Characterization of atypical scrapie cases from Great Britain in transgenic ovine PrP mice

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Twenty-four atypical scrapie cases from sheep with different prion protein genotypes from Great Britain were transmitted to transgenic tg338 and/or TgshpXI mice expressing sheep PrP alleles, but failed to transmit to wild-type mice. Mean incubation periods were 200–300 days in tg338 mice and 300–500 days in TgshpXI mice. Survival times in C57BL/6 and VM/Dk mice were >700 days. Western blot analysis of mouse brain samples revealed similar multi-band, protease-resistant prion protein (PrP(res)) profiles, including an unglycosylated band at ~8–11 kDa, which was shown by antibody mapping to correspond to the ~93–148 aa portion of the PrP molecule. In transgenic mice, the incubation periods, Western blot PrP(res) profiles, brain lesion profiles and abnormal PrP (PrP(Sc)) distribution patterns produced by the Great Britain atypical scrapie isolates were similar and compatible with the biological characteristics of other European atypical scrapie or Nor98 cases.

Scrapie is a transmissible, prion protein (PrP) disorder that causes a progressive degeneration of the central nervous system (CNS) of sheep and goats. Although recognized for centuries, and characterized by the vacuolar pathology the disease produces in the brain, the aetiology of this transmissible spongiform encephalopathy (TSE) as a prion disease only emerged in the 1980s during the biochemical investigation of the nature of the transmissible agent (Prusiner, 1982). Prions are cellular proteins that can transfer metabolic and pathological phenotypes vertically from parent to progeny or horizontally between cells and animals (Wickner, 1996). Scrapie is characterized by the accumulation of the abnormal prion form (PrP(Sc)) of the normal cellular prion protein (PrP(C)) in the CNS or peripheral tissues of sheep and goats. Whereas PrP(C) is completely hydrolysed by proteinase K (PK) under mildly denaturing conditions, a core structure of PrP(Sc) (i.e. PrP(res)) resists degradation due to its differing molecular shape and/or degree of aggregation (Hope et al., 1986). Different alleles of the PrP gene are linked to the susceptibility and disease incubation period of an animal naturally or experimentally exposed to prions (Westaway et al., 1987; Goldmann et al., 1990; Hunter et al., 1996) and their relative effects can change depending on the prion type or strain (Goldmann et al., 1994).

Historically, clinical signs, biological transmission properties and brain pathology have been used to classify scrapie isolates, while more recently categorization based on the electrophoretic mobility and immunochemical

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Supplementary figures are available with the online version of this paper.
properties of the prion form of PrP\(^{C}\) has developed (Beringue et al., 2008). In 1998, a novel type of prion protein disorder was identified in sheep in Norway and termed Nor98 (Benestad et al., 2003). This atypical form of scrapie is found predominantly in sheep carrying PrP genotypes associated with relative resistance to classical scrapie (Moum et al., 2005; Saunders et al., 2006; Lühken et al., 2007). Atypical scrapie prions exhibit a different protease-resistant (PrP\(^{\text{R}}\)) core structure compared with the \(\sim 27–30, 23–26\) and \(21\) kDa triplet profile of classical scrapie (Hope et al., 1999; Baron et al., 2000; Stack et al., 2002), including identification of a prominent low molecular mass band at \(\sim 11\) kDa by Western blot analysis (Buschmann et al., 2004; Klingeborn et al., 2006; Gretzschel et al., 2006; Arsac et al., 2007). The disease has also been detected in goats where it was speculated, based on different PrP\(^{\text{Sc}}\) immunohistochemical (IHC) patterns, that it could be found as several biological types (Seuberlich et al., 2007).

Transmission of atypical scrapie cases from France and Germany, and Nor98 from Norway, showed that the disease could be transferred experimentally to transgenic mice (Le Dur et al., 2005; Arsac et al., 2009; A. B.-B. and M. H. G., personal communication). The purpose of this study was to characterize atypical scrapie cases from sheep identified by active surveillance of British abattoirs during 2002–2003 (Elliott et al., 2005; Everest et al., 2006) and to determine their transmissibility to transgenic and wild-type mice. Tg338 mice [expressing ovine PrP\(^{\text{VRQ}}\)] (Vilotte et al., 2001; Le Dur et al., 2005), TgshpXI mice (expressing ovine PrP\(^{\text{ARQ}}\)) (Kupfer et al., 2006) and wild-type C57BL/6 (Prnp\(^{+/+}\)) and VM/Dk (Prnp\(^{+/-}\)) mice (Bruce et al., 1992, 2002) were selected for bioassay to allow comparison of the clinical, biochemical and pathological transmission characteristics of the British cases.

