POLYMORPHISM IN THE PRNP LOCUS IN PROLIFIC OLKUSKA SHEEP

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Abstract

PCR-RFLP method was used to identify polymorphisms in the 136 (A/V), 154 (R/H), and 171 (R/H/Q) codons of the prion protein gene (PRNP) in 174 ewes and rams of the prolific Olkuska sheep breed from three nucleus flocks. Alanine (A) at codon 136, arginine (R) at codon 154, and arginine (R) or glutamine (Q) at codon 171, responsible for the presence of two alleles (ARR and ARQ) and three genotypes, (ARR/ARR, ARR/ARQ and ARQ/ARQ) were found in the analysed population. In all flocks, ARR allele (the most resistant to scrapie) was the dominant haplotype regardless of sheep sex, and ARR/ARQ genotype had the highest frequency (60.92%). The proportion of the undesirable ARQ/ARQ genotype was only 4.02%. Simulation of genotype distribution for the next generations showed that the mating of ewes with ARR/ARR genotype rams will cause this genotype to appear in 99% of Olkuska ewes already in the sixth generation. However, the study showed no relationship between genotype in the PRPN locus and prolificacy potential of the ewes.

Key words: prolific Olkuska sheep, scrapie, PRPN gene, polymorphism.

Transmissible spongiform encephalopathies (TSE) are neurodegenerative diseases that affect humans and animals. They are characterised by the accumulation of an abnormal isoform of the prion protein (PrPSc) in the central nervous system and show characteristic pathomorphological presentation of brain and cerebral cortex lesions resulting from neuron atrophy and gliosis. Animal TSE include scrapie in sheep, goats, and moufflons. A number of polymorphisms that cause a change in the amino acid chain were observed in the coding portion of the PRNP gene encoding sheep prion protein (2, 16, 20). However, a significant relationship with susceptibility of sheep to scrapie was observed for polymorphisms at codons 136, 154, and 171, whereas the presence of arginine at amino acid position 136, histidine at codon 154, and arginine at codon 171 was found to increase the resistance to this disease. In some sheep, threonine was also found in place of the amino acid encoded at position 136 and lysine at position 171, but these amino acids occur sporadically and are not associated with susceptibility to scrapie (3, 7, 15, 17). Polymorphism in these three codons provides a basis for classifying sheep into five risk groups, with group R1 including scrapie-resistant sheep having the most favourable genotype (ARR/ARR).

The objective of this study was to identify polymorphism in the PRNP gene locus in ewes and rams of the native Olkuska sheep breed, to determine a possible relationship between identified genotypes and high prolificacy of the ewes, and to offer breeders a procedure for reducing the risk of scrapie.

Material and Methods

Polymorphism in the locus of PRNP gene was investigated in 174 ewes and rams of the prolific Olkuska sheep breed from three leading farms that keep purebred Olkuska stock. In 2007, these flocks constituted over 26% of the national population of this breed and most of the new Olkuska flocks established in recent years are based on the breeding material from these leading flocks.

DNA was isolated from full blood based on a MasterPure™ Genomic DNA Purification Kit (Epicentre Technologies, USA) in accordance with the manufacturer’s procedure.
Table 1
Length of PCR products and starters used in PCR reaction

<table>
<thead>
<tr>
<th>Primer</th>
<th>Temperature</th>
<th>Length of PCR products</th>
<th>Restriction enzyme</th>
</tr>
</thead>
<tbody>
<tr>
<td>5' - GTGTACTACAGACCCGTGGA</td>
<td>56°C</td>
<td>144 bp</td>
<td>BseLI (Fermentas)</td>
</tr>
<tr>
<td>5' - TCGTCATATTATCATGTGT (20)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5' - TGTGGCAGGGCTGCTGAGCT</td>
<td>59°C</td>
<td>197 bp</td>
<td>BspHI i BspDI (New England Bio Labs)</td>
</tr>
<tr>
<td>5' - GCACAAAGTTGGTCTGGTGATATAT (12)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5' - TGTGGCAGGGCTGCTGAGCT</td>
<td>59°C</td>
<td>198 bp</td>
<td>BspHI</td>
</tr>
<tr>
<td>5' - GCACAAAGTTGGTCTGGTGATATAT (12)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2
Frequency of genotypes and alleles in locus PRPN (%)

