Short Communication

Genetic polymorphism of β-lactoglobulin in certain Iranian and Russian sheep breeds

Amir Mohammadi[1]*, Mohammad Reza Nassiry[1], Ghorban Elyasi[2], Jalil Shodja[3]

[1]Department of Animal Science, Faculty of Agriculture, Ferdowsi University of Mashhad, P.O. Box 91775-1163 Mashhad, I.R. Iran [2]Animal Science Group, Agriculture Research Center of East Azarbaijan Province, P. O. Box 53555-141, Azarshahr, I.R. Iran [3]Department of Animal Science, Faculty of Agriculture,Tabriz University, P. O. Box 51664, Tabriz, I.R. Iran

Abstract
β-lactoglobulin (Coded by the *β-lg* gene) is the major milk whey protein in ruminants. Studies have shown that this protein is polymorphic in many breeds of sheep as a result of a single base pair substitution in the *β-lg* gene that also gives rise to *Rsa*I restriction fragment length polymorphism (RFLP). Blood samples were collected from 391 animals belonging to 5 Iranian and 6 Russian sheep breeds. BLg5 and BLg3 primers amplified a 452 bp fragment from exon II of the ovine *β-lg* gene. *Rsa*I enzyme was used for restriction analysis of PCR products. Overall, the frequency of alleles *A* and *B* in the studied breeds were stimated as 0.65 and 0.35, respectively. The genotype *BB* was not seen in the Iranian and Russian Karakul, except in the Afshari and Finnish Landrace, other populations were in the Hardy-Weinberg equilibrium.
Keywords: β-Lactoglobulin; Iranian sheep; Russian sheep; PCR-RFLP; Polymorphism.

β-lactoglobulin (coded by the *β-lg* gene) is the major milk whey protein in ruminants. Among specific genes that may affect economically important traits in sheep, the *β-lg* locus has been extensively studied. In ruminants, β-lg consists of a mature polypeptide chain of 162 aa which forms a stable dimer in milk. Three genetic variants of this protein: A, B (Kolde *et al*., 1983; Shlee *et al*., 1993) and C (Erhardt, 1989) have been identified. The genetic variants A and B differ at amino acid position 20, where variant A has a His instead of Thrin variant B (Kolde *et al*., 1983; Anton *et al*., 1999). This is the result of a single base pair substitution in the *β-lg* gene which also gives rise to *Rsa*I restriction fragment lengh polymorphism. Sequence information from alleles A and B revealed an *Rsa*I restriction site in allele *A* but not in allele *B* (Ali *et al.,* 1990). The variant C is a subtype of variant A with a single amino acid exchange of Arg to Glu at position 148 (Erhardt, 1989; Anton *et al*., 1999). Polymorphism of *β*-lg has been detected in several breeds, and studies of the effect of *β-lg* alleles on sheep production traits have given different results. Genotype *BB* is linked with higher milk yield, while *AA* and *AB* genotypes seem to be superior in protein and casein content and crude yield (Bolla *et al*., 1989; Garzon *et al*., 1992). However, other studies failed to detect any effect of the gene on milk production traits (Barillet *et al*., 1993, Recio *et al*., 1997). Nevertheless, Bochkarev (1998) found associations between *β-lg* variant *AB* and higher body weight, while genotype *AA* could be linked with sheep wool density. The aim of the present study was to identify the two genetic variants (*A* and *B*) and three genotypes (*AA*, *AB* and *BB*) of the *β-lg* gene in some Iranian and Russian sheep breeds by PCR-RFLP.

Blood samples were randomly collected from 391 animals of 11 Iranian and Russian sheep breeds (Table 1). DNA was extracted from 100 µl of blood by the guanidine thiocyanate-silica gel method (Boom *et al*., 1989). Quality and quantity of extracted DNA was measured spectrophotometically and on 1% agarose