Caudal medulla samples from 24 cases of atypical scrapie from British sheep were prepared as \(10\%\) (w/v) homogenates in saline for inoculation into tg338, TgshpXI, C57BL/6 and VM/Dk mice. Homogenates were treated with ampicillin (1.25 mg ml\(^{-1}\), 24 h, 4 °C), and gentamicin (0.25 mg ml\(^{-1}\)) if required, to eliminate bacterial contamination. Heavily contaminated inoculum 9 was heat-inactivated (70 °C, 15 min, twice). The presence of abnormal PrP was confirmed in a sample of each inoculum prior to injection (TeSeE rapid assay; Bio-Rad; Table 1).

Transmission studies were carried out at the Veterinary Laboratories Agency (VLA), the Friedrich-Loeffler-Institut (FLI) and the Institut National de la Recherche Agronomique (INRA) in accordance with national legal, ethical and welfare regulations. Groups of 20 tg338, TgshpXI, C57BL/6 and VM/Dk mice at \(\sim 8\) weeks old were inoculated with \(10\%\) (w/v) brain homogenates from atypical scrapie and control cases following isoflurane anaesthesia (VLA). Combined intracerebral (i.c.; right parietal lobe; 20 \(\mu\)l) and intraperitoneal (i.p.; 100 \(\mu\)l) inoculations were conducted. Groups of 12 tg338 (INRA) or 15 TgshpXI mice (FLI) were inoculated i.c. only (20 \(\mu\)l). Inoculations of several atypical scrapie isolates, a classical scrapie case, and a sample from a classical scrapie-free flock (Simmons et al., 2009), were duplicated between laboratories for control purposes. Mice showing clinical signs of disease were euthanized and the brains recovered aseptically for confirmatory studies. Brains were hemisected, with one half frozen for Western blot analysis and the other half fixed in formal saline for neuropathology. Incubation period (IP) was defined as the duration in days post-inoculation (p.i.) to termination where transmission was confirmed; survival times were used where no cases of prion disease were confirmed in an inoculated group.

All 24 atypical scrapie isolates derived from sheep of different genotypes transmitted disease to tg338 and/or TgshpXI mice (Table 1). Affected mice developed a progressive ataxia predominantly affecting hind-limb movement. Clinical signs included weight loss, rough coat, pinched face, hunched posture, lethargy and marked affected gait.

In tg338 mice, the mean IP of 11 transmitted atypical scrapie isolates was 222 days p.i. at VLA (mean range 199–233 days) (Table 1). At INRA, the mean IP for a different set of 11 isolates was 245 days p.i. (mean range 220–274 days). Four isolates common to both inoculation sets demonstrated longer mean IPs at INRA by 16–38 days. The most likely explanation is that mice were euthanized at an earlier time point of the clinical phase at VLA.

In TgshpXI mice, the mean IP of eight transmitted atypical cases was 341 days at VLA (mean range 297–390 days) (Table 1). At FLI, the mean IP of 11 transmitted atypical cases was 383 days (mean range 319–482 days). For duplicated isolates, two demonstrated longer IPs at FLI than VLA by 43–75 days, whereas the third produced similar IPs (319 and 329 days, respectively). Although of little bearing on the scientific interpretation of these data, the number of confirmed transmitted cases per inoculation group was lower at FLI than VLA for unknown reasons.

Inter-site variation in IPs and attack rates preclude combining the data from the three laboratories to calculate any significant effects of sheep donor genotype or inoculum PrP\(^{\text{Sc}}\) content on IPs for these transgenic mouse lines. For the few inocula where a comparison was possible: for the same site, transgenic model and donor genotype, PrP\(^{\text{Sc}}\) levels in inocula (Table 1, mean ELISA absorbance) were inversely correlated with the IP in transgenic mice. In contrast to the subline data for TgOvPrP4 mice reported by Arsac et al. (2009), we did not see a significant effect of sheep donor genotype on IP in tg338 or TgshpXI mice. However, such an effect cannot be ruled out and could be revealed by titration or use of tg338 or TgshpXI sublines with lower expression levels.

