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# Prion protein gene polymorphism and genetic risk evaluation for scrapie in all Turkish native sheep breeds

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**Abstract** The aim of this study was to identify the prion protein (PrP) gene polymorphism in a total of 1,110 healthy sheep from 18 Turkish native sheep breeds. There were nine alleles and 22 genotypes observed based on codons 136, 154, and 171 of the *PrP* gene. The ARQ allele was predominant for all breeds. The most resistant allele to scrapie, ARR, was present in all breeds. The VRQ allele, associated with the highest susceptibility to scrapie, was detected at low frequencies in İvesi (0.06), Kıvırcık (0.021), Sakız (0.010), Karayaka (0.011), Çine Çaparı (0.012), and Güneykaraman (0.017). In general, the ARQ/ARQ genotype was predominant in all breeds. The most resistant genotype to scrapie, ARR/ARR, was found with the frequency lower than 0.180. The most susceptible genotype, VRQ/VRQ, was found in only Kıvırcık. The TRR and TRH alleles and the genotypes of ARR/TRR, ARR/ARK, and ARH/TRH have been found for the first time in Turkish native sheep breeds. According to these results, all breeds belong to risk group R3 followed by R2. It is propounded that the susceptibility to scrapie increased from eastern to western part of Turkey. Our findings of Turkish native sheep breeds with *PrP* gene polymorphisms will assist the sheep breeding program for selection of scrapie resistance genotypes to reduce the risk of scrapie.

**Keywords** Scrapie · *PrP* gene · Polymorphism · Genetic risk · Turkish native sheep

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## Introduction

Scrapie is a fatal, neurodegenerative disease that affects sheep and goats and has been known in Europe for more than 250 years. It is a member of the Transmissible Spongiform Encephalopathies (TSE) group, which also includes Creutzfeldt–Jakob disease (CJD) in humans, bovine spongiform encephalopathy (BSE) in cattle, and chronic wasting disease (CWD) in deer. These are also known as prion diseases, where the neuronal surface glycoprotein known as prion protein (PrP) is converted into its abnormal protease-resistant isoform ($PrP^{Sc}$), which then becomes the infective agent and accumulates in the central nervous system and lymphoid tissues [1].

Studies of naturally and experimentally scrapie-affected sheep have shown that genetic susceptibility to scrapie in sheep is associated with polymorphisms in the *PrP* gene. The DNA sequencing search of thousands of sheep *PrP* gene alleles has started and continues to this day with new polymorphic codons being described regularly. The first codon known to be polymorphic was codon 171, where either arginine (R), histidine (H), or glutamine (Q) could occur. Subsequently two other codons were found to be important: 136 with valine (V) or alanine (A), and 154 with histidine (H) or arginine (R). Many other codons are now known to be polymorphic, but these three codons have been reported to have a linkage with susceptibility or resistance to scrapie in sheep and remain of major

importance [2]. Sheep with VRQ were highly susceptible to scrapie and had a short survival period after challenge with scrapie; whereas sheep with ARR were resistant to scrapie under field and laboratory conditions [2, 3].

In 2001, the National Scrapie Plan (NSP) was put in place to assist in sheep breeding that would reduce the frequencies of the susceptible genotypes in the UK. Within the NSP, there are 15 genotypes listed in five different groups (R1, R2, R3, R4, and R5) related to the known susceptibility to natural scrapie [4]. The European Community Regulation for scrapie breeding programs aims to increase the frequency of ARR allele, which is associated with resistance to scrapie, and to decrease the frequency of VRQ, which is the allele highly susceptible to scrapie [5]. According to these regulations, many European countries have genotyped their sheep breeds for scrapie resistance.

