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Allelic frequency and genotypes of prion protein at codon 136 and 171 in Iranian Ghezel sheep breeds

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PrP genotypes at codons 136 and 171 in 120 Iranian Ghezel sheep breeds were studied using allele-specific PCR amplification and compared with the well-known sheep breeds in North America, the United States and Europe. The frequency of V allele and VV genotype at codon 136 of Ghezel sheep breed was significantly lower than AA and AV. At codon 171, the frequency of allele H was significantly lower than Q and R. Despite the similarities of PrP genotypes at codons 136 and 171 between Iranian Ghezel sheep breeds and some of the studied breeds, significant differences were found with others. Planning of effective breeding control and successful eradication of susceptible genotypes in Iranian Ghezel sheep breeds will not be possible unless the susceptibility of various genotypes in Ghezel sheep breeds to natural or experimental scrapie has been elucidated.

Scrapie was first described in England in 1732,[1] and it is an infectious neurodegenerative fatal disease of sheep and goats belonging to the group of transmissible subacute spongiform encephalopathies (TSEs), along with bovine spongiform encephalopathy (BSE), chronic wasting disease and Creutzfeldt-Jakob disease.[2,3] The term prion, proteinaceous infectious particles, coined by Stanley B. Prusiner, was introduced, and he presents the idea that the causal agent is a protein.[4] Prion proteins are discovered in two forms, the wild-type form (PrP^{c}) and the mutant form (PrP^{Sc}).[5] Although scrapie is an infectious disease, the susceptibility of sheep is influenced by genotypes of the prion protein (PrP) gene.[2,6] Researchers have found that the PrP allelic variant alanine/arginine/arginine (ARR) at codons 136, 154 and 171 is associated with resistance to scrapie in several breeds.[7-14] Most of the sheep populations in the Near East and North African Region (84% of the total population of 255 million) are raised in Iran, Turkey, Pakistan, Sudan, Algeria, Morocco, Afghanistan, Syria and Somalia.[15] In 2003, the Iranian sheep population was estimated at 54,000,000 head. The Ghezel sheep breed, which also is known as Kizil-Karaman, Mor-Karaman, Dugli, Erzurum, Chacra, Chagra, Chakra, Gesel, Gezel, Kazil, Khezel, Khizel, Kizil, Qezel, Qizil and Turkish Brown, originated in northwestern Iran and northeastern Turkey. By considering sheep breeds as one of the main sources of meat, dairy products and related products, a global screening attempt is started in different areas. In compliance with European Union Decision 2003/100/EC, each member state has introduced a breeding program to select for resistance to TSEs in sheep populations to increase the frequency of the ARR allele. A similar breeding program is established in United States and Canada. The Near East and North African Region still needs additional programs to help the global plan of eradication of scrapie-susceptible genotypes. The current study was the first to assess the geographical and molecular variation of codons 136 and 171 polymorphism between Iranian Ghezel sheep breed and well-known sheep breeds.

Polymorphism at codon 136 is associated with susceptibility to scrapie in both experimental and natural models.[10,11,13,16] **Table 1** presents the allelic frequency and percentage of each allele and resulting genotypes at position 136. Alleles A and V were found in 77.5% and 22.5% of studied cases, respectively. The difference between frequencies of alleles is significant ($p < 0.05$). The frequency of possible VV, AV and AA genotypes were 6%, 15%, 39%, respectively. It shows that frequency of VV genotype is significantly lower than AA and AV ($p < 0.05$). No significant differences were found between ewes and rams for the position 136 ($p > 0.50$). Extremely low prevalence of the susceptibility allele, that is, V136, was reported in Greek Chios and Karagouniko sheep[17] and Austrian Carynthian sheep.[18] Swiss White Alpine showed higher frequency of allele V at position 136 than Swiss Oxford Down, Swiss Black-Brown Mountain and Valais Blacknose.[19] Comparison of polymorphism at codon 136 in the current study with some of other breeds (**Table 1**) shows a similar pattern between Montadale breed of Oklahoma sheep,[20] some flock of Hampshire sheep[21] with current study, but the frequency of it is higher than that of some other breeds.

