Euroasia Journal of Mathematics, Engineering, Natural & Medical Sciences
International Indexed and Refereed
ISSN 2667-6702

Insulin-Like Growth Factor 1 (IGF-1) Gene Polymorphism in Some Goat Breeds Reared in Turkey

Türkiye'de Yetiştirilen Bazı Keçi Irklarında İnsülin Benzeri Büyüme Faktörleri-1 (IGF-1) Geni Polimorfizm

Mahamat Ali Amine Ouchar [1*], Birol Dağ [2], İbrahim Aytekin [3]

[1] Institut de Recherche en Elevage pour le Développement, Animal Production and Environment Division, PO Box 433, N'Djamena - Tchad
[2] Selcuk University, Faculty of Agriculture, Department of Animal Science, Postal Code: 42130, Department of Zootechnie, Konya, Türkiye
[3] Selcuk University, Faculty of Agriculture, Department of Animal Science, Postal Code: 42130, Department of Zootechnie, Konya, Türkiye

** Corresponding author: amine.ouchar@gmail.com*

Geliş Tarihi / Received: 03.06.2023
Kabul Tarihi / Accepted: 14.07.2023
Araştırma Makalesi/Research Article
DOI: 10.5281/zenodo.8238374

ABSTRACT

The purpose of this study is to determine *Hae*III polymorphism in the exon 4 and intron 4 region of the Insulin-like Growth Factors -1 (IGF-1) gene which the regulation of tissue growth and muscle development in 6 different goat breeds reared in Turkey. *Hae*III polymorphism in IGF-1 gene (363 bp) was investigated by Restriction Fragment Length Polymorphism PCR-RFLP methodology in a total of 303 goats including 52 Hair, 51 Angora, 50 Kilis, 50 Honamlı, 50 Halep, and 50 Saanen breeds. A and B allele frequencies in Hair, Angora, Honamlı, Halep, Saanen and Kilis breeds were found to be 0.3558 and 0.6442, 0.5784 and 0.4216, 0.2100 and 0.7900, 0.7300 and 0.2700, 0.7800 and 0.2200, 0.2900 and 0.7100, respectively. AA, AB and BB genotype frequencies were found to be 0.250, 0.212 and 0.538 ($P<0.05$), 0.451, 0.255 and 0.294 ($P<0.05$), 0.060, 0.300 and 0.640 ($P>0.05$), 0.600, 0.260 and 0.140 ($P<0.05$), 0.700, 0.160 and 0.140 ($P<0.05$), 0.200, 0.180 and 0.620 ($P<0.05$) respectively. When all breeds were taken into consideration, A and B allel frequencies were found to be 0.4901 and 0.5099, and AA, AB and BB genotype frequencies were found to be 0.376, 0.228 and 0.396 ($P<0.05$). It was determined that populations except for Honamlı goats were in not Hardy-Weinberg equilibrium when both the breed and the general evaluation were made in terms of *Hae*III polymorphism in the exon 4 region of the IGF-1 gene ($P<0.05$).

Keywords: IGF-1, *Hae*III polymorphism, goats, PCR, RFLP

ÖZET

Bu araştırmada amaç dokuların büyümesi ve kas gelişmenin düzenlenmesinde aday gen olarak kabul edilen İnsülin Benzeri Büyüme Faktörleri -1 (IGF-1) geninin ekzon 4 ve İntron 4 bölgesindeki *Hae*III polimorfizmini Türkiye'de yetiştirilen 6 farklı keçi ırkında belirlemektir. Araştırmada 52 baş Kıl, 51 baş Tiftik, 50 baş Honamlı, 50 baş Halep, 50 baş Saanen ve 50 baş Kilis ırkı olmak üzere toplam 303 baş keçide GH1 geni *Hae*III polimorfizmi (363 bç) Restriksiyon Fragman Uzunluk Polimorfizmi (RFLP) yöntemi ile araştırılmıştır. IGF-1 geni *Hae*III enzim kesimi sonucu iki allel (A ve B) ve üç genotip (AA, BB ve AB) belirlenmiştir. Kıl, Tiftik, Honamlı, Halep, Saanen ve Kilis keçi ırkları için A ve B allel frekansları sırasıyla 0.3558 ve 0.6442, 0.5784 ve 0.4216, 0.2100 ve 0.7900, 0.7300 ve 0.2700, 0.7800 ve 0.2200, 0.2900 ve 0.7100 olarak

