Short Communication

Association of N176K and L141F dimorphisms of the PRNP gene with lack of pathological prion protein deposition in placentas of naturally and experimentally scrapie-affected ARQ/ARQ sheep

Cinzia Santucciu,† Caterina Maestrale,† Laura Madau,† Sonia Attene,† Maria Giovanna Cancedda,† Franca Demontis,† Maria Giovanna Tilocca,† Mariangela Saba,† Simona Macciozuc,† Antonello Carta2 and Ciriaco Ligios1

1Istituto Zooprofilattico Sperimentale della Sardegna, 07100 Sassari, Italy
2Research Unit, Genetics and Biotechnology, DIRPA AGRIS Sardinia, 07040 Olmedo, Italy

The placenta is important in the horizontal transmission of the aetiologial agent in scrapie-affected sheep. It has been demonstrated that the placentas of fetuses carrying the dimorphism Q171R of the PRNP gene is resistant to pathological prion protein (PrPSc) accumulation in the placenta. To test whether other PRNP polymorphisms are associated with a lack of placental PrPSc deposition, we carried out a study on 26 naturally and 11 experimentally scrapie-affected ewes with or without clinical signs. PrPSc was detected in the placenta of ARQ/ARQ wild type fetuses by Western blot and immunohistochemical analysis, but not in ARQ/N176K/ARQK141 or, as expected, ARQ/ARR samples. Furthermore, three of four AL141RQ/AF141RQ placentas were also PrPSc negative, suggesting that the dimorphism at codon 141 may also mediate placental deposition of PrPSc. This finding demonstrates for the first time that fetal PRNP polymorphisms, other than those at codon 171, are associated with the lack of placental deposition of PrPSc.

Received 19 February 2010
Accepted 11 May 2010

Scrapie belongs to the family of transmissible spongiform encephalopathies (TSEs) and is a fatal neurodegenerative disorder of sheep and goat. The most important hallmark of all TSEs is the accumulation of an abnormal isoform of prion protein (PrPSc), an isoform of the host-encoded protein cellular prion protein (PrPC) in the central nervous system (Wells & McGill, 1992).

It is commonly known that susceptibility or resistance to the disease is linked to polymorphisms at codons 136 (A or V), 154 (R or H) and 171 (Q, R or H) of the PrPSc-encoding gene (PRNP) (Basler et al., 1986; Hunter, 1997). The susceptibility to scrapie increases after substitution of A with V at codon 136, while replacement of Q with R at codon 171 confers resistance (Hunter, 1997). The ancestral PRNP genotype, believed to be ARQ/ARQ (ARQ/ARQwild type) (Goldmann et al., 2005), increases susceptibility in some breeds, including the Sarda breed (Vaccari et al., 2001; Ligios et al., 2006).

Studies in naturally and experimentally scrapie-affected sheep have shown that these polymorphisms determine characteristic patterns of PrPSc deposition and pathology, such as the accumulation of PrPSc in the placenta, which occurs only when the fetuses carry the QQ171 genotype. The amino acid substitution of Q with R at the fetal PRNP codon 171 confers resistance to PrPSc deposition in the placenta of scrapie-affected sheep carrying either V (Andréoletti et al., 2002) or A (Tuo et al., 2002) at codon 136.

Following large-scale studies of sheep genotyping in different breeds, additional polymorphisms of the PRNP gene have been described, mostly in association with the ARQ allele, although their frequencies are estimated to be very low (Goldmann et al., 2005). It was recently demonstrated that amino acid substitutions at some of these polymorphisms are associated with resistance to scrapie and bovine spongiform encephalopathy (BSE) in ARQ/ARQ sheep (Vaccari et al., 2007, 2009; Laegreid et al., 2008; Maestrale et al., 2009; Saunders et al., 2009). Consequently, it is of interest whether they are also related to PrPSc deposition in the placenta of scrapie-infected ewes. To address this important question, we studied the accumulation of PrPSc in placentas of ARQ/ARQ fetuses with the L141F and N176K dimorphisms from Sarda ewes clinically or preclinically affected with natural or experimental scrapie.

