L, Jeffrey M, Dagleish MP, Goldmann W, Siso S, Eaton SL,**
370 **Martin S, Finlayson J, Stewart P, Steele P, Pang Y, Hamilton S, Reid HW,**
371 **Chianini F.** 2012. Susceptibility to scrapie and disease phenotype in sheep:
372 cross-PRNP genotype experimental transmissions with natural sources.
373 *Veterinary research* **43**:55.

- 374 15. **Raymond GJ, Hope J, Kocisko DA, Priola SA, Raymond LD, Bossers A,**
375 **Ironside J, Will RG, Chen SG, Petersen RB, Gambetti P, Rubenstein R,**
376 **Smits MA, Lansbury PT, Jr., Caughey B.** 1997. Molecular assessment of the
377 potential transmissibilities of BSE and scrapie to humans. *Nature* **388**:285-
378 288.
- 379 16. **Fraser H, Dickinson AG.** 1968. The sequential development of the brain
380 lesion of scrapie in three strains of mice. *Journal of comparative pathology*
381 **78**:301-311.
- 382 17. **Kimberlin RH, Walker C.** 1977. Characteristics of a short incubation model of
383 scrapie in the golden hamster. *The Journal of general virology* **34**:295-304.
- 384 18. **Bruce ME, McConnell I, Fraser H, Dickinson AG.** 1991. The disease
385 characteristics of different strains of scrapie in Sinc congenic mouse lines:
386 implications for the nature of the agent and host control of pathogenesis. *The*
387 *Journal of general virology* **72 (Pt 3)**:595-603.
- 388 19. **Le Dur A, Beringue V, Andreoletti O, Reine F, Lai TL, Baron T, Bratberg**
389 **B, Vilotte JL, Sarradin P, Benestad SL, Laude H.** 2005. A newly identified
390 type of scrapie agent can naturally infect sheep with resistant PrP genotypes.
391 *Proceedings of the National Academy of Sciences of the United States of*
392 *America* **102**:16031-16036.
- 393 20. **Nonno R, Di Bari MA, Cardone F, Vaccari G, Fazzi P, Dell'Omo G, Cartoni**
394 **C, Ingrosso L, Boyle A, Galeno R, Sbriccoli M, Lipp HP, Bruce M,**
395 **Pocchiari M, Agrimi U.** 2006. Efficient transmission and characterization of
396 Creutzfeldt-Jakob disease strains in bank voles. *PLoS pathogens* **2**:e12.
- 397 21. **Collinge J.** 2005. Molecular neurology of prion disease. *Journal of neurology,*
398 *neurosurgery, and psychiatry* **76**:906-919.

- 399 22. **Johnson CJ, Herbst A, Duque-Velasquez C, Vanderloo JP, Bochsler P,**
400 **Chappell R, McKenzie D.** 2011. Prion protein polymorphisms affect chronic
401 wasting disease progression. *PloS one* **6**:e17450.
- 402 23. **Cartoni C, Schinina ME, Maras B, Nonno R, Vaccari G, Di Baria MA,**
403 **Conte M, Liu QG, Lu M, Cardone F, Windl O, Pocchiari M, Agrimi U.** 2005.
404 Identification of the pathological prion protein allotypes in scrapie-infected
405 heterozygous bank voles (*Clethrionomys glareolus*) by high-performance liquid
406 chromatography-mass spectrometry. *Journal of chromatography* **1081**:122-
407 126.
- 408 24. **Westaway D, Zuliani V, Cooper CM, Da Costa M, Neuman S, Jenny AL,**
409 **Detwiler L, Prusiner SB.** 1994. Homozygosity for prion protein alleles
410 encoding glutamine-171 renders sheep susceptible to natural scrapie. *Genes*
411 *& development* **8**:959-969.
- 412 25. **Belt PB, Muileman IH, Schreuder BE, Bos-de Ruijter J, Gielkens AL,**
413 **Smits MA.** 1995. Identification of five allelic variants of the sheep PrP gene
414 and their association with natural scrapie. *The Journal of general virology* **76** (
415 **Pt 3**):509-517.
- 416 26. **Bossers A, de Vries R, Smits MA.** 2000. Susceptibility of sheep for scrapie
417 as assessed by in vitro conversion of nine naturally occurring variants of PrP.
418 *Journal of virology* **74**:1407-1414.
- 419 27. **Bossers A, Schreuder BE, Muileman IH, Belt PB, Smits MA.** 1996. PrP
420 genotype contributes to determining survival times of sheep with natural
421 scrapie. *The Journal of general virology* **77** (**Pt 10**):2669-2673.
- 422 28. **Ikeda T, Horiuchi M, Ishiguro N, Muramatsu Y, Kai-Uwe GD, Shinagawa**
423 **M.** 1995. Amino acid polymorphisms of PrP with reference to onset of scrapie

