GOAT AGOUTI GENE POLYMORPHISM AND ITS ASSOCIATION WITH COAT COLOR IN INDIGENOUS TURKISH GOAT BREEDS

Iraz Akis[1], Kemal Oztabak[1], Feraye Esen Gursel[1], Cemal Un[2]
[1]*Istanbul University, Faculty of Veterinary Medicine, Department of Biochemistry*
34310 Avcilar, Istanbul, Turkey
Phone: 0090 473 70 70/17126, Fax: 0090 212 473 72 41, e-mail: oztabak@istanbul.edu.tr; iraz@istanbul.edu.tr
[2]*Ege University, Faculty of Art and Science, Department of Biology, Izmir, Turkey*

Abstract. Agouti gene has an important effect on pigment synthesis in mammals. It encodes Agouti signaling protein, which stimulates the phaeomelanin synthesis resulting red, yellow coat color. In this study 120 animals from three indigenous Turkish goat breeds were genotyped for 423 G>T polymorphism in exon 4 of Agouti gene using PCR-RFLP method. Two alleles T and G were observed. GG genotype was absent in all breeds. No clear association could be found between coat color and alleles of 423 G>T polymorphism. The genetic diversity for the site analyzed in the study was found to be very low in all breeds. As a conclusion we may say that caprine Agouti gene and its association with coat phenotype should be studied further using new polymorphisms and in a greater number of breeds.
Keywords: Agouti gene, goat, coat color, polymorphism.

OŽKŲ AGOUTI GENO POLIMORFIZMAS IR JO RYŠYS SU TURKIŠKŲ VEISLIŲ OŽKŲ KAILIO SPALVA

Iraz Akis[1], Kemal Oztabak[1], Feraye Esen Gursel[1], Cemal Un[2]
[1]*Biochemijos katedra, Veterinarinės medicinos fakultetas, Stambulo universitetas*
34310 Avcilar, Stambulas, Turkija
tel. 0090 473 70 70/17126; faks. 0090 212 473 72 41; el. paštas: oztabak@istanbul.edu.tr; iraz@istanbul.edu.tr
[2]*Biochemijos katedra, Meno ir mokslo fakultetas, Ageno universitetas, Izmyras, Turkija*

Santrauka. Agouti genas atlieka svarbų vaidmenį žinduolių pigmento sintezėje. Jame užšifruotas Agouti signalinis baltymas, kuris stimuliuoja rudą/geltoną kailio spalvą, lemiančią feomelanino sintezę. Šiame darbe PCR-RFLP metodu buvo ištirtas 120 trijų vietinių turkiškų veislių ožkų 4 eksono Agouti geno 423 G>T polimorfizmas. Buvo stebimi du aleliai – T ir G. GG genotipas nenustatytas nė vienoje iš tirtų veislių. Nenustatytas akivaizdus ryšys tarp kailio spalvos ir 423G>T alelių polimorfizmo. Nustatyta, kad tirtoje teritorijoje genetinė visų ožkų veislių įvairovė labai menka. Remiantis naujais polimorfizmais ir didesniu veislių skaičiumi, ožkų Agouti genas ir jo ryšys su kailio fenotipu turėtų būti tiriamas toliau.
Raktažodžiai: Agouti genas, ožka, kailio spalva, polimorfizmas.

Introduction. Coat color is an easily recognizable trait; therefore it is widely used as a model phenotype to study gene and trait associations (Andersson, 2001). In mammals pigmentation is controlled by two pigments called eumelanin and pheomelanin in melanocytes. The switch of these pigments is regulated by melanocortin 1 receptor (MC1R) and its peptide antagonist Agouti signaling protein (ASIP) (Andersson and Georges, 2004). MC1R is expressed in melanocytes and plays an important role in melanogenesis upon binding to α-melanocyte-stimulating hormone (α-MSH). Molecular studies have shown that exclusive binding of the MC1R by the Agouti protein or by α-MSH signals melanocytes to synthesize either pheomelanin (yellow-red pigment) or eumelanin (black-brown) pigment, respectively (Robbins et al., 1993; Silvers, 1979). ASIP is encoded by Agouti gene which has a key role in pigment synthesis in domestic animals. There are several studies conducted on sequencing, single nucleotide polymorphism (SNP) identification of Agouti gene and its association analysis with coat color variation in different animals (Voisey et al., 2006). ASIP is a high-affinity antagonist of MC1R. Dominant mutations in the noncoding part of the mouse Agouti gene results yellow coat color, because of the antogonizing effect of the agouti protein, which inhibits the binding of α-MSH to MC1R (Barsh, 1996; Voisey and van Daal, 2002). Recessive mutations in the Agouti gene weaken the ASIP activity or decrease the level of Agouti mRNA synthesis and cause darker coat color. A SNP (8818A>G) in the 3' untranslated region of the Agouti gene in humans is associated with decreased levels of ASIP and less pheomelanin production (Voisey et al., 2006).