*Correspondence to: **Amir Mohammadi, M.Sc.**
Tel: +98 511 8795618; Fax: +98 511 8787430
E-mail: amohamadi57@yahoo.com

gel electrophoresis. For amplifying a 452 bp region from exon II, we used specific primers BLg5 (5′-TTGGGTTCAGTGTGAGTCTGG-3′) and BLg3 (5′-AAAAGCCCTGGGTGGGCAGC-3′) as described by Eignatev (1998). 1 µl (50-100 ng) of DNA samples were added to 19 µl of PCR mixtures containing 2 µl PCR buffer (200 mM $(NH_4)_2SO_4$, 0.1 mM Tween 20, 750 mM Tris-HCl pH 8.8), 1.5 mM $MgCl_2$, 0.25 mM of each deoxynucleoside triphosphates (dNTPs), 10 pmol of each primer and 1 U of *OligoTaq* DNA polymerase (IsoGene, Moscow). Amplification reactions were conducted in a Tpersonal thermal cycler (Biometra, USA) with thirty-five cycles at 95ºC for 1 min, 65ºC for 30 sec, and 74ºC for 40 sec followed by a final extension step at 74°C for 8 min. Correctness of PCR was assessed by electrophoresis of each sample (10 µl) on 1.3% (w/v) agarose gel. Samples (3 µl) of each PCR product were incubated for 3 h at 37ºC with 5 U *Rsa*I enzyme, according to manufacturer's instructions (Fermentas, Lithuania). Digestion products were separated by electrophoresis on 8% w/v non-denaturant polyacrylamide gel and visualized after staining with ethithium bromide. Results were recorded by an UVidoc Gel Documentation System (UVitec, UK). PopGen32 software (ver. 1.31) was used to estimate the frequency of allele, genotype and Hardy-Weinberg equilibrium (Yeh and Yang, 2000). An c^2 analysis was performed for each breed to test the goodness of fit to the Hardy-Weinberg equilibrium expectations for the distribution of *β-lg* phenotypes.

After the PCR amplification, enzymatic digestion and gel electrophoresis, DNA from the *AA* homozygotes showed four bands of 175, 170, 66 and 41 bps, while *BB* homozygotes gave three bands of 236, 175 and 41 bp and heterozygotes had all five distinct bands. The distribution of *β-lg* alleles and genotypes are presented in Table 1. The greatest frequency of allele *B* belonged to the Afshari (0.65) followed by Moghani (0.64) and Oparinesky (0.60) breeds, while the lowest frequency of this allele was detected in the Iranian Karakul (0.114) and then in the Finish Landrace (0.14) sheep. The highest frequencies of genotype *BB* were 0.41, 0.40, and 0.35 that were seen in the Moghani, Oparinesky and Afshari sheep, respectively. This genotype was not seen in the two Karakul populations (Iranian and Russian). Afshari (0.62) and Arkharmerino (0.57) breeds had the most frequent *AB* genotype. Except for the Afshari and Finnish Landrace, other populations were in the Hardy-Weinberg equilibrium (HWE) (Table 1). Genetic relationships between breeds based on the information provided from polymorphism of β-lg are represented in a dendrogram (Fig. 1). The smallest genetic distances are observed between the Ghezel and Makoii,

Table 1. The observed frequencies of β-lactoglobulin genotypes and alleles and test for Hardy-Weinburg Equilibrium (HWE) among 11 sheep breeds.

Breed	N	β-lg genotype			β-lg allele		χ^2
		AA	AB	BB	A	B	
Ghezel	32	0.31	0.50	0.19	0.56	0.44	0.000
Arkharmerino	30	0.19	0.57	0.24	0.48	0.52	0.308
Makoii	32	0.27	0.53	0.20	0.53	0.47	0.089
Moghani	29	0.14	0.45	0.41	0.36	0.64	0.065
Afshari	29	0.03	0.62	0.35	0.34	0.65	3.708*
Iranian Karakul	100	0.79	0.21	0.00	0.89	0.11	1.200
Oparineski	20	0.25	0.45	0.40	0.40	0.60	0.560
Merino Saveski	20	0.30	0.45	0.25	0.53	0.47	0.190
Russian Karakul	18	0.44	0.56	0.00	0.72	0.28	2.560
Finnish Landrace	28	0.79	0.14	0.07	0.86	0.14	5.750*
Cross	53	0.75	0.24	0.01	0.84	0.16	1.850
Overall	391	0.475	0.335	0.19	0.65	0.35	28.794

