

PRNP* gene polymorphism in Świniarka sheep in a flock included in the Genetic Resources Conservation Programme

Agata Piestrzyńska-Kajtoch¹, Aldona Kawęcka², Grzegorz Smolucha¹, Anna Miksza-Cybulska², Barbara Rejduch¹

National Research Institute of Animal Production,
¹Department of Genomics and Animal Molecular Biology,
²Department of Animal Genetic Resources Conservation,
ul. Sarego 2, 31-047 Kraków

Scrapie is a fatal neurodegenerative disease which affects sheep and goats. Genotypes of the *PRNP* gene are linked to susceptibility to scrapie. The aim of our study was to investigate the frequency of *PRNP* genotypes in the Świniarka sheep breed, in foundation stock and in young ewes for flock rebuilding (195 animals). The breed is included in the Genetic Resources Conservation Programme. Three alleles (ARR, ARQ and VRQ) and five genotypes (ARR/ARR, ARR/ARQ, ARQ/ARQ, ARR/VRQ and ARQ/VRQ) were found in the group studied. There were differences in allele and genotype frequencies between the foundation stock and the young ewes due to ram selection in the foundation stock. The results confirmed the importance of monitoring scrapie genotypes and selection based on this monitoring.

KEY WORDS: sheep / prion protein gene / *PRNP* / polymorphism / scrapie

Scrapie is a fatal neurodegenerative disease of sheep, goats, and mouflons belonging to the group of spongiform encephalopathies, like BSE in cows, Creutzfeldt-Jakob disease (vCJD) in humans, and Chronic Wasting Disease (CWD) in deer [13]. These diseases are believed to be caused by prion proteins. The partially protease-resistant protein PrP^{sc} (scrapie), which is a pathogenic conformation of the cellular protein PrP^c, is capable of inducing conversion of the normal protein to the pathogenic form. The target site of infection is the central nervous system (CNS). Amyloid deposits in the brain and their accumulation cause tissue degeneration, resulting in the characteristic appearance of spongiform structures [1]. Symptoms of scrapie in sheep are usually observed after a long incubation period (from one to three years), and include nervous behaviour, incre-

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ased sensitivity to noise, problems with milk yield, weight loss, characteristic scratching, tremors, uncoordinated movements, ataxia, and finally death. Infection may be foodborne, and in lambs may occur during birth and lactation. The disease may also appear spontaneously [1].

Two forms of scrapie occur in sheep—classical and atypical. They differ in the electrophoretic pattern of the protein (different number of bands in immunoblot analysis), the glycosylation profile, sensitivity to proteinase digestion, the location of pathological changes in the brain (classical—the brain stem; atypical—the cerebellum and cerebral cortex), the vacuolation profile, and the pattern of prion deposition in the CNS. Furthermore, atypical scrapie has been diagnosed in sheep whose genotypes have been linked in previous studies to high resistance to classical scrapie and BSE [19].

The gene *PRNP* has been mapped in sheep on chromosome 13, and within its coding region polymorphisms have been observed in codons 136, 154 and 171, the occurrence of which is most strongly linked to susceptibility to classical scrapie in sheep. Five alleles having the greatest influence on genetic regulation of resistance to scrapie have been distinguished: ARR ($A_{136}R_{154}R_{171}$), ARQ, AHQ, ARH and VRQ. These alleles determine the occurrence of 15 genotypes responsible for varying degrees of risk of classical scrapie [1, 2, 18]. Animals with genotype ARR/ARR are highly resistant to classical scrapie, while the VRQ/VRQ genotype is considered the least favourable and is associated with high susceptibility to classical scrapie. Atypical scrapie has been linked to the presence of the F allele in codon 141 of the *PRNP* gene (L141F). This form of the disease appears most often in individuals with the ALHQ and AFRQ alleles, but also in sheep with the ALRR allele, which determines resistance to classical scrapie [8, 9, 16]. Monitoring the frequency of genotypes in a population and not allowing animals with genotypes determining vulnerability to scrapie to be used for reproduction is one of the measures recommended by the European Union to prevent the disease.

The aim of the study was to determine the frequency of *PRNP* alleles and genotypes in sheep of the Świniarka breed from a flock included in a genetic resources conservation programme.