The earliest evidence of sheep domestication was found in certain parts of the Near East, with Turkey as an area of major importance. Within the Near East and because of its geographical location at the intersection of Asia and Europe, Anatolia has been a cradle for civilizations since prehistoric times. Data from the numerous Neolithic human settlements found throughout this region strongly point to it as a major domestication centre for livestock species, mainly goats and sheep [6, 7]. Therefore, they must be explored with regard to genetic markers. In Turkey, there have been no official reports about the cases of scrapie, although there are a few studies on identification of *PrP* polymorphism in Turkish native sheep breeds. These studies [8–11] have been carried out in restricted sheep samples and breeds (3, 5, 3, and 2 breeds, respectively). As we did not have scrapie cases and there was no official report regarding scrapie cases in Turkey, we could not carry out a case–control study. The objective of this study was to genotype all 18 Turkish native sheep breeds in order to determine polymorphisms of the *PrP* gene and identify the genetic susceptibility to scrapie.

## Materials and methods

### Sheep

In this study, a total of 1,110 unrelated healthy sheep were randomly (regardless of age and sex) sampled from 18 Turkish native breeds (Fig. 1).

### DNA extraction

Blood samples were collected from the jugular vein into EDTA-containing tubes, transported to the laboratory and stored at −20 °C until genomic DNA extraction, which was carried out using a salting-out method [12].

### PCR assay

The fragment of 771 bp in length, which covered the open reading frame of the *PrP* gene and codons 136, 154, and 171, was amplified by PCR. The amplification reactions were prepared in a final volume of 50 µl containing as follows: 1× PCR buffer, 0.2 mM dNTPs, 1 units *Taq* DNA polymerase, 1.5 mM $MgCl_2$, 20 pmol of forward (5′-ATG GTG AAA AGC CAC ATA GGC AGT-3′) and reverse (5′-CTA TCC TAC TAT GAG AAA AAT GAG-3′) primers suggested by Sipos et al. [13], and 100 ng of genomic DNA. Amplification was performed using an initial


**Fig. 1** Sampling localities of Turkish native sheep breeds on the map of Turkey. *IVE* İvesi (Awassi), *AKK* Akkaraman, *KAK* Kangal Akkaraman, *MRK* Morkaraman, *GNK* Güneykaraman, *KVR* Kıvırcık, *NRD* Norduz, *KRK* Karakaş, *SKZ* Sakız (Chios), *HRK* Herik, *HMS* Hemşin, *DGL* Dağlıç, *KRY* Karayaka, *TUJ* Tuj (Tushin), *CCP* Çine Çaparı, *GOK* Gökçeada (Imroz), *KRG* Karagül (Karakul), *ZOM* Zom

denaturation of 5 min at 95 °C, followed by 40 cycles of 60 s at 95 °C, 60 s at 60 °C and 90 s at 72 °C, and a final extension of 7 min at 72 °C. PCR products resolved by electrophoresis on 2 % agarose gels.

### DNA sequencing

After gel electrophoresis, the amplicons were purified using a Qiamp Mini Kit (QIAGEN, Valencia, CA, USA). The purified samples were sequenced using a Big dye terminator chemistry on an ABI 3100 Avant Automated DNA Sequencer (Applied Biosystems, Foster City, CA, USA). The DNA sequences were analyzed by the Sequencing Analysis Software Version 3.3 (Applied Biosystems, Foster City, CA, USA). DNA sequencing was done by Refgen Biotechnology (www.refgen.com).

### Statistical analysis

Genotype ($X_{ij}$) and gene ($\hat{x}_i$) frequencies were estimated by the following formulas [14];

$$X_{ij} = \frac{n_{ij}}{n} \quad \text{and} \quad \hat{x}_i = \frac{2n_{ii} + \sum n_{ij}}{2n}$$

where $X_{ij}$ is the genotypic frequency of $A_iA_j$; $n_{ij}$, and $n_{ii}$ are the number of individuals for heterozygous ($A_iA_j$) and homozygous ($A_iA_i$) genotypes, respectively; $\hat{x}_i$ is the gene frequency of $A_i$ and $n$ is the total number of individuals sampled from the population.