bulunmuştur. AA, AB ve BB genotip frekansları bakımından ise sırasıyla 0.250, 0.212 ve 0.538 (P<0.05), 0.451, 0.255 ve 0.294 (P<0.05), 0.060, 0.300 ve 0.640 (P>0.05), 0.600, 0.260 ve 0.140 (P<0.05), 0.700, 0.160 ve 0.140 (P<0.05), 0.200, 0.180 ve 0.620 (P<0.05) olarak bulunmuştur. Bütün ırklar dikkate alındığında A ve B allel frekanslarını 0.4901 ve 0.5099, AA, AB ve BB genotip frekanslarını ise 0.376, 0.228 ve 0.396 (P<0.05) olarak tespit edilmiştir. Honamlı keçisi dışındaki ırkların Hardy-Weinberg dengesinde olmadığı belirlenmiştir (P<0.05).

Anahtar Kelimeler: IGF-1, *HaeIII* polimorfizm, keçi, PCR, RFLP

1. INTRODUCTION

The estimated number of goats worldwide is 1 034 406 504 and are reared as 53.3% in Asia, 40.9% in Africa, 3.6% in the USA, 1.6% in EU and 0.4% in the Oceania, respectively. Goats are mostly reared in China, India, Nigeria, Pakistan, Bangladesh, Chad Republic, Sudan, Ethiopia, Mongolia, and Kenya countries, respectively (FAO, 2017). In Turkey, the presence of goats is 10 992 427 heads and approximately 98% of the total goat is made up of hair goats and hybrids and 2% of them is Angora goats. Its share in livestock is 17.35% (Anonim, 2018). When expressed in goat breeding in Turkey comes to mind Hair goat breeding. On the contrary, their production performance is rather low. They are mainly kept on permanent grazing land and dry steppe areas with poor nutritive values in an extensive production system. In recent decades, although there is an attempt to improve their production performance and to develop more sophisticated breeds and genotypes, due to lack of a breeding strategy on a national scale, inadequate nutrition and not having performance recording, there is a big gap between the potential and realized performance of native breeds (Kaymakci et al., 2006). Sheep and goats have an important place in the economy of Turkey and in the nutrition of its people. They convert otherwise unuseable vegetaion on poor grazing lands to meat, milk, fiber and skins. Sheep and goats contribute also indirectly to the export earnings of the country through providing raw materials for the export-oriented carpet, textile, leather and food industries, as in the other Near East countries, sheep meat, sheep milk and sheep-milk products are valuable and generally preferred commodities. Goat meat and goat milk are the main sources of animal protein for the inhabitants of the mountainous regions (Kaymakci and Aşkın, 1997). The increase in yield in farm animals will be possible by improving genotype and/or environment, having knowledge about genetic structure of population increases the success of breeding. In particular, there is a high correlation between the polymorphic properties and the properties emphasized. The presence of criteria such as the feasibility of early detection and non-gender dependence makes a significant contribution to the breeders in selection. To date, many candidate genes have been studied in farm animals and the relationships between these genes and yield characteristics of economic importance have been investigated.