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Farm</th>
<th>I</th>
<th>%</th>
<th>II</th>
<th>%</th>
<th>III</th>
<th>%</th>
<th>Rams</th>
<th>%</th>
<th>Ewes</th>
<th>%</th>
<th>Total</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARR/ARR</td>
<td>31</td>
<td>44.93</td>
<td>8</td>
<td>17.39</td>
<td>22</td>
<td>37.29</td>
<td>11</td>
<td>42.31</td>
<td>50</td>
<td>33.79</td>
<td>64.87</td>
<td>35.06</td>
<td></td>
</tr>
<tr>
<td>ARR/ARQ</td>
<td>34</td>
<td>49.27</td>
<td>35</td>
<td>76.09</td>
<td>37</td>
<td>62.71</td>
<td>14</td>
<td>53.85</td>
<td>92</td>
<td>62.16</td>
<td>65.52</td>
<td>60.92</td>
<td></td>
</tr>
<tr>
<td>ARQ/ARQ</td>
<td>4</td>
<td>5.80</td>
<td>3</td>
<td>6.52</td>
<td>0</td>
<td>0.00</td>
<td>1</td>
<td>3.84</td>
<td>6</td>
<td>4.05</td>
<td>35.13</td>
<td>34.48</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Allele</th>
<th>Total</th>
<th>%</th>
<th>%</th>
<th>%</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARR</td>
<td></td>
<td>69.56</td>
<td>55.43</td>
<td>68.64</td>
<td>69.23</td>
</tr>
<tr>
<td>ARQ</td>
<td></td>
<td>30.44</td>
<td>44.57</td>
<td>31.36</td>
<td>30.77</td>
</tr>
</tbody>
</table>

n – number of animals; investigated population did not reach genetic equilibrium.

Table 3
Distribution of ewes of three genotypes at PRPN locus based on their mean lifetime prolificacy and maximum litter size

<table>
<thead>
<tr>
<th>Mean lifetime prolificacy</th>
<th>Genotype</th>
<th>Maximum litter size</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ARQ/ARQ</td>
<td>ARQ/ARQ</td>
<td>ARR/ARR</td>
</tr>
<tr>
<td>1.00 – 2.00</td>
<td>2</td>
<td>38</td>
<td>18</td>
</tr>
<tr>
<td>2.01 – 3.00</td>
<td>2</td>
<td>25</td>
<td>13</td>
</tr>
<tr>
<td>above 3.01</td>
<td>2</td>
<td>11</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>6</td>
<td>74</td>
<td>34</td>
</tr>
</tbody>
</table>

Fig. 1. The electrophoretic picture of individual genotypes in PRPN locus (2.5% agarose gel). ARR/ARR, ARQ/ARQ, ARR/ARQ - genotype; M – marker Gene Rule™ DNA Ladder Low Range.
Polymorphism at codons 136, 154, and 171 of the *PRPN* gene locus was identified by PCR-RFLP. For each animal, three fragments of the *PRNP* gene (AB373806) of 144, 197, and 198 bp, encoding the corresponding segments of the prion protein, were amplified (Table 1). PCR was performed with 25 µl: Taq DNA polymerase (1.5U Fermentas); dNTP's mix 0.16-0.2 mM; MgCl\(_2\) 2.0 mM; primers 0.12-0.16 µM. PCR products were analysed in 2.5% and restriction enzyme digestion products in 3.5% agarose gel (Nusieve® CTG® agarose, BioWhittaker Molecular Applications).

The results obtained by PCR-RFLP were confirmed by sequencing the 256 bp fragments of the *PRPN* gene in 18 randomly selected sheep, using primers described by Lühken *et al.* (12).

Genetic equilibrium at the analysed locus was determined by the Hardy-Weinberg law. Simulation of genotype distribution for the next generations was performed for each flock assuming random mating and mating to a ram representing R1 risk group (ARR/ARR genotype). The relationship between genotype at the *PRPN* locus and mean and maximum litter size of the ewes was also analysed. The hypothesis was tested using $\chi^2$-square test of independence, separately for each flock and on combined data.

### Results

**Genotyping of sheep at the *PRPN* gene locus.**

The polymorphism in the *PRPN* gene locus was identified only at codon 171 (R and Q). The electrophoretic picture of individual genotypes is shown in Fig. 1. Only two alleles of the gene (ARR and ARQ) and three genotypes (ARR/ARR, ARR/ARQ, and ARQ/ARQ) were identified (Table 2).