The first part of our study was performed on 11 scrapie-affected flocks in which an eradication plan was carried out

†These authors contributed equally to this work.
A supplementary figure is available with the online version of this paper.
according to European regulations. By applying Western blot (WB) and immunohistochemical (IHC) analyses to medulla oblongata (obex) and palatine tonsils of the slaughtered sheep from these flocks, we found 26 naturally scrapie-affected pregnant sheep aged 2–5 years: ARQ/ARQ$_{\text{wild type}}$ (n=24) and AL141RQ/AF141RQ (n=2). From the 26 sheep, of which 13 showed neurological signs and 13 were asymptomatic (Table 1), we collected numerous placental cotyledons that, in the case of twin pregnancy, were taken separately from each of the two placentas. All samples were halved and one part was frozen at $-20^\circ$C, while the other was embedded in paraffin. For $PRNP$ genotyping, spleen DNA was isolated from the fetuses and stored at $-20^\circ$C.

Table 1. Details of the scrapie-affected ewes and fetuses under study, including the $PRNP$ genotype and PrP$_{\text{Sc}}$ distribution by WB and IHC

<table>
<thead>
<tr>
<th>Ewe no.</th>
<th>Scrapie source</th>
<th>$PRNP$ genotype of ewes</th>
<th>Clinical status of ewes</th>
<th>PrP$_{\text{Sc}}$ detection in ewes</th>
<th>Fetus genotype</th>
<th>Fetal gestational age</th>
<th>PrP$_{\text{Sc}}$ in fetal cotyledons</th>
<th>WB</th>
<th>IHC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Natural</td>
<td>ARQ/ARQ$_{\text{wild type}}$</td>
<td>Asymptomatic</td>
<td>+</td>
<td>ARQ/ARR</td>
<td>Early</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Natural</td>
<td>ARQ/ARQ$_{\text{wild type}}$</td>
<td>Asymptomatic</td>
<td>+</td>
<td>ARQ/ARR</td>
<td>Early</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Natural</td>
<td>ARQ/ARQ$_{\text{wild type}}$</td>
<td>Asymptomatic</td>
<td>+</td>
<td>ARQ/ARR</td>
<td>Late</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Natural</td>
<td>ARQ/ARQ$_{\text{wild type}}$</td>
<td>Symptomatic</td>
<td>+</td>
<td>ARQ/ARR</td>
<td>Middle</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Natural</td>
<td>ARQ/ARQ$_{\text{wild type}}$</td>
<td>Symptomatic</td>
<td>+</td>
<td>ARQ/ARR</td>
<td>Late</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Natural</td>
<td>ARQ/ARQ$_{\text{wild type}}$</td>
<td>Symptomatic</td>
<td>+</td>
<td>ARQ/ARR</td>
<td>Late</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Natural</td>
<td>ARQ/ARQ$_{\text{wild type}}$</td>
<td>Symptomatic</td>
<td>+</td>
<td>ARQ/ARR</td>
<td>Late</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Natural</td>
<td>ARQ/ARQ$_{\text{wild type}}$</td>
<td>Symptomatic</td>
<td>+</td>
<td>ARQ/ARR</td>
<td>Late</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Natural</td>
<td>ARQ/ARQ$_{\text{wild type}}$</td>
<td>Symptomatic</td>
<td>+</td>
<td>ARQ/ARR</td>
<td>Late</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Natural</td>
<td>ARQ/ARQ$_{\text{wild type}}$</td>
<td>Symptomatic</td>
<td>+</td>
<td>ARQ/ARR</td>
<td>Late</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Natural</td>
<td>ARQ/ARQ$_{\text{wild type}}$</td>