Insulin-like Growth Factors (IGF-1 and IGF-2) and insulin have a critical role in regulating growth and development in living organisms (Reinecke and Cololet, 1998). Therefore, polymorphisms in the IGF gene have led researchers to use them as candidate genes and to work in this field. The IGFs signaling system of the insulin-like growth factors (IGF) gene, IGF-1 and IGF-2 have distinct receptors. IGF binding proteins (IGFBP-1–IGFBP-6) are a member of six high affinity protein families. Plays an important role in development, growth and reproduction and aging (Miller and Gore, 2001; Li et al., 2009; Lan et al., 2007). The IGF-1 gene is encoded by a single gene located on chromosome 5 (Schibler et al., 1998). Three leading exons (1 w, 1 and 1 a) and three exons (3, 4 and 6), exons 3 and 4, encode the mature IGF-I peptide (Mikawa et al., 1995). IGF-I plays a key role in mammalian growth, lactation and metabolism (Zhang et al., 2008). By stimulating anabolic processes such as cell proliferation, skeleton and hair growth and protein are synthesized. POUIF1

(also called PIT-1 or GHF-1) is mainly expressed in the pituitary gland and plays an important role in the expression of Growth Hormone (GH), Prolactin (PRL) and thyroid stimulating hormone β (TSH-β). Mutation on this gene could possibly result in lack of, GH, PRL and TSH (Cohen et al., 1996; Li et al., 1990). The entire sequence of the Insulin-like Growth Factors (IGF) gene for goats is reached in the GenBank database with access number 80190 bp and NC_030812 (Anonymous, 2017). The 5th chromosome consists of 4 exons and 4 introns (Mikawa et al., 1995). The aim of this study was to determine the IGF-1 gene polymorphism in exon 4 and intron 4 to determine *Hae*III grown in 6 different goat breeds in Turkey.

2. MATERIAL and METHOD

Blood sampling

In this study, a total of 303 blood samples were obtained from 52 Hair goats, 51 Angora goats, 50 Honamlı goats, 50 Halep goats, 50 Saanen goats, and 50 Kilis goats.

Blood sample was collected from jugular vein by using vacutainer tubes containing with 10 ml Ethylene Diamine Tetra Acetic (EDTA) tubes and brought to Selçuk University Faculty of Agriculture Department of Animal Science Biotechnology Laboratory then stored in refrigerator temperature condition set to -20ºC before DNA extraction.

DNA Extraction

The DNA isolation was performed using the QuickGene DNA (DB-S) kit (Fujifilm Corp., Tokyo, Japan). After DNA isolation, all samples were measured on a Nanodrop spectrophotometer (ND1000; NanoDrop Technologies, USA) and DNA concentrations were determined.

Amplification and genotyping of DNA target (PCR-RFLP)

PCR amplification of the 363 bp region in the Exon 4 Intron 4 region of the IGF-1 gene. DNA sequences of the forward and reverse primer used were F=5'-CACAGCGTATTATCCCAC-'3 and R=5'- GACACTATGAGCCAGAAG –3' (Liu et al. 2010). After DNA isolation, all samples were measured on a Nanodrop spectrophotometer (ND1000; NanoDrop Technologies, USA) and DNA concentrations were determined.

The chemicals and concentrations used in the PCR are 10 µL of 2 µL DNA, 1X Master mix, 0.25 µM primers and 5.5 µL ddH2O with a total concentration of 10 µL. The PCR condition starting with initial denaturation temperature at 94 °C for 5 minutes, followed by 35 cycles of denaturation at 94 °C for 40 seconds, annealing at 56 °C for 35 seconds, followed by one cycle of final extension at 72 °C for 10 minutes with using a PCR machine (TC-512 Gradient Thermal Cycler). To determine the polymorphism in the gene region of interest, 10 µL of PCR product, 1 U *Hae*III restriction enzyme, 1X Buffer and 7 µL ddH2O were added and treated for 30 min at 37 ° C. The PCR products were then electrophoresis on 2% agarose gel and visualized on UV transiluminator. To identify the allele variation, PCR products obtained from the target gene was then analyzed by using RFLP with *Hae*III restriction enzymes with the cutting site.

Statistical analysis

POPGENE 32 statistical program was used to analyze whether allele and genotype frequencies and populations were in balance (Yeh et al. 1997).

3. RESULTS and DISCUSSION

Amplification IGF-1 gene

IGF-1 gene region amplified by the method and the genotypes obtained by cutting enzyme *HaeIII* in Figure 1.

