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*Full Length Research Paper*

# Polymorphisms of the prion protein gene Arabi sheep breed in Iran

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**Ovine scrapie is a neurodegenerative disease caused by polymorphisms of the prion protein gene (Prnp); especially the amino acid residue alterations at codons 136, 154, and 174, in sheep have been found to be associated with susceptibility to scrapie disease. We studied Prnp polymorphisms in local sheep of Khuzestan, Iran. In this study, a total of 250 healthy and randomly chosen sheep were investigated. Information from the breeder was considered in order to avoid family connections. The genetic DNA of blood samples was extracted, amplified and sequenced. At codon 136, are two different amino acid residues A and V with frequencies of 98.8 and 1.2%, respectively. In our study, the amino acid residue at 154 in all the prion protein (PrPs) was A. At codon 171, are three different amino acid residues Q, R and H with frequencies of 68.4, 21.8 and 9.8%, respectively. The frequency of the amino acid residues ARQ/ARQ at codons 136, 154, and 171, respectively, which is associated with medium-high susceptibility to scrapie, was 43.2%. The frequencies of genotypes ARQ/ARR, ARQ/ARH, ARQ/VRQ, ARR/ARH and ARH/ARH were 38, 10, 1.2, 5.2 and 2.4, respectively. The scrapie-resistant genotype ARR/ARR was not found. Also, the highly susceptible genotype VRQ/VRQ at these codons were not detected in the tested sheep.**

**In addition, five polymorphism were identified ( G127V, N146S, Y172D, S173N, and V179E ) at different codons of PrP gene.**

**Key words:** Prion protein gene (Prnp), polymorphisms, susceptibility, scrapie.

## INTRODUCTION

Scrapie is an invariably fatal transmissible neurodegenerative disease of sheep and goats (Goldman et al., 2005). This disease has been known in Europe for over250 years. In the United States of America, it was first diagnosed in 1947 in a flock of Suffolk sheep (Rook, 2001; Un et al., 2008). The same neurodegenerative disease in cattle is referred to as bovine spongiform encephalopathy (BSE), and in humans as Creutzfeldt-Jakob disease (CJD). These diseases are collectively called transmissible spongiform encephalopathies (TSE) (Wang et al., 2008).

A characteristic feature of these diseases is typically, a long incubation period (1.5 to 3 years) and progressive clinical deterioration. Typical symptoms include pruritus, excitability, trembling, lack of co-ordination and paralysis at a later stage with eventual death. TSEs are generally characterized by pathogenic prion protein (PrPSc) accumulation in the central nervous system (CNS) in all hosts including man and in lymphoreticular system (LRS) in sheep and goats. PrPSc is a protease-resistant glyco-

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**Abbreviations: BSE,** Bovine spongiform encephalopathy; **CJD,** CreutzfeldtJakob disease; **TSE,** transmissible spongiform encephalopathies; **PrPSc,** pathogenic prion protein; **CNS,** central nervous system; **LRS,** lymphoreticular system; **PrPc,** protease-sensitive isoform; **PrP,** prion protein; **EDTA,** ethylenediaminetetraacetic acid; **PCR,** polymerase chain reaction.

protein derived from a cellular protease-sensitive isoform (PrPc) that is hostencoded by the prion protein gene and is strongly believed to cause TSE (Prusiner, 2004; Baber et al., 2009).

The sheep *PrP* gene is located on chromosome 13 and consists of three exons and two introns. Its open reading frame is located in exon three, coding for a protein product of 256 amino acids. Research has evidenced that polymorphisms within the open reading frame of PrP gene modulate susceptibility and pathology of TSEs (Pocchiari, 1994; Bossers et al., 1999).

Polymorphisms in codons 136 [coding either alanine (A) or valine (V)], 154 [coding arginine (R) or histidine (H)] and 171 [coding R, H or glutamin (Q)] of the *PrP* gene are known to be highly related to the degree of susceptibility or resistance to scrapie in sheep (Vaccari et al., 2001; Tongue et al., 2004; Un et al.,2008). In general, sheep of the ARR/ARR homozygous genotype are known to be highly resistant and those of the VRQ/VRQ genotype are highly susceptible to clinical scrapie (Tongue et al., 2004; Gama et al., 2006). ARQ is predicted to be wild type allele (Elsen et al., 1999). Moreover, many other polymorphisms were identified in ovine *PrP* gene at codons 12, 83, 85, 101, 112, 116, 126, 127, 138, 141, 143, 146, 151, 152, 167, 172, 175, 176, 180, 189, 194, 195, 196, 211, 231, 237 and 241, and yet remain to be associated with scrapie susceptibility (Thorgeirsdottir et al., 2002; Tranulis et al., 1999; Vaccari et al., 2001). Association of the additional polymorphisms with susceptibility or resistance to scrapie disease in sheep is in most cases unknown.