## Results

The *PrP* polymorphisms at codons 136, 154, and 171 were identified using DNA sequencing in all 18 Turkish native sheep breeds. According to our results, nine alleles (ARR, ARQ, ARH, AHQ, VRQ, TRR, TRQ, TRH, and ARK) were observed based on codons 136, 154, and 171 of the *PrP* gene (Table 1). The sequences of these alleles were deposited in GenBank with the accession numbers of JQ248961, JQ248962, JQ248963, JQ248964, JQ248965, JQ248967, JQ248969, JQ248990, and JQ249021. The most frequent allele in each of the 18 breeds was ARQ with the frequencies ranging from 0.445 to 0.757. The ARR allele was present in all breeds, but frequencies were very different. The ARH allele was found in all breeds except Kıvırcık and Gökçeada. The VRQ allele was detected at low frequencies in İvesi, Güneykaraman, Kıvırcık, Sakız, Karayaka and Çine Çaparı. In addition, AHQ, TRR, TRQ, TRH, and ARK alleles were found at low frequencies.

In this study, 22 different genotypes comprising pairs of nine alleles were identified in Turkish native sheep breeds based on codons 136, 154, and 171 of the *PrP* gene (Table 1). The ARQ/ARQ genotype, which is corresponded to a risk score of R3, was detected in all breeds with the frequency ranging from 0.205 to 0.585. The most resistant genotype to scrapie, ARR/ARR, was found with the frequency of 0.180 in Sakız, 0.133 in Herik, and less than 0.100 in other breeds. This genotype was not found in Norduz, Karakaş or Dağlıç. The ARR/VRQ, in risk group R4, was found only in Kıvırcık and Güneykaraman. The VRQ/AHQ and VRQ/ARH, which are members of most susceptible group R5, were found only in Kıvırcık and Sakız, respectively. VRQ/ARQ, which is other R5 member, was found in İvesi, Kıvırcık, Karayaka and Çine Çaparı. The most susceptible genotype to scrapie, VRQ/VRQ, was found only in Kıvırcık. Eight additional genotypes (ARR/TRR, ARR/TRQ, ARQ/TRQ, TRQ/TRQ, ARR/ARK, ARH/ARK, ARQ/ARK, and ARH/TRH) were also found at low frequencies in Turkish native sheep breeds.

## Discussion

The identification of sheep populations for the *PrP* gene polymorphisms is very important for every country. For that reason, many European countries have developed national scrapie control plans and have genotyped their sheep breeds to increase the frequency of the ARR allele and decrease the frequency of the VRQ allele. In recent years, results of *PrP* polymorphisms in different sheep breeds in Europe have been reported, including the characterization of Icelandic [15], Norwegian [16], Irish [17], Italian [18], Austrian [13], British [19], French [20], German [21], Spanish [22], and Portuguese [23] breeds. In Turkey, scrapie control breeding programs have not been established because there is no information about the prevalence of scrapie. Until now, the polymorphism of the *PrP* gene has been reported in limited Turkish native sheep breeds [8–11]. However, in this study, all Turkish native sheep breeds were tested for scrapie and more sheep sampled from many more breed than previous studies in Turkey. As we did not have scrapie cases and there was not any official report about scrapie cases in Turkey, we could not carry out a case–control study. We just tried to genotype all 18 Turkish native sheep breeds in order to determine polymorphisms of the *PrP* gene and identify the genetic susceptibility to scrapie.

In this study, the most frequent allele was ARQ in all breeds (Table 1). This finding is consistent with the other reports in Turkish native sheep breeds [8–11]. Based on these findings, scrapie control breeding programs should be established in Turkey and the ARR should be increased as the ARQ is associated with susceptibility to scrapie. As we mentioned before, ARQ is the predominant allele in İvesi, Hemşin, Tuj, and Çine Çaparı breeds. Therefore, the high

