

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

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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 *RsaI* 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. *RsaI* enzyme was used for restriction analysis of PCR products. Overall, the frequency of alleles A and B in the studied breeds were estimated 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 Thr in 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 *RsaI* restriction fragment length polymorphism. Sequence information from alleles A and B revealed an *RsaI* 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 μ l of blood by the guanidine thiocyanate-silica gel method (Boom *et al.*, 1989). Quality and quantity of extracted DNA was measured spectrophotometrically and on 1% agarose

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gel electrophoresis. For amplifying a 452 bp region from exon II, we used specific primers BLg5 (5'-TTGGGTTTCAGTGTGAGTCTGG-3') and BLg3 (5'-AAAAGCCCTGGGTGGGCAGC-3') as described by Eignatev (1998). 1 μ l (50-100 ng) of DNA samples were added to 19 μ l of PCR mixtures containing 2 μ l PCR buffer (200 mM (NH₄)₂SO₄, 0.1 mM Tween 20, 750 mM Tris-HCl pH 8.8), 1.5 mM MgCl₂, 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 95°C for 1 min, 65°C for 30 sec, and 74°C 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 μ l) on 1.3% (w/v) agarose gel. Samples (3 μ l) of each PCR product were incubated for 3 h at 37°C with 5 U *RsaI* 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 ethidium 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 χ^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 Makooi,

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
Makooi	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

* $p < 0.05$

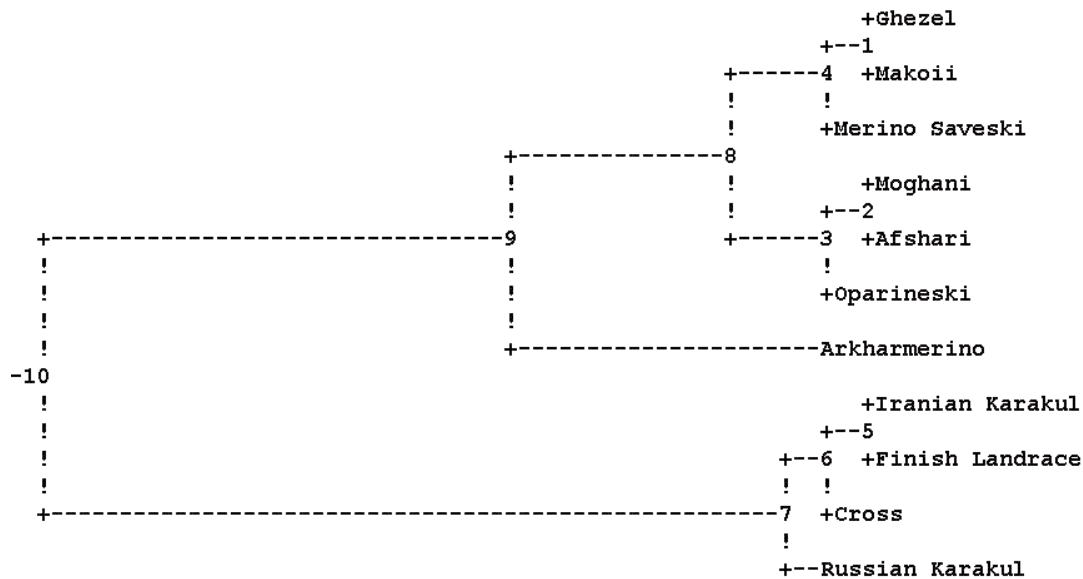


Figure 1. Dendrogram based on Nei and Li (1979). Genetic distance clustered by the unweighted pair group method with arithmetic mean for the β -lg alleles

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.

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