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Table 1. Comparison of PrP allelic and genotype frequencies at codon 136 in different breeds

Breed	A (%)	V (%)	AA (%)	AV (%)	VV (%)	Reference
Iranian Ghezel breeds (n = 120)	77.50	22.5	65.00	25.00	10.00	Current study
Oklahoma sheep (n = 334)						De Silva, et al.[27]
Suffolk	99.24	0.76	98.48	1.52	0.00	
Hampshire	100	0.00	100	0.00	0.00	
Dorset	92.6	7.94	87.30	9.52	3.17	
Montadale	77.66	22.34	59.57	36.17	4.26	
Hampshire (n = 48)	93.75	6.25	88.00	12.00	0.00	Youngs, et al.[21]
German Sheep Breeds (n = 660)	92.89	7.11	87.80	10.47	1.73	Kutzer, et al.[28]
Bleu du Maine	83.47	16.53	69.56	27.83	2.61	
Friesian Milk S.	100	0.00	100	0.00	0.00	
Nolana	90.13	9.87	85.90	8.46	5.64	
Suffolk	100	0.00	100	0.00	0.00	
Texel	90.87	9.13	82.16	17.41	0.43	
Swiss Sheep (n = 200)	92.5	7.5				Gmur, et al.[19]
Swiss Oxford Down	93.00	7.00	-	-	-	
Swiss Black-Brown M.	99.00	1.00	-	-	-	
Valais Blacknose	100	0.00	-	-	-	
Swiss White Alpine	88.00	22.00	-	-	-	
Austrian Sheep (n = 112)	98.95	1.05	98.95	0.00	1.05	Sipos, et al.[18]
Tyrolean mountain sheep	100	0.00	100	0.00	0.00	
Forest sheep	100	0.00	100	0.00	0.00	
Tyrolean stone sheep	100	0.00	100	0.00	0.00	
Carynthian sheep	95.80	4.20	95.80	0.00	4.20	

It has been found that a polymorphism at codon 171 also is associated with susceptibility to experimental scrapie in Cheviot sheep[16] and natural scrapie in Suffolk sheep.[22] As shown in **Table 2**, alleles H and Q with frequencies of 1.67% and 55% were the least and most frequent alleles, respectively. Frequency of allele R was 44.33%. A significant difference was found between allele H and others ($p < 0.05$), but no significant difference was found between alleles Q and R ($p > 0.5$). The frequency of QR and QQ genotypes were the same (36.67%) and significantly higher than RR (23.33%) and RH (3.33) ($p < 0.05$). None of QH or HH genotypes were observed. No significant differences also were found between ewes and rams for the position 171 ($p > 0.50$). It has been shown that the predominate genotype in Columbia breeds is QQ instead of QR in Suffolk, Rambouille and Targhee.[23] They also found that different breeds show different predominant genotypes in ewes and rams.[23] Different PrP genotypes were found at codon 171 in Austrian sheep breeds, but QQ has higher frequency than others.[18] In some kinds of Swiss breeds, allelic frequencies of allele Q was higher than R.[19] Distribution of prion protein codon 171 genotypes in Hampshire sheep revealed that different flocks shows different patterns.[21] The frequency of PrP genotypes at codon 171 in Iranian Ghezel breeds was similar to some sheep breeds, like the Suffolk breed of Oklahoma sheep, but it was completely different from others (**Table 2**).

The association between scrapie susceptibility and polymorphism at codon154 is unclear, and fewer evidences were found that support it.[24,25] So the frequency of different genotypes at codon 154 in Iranian Sheep breeds has not been included in the current study.

In addition to difference in number of included animals and methodology of genotyping, the apparent discrepancies among reported allelic frequency might be caused by the difference in geographical dissemination of sheep breeds and related purity.[26] The deviations from Hardy-Weinberg equilibrium, which were assumed in the current study, were checked using Pearson's chi-squared test or Fisher's exact test. Although the number of animals in this study is acceptable, a population study is still suggested. In conclusion, fairly different patterns of PrP genotypes in this common Near eastern sheep breed are an evidence for geographical variation of molecular susceptibility to scrapie. Because other report from Turkey also has shown a prevalence of genotypes, which is different from western countries,[26] and no reports have been published yet to show which of the genotypes in that breed are actually resistant or susceptible to natural or experimental scrapie, our results is an authentic platform to motivate further studies. Actually, extrapolation of the existing general pattern of susceptibility or resistance for all breeds and current plan of elimination would not be successful unless the susceptible genotypes in the Near East with numerous breeds

Table 2. Comparison of PrP allelic and genotype frequencies at codon 171 in different breeds