<td>Symptomatic</td>
<td>+</td>
<td>ARQ/ARR</td>
<td>Late</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Natural</td>
<td>ARQ/ARQ$_{\text{wild type}}$</td>
<td>Symptomatic</td>
<td>+</td>
<td>ARQ/ARR</td>
<td>Late</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Natural</td>
<td>ARQ/ARQ$_{\text{wild type}}$</td>
<td>Symptomatic</td>
<td>+</td>
<td>ARQ/ARR</td>
<td>Late</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Natural</td>
<td>ARQ/ARQ$_{\text{wild type}}$</td>
<td>Asymptomatic</td>
<td>+</td>
<td>ARQ$<em>{\text{N176/K}}$/ARQ$</em>{\text{K176}}$</td>
<td>Early</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Natural</td>
<td>ARQ/ARQ$_{\text{wild type}}$</td>
<td>Asymptomatic</td>
<td>+</td>
<td>ARQ$<em>{\text{N176/K}}$/ARQ$</em>{\text{K176}}$</td>
<td>Late</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>Natural</td>
<td>ARQ/ARQ$_{\text{wild type}}$</td>
<td>Asymptomatic</td>
<td>+</td>
<td>ARQ$<em>{\text{N176/K}}$/ARQ$</em>{\text{K176}}$</td>
<td>Late</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>Natural</td>
<td>ARQ/ARQ$_{\text{wild type}}$</td>
<td>Asymptomatic</td>
<td>+</td>
<td>ARQ$<em>{\text{N176/K}}$/ARQ$</em>{\text{K176}}$</td>
<td>Late</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>Experimental</td>
<td>ARQ/ARQ$_{\text{wild type}}$</td>
<td>Symptomatic</td>
<td>+</td>
<td>ARQ$<em>{\text{N176/K}}$/ARQ$</em>{\text{K176}}$</td>
<td>Partum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>Experimental</td>
<td>ARQ/ARQ$_{\text{wild type}}$</td>
<td>Symptomatic</td>
<td>+</td>
<td>ARQ$<em>{\text{N176/K}}$/ARQ$</em>{\text{K176}}$</td>
<td>Partum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>Experimental</td>
<td>ARQ/ARQ$_{\text{wild type}}$</td>
<td>Symptomatic</td>
<td>+</td>
<td>AL$<em>{141}$/AF$</em>{141}$/ARQ$_{\text{RQ}}$</td>
<td>Partum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>Natural</td>
<td>AL$<em>{141}$/RQ/AF$</em>{141}$/RQ</td>
<td>Asymptomatic</td>
<td>+</td>
<td>AL$<em>{141}$/RQ/AF$</em>{141}$/RQ</td>
<td>Late</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>Natural</td>
<td>ARQ/ARQ$_{\text{wild type}}$</td>
<td>Asymptomatic</td>
<td>+</td>
<td>AL$<em>{141}$/RQ/AF$</em>{141}$/RQ</td>
<td>Late</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>Natural</td>
<td>ARQ/ARQ$_{\text{wild type}}$</td>
<td>Asymptomatic</td>
<td>+</td>
<td>AL$<em>{141}$/RQ/AF$</em>{141}$/RQ</td>
<td>Late</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>Natural</td>
<td>ARQ/ARQ$_{\text{wild type}}$</td>
<td>Asymptomatic</td>
<td>+</td>
<td>ARQ/ARR</td>
<td>Middle</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>Natural</td>
<td>ARQ/ARQ$_{\text{wild type}}$</td>
<td>Asymptomatic</td>
<td>+</td>
<td>ARQ/ARR</td>
<td>Middle</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>Natural</td>
<td>ARQ/ARQ$_{\text{wild type}}$</td>
<td>Asymptomatic</td>
<td>+</td>
<td>ARQ/ARR</td>
<td>Middle</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>Natural</td>
<td>ARQ/ARQ$_{\text{wild type}}$</td>
<td>Asymptomatic</td>
<td>+</td>
<td>ARQ/ARR</td>
<td>Late</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>Natural</td>
<td>AL$<em>{141}$/RQ/AF$</em>{141}$/RQ</td>