* $p<0.05$

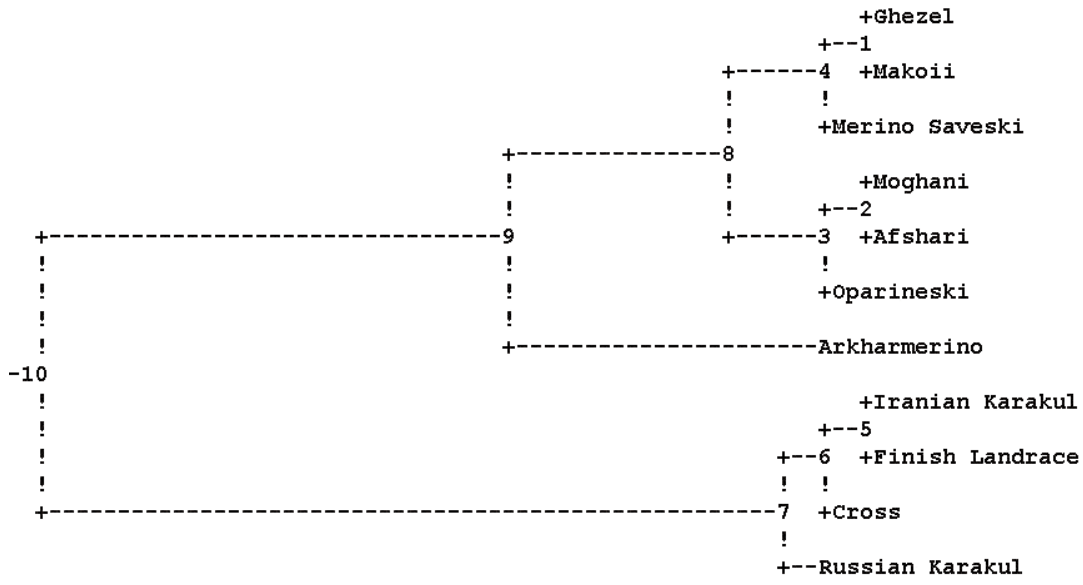

Figure 1. Dendrogram based on Nei and Li (1979). Genetic distance clustered by the unweighted pair group method with arithmetic mean for the *β-lg* alleles

Moghani and Afshari, and Iranian Karakul and Finish Landrace. Iranian Karakul and Finish Landrace with Cross (Finish Landrace X Romney Marsh X Texel) and the Russian Karakul were clustered on a different branch from the other breeds.

Results of the present study have provided more information on polymorphism of the ovine β-lactoglobulin. The frequency of *AB* genotype in almost all studied breeds (8 out of 11) was higher than other genotypes. Recently similar results for the *AB* genotype of ovine β-lactoglobulin in Pag ewes (Croatia) were reported by Vlatka *et al*. (2002). Overall, the frequency of alleles *A* and *B* in the breeds of the current study were estimated as 0.65 and 0.35, respectively. Kucinskiene *et al*. (2005) also reported comparable results in the Lithuanian Blackface (*A*: 0.52, *B*: 0.48) and Lithuanian Native Coarsewooled breeds (*A*: 0.69, *B*: 0.31). Similar findings for allele *A* were obtained by Barillet *et al*. (2005) who reported its frequency at 0.64, 0.68 and 0.58 in the French Lacaune, Spanish Segurena and Merino breeds, respectively. Anton *et al.* (1998) also reported a very low frequency for allele *B* in the Hungarian dairy sheep breeds. Also the frequency of 0.58 for allele *A* and 0.41 for allele *B* has been reported in Awassi and Morkaraman breeds of sheep (Recio *et al*., 1997). Nevertheless, others have reported reverse findings. For example, Di Stasio *et al*. (1997) reported that the frequencies of alleles *A* and *B* in the Valle del belice breed are 0.35 and 0.65, respectively, or in the study of Barillet *et al*. (2005), the frequencies of allele *A* in the Italian Sarda, Spanish Churra, and Spanish Manchega breeds were reported as 0.27, 0.32, and 0.32, respectively, which is much lower than those for allele *B*. However, it can be concluded that allele *A* and genotype *AB* are prevalent in sheep populations worldwide. The higher frequencies of the *AA* and *AB* genotypes could be explained by the fact that sheep have been mainly reared for dairy products rather than milk yield or at least for both purposes. These data provide evidence that Iranian and Russian sheep breeds are showing good variability, which opens interesting prospects for future selection programs, especially marker assisted selection between different genotypes of milk and cheese characteristics, and also for preservation strategies.