- 424 in Suffolk and Corriedale sheep in Japan. The Journal of general virology **76** (
425 **Pt 10**):2577-2581.
- 426 29. **Saunders GC, Lantier I, Cawthraw S, Berthon P, Moore SJ, Arnold ME,**
427 **Windl O, Simmons MM, Andreoletti O, Bellworthy S, Lantier F.** 2009.
428 Protective effect of the T112 PrP variant in sheep challenged with bovine
429 spongiform encephalopathy. The Journal of general virology **90**:2569-2574.
- 430 30. **Tan BC, Alejo-Blanco AR, Goldmann W, Stewart P, Gill AC, Graham JF,**
431 **Manson JC, McCutcheon S.** 2010. Codon 141 in ovine PRNP gene
432 modulates incubation time in sheep orally infected with BSE. Prion **4**:195.
- 433 31. **EU.** 2003. Commission Decision of 13 February 2003 laying down minimum
434 requirements for the establishment of breeding programmes for resistance to
435 transmissible spongiform encephalopathies in sheep. Official Journal of the
436 European Union **41**:41-45.
- 437 32. **Melchior MB, Windig JJ, Hagens T, Bossers A, Davidse A, van**
438 **Zijderveld FG.** 2010. Eradication of scrapie with selective breeding: are we
439 nearly there? BMC veterinary research **6**:24.
- 440 33. **Jacobs JG, Bossers A, Rezaei H, van Keulen LJ, McCutcheon S,**
441 **Sklaviadis T, Lantier I, Berthon P, Lantier F, van Zijderveld FG, Langeveld**
442 **JP.** 2011. Proteinase K-resistant material in ARR/VRQ sheep brain affected
443 with classical scrapie is composed mainly of VRQ prion protein. Journal of
444 virology **85**:12537-12546.
- 445 34. **Morel N, Andreoletti O, Grassi J, Clement G.** 2007. Absolute and relative
446 quantification of sheep brain prion protein (PrP) allelic variants by matrix-
447 assisted laser desorption/ionisation time-of-flight mass spectrometry. Rapid
448 communications in mass spectrometry : RCM **21**:4093-4100.

- 449 35. **Garcia-Crespo D, Juste RA, Hurtado A.** 2005. Selection of ovine
450 housekeeping genes for normalisation by real-time RT-PCR; analysis of PrP
451 gene expression and genetic susceptibility to scrapie. *BMC veterinary*
452 *research* **1**:3.
- 453 36. **Priem J, Langeveld JP, van Keulen LJ, van Zijderveld FG, Andreoletti O,**
454 **Bossers A.** 2014. Enhanced virulence of sheep-passaged bovine spongiform
455 encephalopathy agent is revealed by decreased polymorphism barriers in
456 prion protein conversion studies. *Journal of virology* **88**:2903-2912.
- 457 37. **Houston F, Goldmann W, Chong A, Jeffrey M, Gonzalez L, Foster J,**
458 **Parnham D, Hunter N.** 2003. Prion diseases: BSE in sheep bred for
459 resistance to infection. *Nature* **423**:498.
- 460 38. **Jacobs JG, Sauer M, van Keulen LJ, Tang Y, Bossers A, Langeveld JP.**
461 2011. Differentiation of ruminant transmissible spongiform encephalopathy
462 isolate types, including bovine spongiform encephalopathy and CH1641
463 scrapie. *The Journal of general virology* **92**:222-232.
- 464 39. **Langeveld JP, Jacobs JG, Erkens JH, Baron T, Andreoletti O, Yokoyama**
465 **T, van Keulen LJ, van Zijderveld FG, Davidse A, Hope J, Tang Y, Bossers**
466 **A.** 2014. Sheep prions with molecular properties intermediate between
467 classical scrapie, BSE and CH1641-scrapie. *Prion* **8**:296-305.
- 468 40. **Thuring CM, Erkens JH, Jacobs JG, Bossers A, Van Keulen LJ, Garssen**
469 **GJ, Van Zijderveld FG, Ryder SJ, Groschup MH, Sweeney T, Langeveld**
470 **JP.** 2004. Discrimination between scrapie and bovine spongiform
471 encephalopathy in sheep by molecular size, immunoreactivity, and glycoprofile
472 of prion protein. *Journal of clinical microbiology* **42**:972-980.

