Kafkas Univ Vet Fak Derg
21 (4): 535-538, 2015
DOI: 10.9775/kvfd.2014.12885

Kafkas Universitesi Veteriner Fakultesi Dergisi
Journal Home-Page: http://vetdergi.kafkas.edu.tr
Online Submission: http://vetdergikafkas.org

Research Article

Polymorphism of the Kap 1.1, Kap 1.3 and K33 Genes in Chios, Kivircik and Awassi [1]

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Article Code: KVFD-2014-12885 Received: 29.12.2014 Accepted: 05.02.2015 Published Online: 05.02.2014

Abstract

Keratin-associated proteins are major structural components of hair and wool fibres. They also play an important role in determining the properties of the fibre. This study is designed to examine variation in the genes encoding key keratin and keratin –associated proteins in Chios, Kivircik and Awassi sheep. Variation at these loci has the potential to be developed as genetic markers deal with wool traits. Blood samples were taken into 2 ml sterilized tubes with EDTA from vena jugularis for having genomic DNA samples. Genomic DNA isolation was obtained by standard salting out method. Regions were amplified for the determination of KAP 1.1, KAP 1.3 and K33 polymorphisms in DNA samples by using PCR. Gene polymorphisms and chi-square test were used to determine whether the populations are in Hardy-Weinberg equilibrium using by POPGENE32 software. Results of the sequence analysis of the regions were evaluated by using MEGA 5. The results have shown a possibility to improve the quality of the wool traits on those local species by doing selection trials targeting on those mentioned genes.

Keywords: *KAPs, Sequencing, Turkish sheep, Wool trait*

Sakız, Kıvırcık ve İvesi Koyun Irklarında KAP 1.1, KAP 1.3 ve K33 Gen Polimorfizmlerinin Belirlenmesi

Özet

Kreatin ilişkili proteinler, yün ve kıl yapısının komponentleridir. Lifli yapının özelliklerinin belirlenmesinde önemli rol oynarlar. Yapılan bu çalışmada amaç, Sakız, Kıvırcık ve İvesi koyun ırklarında kreatin ve kreatin ilişkili proteinleri kodlayan genlerdeki varyasyonu incelemektir. Bu lokuslardaki olası varyasyonlar, yün verimi ile ilişkili genetik markör oluşturabilir. Kan örnekleri 2 ml'lik EDTA'lı tüplere vena jugularis den alınmıştır. Standart salting out yöntemiyle genomik DNA izole edilmiştir. KAP 1.1, KAP 1.3 ve K33 için bölgeleri belirlemek için PCR yöntemi kullanılmıştır. Elde edilen sonuçler POPGEN 32 istatistik programı ile değerlendirilmiştir. Sekans analizi sonuçları için MEGA 5 programı kullanılmıştır. Sonuçlar göstermiştir ki, çalışmadaki yerli ırklarda, söz konusu genlere odaklı seleksiyon uygulamalarının , yün verim ve kalitesini geliştirmek için önem oluşturabilir.

Anahtar sözcükler: *KAPs, Sekans, Yerli koyun ırkları, Yün verimi*

INTRODUCTION

Most of the proteins in hair and wool are categorized in two types; keratin intermediate filament-forming proteins (known as keratins) and keratin-associated proteins (KAPs). The studies show that there has been an increase in the number of KAP genes defined in humans and other species and progressively accounts of variation in these genes [1]. Keratin - associated proteins are a structural constituent of hair and wool fibres. They play a critical role in determining the physic-mechanical properties of hair and wool fibres [2-5].

The wool fibre consists of three main parts, the cuticle, the cortex and medulla [6]. The cortex includes 90% of the wool fibre. It also consists of filamentous microfibrils [7,8]. Keratin intermediate filaments are formed by microfibrils. KAPs have 3 groups based on amino acids compositions; the high-sulphur proteins (KAP 1.n, KAP 2.n, and KAP 3.n),

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ultra high sulphur proteins (KAP 4.n, KAP 5.n, and KAP 10.n) and the last one; high glycine tyrosine proteins (KAP 6.n, KAP7.n, KAP 8.n) [7,9,10]. KAPs are encoded by a large number of genes which are polymorphic [11]. The KAP genes between two and nine alleles have been determined [12].