**Table 1** The genotype and allele frequencies of Turkish native sheep breeds

| NSP[a] | | IVE $n = 100$ | AKK $n = 100$ | KAK $n = 100$ | MRK $n = 100$ | GNK $n = 30$ | KVR $n = 140$ | NRD $n = 35$ | KRK $n = 35$ | SKZ $n = 50$ | HRK $n = 45$ | HMS $n = 55$ | DGL $n = 40$ | KRY $n = 45$ | TUJ $n = 45$ | CCP $n = 40$ | GOK $n = 50$ | KRG $n = 50$ | ZOM $n = 50$ | TOTAL $n = 1,110$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Genotype frequency* | | | | | | | | | | | | | | | | | | | |
| R1 | ARR/ARR | 0.030 | 0.040 | 0.100 | 0.080 | 0.069 | 0.093 | | | 0.180 | 0.133 | 0.036 | | 0.023 | 0.044 | 0.049 | 0.041 | 0.104 | 0.040 | 0.064 |
| R2 | ARR/AHQ | | 0.010 | | | 0.034 | 0.029 | | | | | | | | | | 0.020 | | | 0.006 |
| R2 | ARR/ARH | 0.040 | 0.160 | 0.060 | 0.030 | | | | | 0.120 | | | 0.026 | 0.023 | 0.022 | 0.024 | | 0.083 | 0.020 | 0.040 |
| R2 | ARR/ARQ | 0.040 | 0.230 | 0.220 | 0.220 | 0.241 | 0.407 | 0.706 | 0.703 | 0.260 | 0.222 | 0.255 | 0.359 | 0.432 | 0.089 | 0.171 | 0.367 | 0.292 | 0.260 | 0.281 |
| R3 | AHQ/AHQ | | | | | | 0.014 | | | | | | | | | | | | | 0.002 |
| R3 | AHQ/ARH | | | | | | | | | | | | | | | | | | | 0.000 |
| R3 | AHQ/ARQ | | | 0.020 | | 0.034 | 0.093 | | | 0.020 | | | 0.026 | | | | 0.020 | | | 0.017 |
| R3 | ARH/ARH | 0.090 | 0.070 | 0.130 | | | | | 0.027 | | 0.022 | 0.018 | | | | | | 0.021 | 0.020 | 0.031 |
| R3 | ARH/ARQ | 0.200 | 0.080 | 0.090 | 0.040 | 0.069 | | 0.029 | | 0.100 | 0.156 | 0.109 | 0.051 | 0.023 | 0.222 | 0.122 | | 0.104 | 0.220 | 0.087 |
| R3 | ARQ/ARQ | 0.530 | 0.410 | 0.270 | 0.520 | 0.276 | 0.329 | 0.265 | 0.270 | 0.260 | 0.422 | 0.564 | 0.410 | 0.205 | 0.489 | 0.585 | 0.551 | 0.375 | 0.360 | 0.401 |
| R4 | ARR/VRQ | | | | | 0.034 | 0.007 | | | | | | | | | | | | | 0.002 |
| R5 | VRQ/AHQ | | | | | | 0.007 | | | | | | | | | | | | | 0.001 |
| R5 | VRQ/ARH | | | | | | | | | 0.020 | | | | | | | | | | 0.001 |