Most of the analysed sheep were either ARR/ARQ heterozygotes (60.92%) or ARR/ARR homozygotes (35.06%). Frequency of genotypes varied between flocks; however, the differences were non-significant. The ARR/ARR (favourable) genotype was most frequent on farm I (44.93%) and least frequent on farm II (17.39%). Sex had no effect on the frequency of genotypes, although ARR/ARQ was more frequent in ewes (62.16%) and ARR/ARR in rams (42.31%).

**Simulation of mating.** Simulation of genotype distribution for the next generations (1-6) with random mating of ewes and rams showed that in all flocks this mating may increase the frequency of animals with the least favourable genotype (ARQ/ARQ), representing risk group R3. The use of ARR/ARR rams for mating would gradually increase the frequency of this favourable genotype in the next generations and the ARR/ARQ genotype would not occur in the first generation. It was found that in the sixth generation sheep with the ARR/ARR genotype on farms II, I, and III would form 98.6%, 99.0%, and 99.0% of the flock, respectively, while the proportion of sheep with the ARR/ARQ genotype would decrease to a small percentage of animals (Fig. 2).

**Relationship between genotype at the *PRPN* locus and prolificacy of the ewes.** The hypothesis that the genotype at the *PRPN* locus has no effect on lifetime prolificacy of the ewes or their maximum litter size was tested for each flock separately, and using pooled data (Table 3). No relationship was found between the analysed traits.

### Discussion

The analysis of polymorphism in prolific Olkuska sheep at amino acid positions 136, 154, and 171 of the polypeptide chain showed that codons 136 and 154 are monomorphic. Alanine (A), which determines resistance to scrapie, was present at codon 136; and arginine (R), which is associated with susceptibility to this disease, was present at codon 154 (3, 15). Dimorphism associated with a single nucleotide substitution was only found at codon 171, which causes the coding of arginine (R) in some animals and of glutamine (Q) in others.

The presence of alanine at codon 136 in all of the studied sheep shows the affinity of Olkuska sheep with the native breeds Wrzosówka (Polish Heath Sheep) and Polish Mountain Sheep; other breeds of sheep found in Poland carry alanine or valine (14, 21). Following the classification of breeds into “valine” and “alanine” groups, the prolific Olkuska sheep belongs to the
“alanine” group along with Berrichone and Suffolk (10). In all Polish sheep breeds in which polymorphism at codon 154 was determined, arginine was the dominant amino acid, whereas in the analysed flocks of Olkuska sheep this is the only amino acid encoded in this region of the \textit{PrP} gene. In native breeds such as Świniarka, Wrzosówka, Żelaźnińska, Kamieniecka, Pomorska, and Polish Mountain Sheep, histidine only appears with alanine at codon 136 and glutamine at codon 171. It is known that codon 171 has the highest number of amino acid variants (arginine, glutamine, and histidine), but histidine is very rare, and Pomorska sheep is the only Polish breed in which it was identified (14, 21). Seven haplotypes were identified in European sheep breeds: ARR, ARH, ARQ, AHQ, VRQ, VRR, and AHR (16), while VRR and AHR alleles are sporadically found in Germany and Slovakia (1). In the studied Olkuska sheep flocks, the most favourable allele (ARR) was observed in 65.5% of the sheep, similarly to other domestic breeds of sheep investigated by Niźnikowski et al. (14) and Wiśniewska and Mrockowski (21), who found very high frequency of this allele in Suffolk, Polish Merino, and Kamieniecka sheep (60%, 56.5%, and 51.6%, respectively). Lower frequency was detected in Żelaźnińska and Wrzosówka sheep at 46.8% and 41.09%, respectively. The lowest frequency (about 40%) of ARR allele was observed in Pomorska and Polish Mountain Sheep (14).

There were no differences in the frequency of the ARR allele related to sex. A similar situation was reported in Kamieniecka, Pomorska, and Berrichone sheep. In the latter breed, the frequency of the ARR allele in rams (originating from one farm) was 100%, much in excess of the frequency observed in the rams of other breeds kept in Poland: Blackheaded (88.5%), Suffolk (66.7%), Kamieniecka (60%), Pomorska (59.1%), Polish Merino and Wrzosówka (40.9%), Żelaźnińska (27.8%), and Polish Mountain Sheep (22.2%) (12, 21). The fact that the ARR allele has got high frequency in rams of Olkuska sheep breed, as well as other sheep breeds kept in Poland, indicates the possibility that selection for minimising the presence of undesirable alleles has been efficient (14, 12).