- 473 41. **Thuring CM, van Keulen LJ, Langeveld JP, Vromans ME, van Zijderveld**
474 **FG, Sweeney T.** 2005. Immunohistochemical distinction between preclinical
475 bovine spongiform encephalopathy and scrapie infection in sheep. *Journal of*
476 *comparative pathology* **132**:59-69.
- 477 42. **Jeffrey M, Gonzalez L, Chong A, Foster J, Goldmann W, Hunter N, Martin**
478 **S.** 2006. Ovine infection with the agents of scrapie (CH1641 isolate) and
479 bovine spongiform encephalopathy: immunochemical similarities can be
480 resolved by immunohistochemistry. *Journal of comparative pathology* **134**:17-
481 29.
- 482 43. **Feraudet C, Morel N, Simon S, Volland H, Frobert Y, Creminon C, Vilette**
483 **D, Lehmann S, Grassi J.** 2005. Screening of 145 anti-PrP monoclonal
484 antibodies for their capacity to inhibit PrPSc replication in infected cells. *The*
485 *Journal of biological chemistry* **280**:11247-11258.
- 486 44. **Harmeyer S, Pfaff E, Groschup MH.** 1998. Synthetic peptide vaccines yield
487 monoclonal antibodies to cellular and pathological prion proteins of ruminants.
488 *The Journal of general virology* **79 (Pt 4)**:937-945.
- 489 45. **Demart S, Fournier JG, Creminon C, Frobert Y, Lamoury F, Marce D,**
490 **Lasmezas C, Dormont D, Grassi J, Deslys JP.** 1999. New insight into
491 abnormal prion protein using monoclonal antibodies. *Biochemical and*
492 *biophysical research communications* **265**:652-657.
- 493 46. **Slootstra JW, Puijk WC, Ligtoet GJ, Langeveld JP, Meloen RH.** 1996.
494 Structural aspects of antibody-antigen interaction revealed through small
495 random peptide libraries. *Molecular diversity* **1**:87-96.
- 496 47. **Rezaei H, Marc D, Choiset Y, Takahashi M, Hui Bon Hoa G, Haertle T,**
497 **Grosclaude J, Debey P.** 2000. High yield purification and physico-chemical

- 498 properties of full-length recombinant allelic variants of sheep prion protein
499 linked to scrapie susceptibility. *European journal of biochemistry / FEBS*
500 **267**:2833-2839.
- 501 48. **Goldmann W, Hunter N, Foster JD, Salbaum JM, Beyreuther K, Hope J.**
502 1990. Two alleles of a neural protein gene linked to scrapie in sheep.
503 *Proceedings of the National Academy of Sciences of the United States of*
504 *America* **87**:2476-2480.
- 505 49. **Jeffrey M, Martin S, Barr J, Chong A, Fraser JR.** 2001. Onset of
506 accumulation of PrPres in murine ME7 scrapie in relation to pathological and
507 PrP immunohistochemical changes. *Journal of comparative pathology* **124**:20-
508 28.
- 509 50. **Langeveld JP, Jacobs JG, Erkens JH, Bossers A, van Zijderveld FG, van**
510 **Keulen LJ.** 2006. Rapid and discriminatory diagnosis of scrapie and BSE in
511 retro-pharyngeal lymph nodes of sheep. *BMC veterinary research* **2**:19.
- 512 51. **Ryder SJ, Dexter GE, Heasman L, Warner R, Moore SJ.** 2009.
513 Accumulation and dissemination of prion protein in experimental sheep
514 scrapie in the natural host. *BMC veterinary research* **5**:9.
- 515 52. **Fediaevsky A, Tongue SC, Noremark M, Calavas D, Ru G, Hopp P.** 2008.
516 A descriptive study of the prevalence of atypical and classical scrapie in sheep
517 in 20 European countries. *BMC veterinary research* **4**:19.
- 518 53. **Diaz C, Vitezica ZG, Rupp R, Andreoletti O, Elsen JM.** 2005. Polygenic
519 variation and transmission factors involved in the resistance/susceptibility to
520 scrapie in a Romanov flock. *The Journal of general virology* **86**:849-857.
- 521 54. **Cartoni C, Schinina ME, Maras B, Nonno R, Vaccari G, Di Bari M, Conte**
522 **M, De Pascalis A, Principe S, Cardone F, Pocchiari M, Agrimi U.** 2007.