| R5 | VRQ/ARQ | 0.010 | | | | | 0.014 | | | | | | | 0.023 | | 0.024 | | | | 0.004 |
| R5 | VRQ/VRQ | | | | | | 0.007 | | | | | | | | | | | | | 0.001 |
| n.c. | ARR/TRR | | | 0.010 | | | | | | | | | | | | | | | | 0.001 |
| n.c. | ARR/TRQ | | | 0.060 | | 0.069 | | | | 0.020 | 0.022 | | | 0.045 | | | | | | 0.011 |
| n.c. | ARQ/TRQ | 0.030 | | 0.020 | 0.090 | 0.172 | | | | 0.020 | | | 0.077 | 0.205 | | | | | | 0.029 |
| n.c. | TRQ/TRQ | | | | | | | | | | | | | 0.023 | | | | | | 0.001 |
| n.c. | ARR/ARK | | | | | | | | | | | | 0.026 | | | | | | | 0.001 |
| n.c. | ARH/ARK | | | | 0.020 | | | | | | | 0.018 | | | 0.022 | | | | | 0.004 |
| n.c. | ARQ/ARK | 0.030 | | | | | | | | | 0.022 | | 0.026 | | 0.111 | 0.024 | | 0.021 | 0.080 | 0.014 |
| n.c. | ARH/TRH | | | 0.020 | | | | | | | | | | | | | | | | 0.002 |
| | *Allele frequency* | | | | | | | | | | | | | | | | | | | |
| | ARR | 0.059 | 0.240 | 0.275 | 0.205 | 0.259 | 0.315 | 0.353 | 0.351 | 0.380 | 0.256 | 0.165 | 0.205 | 0.273 | 0.100 | 0.146 | 0.235 | 0.292 | 0.180 | 0.234 |
| | ARQ | 0.706 | 0.565 | 0.445 | 0.695 | 0.535 | 0.586 | 0.632 | 0.622 | 0.460 | 0.622 | 0.745 | 0.679 | 0.546 | 0.700 | 0.757 | 0.745 | 0.583 | 0.640 | 0.619 |
| | ARH | 0.206 | 0.190 | 0.215 | 0.045 | 0.034 | | 0.015 | 0.027 | 0.120 | 0.100 | 0.081 | 0.038 | 0.022 | 0.133 | 0.073 | | 0.115 | 0.140 | 0.097 |
| | AHQ | | 0.005 | 0.010 | | 0.034 | 0.078 | | | 0.010 | | | 0.013 | | | | 0.020 | | | 0.014 |
| | VRQ | 0.006 | | | | 0.017 | 0.021 | | | 0.010 | | | | 0.011 | | 0.012 | | | | 0.005 |
| | TRR | | | 0.005 | | | | | | | | | | | | | | | | 0.001 |
| | TRQ | 0.012 | | 0.040 | 0.045 | 0.121 | | | | 0.020 | 0.011 | | 0.039 | 0.148 | | | | | | 0.021 |
| | TRH | | | 0.010 | | | | | | | | | | | | | | | | 0.001 |
| | ARK | 0.012 | | | 0.010 | | | | | | 0.011 | 0.009 | 0.026 | | 0.067 | 0.012 | | 0.010 | 0.040 | 0.009 |