<td>Asymptomatic</td>
<td>+</td>
<td>ARQ/ARR</td>
<td>Early</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>Natural</td>
<td>ARQ/ARQ$_{\text{wild type}}$</td>
<td>Symptomatic</td>
<td>+</td>
<td>ARQ/ARR</td>
<td>Middle</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>Natural</td>
<td>ARQ/ARQ$_{\text{wild type}}$</td>
<td>Symptomatic</td>
<td>+</td>
<td>ARQ/ARR</td>
<td>Middle</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>31</td>
<td>Natural</td>
<td>ARQ/ARQ$_{\text{wild type}}$</td>
<td>Symptomatic</td>
<td>+</td>
<td>ARQ/ARR</td>
<td>Middle</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>32</td>
<td>Experimental</td>
<td>ARQ/ARQ$_{\text{wild type}}$</td>
<td>Symptomatic</td>
<td>+</td>
<td>ARQ/ARR</td>
<td>Partum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>33</td>
<td>Experimental</td>
<td>ARQ/ARQ$_{\text{wild type}}$</td>
<td>Symptomatic</td>
<td>+</td>
<td>ARQ/ARR</td>
<td>Partum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>34</td>
<td>Experimental</td>
<td>ARQ/ARQ$_{\text{wild type}}$</td>
<td>Symptomatic</td>
<td>+</td>
<td>ARQ/ARR</td>
<td>Partum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>Experimental</td>
<td>ARQ/ARQ$_{\text{wild type}}$</td>
<td>Symptomatic</td>
<td>+</td>
<td>ARQ/ARR</td>
<td>Partum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>36</td>
<td>Experimental</td>
<td>ARQ/ARQ$_{\text{wild type}}$</td>
<td>Symptomatic</td>
<td>+</td>
<td>ARQ/ARR</td>
<td>Partum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>37</td>
<td>Experimental</td>
<td>ARQ/ARQ$_{\text{wild type}}$</td>
<td>Symptomatic</td>
<td>+</td>
<td>ARQ/ARR</td>
<td>Partum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>38</td>
<td>Experimental</td>
<td>ARQ/ARQ$_{\text{wild type}}$</td>
<td>Symptomatic</td>
<td>+</td>
<td>ARQ/ARR</td>
<td>Partum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>39</td>
<td>Experimental</td>
<td>ARQ/ARQ$_{\text{wild type}}$</td>
<td>Symptomatic</td>
<td>+</td>
<td>ARQ/ARR</td>
<td>Partum</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The second part of the study was carried out on 14 lambs aged 23 days, which were inoculated with 25 ml of a 10% pooled-brain homogenate from eight scrapie-infected ARQ/ARQ sheep (scrapie-affected status confirmed by IHC and WB). Eleven of the 14 experimentally inoculated sheep had the ARQ/ARQ<sub>wild</sub> type genotype, while three were ARQN<sub>176</sub>/ARQK<sub>176</sub>. At 15 months of age, these lambs were naturally inactivated by two rams, one carrying the AL<sub>141</sub>RQ/AF<sub>141</sub>RQ and the other the ARQN<sub>176</sub>/ARQK<sub>176</sub> genotype, in order to produce ARQ/ARQ fetuses with or without these allelic variants. From these sheep, the placenta was collected and several cotyledons were analysed for PrP<sup>S<sub>c</sub></sup> by WB and IHC. DNA was isolated from the blood of the newborn lambs to determine the entire PRNP gene sequence. By performing WB and IHC analysis on lymphoid and nervous tissues, the scrapie status was assessed in both the neurologically sick (slaughtered by 1 month post-partum) and clinically healthy ewes (slaughtered at later dates).