Although information regarding sheep scrapie-associated {136 (A/V), 154 (R/H) and 171 (Q/R,H)} as well as other prion protein (PrP) amino acid polymorphisms has been documented in breeds from different countries, no study on PrP variability in Iranian sheep breeds has so far been undertaken. The present study was therefore undertaken to determine PrP genotype profiles of Iranian local sheep in Khuzestan province in order to estimate the degree of resistance of these animals to natural/typical scrapie.

## MATERIALS AND METHODS

### Animals

In this study, a total of 250 healthy and randomly chosen sheep were investigated. Information from the breeder was considered in order to avoid family connections. Blood from sheep were collected into tubes containing ethylenediaminetetraacetic acid (EDTA).

### DNA isolation and amplification

Genomic DNA was isolated from the EDTA-treated blood by using salting-out procedure (Javanrouh et al., 2006) after thawing the blood samples. The extracted DNA was then subjected to a polymerase chain reaction (PCR). *PrP* coding region was amplified with following primers: forward primer 5_-GGAGGCTGGGGTCA AGGT-3_ and reverse primer 5_-GTGGTGGTGACTGTGTTG-3_(Van poucke et al., 2005).

PCR was performed in a total volume of 20 µl containing 100 to 150 ng DNA, 500 nM of each primer, 0.8 mM dNTPs, 2 mM $MgCl_2$, buffer PCR and 0.5 U BioTaq polymerase. The PCR program consists of a DNA denaturation step (5 min at 95°C), followed by 30 amplification cycles (denaturation for 30 s at 95°C, annealing for 30 s at 54°C and extension for 1 min at 63°C), and a final extension step for 10 min at 72°C (Van poucke et al., 2005). Amplification products were visualized after electrophoresis on a 2% agarose gel with along-wavelength UV transilluminator and eluted in 20 µl with the Genecleen II kit (Bio 101, USA). Approximately, 200 ng of this purified PCR product was used in combination with 2 pmol of forward primer, sequencing buffer and Big dye (Applied Biosystems, USA) for a direct sequencing reaction. The reaction, consisting of 30 cycles (10 s at 94°C, 5 s at 50°C and 4 min at 60°C), was performed in the T3 Termocycler (Ettenford, Germany).

### Genotyping

The sequencing PCR products were washed with 70% ethanol and resolved on an ABI 3130 genetical analyzer (Applied Biosystems, Inc., Foster City, CA). Single nucleotide polymorphisms in different codons of the PrnP gene were checked directly by using the Chromaspro ( 1.5 ) software.

### Statistical analysis

Genotypic frequencies were calculated from the direct counting of genotypes as follows:

$$fij = \frac{nij}{N}$$

Where, *n*ij is the number of animals with the genotype of ij and *N* is the number of total animals.

$\chi^2$ tests and allele frequencies were carried out by using PopGene software (Yeh et al., 2000). $\chi^2$ tests were conducted for each codon to evaluate possible deviations from Hardy-Weinberg equilibrium.

## RESULTS AND DISCUSSION

The analyzed amino acid sequences of the PrP genes in the 250 sheep belonging to Arabi sheep breed in Iran, Iran showed that R was invariant at codon 154 , whereas polymorphisms were present at each of the codons 136 and 171. In addition, five different polymorphisms were identified at codons 127, 146, 172, 173, and 179.