[a] Risk group of the genotypes by National Scrapie Plan (NSP)

*n.c.* not classified in any risk group by National Scrapie Plan

*IVE* İvesi (Awassi), *AKK* Akkaraman, *KAK* Kangal Akkaraman, *MRK* Morkaraman, *GNK* Güneykaraman, *KVR* Kıvırcık, *NRD* Norduz, *KRK* Karakaş, *SKZ* Sakız (Chios), *HRK* Herik, *HMS* Hemşin, *DGL* Dağlıç, *KRY* Karayaka, *TUJ* Tuj (Tushin), *CCP* Çine Çaparı, *GOK* Gökçeada (Imroz), *KRG* Karagül (Karakul), *ZOM* Zom

frequency of ARQ and low frequency of ARR represent the main problem faced by breeding programs aimed at increasing the frequency of ARR. In addition, most reports in other countries showed that the ARQ allele was the most frequent allele in their sheep breeds [13, 15, 18, 21–30]. For that reason, the ARQ allele is thought to be wild-type allele and to represent the ancestral form of the *PrP* gene. Our findings seem to support to this idea.

The frequency of the ARR allele was quite low in İvesi (Awassi), which is a regional transboundary breed in Middle East (Table 1). This issue is crucial because if the ARR allele was absent in any breed, the only way to create a strain genetically resistant to scrapie would be to introgress the gene of interest from other breeds, thus threatening the original breed. The frequency of ARR found in this study was almost similar to those reported by Gootwine et al. [27] and by Frootan et al. [11] in İvesi. The VRQ allele, which is associated with the highest susceptibility to scrapie, was detected at low frequencies in İvesi, Güneykaraman, Kıvırcık, Sakız, Karayaka, and Çine Çaparı. Four sheep were found as heterozygous and one sheep was found as homozygous for VRQ allele in Kıvırcık. In previous studies, VRQ allele was also detected at low frequencies in Kıvırcık [8, 10], in İvesi [11], in Sakız [8, 10], and in Karayaka [9]. The VRQ allele has been reported for the first time in Güneykaraman and Çine Çaparı in this study. Even if the VRQ allele was found at low frequency in six Turkish sheep breeds, eradication of the VRQ allele in these particular breeds may be implemented to avoid further losses of genetic variability.

Four rare alleles (TRR, TRQ, TRH, and ARK), which were not classified in any risk group by NSP, were also observed in this study. While TRQ and ARK have been previously reported [8, 9, 10, 11], TRR and TRH have been found for the first time in Turkish native sheep breeds. While TRR and TRH were found in 1 and 2 sheep, respectively, TRQ and ARK were detected in 46 and 21 sheep, respectively, in 1,110 Turkish native sheep. Among the rare alleles found in this study, TRQ showed a higher frequency (0.148) in Karayaka (Table 1). Alvarez et al. [9] has also reported that the frequency of the TRQ was found as 0.158 in Karayaka. This breed, which is pure breed reared in northeast part of Turkey, is different from other breeds with regard to the frequency of the TRQ allele. However, we do not yet have information about relationship between the frequency of the TRQ and the origin of this breed.

According to our results, the ARQ/ARQ genotype was the predominant genotype in all breeds except for Kıvırcık, Norduz, Karakaş, and Karayaka breeds (Table 1). This finding is consistent with the other reports concerning Turkish native sheep breeds [8–11] as well as report on breeds found in other countries [13, 15, 21–27]. The most resistant genotype, ARR/ARR, was detected in all breeds, except for in Norduz, Karakaş, and Dağlıç. This is the primary obstacle faced by breeding programs which aim to increase the frequency of the ARR. Therefore, such breeding programs should be implemented for these breeds. The AHQ/ARH genotype, a member of R3, was not detected in any breeds. Ün et al. [8], Alvarez et al. [9] and Frootan et al. [11] have also reported that they did not find the AHQ/ARH genotype in Turkish native sheep breeds. Among eight additional genotypes, which were not classified in any risk group by NSP, ARQ/TRQ was found most common (Table 1). The genotypes of ARR/TRR, ARR/ARK, and ARH/TRH have been found for the first time in Turkish native sheep breeds.

When high-sensitivity groups R4 and R5 were combined as VRQ-carrying genotypes, Güneykaraman and Kıvırcık had 3.4 and 3.5 % of the sheep in these categories, respectively (Table 2). The Kıvırcık breed was found to have five sheep carrying the VRQ allele, with one of the five carrying the VRQ/VRQ genotype. Scrapie control breeding programs may be established for Kıvırcık to eliminate the VRQ-carrying genotypes.

All breeds except Norduz, Karakaş, and Karayaka belong to the risk group R3. The prevalence of sheep in risk group R3 was estimated at 53.7 % in Turkish native sheep breeds (Table 2). Compared with the other breeds studied in this study, the breed most susceptible to scrapie among Turkish native sheep breeds is Kıvırcık, reared in the western part of Turkey, because 2.8 % of the animals were in the high susceptible group. While 4 breeds (Güneykaraman, Kıvırcık, Sakız, and Çine Çaparı) from western part of Turkey had the sheep classified in high-susceptible groups (R4 and R5), almost none of the breeds (Kangal Akkaraman, Morkaraman, Norduz, Karakaş, Hemşin, Tuj, and Zom) from eastern part of Turkey had the sheep classified in high-susceptible groups (Table 2). Karami et al. [31] and Babar et al. [32] have reported that sheep in high-susceptible groups were not found in Iran and Pakistan, the eastbound neighbors of Turkey. Guan et al. [33] and Wang et al. [34] have also reported that there were no sheep with VRQ/VRQ in China or Mongolia, which are East Asian countries. In this study, it is propounded that the susceptibility to scrapie increased from eastern to western part of Turkey. Further research is needed to argue and confirm this assumption. Moreover, it was found that Kangal Akkaraman might be thought the highest variable breed among Turkish native sheep breeds as this breed had all alleles except VRQ and ARK (Table 1).