References

Ali S, McGlenaghan M, Simons JP, Clark AJ (1990). Characterization of the alleles encoding ovine β-lactoglobulin A and B. *Gene* 91: 202-207.

Anton Z, Istvan K, Attila M, Sandor F, Andras L (1998). Genetic polymorphisms of milk proteins in Hungarian dairy sheep breeds and crosses, Sheep and Goat Production in Central and Eastern European Countries, Hungary, *REU Technical Series* 50: 224-226.

Barrilet F, Arranz JJ, Carta A (2005). Mapping quantitative trait loci for milk production and genetic polymorphisms of milk proteins in dairy sheeps. *Genet Sel Evol*. 37 (Suppl. 1): S109-S121.

Barillet F, Sanna S, Boichard D, Astruc JM, Carta M, Casu S (1993). Genetic evaluation of the Lacaune, Manech and Sarda dairy sheep with animal model. *J Anim Prod* 1(Suppl): 580-607.

Bochkarev VV, (1998). The molecular analysis of *ß-lactoglobulin* locus in the different sheep breeds. *PhD. Thesis*. Moscow University.

Bolla P, Caroli A, Mezzelani A, Rizzi R, Pagnacco G, Fraghi A, Casu S (1989). Milk protein markers and production in sheep. *Anim Gen.* 20: 78.

Boom R, Sol CJA, Salimans MMM, Jansen CL, Wertheim-Van Dillen PME, Van Der Noordaa J (1989). Rapid and simple method for purification of nucleic acids. *J Clin Microbiol.* 28: 495-503.

Di Stasio I, Portolano B, Todaro M, Fiandra P, Giaccone P, Finocchiaro R, Alicata ML (1997). Effect of ovine β-lactoglobulin phenotype on cheese yield and composition. In: *Milk Protein Polymorphism*. International Dairy Federation. Brussels, Belgium. P: 324-327.

Eignatev G (1998). Genetic polymorphisms of milk proteins in Russian sheep breeds and crosses. *PhD. Thesis*, Moscow University.

Erhardt G (1989). Evidence for a third allele at the *β-lactoglobulin* (*BLg*) locus of sheep milk and its occurrence in different breeds. *Anim Genet.* 20: 197-204.

Garzon AI, Martinez J (1992). β-lactoglobulin in Manchega sheep breed: Relationship with milk technological index in handcraft manufacture of manchego cheesse. *XXIII Int Conf Anim Genet*. Thenameis OK. PP: 137.

Kolde HJ, Braunitzer G (1983). The primary structure of ovine β-lactoglobulin. *Milckwissenschaft* 38: 70-72.

Kurcinskiene J, Vagonis G, Maleviciute J, Tapio I (2005). Genetic polymorphism of β-lactoglobulin in Lithuanian Blackface and Lithuanian Native Coarswooled sheep. *Vet Zootech.* 29: 90-92.

Nei M, Li WH (1979). Mathematical model for studying genetic variation in terms of the restriction endonucleases. *Proc Nation Acad Sci USA.* 76: 5269-5273.

Recio I, Fernandez-Fournier A, Martin-Alvarez PJ, Ramos M (1997). β-lactoglobulin polymorphism in ovine breeds: Influence of cheese making properties and milk composition. *Lait.* 77: 259-265.

Shlee P, Krause I, Rottmann O (1993). Genotyping of ovine β-LG A and B using the PCR. *Arch Tierz.* 36: 519-523.

Vlatka CC, Feligini M, Lukac-Havranek J, Curik I, Guiseppe E (2002). Genetic polymorphism of β-lactoglobulin in native sheep from the Island of Pag. *Food Technol Biotechnol.* 40: 75-78.

Yeh FC, Yang, RC (2000). PopGen computer program (ver. 1.31) microsoft windows based freeware for population genetic analysis. http://www.Ualberta.ca/fyeh.