At VLA, inoculation of 11 atypical scrapie isolates into C57BL/6 and VM/Dk mice failed to produce a single confirmed case of transmitted disease, with all groups of...
### Table 1. IP analysis of atypical scrapie cases inoculated into transgenic and wild-type mice

ND, Not done; NA, not applicable. Cases 5, 6, 26, 45, 25 and 24 were inoculated at both VLA and INRA, and cases 7, 27, 45, 25 and 24 were inoculated at both VLA and FLI.

<table>
<thead>
<tr>
<th>Inoculum PrP genotype</th>
<th>Mean ELISA OD</th>
<th>tg338 (VLA) N/N±</th>
<th>Mean IP ± SEM</th>
<th>tg338 (INRA) N/N±</th>
<th>Mean IP ± SEM</th>
<th>TgshpXI (VLA) N/N±</th>
<th>Mean IP ± SEM</th>
<th>TgshpXI (FLI) N/N±</th>
<th>Mean IP ± SEM</th>
<th>C57BL/6 (VLA) N/N±</th>
<th>Mean IP ± SEM</th>
<th>VM/Dk (VLA) N/N±</th>
<th>Mean IP ± SEM</th>
<th>Survival time‡</th>
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<td><strong>Atypical scrapie cases</strong></td>
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<td>0.368§</td>
<td>19/20</td>
<td>210 ± 3</td>
<td>ND</td>
<td>18/20</td>
<td>327 ± 14</td>
<td>ND</td>
<td>0/20</td>
<td>811</td>
<td>0/18</td>
<td>778</td>
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<td>ND</td>
<td>14/15</td>
<td>341 ± 14</td>
<td>ND</td>
<td>0/18</td>
<td>860</td>
<td>0/20</td>
<td>778</td>
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<td>13/19</td>
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<td>229 ± 5</td>
<td>10/10</td>
<td>247 ± 12</td>
<td>ND</td>
<td>0/20</td>
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<td>359 ± 7</td>
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<td>15/15</td>
<td>297 ± 7</td>
<td>ND</td>
<td>0/20</td>
<td>852</td>
<td>0/20</td>
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<td>16/20</td>
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<td>438 ± 4</td>
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<td>ND</td>
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<td>251 ± 5</td>
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<td>—</td>
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<td>12/12</td>
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<td>17/20</td>
<td>233 ± 6</td>
<td>ND</td>
<td>15/18</td>
<td>329 ± 32</td>
<td>6/13</td>
<td>319 ± 10</td>
<td>0/19</td>
<td>804</td>
<td>0/19</td>
<td>734</td>
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<td>1.3</td>
<td>—</td>
<td>ND</td>
<td>ND</td>
<td>11/15</td>
<td>356 ± 2</td>
<td>ND</td>
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<td>—</td>
<td>ND</td>
<td>12/12</td>
<td>239 ± 6</td>
<td>ND</td>
<td>—</td>
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<td>0.497</td>
<td>—</td>
<td>ND</td>
<td>ND</td>
<td>5/15</td>
<td>334 ± 28</td>
<td>ND</td>
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<td>—</td>
<td>ND</td>
<td>12/12</td>
<td>274 ± 9</td>
<td>ND</td>
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<td>ND</td>
<td>ND</td>
<td>8/15</td>
<td>341 ± 16</td>
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<tr>
<td>39 ARR/ARR</td>
<td>1.074</td>
<td>—</td>
<td>ND</td>
<td>11/11</td>
<td>227 ± 9</td>
<td>ND</td>
<td>5/14</td>
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<td>0.108</td>
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<td>ND</td>
<td>ND</td>
<td>12/15</td>
<td>482 ± 33</td>
<td>ND</td>
<td>—</td>
<td>ND</td>
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<td>ND</td>
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<td>0.835</td>
<td>19/19</td>
<td>213 ± 3</td>
<td>11/11</td>
<td>251 ± 7</td>
<td>ND</td>
<td>8/14</td>
<td>422 ± 29</td>
<td>0/18</td>
<td>700</td>
<td>0/20</td>
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<td>ND</td>
<td>12/12</td>
<td>256 ± 6</td>
<td>ND</td>
<td>—</td>
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<td>0.505</td>
<td>20/20</td>
<td>229 ± 4</td>
<td>ND</td>
<td>19/20</td>
<td>379 ± 16</td>
<td>3/15</td>
<td>454 ± 21</td>
<td>0/20</td>
<td>700</td>
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<td>2.459§</td>
<td>16/16</td>
<td>391 ± 22</td>
<td>9/9</td>
<td>267 ± 3</td>
<td>8/9</td>
<td>519 ± 26</td>
<td>9/15</td>
<td>516 ± 33</td>
<td>4/20I</td>
<td>622 ± 28</td>
<td>1/19I</td>
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<td><strong>Negative control</strong></td>
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<td>24 ARR/ARR</td>
<td>0.020§</td>
<td>3/14</td>
<td>NA</td>
<td>0/12</td>
<td>NA</td>
<td>ND</td>
<td>ND</td>
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<td>891</td>
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<td>797</td>
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mice demonstrating survival times of >700 days (Table 1). Furthermore, these wild-type models are also inefficient monitors for natural scrapie and with the classical scrapie-positive control used here exhibited poor attack rates of 4/20 (C57BL/6) and 1/19 (VM/Dk), with a range of IPs of 540–666 and 700 days, respectively (Table 1).