In conclusion, all Turkish native sheep breeds in this study were identified for the *PrP* polymorphism. Allele frequencies at the PrP locus showed a predominance of the ARQ allele, although ARR is present in all breeds. The

**Table 2** Distributions of scrapie risk groups in Turkish native sheep breeds

| NSP[a] | IVE (%) | AKK (%) | KAK (%) | MRK (%) | GNK (%) | KVR (%) | NRD (%) | KRK (%) | SKZ (%) | HRK (%) | HMS (%) | DGL (%) | KRY (%) | TUJ (%) | CCP (%) | GOK (%) | KRG (%) | ZOM (%) | TOTAL (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| R1 | 3.0 | 4.0 | 10.0 | 8.0 | 6.9 | 9.3 | | | 18.0 | 13.3 | 3.6 | | 2.3 | 4.4 | 4.9 | 4.1 | 10.4 | 4.0 | 6.4 |
| R2 | 8.0 | 40.0 | 28.0 | 25.0 | 26.7 | 43.6 | 70.6 | 70.3 | 38.0 | 22.2 | 25.5 | 38.5 | 45.5 | 11.1 | 19.5 | 38.7 | 37.5 | 28.0 | 32.7 |
| R3 | 82.0 | 56.0 | 51.0 | 56.0 | 40.3 | 43.6 | 29.4 | 29.7 | 38.0 | 60.0 | 69.1 | 48.7 | 22.8 | 71.1 | 70.7 | 57.1 | 50.0 | 60.0 | 53.7 |
| R4 | | | | | 3.4 | 0.7 | | | | | | | | | | | | | 0.2 |
| R5 | 1.0 | | | | | 2.8 | | | 2.0 | | | | 2.3 | | 2.4 | | | | 0.7 |
| n.c. | 6.0 | | 11.0 | 11.0 | 24.1 | | | | 4.0 | 4.4 | 1.8 | 12.9 | 27.3 | 13.3 | 2.4 | | 2.1 | 8.0 | 6.3 |

[a] Risk group of the genotypes by National Scrapie Plan (NSP)

*n.c.* not classified in any risk group by National Scrapie Plan

*IVE* İvesi (Awassi), *AKK* Akkaraman, *KAK* Kangal Akkaraman, *MRK* Morkaraman, *GNK* Güneykaraman, *KVR* Kıvırcık, *NRD* Norduz, *KRK* Karakaş, *SKZ* Sakız (Chios), *HRK* Herik, *HMS* Hemşin, *DGL* Dağlıç, *KRY* Karayaka, *TUJ* Tuj (Tushin), *CCP* Çine Çaparı, *GOK* Gökçeada (Imroz), *KRG* Karagül (Karakul), *ZOM* Zom

VRQ allele, associated with high susceptibility to scrapie, had the frequency ranging between 0.006 and 0.021 in İvesi, Güneykaraman, Kıvırcık, Sakız, Karayaka, and Çine Çaparı. According to our results, all breeds belong to the risk group R3 followed by R2. Selection for genetic resistance to scrapie through fixation of the ARR allele is possible, but should be conducted carefully to avoid further losses of genetic variation in breeds generally endangered of extinction. Our findings of sheep breeds with *PrP* polymorphisms will assist the sheep breeding programs for selection of scrapie resistant genotypes to reduce the risk of scrapie in Turkey.

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**Conflict of interest** The authors declare that they have no competing interests.

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