PrP genotypes at codon 172										
Breed	Allelic frequency			Genotypes						Reference
	Q	R	H	RR	QR	QQ	QH	RH	HH	
Iranian Iranian Ghezel breeds (n = 120)	55.00	43.33	1.67	23.33	36.67	36.67	0.00	3.33	0.00	Current study
Oklahoma sheep (n = 334)										De Silva, et al.[20]
Suffolk	40.95	59.05	0.00	37.07	43.97	18.97	0.00	0.00	0.00	
Hampshire	51.89	48.11	0.00	21.70	52.83	25.47	0.00	0.00	0.00	
Dorset	67.75	31.25	0.00	7.95	46.59	45.45	0.00	0.00	0.00	
Montadale	62.96	37.04	0.00	14.81	44.44	40.74	0.00	0.00	0.00	
Hampshire (n = 201)	72.14	26.60	1.26	5.00	42.00	50.00	2.00	1.00	0.00	Youngs, et al.[21]
German Sheep Breeds (n = 660)										Kutzer, et al.[28]
Bleu du Maine	37.8	62.2	0.00	46.96	30.44	22.6	0.00	0.00	0.00	
Friesian Milk S.	90.45	8.9	0.65	1.27	15.3	82.8	0.00	0.00	0.64	
Nolana	42.3	57.8	0.00	36.62	42.26	21.13	0.00	0.00	0.00	
Suffolk	68.4	27.6	4.0	16.1	21.84	55.17	4.6	1.15	1.15	
Texel	55.35	29.7	14.9	12.56	26.83	36.36	11.25	7.36	5.63	
Swiss Sheep (n = 200)										Gmur, et al.[19]
Swiss Oxford Down	32.00	68.00	-	-	-	-	-	-	-	
Swiss Black-Brown M.	70.00	30.00	-	-	-	-	-	-	-	
Valais Blacknose	85.00	15.00	-	-	-	-	-	-	-	
Swiss White Alpine	27.00	73.00	-	-	-	-	-	-	-	
Austrian Sheep (n = 112)										Sipos, et al.[18]
Tyrolean mountain sheep	74.30	25.80	0.00	2.90	45.70	51.40	0.00	0.00	0.00	
Forest sheep	77.00	19.20	3.80	11.50	15.40	69.20	0.00	0.00	3.80	
Tyrolean stone sheep	81.50	14.80	3.70	0.00	29.60	62.90	7.40	0.00	0.00	
Carynthian sheep	72.80	23.00	4.20	4.20	41.70	13.00	8.40	0.00	0.00	

will be identified. Hence, the current study could be used as an important pilot study for further investigation.

Genomic DNA was isolated from fresh EDTA-treated blood of 120 healthy, randomly chosen sheep of Iranian Ghezel sheep breeds using a mammalian blood DNA isolation kit (Bioflux, Japan). The allelic frequencies of prion protein codons 171 and 136 were determined by allele-specific PCR amplifications using scrapie susceptibility test kit (Elchrom Scientific AG). Primer sets were designed by manufacturer to amplify specific gene targets according to possible genotypes of positions 136 and 171.

The amplification reactions were performed using iCycler™ (BioRad Inc.,), and PCR products (**Table 3**) were analyzed using Origin™ electrophoresis apparatus and Spreadex EL 500 gels (Elchrom Scientific AG) at 55°C for 50 min. The gels were visualized by Syber Gold staining for 45 min.

Table 3. PCR product size of different alleles

Position	Genotype	Fragment size
136	A	133
136	V	139
171	H	170
171	Q	247
171	R	155

References

1. McGowan J. Scrapie in sheep. Scott J Agric 1922; 5:365-75.
2. Hunter N. PrP genetics in sheep and the applications for scrapie and BSE. Trends Microbiol 1997; 5:331-4.
3. Hunter N. Scrapie: uncertainties, biology and molecular approaches. Biochim Biophys Acta 2007; 1772: 619-28.
4. Prusiner SB. Novel proteinaceous infectious particles cause scrapie. Science 1982; 216:136-44.
5. Prusiner SB, DeArmond SJ. Molecular biology and pathology of scrapie and the prion diseases of humans. Brain Pathol 1991; 1:297-310.
6. Hunter N, Cairns D, Foster JD, Smith G, Goldmann W, Donnelly K. Is scrapie solely a genetic disease? Nature 1997; 386:137.
7. Belt PB, Muileman IH, Schreuder BE, Bos-de Ruijter J, Gielkens AL, Smits MA. Identification of five allelic variants of the sheep PrP gene and their association with natural scrapie. J Gen Virol 1995; 76:509-17.
8. Bossers A, Schreuder BE, Muileman IH, Belt PB, Smits MA. PrP genotype contributes to determining survival times of sheep with natural scrapie. J Gen Virol 1996; 77:2669-73.
9. Clouscard C, Beaudry P, Elsen JM, Milan D, Dussaucy M, Bounneau C, et al. Different allelic effects of the codons 136 and 171 of the prion protein gene in sheep with natural scrapie. J Gen Virol 1995; 76:2097-101.
10. Goldmann W, Hunter N, Foster JD, Salbaum JM, Beyreuther K, Hope J. Two alleles of a neural protein gene linked to scrapie in sheep. Proc Natl Acad Sci USA 1990; 87:2476-80.
11. Hunter N, Goldmann W, Benson G, Foster JD, Hope J. Swaledale sheep affected by natural scrapie differ significantly in PrP genotype frequencies from healthy sheep and those selected for reduced incidence of scrapie. J Gen Virol 1993; 74:1025-31.