Gene expression can be affected by the structure and function of the encoded proteins [13]. It may also be affected by the variation in KAP genes and as a result of that wool traits are influenced. Wool quality might be improved if we can identify the KAP genes which affect wool traits. On the other side only 16 functional genes have been identified until now so this number of sheep KAP genes, may not be sufficient to improve the wool quality. Still many genes have not been determined in human which are homologues in the sheep genome [14-16].

MATERIAL and METHODS

One hundred and thirty five Chios, Awassi and Kivircik sheep were investigated. Information from the breeder producer was considered in order to avoid family connections. Blood from sheep was collected into tubes containing EDTA. Genomic DNA was isolated by using salt-out method [17].

The primers used to amplify the KAP 1.1 locus were designed from a published gene sequence, Gen Bank nos. AY835603- AY835605 to amplify a 311 bp fragment of the KAP1.1. and F :5´-CAACCCTCCTCTCAACCCAACTCC-3′, R :5´-CGCTGCTACCCACCTGGCCAT A-3´.

The KAP 1.3 locus were designed from a published sequence, Gen Bank nos.AY835589-AY835597 to amplify a 598 bp fragment of the KAP 1.3; F :5´-GGGTGGAACAA GCAGACCAAACTC-3′, R :5´- TAGTTTGTTGGGACTGTACACT GGC-3´. The K33 locus were designed from a published sequence, Gen Bank nos.AY835598-AY835602 to amplify a 480 bp fragment of the K33;

F:5´-CACAACTCTGGCTTGGTGAACTTG-3′,

R:5´- CTTAGCCATATCTCGGATTCCCTC-3´ [18].

Amplification consisted of; initial denaturation 95°C for 1 min, followed by 30 cycles of 95°C for 30 s, annealing at 65°C for 30 s and extension at 72°C for 30 s with a final extension of 72°C for 7 min.

The Polymerase Chain Reaction (PCR) volume of 25 µl contained; 1 U Taq DNA Polymerase (Fermentas Life Sci., Canada), 2-2.5 mL 10X PCR buffer, 1.5 mM $MgCl_2$, 50-100 ng genomic DNA, 100mM dNTP (TaKaRa Biotechnology Co., Ltd., Japan), and 10 pmol of each primer [18]. PCR products was visualised after electrophoresis on a 2% agarose gel with a long-wavelength UV transilluminator (Thermo Fisher Scientific, Germany).

Sequencing was performed by using an ABI-3100 sequencer (PE Biosystems) and the BiyDye™ terminator cycle sequencing kit after the purification of the PCR products. Forward primer was used to sequence the PCR products. Single nucleotide polymorphisms (KAP1.1) in codons 74, 111, 177, 207, 241, 262, 289 were checked directly. For KAP 1.3; codons 53, 60, 66, 67, 160, 178, 184, 232, 241, 264, 313, 337, 352, 364, 380, 486, 557, 598 and for K33; codons 127, 160, 184, 208, 223, 251, 307, 308, 340 were checked directly.

Genotype and allele frequencies of each polymorphism were calculated by using the PopGene 32 software program and also the chi-square tests (x^2) was used to check whether the populations were in Hardy-Weinberg equilibrium using PopGene32 software [19].

Statistical Analysis

Results of the sequence analysis of the regions were evaluated by using MEGA 5 (Mega Software, USA www.megasoftware.net). MEGA5 is a collection of maximum likelihood (ML) analyses for inferring evolutionary trees, selecting best-fit substitution models (nucleotide or amino acid), inferring ancestral states and sequences (along with probabilities), and estimating evolutionary rates site-by-site [20].

RESULTS

Information on product size, genotype frequencies and allele frequencies for KAP 1.1 gene, KAP 1.3 gene and K33 gene are listed in *Table 1*.

KAP 1.1: Three amplimers of different length were obtained and designated A, B and C. The length of amplimers was 341, 311 and 281 bp respectively. For KAP 1.1 gene, the obtained results indicated that A and B alleles frequencies were higher in Awassi than the others. It was found that C allele frequency was higher in Chios than the Awassi and Kivircik.