WB for PrP<sup>S<sub>c</sub></sup> detection on the frozen samples, IHC staining for PrP<sup>S<sub>c</sub></sup> visualization on the paraffin embedded samples and PRNP gene sequencing were performed as previously described by the authors (Ligios et al., 2006).

To estimate the relative amount of PrP<sup>S<sub>c</sub></sup> µg<sup>-1</sup>, we compared the intensity of the WB signal of some tested cotyledons to a standard curve generated from serial brain dilutions. To do this we used the above-mentioned WB procedure, in which the quantification of the total loaded brain and cotyledon proteins was performed with the BCA Protein Assay kit (23-225 BCA Protein Assay kit; Pierce). The PrP<sup>S<sub>c</sub></sup> bands detected by WB analysis from the cotyledons were quantified by densitometric analysis (Chemidoc Imager System; Bio-Rad) as a proportion of signal per mg of total brain protein loaded. Tissue samples were taken from two different cotyledons. For each sample, the WB was repeated three times (Supplementary Fig. S1, available in JGV Online). The mean of the values obtained in the three different WB was used to define the final value.

After slaughtering of the 26 sheep with natural scrapie, the fetuses (n=29) were distributed by morphological evaluation into three different gestational ages, namely early (1–50 days), middle (51–100 days) and late (101–150 days) (Table 1).

DNA sequencing of PRNP established that 16 of the 29 fetuses had the genotype ARQ/ARQ with (n=7) or without (n=9) additional mutations, while the remaining 13 were of the ARQ/ARR genotype (Table 1).

Eight of the nine placenta from the ARQ/ARQ<sub>wild</sub> type fetuses displayed PrP<sup>S<sub>c</sub></sup> with substantial individual variation by WB (Fig. 1a) and IHC, while no PrP<sup>S<sub>c</sub></sup> was observed in those from ARQ/ARR fetuses. All but one of the seven ARQ/ARQ fetal placentas carrying the dimorphism N176K and/or L141F did not display PrP<sup>S<sub>c</sub></sup> deposition (Table 1 and Fig. 1a).

An AL<sub>141</sub>RQ/AF<sub>141</sub>RQ fetal placenta showed a weak PrP<sup>S<sub>c</sub></sup> WB signal in a dizygotic twin pregnancy in which the other placenta carried the ARQ/ARQ<sub>wild</sub> type genotype (sheep no. 23, see Table 1). In this case, PrP<sup>S<sub>c</sub></sup> densitometric quantification indicated that the amount of PrP<sup>S<sub>c</sub></sup> detected in the L141F fetal placenta was approximately eightfold less than in the ARQ/ARQ<sub>wild</sub> type fetal placenta (Fig. 1b). No PrP<sup>S<sub>c</sub></sup> staining was evident by IHC in this L141F placenta, again suggesting that there was a low deposition.

Of the 14 experimentally scrapie-inoculated sheep, only those with the ARQ/ARQ<sub>wild</sub> type genotype (n=11) developed clinical signs of scrapie after a mean incubation period of 600 (SD ± 20) days and they were confirmed to be scrapie-affected sheep by using WB and IHC. Of the three clinically healthy ARQN<sub>176</sub>/ARQK<sub>176</sub> sheep, two were sacrificed at 800 and one at 1800 days post-inoculation. There was no PrP<sup>S<sub>c</sub></sup> deposition in the lymphoid or nervous tissues of any of these three animals.

Among the fetuses derived from the 11 ARQ/ARQ experimentally scrapie-affected sheep all those with the ARQ/ARQ<sub>wild</sub> type genotype (n=8) had PrP<sup>S<sub>c</sub></sup> deposits in the placenta. In contrast, two ARQN<sub>176</sub>/ARQK<sub>176</sub> (from sheep nos 18 and 19) and one AL<sub>141</sub>RQ/AF<sub>141</sub>RQ fetus (from sheep no. 20) were resistant to placental PrP<sup>S<sub>c</sub></sup> deposition (Table 1). Six of the lambs from the 14 sheep were kept...
together in the same sheepfold and monitored over a 45 month period. Interestingly, the ARQ/ARQ<sub>wild</sub> type lambs (from sheep nos 32, 33 and 34) developed scrapie at 19–27 months of age, while those carrying the ARQ<sub>176</sub>/ARQ<sub>176</sub> and AL<sub>141</sub>RQ/AF<sub>141</sub>RQ genotypes (from sheep nos 18, 19 and 20) were still clinically healthy at 45 months of age.