In Western blot analysis detection of atypical scrapie PrPres, frozen brain samples were ribolysed (Omigene) in homogenization buffer to produce 20% (w/v) homogenates, which were extracted, treated with PK and analysed according the manufacturer’s instructions (TeSeE Western blot; Bio-Rad).

Western blot analysis of brain from tg338 mice clinically affected by Great Britain (GB) atypical scrapie cases showed very similar PrPres profiles, with immuno-reactive bands at ~31, 26.6, 24 and 18 kDa as well as a prominent low molecular mass band at ~8–11 (10.6) kDa, as detected by monoclonal antibody Sha31 (Fig. 1, lanes 1–10).

These profiles were similar to or indistinguishable from those of Nor98/atypical scrapie and the French discordant cases transmitted to the same transgenic line and differed considerably from the triple band profile of classical scrapie in sheep (Fig. 1, lane 11) or in TgshpXI or tg338 mice (data not shown). Whereas PrPres was detected by immunoblotting in the spleen of tg338 mice infected with classical scrapie (data not shown), PrPres was not detectable in the spleen of tg338 mice infected with atypical scrapie at terminal disease, which was similar to the findings of Le Dur et al. (2005). Western blot PrPres profiles obtained for TgshpXI mouse brain affected by atypical scrapie (data not shown) were essentially similar to tg338 profiles.

To deglycosylate PrPres, PK-treated tg338 brain extracts were treated with methanol (7 vols, −20 °C, 2 h) and centrifuged (13 000 g, 30 min) to precipitate proteins, which were resuspended in deionized water and treated with peptide N-glycosidase F (PNGaseF; New England Biolabs), at 37 °C overnight, according to the manuf-
turer’s recommendations. Methanol-precipitated pellets of deglycosylated proteins were analysed by Western blotting (as above).