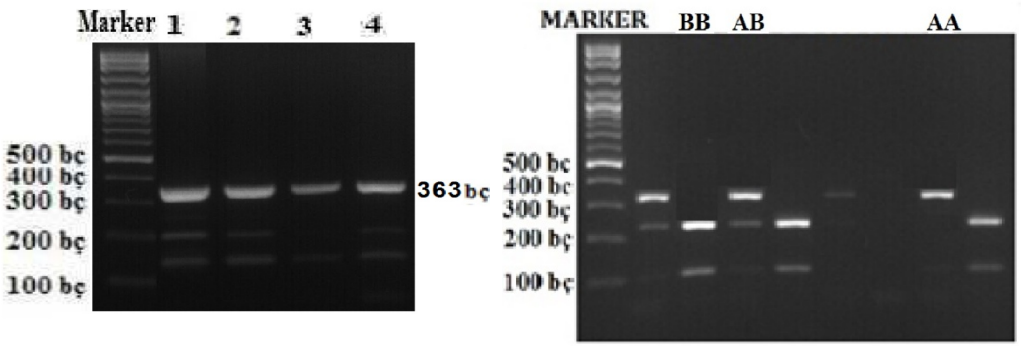

Figure1. Visualization, PCR-RFLP of IGF-1 Gene Exon 4 Intron 4, PCR product (left) and (Right) product, Lane M = DNA Marker, Uncutting PCR product (363 bp), *Hae*III cutting size, AA genotype (363 bp), BB genotype (264- 99 bp) ve AB genotip (363-264-99 bp), M: 100 bp DNA Ladder.

Genotype and allele frequency and Hardy Weinberg equilibrium

The IGF-1/ *Hae*III polymorphisms in terms of genotype and allel frequencies in six of diferent goat breeds of Turkey were given in Table 1.

The statistical analysis result obtained in this study showed that the breeds which detected have generalty 2 alleles (A and B), and 3 genotypes (AA, AB and BB). The 303 under-head race goats IGF-1 / *Hae*III polymorphism as a result of 114 AA, 69 AB and 120 BB genotype breeds with identified as the number of animals (Table 1). A and B allele are the frequencies 0.4901and 0.5099 respectively. The frequencies for AA, AB and BB genotypes were 0.376, 0.228 and 0.396, respectively. Also, Genotype and alleles frequencies distributions of the breeds in the study were found and except for Honamlı breed, they were not in Hardy-Weinberg equilibrium ($P<0.05$).

Table 1: The IGF-1/ *Hae*III polymorphisms in terms of genotype and allele frequencies in different six goat breeds of Turkey.

Breeds	N	Genotype frequencies			Allele frequencies		He	χ^2
		AA (n)	AB (n)	BB (n)	A	B		
Hair goats	52	0.250(13)	0.212(11)	0.538(28)	0.3558	0.6442	0.458	15.080*
Angora goats	51	0.451(23)	0.255(13)	0.294(15)	0.5784	0.4216	0.488	11.620*
Honamlı goats	50	0.060(3)	0.300(15)	0.640(32)	0.2100	0.7900	0.332	0.459

Halep goats	50	0.600(30)	0.260(13)	0.140(7)	0.7300	0.2700	0.394	5.795*
Saanen goats	50	0.700(35)	0.160(8)	0.140(7)	0.7800	0.2200	0.343	14.247*
Kilis goats	50	0.200(10)	0.180(9)	0.620(31)	0.2900	0.7100	0.412	15.843*
Total	303	0.376(114)	0.228(69)	0.396(120)	0.4901	0.5099	0.500	89.793*