IHC analysis showed that PrP<sub>Sc</sub> deposition was granular, often coalescing, or in plaque-like formations in the cotyledon (Fig. 2a, b). Interestingly, PrP<sub>Sc</sub> deposition was clearly demonstrated in the placenta collected post-partum, even when autolysis was evident (Fig. 2b).

In this work we confirm, as previously described in other sheep breeds carrying V (Andrèoletti <em>et al.</em>, 2002) or A (Tuo <em>et al.</em>, 2002) at codon 136, that the placenta of fetus with the Q171R dimorphism do not accumulate PrP<sub>Sc</sub> (Tuo <em>et al.</em>, 2002; Andrèoletti <em>et al.</em>, 2002; Caplazi <em>et al.</em>, 2004; Ersdal <em>et al.</em>, 2005; Alverson <em>et al.</em>, 2006; Lacroux <em>et al.</em>, 2007). Furthermore, for the first time, we have extended these results to demonstrate that besides the Q171R dimorphism, N176K is also associated with the lack of accumulation of PrP<sub>Sc</sub> in the placenta of scrapie-affected ARQ/ARQ sheep, since six ARQ<sub>176</sub>/ARQ<sub>176</sub> fetuses derived from six ARQ/ARQ<sub>wild</sub> type sheep did not display PrP<sub>Sc</sub> accumulation in the placentas (Table 1).

Our study corroborates previous findings, that reported that the allele ARQ<sub>176</sub> was associated with resistance to classical scrapie in the Sarda breed sheep orally challenged with BSE and scrapie (Vaccari <em>et al.</em>, 2007) or exposed to natural scrapie (Maestrale <em>et al.</em>, 2009; Vaccari <em>et al.</em>, 2009). These recent findings are also confirmed in this study, in which the three ARQ<sub>176</sub>/ARQ<sub>176</sub> sheep experimentally infected with scrapie and the two ARQ<sub>176</sub>/ARQ<sub>176</sub> offspring (from sheep nos 18 and 19) did not develop scrapie.

Several descriptive data suggest that PrP<sub>Sc</sub> is absent in the fetus tissues. Scrapie infection may occur from the environment after birth. In such cases the infected placenta may contribute to the shedding of the aetiological agent (Andrèoletti <em>et al.</em>, 2002). Our study did not explore whether PrP<sub>Sc</sub> was present in the fetal tissues. However, the results obtained in the follow-up on the N176K offspring allow us to state that their dimorphism was related to scrapie resistance because they were exposed to natural scrapie during the peri-partum period and did not become infected, unlike the ARQ/ARQ<sub>wild</sub> type lambs.

It has been shown that placental cotyledons with the ARR allele are negative for PrP<sub>Sc</sub> accumulation (Andrèoletti <em>et al.</em>, 2002) regardless of whether they were heterozygous with the ARQ or VRQ alleles. In this study, we do not have data to show whether the ARQK<sub>176</sub> allele is associated with the absence of PrP<sub>Sc</sub> in cotyledons of genotypes with V at the 136 codon (i.e. ARQ<sub>176</sub>/VRQ<sub>176</sub>).

In ARQ/ARQ sheep, Vaccari <em>et al.</em> (2009) found that a lower risk of classical scrapie was associated with the L141F dimorphism, although the data were only considered to be statistically significant in some flocks. Maestrale <em>et al.</em> (2009) hypothesized that there was an association of this dimorphism with a longer incubation period because they found that, among the scrapie-affected ARQ/ARQ sheep carrying the L141F dimorphism, the number of the asymptomatics was statistically higher than that of the symptomatics.