Agouti alleles affecting coat color have been identified in some domestic animals (Mao et al., 2010). In exon 2 of the horse Agouti gene a homozygous deletion of 11 bp has been found which is associated with recessive black coat color (Stefan et al., 2001). An association between black coat color and 2 bp deletion in Agouti gene was observed in domestic cats (Eizirik et al., 2003). Girardot et al. (2005) could not find any association between coding region of Agouti gene and coat color in cattle.

In goat Agouti gene 10 alleles were identified associated with 10 color patterns. The most common

dominant allele is the allele for white color and the other nine alleles are codominant (Adalsteinsson et al., 1994). Several SNP's have been identified in goat Agouti gene and some of them were claimed to be associated with coat color (Tang et al. 2009, Li et al. 2010, Tang et al. 2008, Badaoui et al. 2011).

Objective. The aim of this study was to analyze the 423 G>T polymorphism in exon 4 in three indigenous Turkish goat breeds and discuss the relation between this polymorphism and coat color.

Materials and methods. Blood samples were taken from 120 individuals of three indigenous goat breeds in Turkey, Anatolian Black Goat (mainly black and white), Angora Goat (white) and Kilis Goat (black). Genomic DNA samples were isolated by using standard salt-out method (Miller et al. 1998).

The animals were genotyped for 423 G>T polymorphism in exon 4 of goat Agouti gene. PCR reaction was performed in a reaction volume of 25 µl using 1 U Taq DNA polimerase (Fermantas Life Sciences, Canada), 2-2.5 µl 10X PCR buffer, 1.5mM $MgCl_2$, 50-100ng genomic DNA, 100µM dNTP (Takara, Biotechnology Co, Ltd, Japan) and 10 pmol of each primer. The primer sequences used for the 178 bp fragment, containing a polymorphic *Ban*II restriction site, were 1: 5'- ACA GAG AAA GGC TCC GAT GA -3', 2: 5' -TCA GCA GGT GGG GTT GAG-3' Amplification was carried out for 94 ^{0}C for 5 min; 35 cycles of 94 ^{0}C for 30 s, 56 ^{0}C for 30 s, 72 ^{0}C for 30 s; and a final extension at 72 ^{0}C for 5 min.

For RFLP analysis 10 µl of the PCR products were digested with 10 units of *Ban*II restriction enzyme at 37 ^{0}C overnight. The digested DNA fragments were separated by electrophoresis in 2% agarose gel including ethidium bromide and visualized under UV light.

PopGene32 software program was used to calculate genotype and allele frequencies, to test Hardy-Weinberg equilibrium and to do a neutrality test. Heterozygosity, effective number of alleles, and Shannon's Index of genetic diversity of the populations also were calculated using PopGene32 software.

Results. Digestion of the 178 bp fragment in exon 4 of Agouti gene with *Ban*II restriction enzyme revealed a polymorphism with two alleles. G allele was characterized by bands of 157 bp and 21 bp. T allele was characterized by a single band of 178 bp. Genotype and allele frequencies of 423 G>T polymorphism are given in Table 1. In all of the three breeds, T allele had a higher frequency varying between 81.25 to 93.75 %. TT genotype is most observed genotype in all breeds. No GG genotype was detected in the study. All of the populations were found to be at Hardy-Weinberg equilibrium. Statistical value of evolutional power (F) was calculated by Ewens-Watterson neutrality test and found to be between normal boundaries in indigenous goat breeds (Table 2). Values for heterozygosity, Shannon's index and effective alleles indicating genetic diversity are showed in Table 3. The genetic diversity for the 423 G>T site analyzed in the study is found to be very low in three indigenous breeds.