χ^2: Hardy-Weinberg equilibrium (HWE) test; He: expected heterozigosity; *P<0.05

IGF-I gene in terms of *Hae*III polymorphism, genotypes AA, AB and BB were determined in Hair, Angora, Honamlı, Halep, Saanen and Kilis goats. Also researchers, first, by Liu et al. (2010), investigated the association between polymorphisms in insulin-like growth factor-1 (IGF-1) and cashmere traits data with two Xinjiang local goat breeds. The frequencies of genotype AA in two goat breeds (Xinjiang goat, Nanjiang cashmere goat) were 0.487 and 0.277. Genotype BB was 0.274 and 0.486. Genotype AB was 0.239 and 0.237. The polymorphisms of the IGF-1 gene were associated with cashmere yield, fiber diameter, body weight in cashmere goat; Qiong et al. (2011) 776 samples of three Xinjiang local goat breeds (Xinjiang, Bogeda Cashmere and Nanjiang Cashmere Goat, obtained results showed that the frequencies of genotype AA were 0.414, 0.591 and 0.319. Genotype AB was 0.000, 0.126 and 0.029. Genotype BB were 0.586, 0.241 and 0.597 and genotype AC were 0.000, 0.042 and 0.055 for Xinjiang, Bogeda Cashmere and Nanjiang Cashmere Goat breeds, respectively. Alakilli et al. (2012) found that the allelic frequencies of İGF-1 for A, B as 0.731 and 0.269 in Barki; 0.432 and 0.568 in Zaribi; 0.615 and 0.385 in Ardi and 0.473 and 0.527 in Masri goat breeds. Alakilli et al. (2012) reported that the three genotypes as AA, AB and BB four the goat breeds (Barki, Zaribi, Ardi and Masri) of Egypt and Saudi Arabia. Kurdistani et al. (2013) in a study they had used Markham and Kurdi goat breeds of goats grown in Iran on which A G seen mutation occurred. Polymorphism has been shown to have some effects on animals. Shareef et al. (2018) stated that the Beetal goats vared in body weight in their study the IGF-1 gene was found polymorphic and following three genotypes (AA, AB, and BB) were detected in tested animals with the frequency of mutant (B) and wild-type (A) alleles as 0.35 and 0.65 respectively.

Hair, Angora, Honamlı, Halep, Saanen and Kilis goats were observed Genotype Frequencies values respectively 0.250, 0.212 ve 0.538; 0.451, 0.255 ve 0.294; 0.060, 0.300 ve 0.640; 0.600, 0.260 ve 0.140; 0.700, 0.160 ve 0.140 ve 0.200, 0.180 ve 0.620; expected Genotype Frequencies values respectively 0.13, 0.46 ve 0.42; 0.45, 0.255 ve 0.294; 0.04, 0.33ve 0.62; 0.53, 0.39 ve 0.07; 0.61, 0.34 ve 0.05; 0.08, 0.41ve 0.50; as determined. Except for Honamlı, Hair, Angora, Honamlı, Halep, Saanen and Kilis breeds were observed not in Hardy-Weinberg balance (P<0.05).

The *Hae*III polymorphism of IGF-I gene of Hair, Angora, Honamlı, Halep, Saanen and Kilis goats, and the genetic structure and allele frequencies of these polymorphisms were determined.

4. CONCLUSION

As a result, goat breeds reared in Turkey IGF-1 /*Hae*III polymorphism in terms of the three genotypes AA, AB and BB, genotypic frequency respectively 0.250, 0.212 and 0.538 in Hair goats, 0.451, 0.255 and 0.294 in Angora goats, 0.060, 0.300 and 0.640 in Honamlı goats, 0.600, 0.260 and 0.140. In Halep goats, 0.700, 0.160 and 0.140 in Saanen goats and 0.200, 0.180 and 0.620 in Kilis

Goats has revealed. Polymorphism in IGF-1 Exon 4 Intron 4, *Hae*III section in Hair, Ankara goat, Honamlı, Halep, Saanen and Kilis goat breeds detect genetic polymorphism of PCR-RFLP method. The chi-square test showed that the genotypes except for Honamlı were not under the Hardy-Weinberg equilibrium, indicating that IGF-1 / *Hae*III Exon 4 Intron 4 was indirectly selected for goat breeds (P>0.05).

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