KAP 1.3: Nine amplimers of different length were obtained and designated A-I. The results noticed that the C allele was the highest in Awassi, the G allele was the highest in Kivircik. Both of the A and D alleles were not determined in all three breed.

K33: Five unique SSCP banding were observed and designated A-E. The D allele was not obtained in all three breeds. The results indicated that the A allele was the highest in Chios and the B allele, the C allele and the E allele were the highest in Kivircik.

DISCUSSION

This study reports three alleles at the KAP 1.1 locus. The length of the amplimers from KAP 1.1 alleles A, B and C were 341, 311, 281 bp respectively. The length

***Table 1.** Loci, product size, genotypes and allele frequencies for polymorphisms in three candidate genes*
***Table1.** Üç aday genin, polymorfizimde lokus, ürün, genotip ve allel frekans verileri*

Locus	Breed	n	Product Size	Genotype Frequency (%)									Allele Frequency (%)									(χ^2)
KAP 1.1			311	AA	AB	BB	CC						A	B	C							
	Sakız	45		7	8	20	10						0.24	0.53	0.23							56.67***
	Kıvırcık	45		1	9	27	8						0.12	0.7	0.18							47.70***
	İvesi	45		10	10	24	1						0.33	0.64	0.03							100.58***
KAP 1.3			598	AA	BB	CC	DD	EE	FF	GG	HH	II	A	B	C	D	E	F	G	H	I	
	Sakız	45			8	15		3	2	6	4	7		0.18	0.34		0.06	0.05	0.13	0.09	0.15	308.00***
	Kıvırcık	45				11		3		25	3	3			0.24		0.07		0.55	0.07	0.07	207.00***
	İvesi	45				34				6	4	1			0.75				0.14	0.08	0.03	188.57***
K33			480	AA	BB	CC	DD	EE					A	B	C	D	E					
	Sakız	45		41		3		1					0.92	0.01	0.07							53.43***
	Kıvırcık	45		35		5		5					0.81	0.04	0.11		0.04					108.92***
	İvesi	45		38		3		4					0.87	0.03	0.07		0.03					112.00***

*[1] Hardy-Weinberg equilibrium, *** P≤0.001*

polymorphism of the KAP 1.1 gene determined in this investigation has previously been reported in Romney sheep and in Merino sheep [18,21]. Our results showed that A and B alleles frequencies were higher in Awassi than the others and also the C allele frequency was the highest in Chios breed.

Five alleles were identified at the K33 locus in Merino sheep which was reported by Itenge *et al.*[18]. By the way Roger *et al.*[21] determined a diallelic polymorphism at this same loci. In our study, the D allele wasn't obtained in all three breed. Addition to this, the E allele was not obtained for Chios sheep.

Itenge *et al.*[18] reported eight alleles (except for B allele) in a 598 bp KAP 1.3 amplimer from Merino sheep. The results found by Powell *et al.*[22] also supported these findings. Roger *et al.*[21] determined six alleles from Romney sheep. In this study we obtained seven alleles for Chios sheep, five alleles for Kivircik sheep and four alleles for Awassi sheep in a 598 bp KAP 1.3 amplimer. Both of the A and D alleles were not determined in all of them. Our investigation showed that the C allele was the highest in Awassi, 0.75 by the way 0.34 for Chios and 0.24 for Kivircik, the G allele was the highest in Kivircik whereas the G allele was 0.13 in Chios and 0.14 in Awassi.

As a conclusion it has been known that Kivircik sheep breed has been preferred for wool quality and Awassi sheep breed has been preferred for wool trait among the domestic sheep breed in Turkey. Their wool quality ranges from carpet to medium-wool quality [23]. It might be a relationship between these alleles and wool quality and wool product. To support this suggestion, further linkage analyses are necessary. This will allow future analysis of how these genes may affect wool traits.

The allele numbers at the KAP1.3 and the K33 locus in Chios, Awassi and Kivircik were determined lower than the Merino sheep and Romney sheep breed. It might be suggested to make further trials on those loci to possible improvement on wool quality and wool trait in those breeds.

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