Table 1. **Distribution of genotypes and allele frequencies of 423G>T polymorphism in three goat breeds**

Breed	n[1]	Genotype						Allele Frequency (%)		Chi-square test	
		TT		TG		GG		T	G	χ^2	P
		Ob[2]	Ex[3]	Ob	Ex	Ob	Ex				
A[4]. Black	40	35	35.1266	5	4.7468	0	0.1266	93.75	6.25	0.1405	0.7077
Kilis	40	25	26.3291	15	12.3418	0	1.3291	81.25	18.75	1.9688	0.1606
Angora	40	30	30.5696	10	8.8608	0	0.5696	87.5	12.5	0.7267	0.3940

[1]number of animals, [2]observed values, [3]expected values, [4]Anatolian

Discussion. There are many candidate genes involved in pigment synthesis in goats, therefore the underlying molecular factors of coat color genetics is not yet clarified (Badaoui et al., 2011). One of these genes is caprine *Agouti*, which has been analyzed by several researchers (Tang et al., 2008, Fontanesi et al. 2009b, Li et al., 2010, Badaoui et al. 2011) No clear relationship could be found between the detected polymorphisms of caprine Agouti gene and coat phenotype (Badaoui et al., 2011). Comparison of the coat colors and allele frequencies of the goat breeds analyzed in this study does not lead to an association between 423 G>T polymorphism in exon 4 and coat color in three indigenous breeds of Turkey. Tang et al. (2008) suggested that T allele of this polymorphism might be related to black coat color according to the results of the study on indigenous Chinese goat breeds. In our study Anatolian Black breed with black and white coat color has the highest T allele frequency (93.75%). T allele has also higher frequencies than G allele in Kilis (black coat) and Angora (white coat) breeds, 81.25% and 87.5%, respectively. The results of Ewens-Watterson neutrality test showed that G>T polymorphism is a neutral variant. The observed F (statistical values of evolutional power) values have been found to be between normal ranges (Table 3). The low frequencies of G allele and the absence of GG genotype in all of the three breeds with different coat colors can be a result of phylogeographic structure of these breeds. In a study on Agouti gene of Spanish and Italian goat breeds, authors analyzed the relationship of 376 T>G polymorphism in exon 4 and coat color variation. No association could be found in 12 goat breeds (Badaoui, 2011). We suggest that polymorphisms in Agouti gene should be analyzed further in various goat breeds.

Table 2. **Ewens-Watterson test for neutrality of 423 G>T polymorphism in three goat breeds**

Breed	F[1]			Mean	SE[2]	95% confidence interval	
	Observed F	Min. F	Max. F			Lower bound	Upper bound
Anatolian Black	0.8828	0.5	0.9753	0.8040	0.0269	0.5012	0.9753
Kilis	0.6953	0.5	0.9753	0.8071	0.0261	0.5028	0.9753
Angora	0.7812	0.5	0.9753	0.7991	0.0275	0.5028	0.9753

[1]statistical value of evolutional power, [2]standard error

Table 3. **Heterozygosity of 423 G>T polymorphism in three goat breeds**

Breed	Heterozygosity		I[1]	N_e[2]
	Observed	Expected		
Anatolian Black	0.125	0.1187	0.2338	1.1327
Kilis	0.375	0.3085	0.4826	1.4382
Angora	0.25	0.2215	0.3768	1.28

[1]Shannon's Index, [2]effective number of alleles

Angora breed with white coat color has even higher T allele frequency (87.5%) compared to Kilis breed's T allele frequency (81.25%). There are some postulations about high T allele frequency and white coat color. There are several loci controlling coat color in goats; Agouti locus, color dilution factor locus, locus of interaction white, white spot locus and extension locus. The locus of interaction white may have an epistatic gene which could cause white coat color in goats (Chang 1999). T allele frequencies in Liaoning Cashmere goat (90%), Inner Mongolian Cashmere goat (93%) and Boer goat (88%) with white coat color can be examples for this hypothesis (Tang et al. 2008). This epistatic gene might play a role in white coat color of Angora goats, too.

The results (heterozigosity values, Shannon's Index and number of effective alleles) show that the genetic diversity is very low at the studied site in Angora, Anatolian Black and Kilis breeds (Table 3). The results of the mt-DNA analysis of these three indigenous breeds (unpublished data) denoted the high genetic variation in these populations. Only Angora breed could have been selected for coat color, so we cannot explain this low values for heterozygosity with artificial selection in the two other breeds. This site can be linked to a mutation which has been selected in the goat populations.

Conclusion. No clear relationship could be observed between the 423G>T polymorphism in exon 4 of caprine Agouti gene and coat phenotype in three Turkish indigenous breeds. The genetic diversity was to be found very low at the analyzed site. As a conclusion we may say that caprine Agouti gene and its association with coat color should be studied further using new polymorphisms and in a greater number of breeds.

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