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Diversity of the B-Lactoglobulin Genotypes in Cypriot Sheep Breeds

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Abstract

β-lactoglobulin is the major whey protein in ruminant milk. In sheep, three genetic variants have been found (A, B and C) which differ from each other by a single base substitution within exon II of the gene. Various reports have indicated that the B variant is associated with higher milk yields. In this study, we have assessed the genotypic frequencies of both A and B variants in samples from Chios and Cyprus fat-tailed sheep. Although both alleles are present in ovine samples, both the allelic frequencies and genotypic distributions were significantly different between the two sheep breeds, thus reflecting a major intra-species difference regarding the genotype of this gene.

Keywords: Chios sheep; Cyprus fat-tailed; Milk production traits; β-lactoglobulin

Introduction

Genetic polymorphisms in milk protein genes have received considerable interest in the dairy industry with particular emphasis being placed on their correlation with milk production traits to facilitate their use in marker assisted selection breeding.

β-lactoglobulin is the major whey protein in ruminant milk. Although its precise physiological role remains uncertain, it is known to be capable of binding and transporting small hydrophobic molecules such as retinol and short chain fatty acids.

To date, three significant polymorphic variants have been identified in sheep, termed A, B and C, where variants A and B differ by a T to C substitution in exon II of the gene leading to a substitution of a tyrosine to a histidine residue at position 20 in the amino acid sequence [1]. The rare C variant is a subtype of variant A and results from a single exchange of a glutamine to arginine residue at amino acid position 148 [2].

Allelic frequencies are varied and have been reported in a number of sheep breeds ranging from 0.39 - 0.78 for the A-allele and 0.22-0.61 for the B-allele [3-6], although a dramatic skewing in favour of the A-allele (0.98) was reported in the Turkish Sakiz breed [3]. The frequency of the C-variant however is much rarer. It is either totally absent in some breeds or demonstrates very low frequency (0.001-0.002) in others (Ramos, 2009), thereby limiting its use in assisted breeding programs.

Although the effect of β-lactoglobulin alleles on milk production traits in sheep have been extensively studied, they have however yielded conflicting results and as such, no clear and definitive associations have been established to date. Significant relationships have been reported between the β-lactoglobulin polymorphism and milk production data with higher milk yields being associated with variant B [7,8] and ewes homozygous for the A variant produced less milk [6]. Similarly, whereas animals homozygous for the B variant produce a higher milk output, those carrying at least one copy of the A-allele were associated with higher milk protein and casein content [9]. In contrast to these findings, others failed to detect any influence of the gene polymorphism on milk production traits in sheep [10-12]. These inconsistencies may be attributed to breed differences, population size and the methods of statistical analysis employed.

With the aim of identifying genetic markers associated with milk production data in Cypriot small ruminant breeds in the future, in this study we genotyped β-lactoglobulin A and B alleles in the two major purebred sheep breeds of Cyprus.

Genomic DNA was isolated from 386 Chios sheep and 40 Cyprus fat tailed sheep, using the Genomic DNA Blood kit

(Macherey-Nagel), according to the manufacturers' instructions and DNA quality and quantity estimated by UV absorption at 260 and 280nm.

To amplify the region of the β-lactoglobulin gene containing the polymorphism, primers were designed using the online program primer3 (http://frodo.wi.mit.edu/primer3/input.htm) against the published ovine β-lactoglobulin GenBank sequence X12817.

PCR reactions were set up in a final volume of 25μl using 25ng genomic DNA, 1.5mM MgCl2, 0.4μM BLG-F (5'-AATTTTCCCCACCTCCAGCC-3'), 0.4μM BLG-R (5'-GGAGTGGGGGTTCCATGTTG-3') and 1U Taq DNA polymerase (Qiagen). Following an initial 5 minute denaturation step at 95 °C, the PCR reactions were subject to 30 cycles of 94 °C for 30s, 60 °C for 30s and 72 °C for 30s, and a final elongation step for 5 minutes at 72 °C. For detection of the two variants A and B, the 406bp fragments generated were digested for 2 hours at 37 °C with 8U of RsaI and digestion products resolved on 2% agarose gels. Expected fragment sizes were as follows: A-variant, 247, 93, 66bp; B-variant, 313, 93bp.

Table 1: β-lactoglobulin genotypic distribution and allelic frequencies in Chios and Cyprus Fat-tail sheep.

		β-Lactoglobulin Genotypea			Allelic Frequency		
Breed	**No. of Animals**	**AA**	**AB**	**BB**	**A-allele**	**B-allele**	**(χ2)b**
Chios Sheep	246	140 (142.91)	95(89.18)	11 (13.91)	0.762	0.238	1.05 NS
Cyprus Fat-Tail Sheep	40	5(5.39)	19 (18.22)	15(15.39)	0.372	0.628	0.07 NS

[a]Observed values with predicted values from Hardy-Weinberg equilibrium analysis below in brackets.

[b]Chi-square significance testing for deviation of observed from predicted values.

Table 2: Comparison of β-lactoglobulin genotypic distribution and allelic frequencies between Chios and Cyprus Fat-tail sheep.

	β-Lactoglobulin Genotype			Allelic Frequency	
Chios Sheep	**AA[a]**	**AB[b]**	**BB[c]**	**A-allele[a]**	**B-allele[a]**
	56.9%	38.6%	4.5%	0.762	0.238
Cyprus Fat-Tail Sheep	12.8%	48.7%	38.5%	0.372	0.628

[a]P<0.001, [b]P<0.05

The results of the allelic frequencies of the A and B variants and genotypic distribution in Cypriot sheep breeds are presented in (Tables 1 & 2). In concordance to other studies [13,14], a clear inter-species difference was observed with both variants being detected in sheep. Statistically significant differences were observed between individual sheep breeds (p<0.001). In Chios sheep, the A allele was more predominant (allele frequency: 0.762), whilst the frequency of the B allele was higher in the Cyprus fat-tailed breed (allele frequency: 0.628). The frequency of the B allele for the β-lactoglobulin gene in Cyprus fat-tailed sheep is much higher compared to most other sheep breeds [3,15,16] However, in both breeds the genotypic frequencies were found to be in Hardy-Weinberg equilibrium, suggesting that selection may not have affected the allelic frequencies of β-lactoglobulin. Since Cyprus is an island and no imports of animals from those breeds take place, it is likely that the differences between the two breeds reflect founder effects. In the case of Cyprus fat-tailed sheep genetic drift due to the small size of the population is likely to have affected the observed frequencies [17].

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