- 523 Quantitative profiling of the pathological prion protein allotypes in bank voles
524 by liquid chromatography-mass spectrometry. *Journal of chromatography. B,*
525 *Analytical technologies in the biomedical and life sciences* **849**:302-306.
- 526 55. **Principe S, Maras B, Schinina ME, Pocchiari M, Cardone F.** 2008.
527 Unraveling the details of prion (con)formation(s): recent advances by mass
528 spectrometry. *Current opinion in drug discovery & development* **11**:697-707.
- 529 56. **Silvestrini MC, Cardone F, Maras B, Pucci P, Barra D, Brunori M,**
530 **Pocchiari M.** 1997. Identification of the prion protein allotypes which
531 accumulate in the brain of sporadic and familial Creutzfeldt-Jakob disease
532 patients. *Nature medicine* **3**:521-525.
- 533 57. **Cardone F, Principe S, Schinina ME, Maras B, Capellari S, Parchi P,**
534 **Notari S, Di Francesco L, Pologgi A, Galeno R, Vinci R, Mellina V, Almonti**
535 **S, Ladogana A, Pocchiari M.** 2014. Mutant PrPCJD prevails over wild-type
536 PrPCJD in the brain of V210I and R208H genetic Creutzfeldt-Jakob disease
537 patients. *Biochemical and biophysical research communications* **454**:289-294.
- 538 58. **Chen SG, Parchi P, Brown P, Capellari S, Zou W, Cochran EJ, Vnencak-**
539 **Jones CL, Julien J, Vital C, Mikol J, Lugaresi E, Auttilio-Gambetti L,**
540 **Gambetti P.** 1997. Allelic origin of the abnormal prion protein isoform in
541 familial prion diseases. *Nature medicine* **3**:1009-1015.
- 542 59. **Tagliavini F, Prelli F, Porro M, Rossi G, Giaccone G, Farlow MR, Dlouhy**
543 **SR, Ghetti B, Bugiani O, Frangione B.** 1994. Amyloid fibrils in Gerstmann-
544 Straussler-Scheinker disease (Indiana and Swedish kindreds) express only
545 PrP peptides encoded by the mutant allele. *Cell* **79**:695-703.

- 546 60. **Capellari S, Cardone F, Notari S, Schinina ME, Maras B, Sita D, Baruzzi A,**
547 **Pocchiari M, Parchi P.** 2005. Creutzfeldt-Jakob disease associated with the
548 R208H mutation in the prion protein gene. *Neurology* **64**:905-907.
- 549 61. **Kitamoto T, Yamaguchi K, Doh-ura K, Tateishi J.** 1991. A prion protein
550 missense variant is integrated in kuru plaque cores in patients with
551 Gerstmann-Straussler syndrome. *Neurology* **41**:306-310.
- 552 62. **Parchi P, Chen SG, Brown P, Zou W, Capellari S, Budka H, Hainfellner J,**
553 **Reyes PF, Golden GT, Hauw JJ, Gajdusek DC, Gambetti P.** 1998. Different
554 patterns of truncated prion protein fragments correlate with distinct phenotypes
555 in P102L Gerstmann-Straussler-Scheinker disease. *Proceedings of the*
556 *National Academy of Sciences of the United States of America* **95**:8322-8327.
- 557 63. **Monaco S, Fiorini M, Farinazzo A, Ferrari S, Gelati M, Piccardo P,**
558 **Zanusso G, Ghetti B.** 2012. Allelic origin of protease-sensitive and protease-
559 resistant prion protein isoforms in Gerstmann-Straussler-Scheinker disease
560 with the P102L mutation. *PloS one* **7**:e32382.
- 561 64. **Crowell J, Hughson A, Caughey B, Bessen RA.** 2015. Host determinants of
562 prion strain diversity independent of prion protein genotype. *Journal of*
563 *virology*.
- 564 65. **Rigter A, Bossers A.** 2005. Sheep scrapie susceptibility-linked
565 polymorphisms do not modulate the initial binding of cellular to disease-
566 associated prion protein prior to conversion. *The Journal of general virology*
567 **86**:2627-2634.
- 568 66. **Goldmann W, Hunter N, Martin T, Dawson M, Hope J.** 1991. Different forms
569 of the bovine PrP gene have five or six copies of a short, G-C-rich element

570 within the protein-coding exon. The Journal of general virology **72 (Pt 1):**201-

571 204.