### Allele frequencies

At codon 136, are two different amino acid residues A and V with frequencies of 98.8 and 1.2%, respectively. Valine at 136 was reportedly associated with susceptibility to scrapie. On the contrary, Alanin at 1 36 was reported to be related to resistance to scrapie (Elsen et

**Table 1.** Allele and their frequencies at codons 136, 154 and 171, of the prion protein gene in Arabi sheep breed in Iran

| Allele | Frequency (%) |
|---|---|
| ARQ | 67.8 |
| VRQ | 0.6 |
| ARR | 21.6 |
| ARH | 10 |

**Table 2.** Genotypes and their frequencies at codons 136, 154 and 171, which are associated with susceptibility or resistance to scrapie, of the prion protein gene in Arabi sheep breed in Iran

| Risk group | Genotype | Frequency (%) |
|---|---|---|
| 1 | ARR/ARQ | 38 |
| 2 | ARR/ARH | 5.2 |
| 2 | ARQ/ARQ | 43.2 |
| 2 | ARQ/ARH | 10 |
| 2 | ARH/ARH | 2.4 |
| 3 | ARQ/VRQ | 1.2 |

1, Associated with partial resistance to scrapie; 2, associated with medium-high susceptibility to scrapie; 3, associated with high susceptibility to scrapie.

al., 1999; Wang et al., 2008). Although the importance of amino acid residue at codon 154 is not fully understood, H at this position has a positive relationship with resistance to scrapie (Elsen et al., 1999). In our study, the amino acid residue at 154 in all the PrPs was A. At codon 171, are three different amino acid residues Q, R and H with frequencies of 68.4, 21.8 and 9.8%, respectively. The low frequency of 171H and high frequency of 171Q were consistent with previously reported sheep PRNP polymorphisms (Desilva et al., 2003; Gombojav et al., 2004; Zhang et al., 2004). Frequencies of the 4 alleles investigated (i.e., ARR, ARQ, VRQ, ARH) in the native sheep are presented in Table 1. ARQ allele was predominant genotype in sample sheep, as the predominant allele in the majority of the studies is ARQ allele (Elsen et al., 1999; Gama et al., 2006; un et al., 2008), ARQ allele is predicted to be wild-type allele of the PrP gene.

The frequencies of ARQ allele was 67.8% (Table 1). Also, the frequency of sheep that carried the allele of ARQ was 91.4%. This allele is associated with medium-high susceptibility to scrapie. The high susceptibility allele VRQ was not found in sample sheep.

## Genotypic frequencies

The polymorphisms were detected in the prion gene which was made up 6 different genotypes: ARR/ARQ, ARQ/AHQ, ARQ/ARQ, ARQ/ARH, ARH/ARH, and ARQ/VRQ (Table 2). Different genotypes were classified in three risk groups according to Tongue et al. (2004). Overall, the more frequent genotype was ARQ/ARQ, followed by ARR/ARQ. The frequency of the amino acid residues ARQ/ARQ at codons 136, 154 and 171, respectively, which is associated with medium-high susceptibility to scrapie, was 43.2%. In our study, highly susceptible genotype VRQ/VRQ was not detected. Most of the investigated sheep belongs to the risk groups 1 and 2. As scrapie disease has not been found in native sheep till now, it was a major point of interest, whether this could be linked to the PrP genotype. In Arabi sheep breed in Iran, the distribution of genotype for codon 136 was in agreement with a situation of Hardy-Weinberg equilibrium.This population was monomorphic for PrP codon 154; while deviated from Hardy-Weinberg equilibrium ($P > 0.05$) for codon 171. The observed ARQ/ARQ and ARQ/ARR genotype frequencies were slightly higher than expected frequencies.

## Additional polymorphism

After the sequencing process of 250 sheep, five different polymorphisms were identified: G127V, N146S, Y172D, S173N, and V179E (Table 3).The relationship between these polymorphisms and scrapie disease is yet to be investigated. The variability of PrP gene in native sheep

**Table 3. Additional polymorphisms of the PrP gene in Arabi sheep breed in Iran.**

| Additional polymorphism | Haplotype polymorphism | Heterozygote/ Homozygote | n |
|---|---|---|---|
| G127V | ARQ/ARQ | Heterozygote | 3 |
| N146S | ARQ/ARQ | Heterozygote | 4 |
| Y172D | ARR/ARQ | Heterozygote | 3 |
| S173N | ARQ/ARQ | Heterozygote | 3 |
| | ARQ/ARQ | Homozygote | 2 |
| V179E | ARQ/ARQ | Heterozygote | 7 |
| | ARQ/ARQ | Homozygote | 2 |
| | ARR/ARQ | Heterozygote | 5 |
| | ARQ/ARH | Heterozygote | 2 |

of Khuzestan is very high. This highly variable PrP gene of sheep may also be profitable for breeding programmes.

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