Material and methods

The study was conducted on a flock of sheep of the native Świniarka breed in Subcarpathia (Podkarpacie). A total of 195 sheep were analysed: 121 ewes from the foundation stock, 11 breeding rams, and 63 young ewes designated for flock rebuilding. The study was carried out in 2012 and 2014. After the first year of the study the rams from the foundation stock underwent selection for reducing the occurrence of the unfavourable VRQ allele.

DNA was isolated from blood samples with a Wizard Genomic DNA Purification Kit (Promega) according to the protocol included with the kit. A modification of the method proposed by Garcia-Crespo et al. [4] was used to identify codons 136, 154 and 171 of the *PRNP* gene. For analysis of codons 136 and 154, PCR products were subjected to an enzyme reaction with the enzyme BspHI at 37°C, followed by electrophoresis in agarose gel stained with MidoriGreen DNA Stain (Nippon Genetics). The results were viewed

in UV light on a transilluminator. The allele discrimination method was used to identify codon 171, using fluorescence-marked TaqMan MGB probes (Life Technologies) in the StepOnePlus Real-time PCR System. The allele discrimination method was also used to identify codon 141, with primers and probes for PCR designed in PrimerExpress software (Life Technologies). The results for the four codons obtained by the two methods were combined to establish individual genotypes of the *PRNP* gene for the sheep.

The results were analysed using StepOne Software v2.2.2. (Life Technologies) coupled with the StepOnePlus Real-time PCR System, Excel spreadsheets, and Statistica software (v.12) for the chi-square test.

Results and discussion

Three alleles, ARR, ARQ and VRQ (Tab. 1) and five genotypes (Fig.) were noted in the whole group of sheep (195 individuals). The ARR allele, which is the most favourable in terms of susceptibility to classical scrapie, was most common (71.28%). The frequency of the VRQ allele determining high susceptibility to classical scrapie was 10.77%, and was 2.5 times higher in ewes than in rams. The frequency of the ARR/ARR genotype, which determines resistance to classical scrapie, was 48.72%. The ARR/ARQ and ARR/VRQ genotypes were less common. The frequency of the remaining genotypes, ARQ/ARQ and ARQ/VRQ, was low, at 1.03% and 5.13%, respectively. The results of the analysis indicate a relatively high percentage of individuals in the flock with the most favourable genotype, as it was noted in nearly half of the sheep tested.

Similar results regarding the most common genotypes were obtained in an earlier study conducted in 2005-2009 at the National Research Institute of Animal Production [15] on more than a dozen breeds and crossbreeds. The authors demonstrated the occurrence of 6 alleles and 17 genotypes in Polish sheep populations, with three genotypes occurring with the greatest frequency, i.e. ARR/ARQ, ARR/ARR and ARQ/ARQ, and the remaining genotypes with a frequency of under 5%. Jasik and Reichert [6], analysing polymorphism of the prion protein gene *PRNP* in Świniarka sheep, found only two genotypes in this breed,

Table 1
PRNP allele frequency (%) in the entire group studied

Allele	Frequency (%)		
	entire group	ewes	rams
ARR	71.28	71.20	72.73
ARQ	17.95	17.66	22.73
VRQ	10.77	11.14	4.55
Number of animals	195	184	11