572

573 FIGURE LEGENDS

574 Figure 1: PrP allotype fraction estimates in PrP^{res} from brain of PrP scrapie and BSE
575 infected sheep with different *PRNP* genotypes. a, Western blot of scrapie and BSE
576 PrP^{res} samples of infected sheep with heterozygous and homozygous genotypes as
577 tested with the L42-SAF84 antibody combination. Lanes: 1 and 8, rec-ovinePrP; 2
578 and 9, molecular mass standards; 3-5, VRQ/VRQ sheep with scrapie; 6-7 ARR/VRQ
579 sheep with scrapie; 10-12, VRQ/VRQ sheep with BSE; 13-14, ARR/VRQ with BSE;
580 15-16 ARR/ARR sheep with BSE. Blotting procedures followed the triplex WB
581 method as described (38, 39). Tissue equivalents per each brain sample applied
582 were 0.5 mg per lane. b, VRQ- or ARQ-PrP and ARR-PrP allotype fractions per
583 genotype group of sheep with scrapie or BSE. Genotypes are given for PrP-amino
584 acid residue positions 136, 154 and 171; XRQ means combined data from either
585 three (scrapie: ARQ/ARQ, VRQ/VRQ, ARQ/VRQ) or two genotypes (BSE:
586 ARQ/ARQ, VRQ/VRQ) respectively. The results of the two antibody combinations –
587 SAF84/L42 and SAF84/Sha31 - are presented and appeared very similar. Bar fillings:
588 black represent the VRQ- and/or ARQ-PrP fraction, open the ARR-PrP fraction. The
589 number within the bars reflect the average XRQ-PrP fraction, and vertical lines the
590 standard deviation of the XRQ fraction. Individual sample numbers are given as n=#.

591

592 Figure 2: Probing the VRQ-PrP allotype level between input and calculated output
593 level in PrP^{res} samples in dose response mixing experiments. See Methods section
594 for design of experiment. For both duplex antibody combinations similar concave
595 curves were obtained. These hollow curves were used for calculation of the final
596 data in Figure 1b. Thus a sample with an output value of 20, 40, 60 or 80% VRQ-PrP
597 allotype, yielded in case of the SAF84/L42 combination respectively 30, 55, 72, and

598 87% and for the SAF84/Sha31 29, 51, 67 and 86% VRQ-PrP. The inset presents the
599 values of the calculated regression lines derived from the data points.

600

601 Fig. 3: Relation between PrP^{res} concentration and VRQ-PrP level of ARR/VRQ sheep
602 brain. For individual samples from ARR/VRQ sheep the PrP concentration in the
603 samples was calculated using recPrP as standard in both blots probed with the
604 SAF84/L42 (closed circles) and SAF84/Sha31 (open triangles) antibody combination
605 (see Methods section). The VRQ-PrP levels were in all individual samples around 1
606 in the scrapie samples and 0.5 in the BSE samples. The linear regression formulae
607 for the two antibody combinations data point to near horizontal curves, indicative for
608 absence of a concentration effect on the Fr(171Q-VRQ) values in the triplex-WB
609 methodology used.

610

611 **Table I: Sheep genotypes, TSE type tissues, laboratory origin and breed^a**

612

TSE	genotype	# of cases	lab source	breed
i.c. 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-WageningenUR	Texel-cross breed
	VRQ/VRQ	2	CVI-WageningenUR	Texel-cross breed
	ARQ/ARQ	4	CVI-WageningenUR	Texel-cross breed
	ARQ/VRQ	4	CVI-WageningenUR	Texel-cross breed

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

617 ^b i.c., intracerebral infection.

618 ^c Publication of detailed study in preparation (Houston and Hunter).

619

620

621

622 **Table II. Susceptibility dependence on TSE/prion type and host PrP**
 623 **polymorphism^a.**

624

disease type	PrP allotype susceptible to acquire		
	disease type		
	most	medium	least
BSE	wt	V ₁₃₆	R ₁₇₁
classical scrapie	V ₁₃₆	wt	R ₁₇₁
atypical/Nor98 scrapie	wt	R ₁₇₁	V ₁₃₆

625 ^a Susceptibility is presented in a qualitative way for the single amino acid allotype.

626 Wild type represents the A₁₃₆R₁₅₄Q₁₇₁ allele. Data about BSE are from experimental

627 infections, classical scrapie from natural and experimental infections, atypical/Nor98

628 scrapie from active monitoring in a number of European countries.

629

630