12. Hunter N, Foster JD, Goldmann W, Stear MJ, Hope J, Bostock C. Natural scrapie in a closed flock of Cheviot sheep occurs only in specific PrP genotypes. Arch Virol 1996; 141:809-24.
13. Laplanche JL, Chatelain J, Westaway D, Thomas S, Dussaucy M, Brugere-Picoux J, et al. PrP polymorphisms associated with natural scrapie discovered by denaturing gradient gel electrophoresis. Genomics 1993; 15:30-7.
14. Gombojav A, Ishiguro N, Horiuchi M, Serjmyadag D, Byambaa B, Shinagawa M. Amino acid polymorphisms of PrP gene in Mongolian sheep. J Vet Med Sci 2003; 65:75-81.
15. Sidahmed AE, Abdouli A, Hassani M, Nourallah M. Report No. 30: Sheep Production Systems in the Near East and North Africa Region. International Found for Agriculture Devolopment 1997.
16. Goldmann W, Hunter N, Smith G, Foster J, Hope J. PrP genotype and agent effects in scrapie: change in allelic interaction with different isolates of agent in sheep, a natural host of scrapie. J Gen Virol 1994; 75:989-95.
17. Billinis C, Psychas V, Leontides L, Spyrou V, Argyroudis S, Vlemmas I, et al. Prion protein gene polymorphisms in healthy and scrapie-affected sheep in Greece. J Gen Virol 2004; 85:547-54.
18. Sipos W, Kraus M, Schmoll F, Achmann R, Baumgartner W. PrP genotyping of Austrian sheep breeds. J Vet Med A Physiol Pathol Clin Med 2002; 49:415-8.
19. Gmur A, Gaillard C, Dolf G. Characterization of the prion protein gene (PRNP) region in Swiss sheep breeds. J Anim Breed Genet 2004; 121:216-20.
20. DeSilva U, Guo X, Kupfer DM, Fernando SC, Pillai AT, Najar FZ, et al. Allelic variants of ovine prion protein gene (PRNP) in Oklahoma sheep. Cytogenet Genome Res 2003; 102:89-94.
21. Youngs CR, Purdy AK, Mickelson JR. A Preliminary Report on the Frequency of Scrapie Susceptibility Alleles in Hampshire Sheep. Iowa: Iowa State University 1997.
22. Westaway D, Zuliani V, Cooper CM, Da Costa M, Neuman S, Jenny AL, et al. Homozygosity for prion protein alleles encoding glutamine-171 renders sheep susceptible to natural scrapie. Genes Dev 1994; 8: 959-69.
23. Alexander BM, Stobart RH, Russell WC, O'Rourke KI, Lewis GS, Logan JR, et al. The incidence of genotypes at codon 171 of the prion protein gene (PRNP) in five breeds of sheep and production traits of ewes associated with those genotypes. J Anim Sci 2005; 83:455-9.
24. Elsen JM, Amigues Y, Schelcher F, Ducrocq V, Andreoletti O, Eychenne F, et al. Genetic susceptibility and transmission factors in scrapie: detailed analysis of an epidemic in a closed flock of Romanov. Arch Virol 1999; 144:431-45.
25. Thorgeirsdottir S, Georgsson G, Reynisson E, Sigurdarson S, Palsdottir A. Search for healthy carriers of scrapie: an assessment of subclinical infection of sheep in an Icelandic scrapie flock by three diagnostic methods and correlation with PrP genotypes. Arch Virol 2002; 147:709-22.
26. Un C, Oztabak K, Ozdemir N, Akis I, Mengi A. Genotyping of PrP gene in native Turkish sheep breeds. Small Ruminant Res 2008; 74:260-4.
27. DeSilva U, Guo X, Kupfer DM, Fernando SC, Pillai ATV, Najar FZ, et al. Allelic variants of ovine prion protein gene (PRNP) in Oklahoma sheep. Cytogenetic and Genome Research 2003; 102:89-94.
28. Kutzer T, Pfeiffer I, Brenig B. Identification of new allelic variants in the ovine prion protein (PrP) gene. J Anim Breed Genet 2002; 119:201-8.