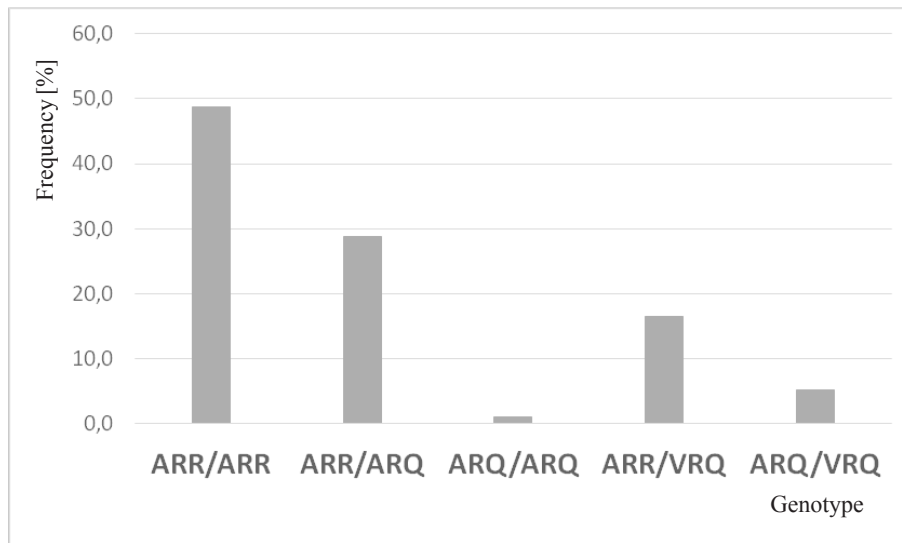


Fig. Frequency of *PRNP* genotypes in the entire group studied

ARR/ARR (70%) and ARR/ARQ (30%), while Niżnikowski et al. [11] also detected the ALHQ and VLRQ alleles in this breed. Other studies conducted on ewes and rams of the native Olkuska breed demonstrated the occurrence of the ARR/ARQ genotype in 60% of individuals tested; the remaining individuals were homozygotic, with the ARR/ARR genotype [7]. Niżnikowski et al. [10], in a study conducted in north-eastern Poland, also found a high frequency of ARR/ARR and ARR/ARQ genotypes in Zelaznenska and Polish Lowland sheep. In Corriedale sheep, which is a lowland breed, the authors [10] identified 11 genotypes, with a higher frequency of genotypes susceptible to scrapie (VRQ/ARR) than resistant ones (ARR/ARR). In Greece, Ekateriniadou et al. [3], analysing *PRNP* po-

Table 2
PRNP allele frequency (%) in the foundation stock of ewes and rams

Allele	Frequency (%)		
	foundation stock	ewes	rams
ARR	68.56	68.18	72.73
ARQ	18.56	18.18	22.73
VRQ	12.88	13.64	4.55
Number of animals	132	121	11

Table 3
Frequency of *PRNP* genotypes (%) in the foundation stock of ewes and rams

Genotype	Total	Ewes	Rams
ARR/ARR	43.18	42.15	54.55
ARR/ARQ	30.30	29.75	36.36
ARQ/ARQ	0.76	0.83	0.00
ARR/VRQ	20.45	22.31	0.00
ARQ/VRQ	5.30	4.96	9.09

lymorphism in over a dozen native Greek breeds of sheep, found that the frequency of the ARQ allele in the population was 50%, as compared to 36% for the ARR allele and a low frequency for the ARH, AHQ and VRQ alleles.

In the foundation stock analysed in the present study the frequency of the unfavourable VRQ allele was three times higher in ewes than in rams (Tab. 2). The differences between frequencies of alleles in ewes and rams were statistically significant ($\chi^2=7.5$; $P=0.024$).

All five genotypes were identified in the animals in the foundation stock (Tab. 3). Individuals with the ARR/ARR genotype made up the highest percentage of the flock (43.18%). This was 42.15% for ewes and 54.55% for rams. The second most frequent genotype was ARR/ARQ (30.30%); the percentage was 29.75% for ewes and 36.36% for rams. The frequency of the ARR/VRQ genotype was high. Ewes with this genotype accounted for a fifth of the entire pool, while among rams it was not noted at all. The percentage of the remaining genotypes was small: 5.30% for ARQ/VRQ and only 0.76% for ARQ/ARQ. The differences observed in the frequencies of genotypes between ewes and rams in the foundation stock were highly statistically significant ($\chi^2=31.7$; $P=0.000002$).

The ARQ/ARQ and ARR/VRQ genotypes were not detected in the Świniarka rams analysed in the study. Similar observations for rams of the native Kamieniecka breed were reported by Szkudlarek et al. [17]. Niżnikowski et al. [12] detected three alleles and three genotypes in rams of the native Zelaznenska breed and only two genotypes (ARR/ARR and ARR/ARQ) in Polish Heath rams in the first year of observations on a farm conducting conservation breeding on these breeds. In the third year, the authors found only the ARR allele in breeding rams, which was the result of intentional breeding work [12]. Similar selection was carried out in the Świniarka foundation stock analysed in the present study, subject to *PRNP* genotype monitoring, focusing primarily on not allowing rams with the VRQ allele to be used for reproduction, which resulted in the differences observed in the frequency of genotypes and alleles between ewes and rams.