In our study, three of four AL<sub>141</sub>RQ/AF<sub>141</sub>RQ fetal placentas (from sheep nos 20, 21 and 22) did not have PrP<sub>Sc</sub>, while in the other (sheep no. 23), which shared the uterus with another placenta carrying the ARQ/ARQ<sub>wild</sub> genotype, a weak-positive WB signal for PrP<sub>Sc</sub> was detected. Alverson <em>et al.</em> (2006) reported that although PrP<sub>Sc</sub> deposition in the ARQ/ARR placenta is not noted, when it is located in a unique uterine horn with an ARQ/ARQ fetus, deposition of PrP<sub>Sc</sub> is observed. In this last study it was also demonstrated that PrP<sub>Sc</sub> accumulation in the placenta of resistant genotypes may depend on the sharing of fetal blood between the fetuses. We suggest that a similar phenomenon occurred in our positive AL<sub>141</sub>RQ/AF<sub>141</sub>RQ placenta (from sheep no. 23) because during the sampling we assessed that both the fetuses resided in the same uterine horn. Taken together, these data suggest that in ARQ/ARQ fetal placentas the L141F dimorphism may be associated with the absence of PrP<sub>Sc</sub>.

Among the three sheep with the PrP<sub>Sc</sub>-negative AL<sub>141</sub>RQ/AF<sub>141</sub>RQ placentas, we observed that one of them (sheep no. 22) lacked PrP<sub>Sc</sub> in the tonsil and another (sheep no. 21) carried the L141F dimorphism (Table 1). We cannot rule out that these two conditions may have an additional influence in determining the lack of PrP<sub>Sc</sub> accumulation in the placenta. Lack of PrP<sub>Sc</sub> in placentas has, indeed, been reported in VRQ/VRQ placenta.

![Fig. 2. PrP<sub>Sc</sub> detected by IHC in the placenta of scrapie-affected Sarda sheep. (a) IHC analysis of a ARQ/ARQ<sub>wild</sub> type cotyledon from scrapie-affected sheep. (b) The same analysis in a ARQ/ARQ<sub>wild</sub> type cotyledon from a placenta collected post-partum. F99 was the primary antibody used. Bar, 50 μm.](http://vir.sgmjournals.org)
fetus from scrapie-contaminated ARR/VRQ dams, in which PrPSc did not accumulate in the lymphoid tissues (Lacroix et al., 2007), as has the fact that maternal L141F might also modulate placental PrPSc deposition because it is associated with scrapie resistance (Vaccari et al., 2009; Maestrale et al., 2009).

Interestingly, the only scrapie-affected sheep with a PrPSc-negative ARQ/ARQwild type fetal placenta was an early gestational age AL141RQ/AF141RQ sheep (no. 28). This last observation may additionally suggest that the maternal L141F dimorphism is associated with the lack of placental PrPSc deposition, although absence of PrPSc was described in ARQ/ARQ fetal placentas at an early period of gestation (Tuo et al., 2002).

However, because of the low number of examined placentas in our study, further experiments are required before assessing whether the presence of L141F in the maternal ARQ/ARQ genotype is associated with placental PrPSc accumulation.

In ARQ/ARQ sheep other additional dimorphisms, which are thought to reduce the risk of having scrapie and/or BSE, namely M112T (Laegreid et al., 2008; Saunders et al., 2009), P168L (Goldmann et al., 2006), M137T (Vaccari et al., 2009; Maestrale et al., 2009) and I142K (Vaccari et al., 2007), may also give resistance to PrPSc deposition in the placenta. These observations have important scientific implications. These are that the epidemiological model delineated to estimate the spreading of scrapie agent during the lambing period and the ongoing studies on scrapie pathogenesis in ARQ/ARQ sheep should take into account these allelic variants in order to ensure accurate interpretation of the data obtained.

To improve scrapie resistance in the European ovine population, the breeding programme is designed to increase the ARR allele frequency. Although the N176K frequency is considered low (3.5%) in breeding rams of Sarda sheep (C. Maestrale, unpublished data), our results suggest that the increase of the ARQK176 allele in the ovine ARQ/ARQ population may be considered as an additional strategy for such a breeding programme, while the increase of the AF141RQ allele is inadvisable, as it is considered to be a risk factor for having atypical scrapie (Moum et al., 2005).

Acknowledgements

We are grateful to M. M. Simmons and O. Winson for the revision of this manuscript and to A. J. Lepedda and A. Lai for the essential technical assistance. This work was supported by IZS SA 05/07 RC of the Italian Ministero della Salute.

References