Epitope mapping of atypical scrapie PrP\textsuperscript{res} (before and after removal of N-linked glycans by PNGaseF; Supplementary Fig. S1a–i, available in JGV Online) produced by transmission of isolate 6 (ARR/AHQ) to tg338 mice was consistent with the \~8–10 kDa PrP\textsuperscript{res} fragment being a polypeptide of \~60 aa. This fragment is likely to correspond to codons \~93–148 of the ovine PrP gene and agrees with data obtained by others of the fragmentation of atypical scrapie PrP\textsuperscript{Sc} in brains of sheep expressing mainly AF\textsuperscript{141}RQ, ARR, AHQ or AL\textsuperscript{141}RQ PrP gene alleles (Klingeborn et al., 2006; Gretzschel et al., 2006; Arsac et al., 2007). As tg338 mice overexpress PrP\textsuperscript{VRQ} and TgshpXI mice overexpress the ALRQ allotype, the similarity of the molecular stability profiles of atypical scrapie PrP\textsuperscript{res} clearly shows independence of the PrP genotype and represents a true strain characteristic distinguishing atypical scrapie from its classical counterpart. PrP\textsuperscript{res} was not detected in C57BL/6 and VM/Dk mice inoculated with atypical scrapie isolates (data not shown), which was consistent with the report of non-transmission of the disease to wild-type mice by Le Dur et al. (2005). For comment on the results obtained with negative control inoculum 24 (Table 1) see Supplementary Fig. S2 (available in JGV Online) for details.

Fixed, paraffin-embedded mouse brains were sectioned at the levels of basal ganglia, thalamus, superior colliculus and medulla. Sections were cut and stained with haematoxylin and eosin, according to standard protocols (http://www.defra.gov.uk/vla/science/docs/sci_tse_rl_prp_ihc.pdf) for histopathology and lesion profiling. Vacuolation was assessed semi-quantitatively in nine grey and three white matter brain areas. For each area, the average degree of vacuolar severity from mice inoculated with a specific TSE source was plotted against the corresponding brain area to produce lesion profiles (Fraser & Dickinson, 1968). IHC was performed according to standard protocols (as above) at the same coronal levels as for histopathology, using serial sections, with the detection of abnormal PrP by C-terminal anti-PrP polyclonal antibody Rb486.

Lesion profiles of the atypical scrapie cases derived from \(>10\) clinically affected tg338 mice per inoculation group are presented (Fig. 2a). A greater vacuolation intensity in four grey and white matter areas (G5, G6, G8 and W3) was revealed. These profiles differed from those of classical scrapie in tg338 mice (Thackray et al., 2008) and agreed with published profiles of Nor98 and the French discordant...
In IHC analysis of tg338 mice, comparison of the PrPSc distribution in coronal brain sections to Nor98 and the French discordant cases revealed that the GB atypical cases (Fig. 2c) were indistinguishable from Nor98 and Nor98-like cases reported from other countries, shown by histoblot analysis (Le Dur et al., 2005). PrPSc patterns in tg338 mice inoculated at VLA and INRA were also indistinguishable.

In TgshpXI mice, vacuolation, IHC and Western blot analysis were used to diagnose atypical scrapie. IHC analysis revealed that TgshpXI mice inoculated with atypical GB cases had a similar PrPSc distribution restricted mainly to the hippocampus and cerebellum (Fig. 2d). Compared with tg338 (Fig. 2c), the distribution and intensity of PrPSc staining in TgshpXI brains were restricted.

In recent years, rapid surveillance testing of healthy slaughter animals in Europe has led to the discovery of apparently novel cattle and sheep prion diseases. During 2002–2003 in GB, biochemical testing of sheep brainstems and designation of a new type, Nor98. However, at the time this study was initiated little was known about the diversity or transmissibility of atypical scrapie, or whether (like variant Creutzfeldt–Jakob disease in humans) cases had emerged as a consequence of cross-species transmission of BSE from cattle to sheep.

IHC mapping of abnormal PrP in Swiss atypical scrapie cases had suggested there was biological variation in the characteristics of this disease in sheep and goats (Seuberlich et al., 2007), and investigations of the biochemical characteristics of PrPSc from GB cases indicated there could be several molecular types (Everest et al., 2006). Here, we present the transmission of 24 independent isolates to transgenic mice expressing different ovine PrP allotypes, the similarities of these isolates in their biochemical and immunohistological profiles in mouse brain, and their universal failure to transmit to strains of wild-type mice expressing physiological levels of murine PrP. The GB cases present examples of transmissible prion diseases with similar if not identical biology, pathology and biochemistry and our data confirm studies in France (Le Dur et al., 2005; Arsac et al., 2007, 2009) and Germany (Gretzschel et al., 2006) showing that European atypical scrapie cases differ in phenotype from experimental BSE in sheep, behave as if due to a single prion strain and resemble Nor98 (Benestad et al., 2003).

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References