The need for breeding work making use of the results of *PRNP* genotyping in Świniarka sheep has previously been pointed out by Niżnikowski et al. [11]. Owing to the selection carried out in rams, the frequency of the ARR allele in the rebuilding flock of ewes analysed in the present study (Tab. 4) was higher than in the ewes in the foundation stock. The ARR/ARR genotype (Tab. 5) appeared more often in the group of older animals, while

Table 4
PRNP gene allele frequency (%) in young ewes for flock rebuilding

Allele	Ewes
ARR	76.98
ARQ	16.67
VRQ	6.35

Table 5
 Frequency of *PRNP* genotypes (%) in young ewes for flock rebuilding

Genotype	Ewes
ARR/ARR	60.32
ARR/ARQ	25.40
ARQ/ARQ	1.59
ARR/VRQ	7.94
ARQ/VRQ	4.76
Number of animals	63

lower frequency was noted for the ARR/VRQ (7.94%) and ARQ/VRQ (4.76%) genotypes. The frequency of the ARR/ARQ genotype in the young ewes was 5 percentage points (p.p.) lower than in the foundation stock. The differences observed in genotype frequencies between the ewes of the foundation stock and the young ewes were highly statistically significant ($\chi^2=32.64$; $P=0.000001$). The frequency of the ARQ allele decreased by only 2 p.p., whereas the frequency of the VRQ allele decreased twofold. However, the differences between the allele frequencies of the foundation ewes and the young ewes were not statistically significant ($\chi^2=5.16$; $P=0.076$).

It can be concluded from the analyses that the absence of the ARR/VRQ genotype in the rams of the Świniarka foundation stock and the fact that rams with the VRQ allele were not used for reproduction resulted in a reduction in the occurrence of the VRQ allele in the offspring in the flock of young ewes. This is particularly evident in the reduction in the frequency of the ARR/VRQ genotype from 22.31% in the foundation ewes to 7.94% in the young generation of ewes, which is a highly favourable effect. However, the genotype most susceptible to scrapie, i.e. ARQ/VRQ, was not fully eliminated from the flock. To eliminate this genotype it would be necessary to completely stop using individuals (of either sex) with even one unfavourable VRQ allele for reproduction. Similar conclusions have been drawn on the basis of selection taking into account the VRQ allele in the Romanowska sheep breed [14].

Depending on the *PRNP* genotype, sheep are classified into five risk groups (classes) for classical scrapie: G1, G2, G3, G4 and G5, where G1 designates the lowest risk and G5 the highest [2, 18]. In all animals evaluated (Tab. 6), the frequency of the ARR/ARR genotype,

Table 6

Genotype frequencies (%) for the *PRNP* gene in relation to the class of resistance/susceptibility to classical scrapie in all examined sheep

Risk class	Genotype	Frequency (%)
G1	ARR/ARR	48.72
G2	ARR/ARQ	28.72
G3	ARQ/ARQ	1.03
G4	ARR/VRQ	16.41
G5	ARQ/VRQ	5.13

which corresponds to the first risk class for scrapie (G1), i.e. the lowest susceptibility to the disease, was highest. Genotypes with the VRQ allele (groups G4 and G5), characterizing sheep with the highest risk of contracting classical scrapie, occurred with frequencies of 16.41% and 5.13%. As in the present study, Gruszecki et al. [5] also observed the occurrence of genotypes belonging to all five risk classes, but nearly half of the animals had the ARR/ARQ genotype.

In all the sheep analysed (195 individuals) only the L allele was observed in codon 141. The F allele, suspected of being associated with susceptibility to atypical scrapie, was not observed in any animal. The same results for this breed were obtained by Niżnikowski et al. [11].

The results of the study indicate that the direction of breeding in the flock is appropriate, and confirm the importance of monitoring *PRNP* u genotypes in sheep. In the case of the young ewes, an increase was observed in the frequency of genotypes containing the ARR allele and a decrease in the frequency of the VRQ allele in comparison with the foundation stock. The results also demonstrate the advisability of selection of breeding rams, which improves the frequency of the most favourable alleles.

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