In the analysed sheep population, frequency of the ARQ allele, which is less favourable in terms of resistance to scrapie, was 34.48%. A similar result was observed for the flocks of Kamieniecka and Suffolk sheep (35.5%). In other domestic breeds, this allele had a higher frequency: 53.2% in Polish Mountain Sheep, 43.6% in Żelaźnińska sheep, 41.9% in Polish Merino, 38.7% in Pomorska, and 38.7% in Wrzosówka. Frequency of the ARQ allele in old native breeds such as the Polish Mountain Sheep is relatively high because it is a “wild allele”. It is worth noting that no VRQ allele, which determines the susceptibility to scrapie, has been found in prolific Olkuska sheep, as well as in Żelaźnińska, Blackheaded, Berrichone, and Polish Mountain Sheep. These findings are indicative that the domestic populations are considerably resistant to scrapie (12, 14, 21).

Compared to other breeds, the prolific Olkuska sheep is characterised by a low variation in haplotype frequency, which suggests high homozygosity at the \textit{PRPN} locus; this confirms the relatively high inbreeding of the breed (5). The ARR allele is also frequent in the Slovakian populations of Dorset (55.9%), Suffolk (66%), and Merino sheep (60%) (9, 19). In most European sheep, the ARQ allele is the dominant allele and its frequency is high in many breeds in Italy, Spain, and the Great Britain. The observed distribution of genotypes (ARR/ARR, ARR/ARQ, and ARQ/ARQ) was similar in Olkuska rams and ewes. An identical series of triplets coding for the same amino acids was found in Blackheaded sheep: ARR/ARR – 62.0% in ewes and 76.9% in rams, ARR/ARQ – 36.6% in ewes and 23.1% in rams; ARQ/ARQ – only 1.4% in ewes (21). Low genotype diversity was also found in Poll Dorset sheep in England, where respective genotype frequency was 12%, 40%, and 49%, and in the Vandeen breed in Ireland: 1.8%, 22.8%, and 75.2%, respectively (8, 15).

ARR/ARQ heterozygotes, representing risk group R2, dominated in all the flocks of prolific Olkuska sheep. The fact that many animals carry this genotype results from a failure to genotype rams and the use of ARR/ARQ or ARQ/ARQ males for mating. Predominance of the ARR/ARQ genotype was also observed in the breeds of Polish Merino (54.1%) and Żelaźnińska sheep (about 60%), Kamieniecka and Mountain (about 50%), Suffolk (46.7%), and Wrzosówka (42%) (14, 20). Similarly to the analysed Olkuska sheep, the high frequency of ARR/ARQ heterozygotes was found in Tsigai (43%) and Suffolk (48%) in Slovakia, in Charollaise (60%) in Ireland, and in Texel (24.9%) in Great Britain. Simulation of genotype distribution showed that random selection of rams for mating will reduce the frequency of the desirable ARR/ARR genotype and gradually increase the number of sheep with the wild ARR/ARR genotype in all the flocks. However, mating using rams of the ARR/ARR genotype will eliminate the ARQ/ARQ genotype already in the first generation. In the next generations, ARR/ARQ heterozygotes will decrease considerably in frequency, and the rate of this reduction will depend on the initial frequency of genotypes in the flock. In the sixth generation on farm I, the frequency of ARR/ARR genotype will be reaching 99.04%. This finding supports the results of a simulation by Wiśniewska and Mrockowski (21) for the domestic breeds Polish Merino, Polish Mountain Sheep, Suffolk, Berrichone, and Blackheaded, which showed that ARR/ARR frequency in the 5th generation would approach 100%. It was predicted that in the German sheep breeds obtaining animals representing risk group R1 will take from six to nine generations (6).

No relationship between the genotype at the \textit{PRPN} locus and reproductive potential of Olkuska sheep.
indicates that the major gene affecting high prolificacy segregating in this population has no connection with the PRPN locus (13). It also supports the findings of other authors, who demonstrated no negative effect of selection for enhancing the frequency of ARR/ARR genotype on reproductive performance of ewes (11, 18). Genotypes at the PRPN locus should be identified in all flocks of the active sheep population, especially in rams. Although the ARR allele has recently been shown not to protect from atypical scrapie, ARR/ARR sheep are largely resistant to the infectious agent being converted to the TSE-inducing pathogen, which reduces the infection risk for humans.

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References