

RESEARCH ARTICLE

Genetic Variation in *HIAT1* and *IGF1R* Genes in Some Goat Populations Reared in Türkiye

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Abstract

This study aimed to assess whether genetic variants of the *HIAT1* and *IGF1R* genes could be integrated into future marker-assisted selection (MAS) studies in three native Turkish goat populations known as Hair (HAI), Honamlı (HNM), and Kabakulak (KBK) to improve meat traits. In terms of the *HIAT1* polymorphism, the frequency of the I allele ranged from 0.06 (KBK) to 0.35 (HNM), while the D allele frequency ranged from 0.65 (HNM) to 0.94 (KBK). No animals with the II genotype for the *HIAT1* gene polymorphism were identified in KBK goats, while one animal carried this genotype in HAI goats. Regarding the *IGF1R* gene polymorphism, the frequency of the A allele ranged from 0.72 (HAI) to 0.92 (HNM), while the T allele frequency varied between 0.08 (HNM) and 0.28 (HAI). Genetic distance-based phylogenetic tree separated HNM goats from KBK and HAI. The literature pointed out that animals with II and AT genotypes for the *HIAT1* and *IGF1R* genes, respectively, are superior in terms of meat traits. Therefore, this study implies that the *HIAT1* gene is promising for MAS studies in KBK goats, while *IGF1R* variation could be utilized to improve meat traits in all goat populations in the future.

Introduction

The domestication process of the goat (*Capra hircus*) is believed to have been achieved approximately 10,000 years ago in the Fertile Crescent, which includes the eastern part of Türkiye (Zeder and Hesse, 2000). Following domestication, goats have been an important livestock species for farmers to produce meat, milk, and leather (Degen, 2007). Compared to other ruminants, goats are able to effectively utilize marginal areas such as mountainous, hilly, and forested regions. Indeed, intensive goat breeding has become a way of life known as nomadic culture in the Taurus Mountains and their extensions located between the Mediterranean and Southeastern Anatolia (Alkan and Ugur, 2015; Daskiran *et al.*, 2018).

Up-to-date statistics of the Food and Agriculture Organization of the United Nations (FAO) confirm that

Türkiye ranks first in Europe in terms of population size by owning approximately 10.3 million goats (FAO, 2023). Angora, Hair (HAI), Honamlı (HNM), Kilis, and Norduz are native Turkish goat breeds in which HAI is the most widely distributed and common breed, due to constituting about 90% of the country's goat population (Demir, 2024). It is noteworthy that HAI goats possess several subgroups, such as Çandır, Kabakulak (KBK), and Pavga, which differ significantly regarding body size, fertility, and milk yield. The breeding area of the KBK genotype extends to the Fethiye district of Muğla province, including the Elmalı and Kaş districts of Antalya province (Karslı *et al.*, 2020; Aslan *et al.*, 2022). The HNM breed, which is distinguished by its characteristic convex nose structure (where the lower jaw is longer than the upper jaw), is bred in the Teke region, which includes the Isparta, Burdur, and Antalya provinces, areas where nomadic populations are dense (Elmaz *et al.* 2012; Daskiran *et al.*, 2018; Aslan *et al.*, 2022).

In Türkiye, goat breeding is primarily practiced to produce meat, while no systematic selection studies focused on improving meat yield are carried out by farmers. On the other hand, traditional breeding studies relying on breeding values calculated from pedigree records and phenotypic data have been utilized to improve economically important traits worldwide. Thanks to this kind of breeding efforts over the past 80 years, significant increases in economic yields have been achieved in livestock breeds. However, contemporary approaches such as marker-assisted selection (MAS) and genomic selection offer new opportunities to enhance various yield traits in livestock. MAS, which involves the study of one or a few major genes in addition to traditional breeding methods, can complement these practices. By integrating MAS with classical breeding, the success of selection and genetic progress can be enhanced.

Numerous candidate genes, including insulin-like growth factor receptor (*IGFR*) and hippocampus abundant transcript 1 (*HIAT1*), have been reported to be integrated into MAS studies since they were directly associated with growth and body weight in numerous goat breeds (Pehlivan, 2019). Indeed, during myogenesis, *IGFs* control and stimulate protein synthesis, cell division, hypertrophy, and proliferation (Mohammadabadi *et al.*, 2021). *IGF* and *IGFR* genes also play crucial roles in growth, development, muscle formation, and metabolic processes in livestock. The *HIAT1* gene, on the other hand, is involved in both the molecular function of transporter activity and transmembrane transport. Indel mutations on the *HIAT1* gene have been reported to be associated with growth traits in goats and sheep (Gao *et al.*, 2020; Luo *et al.*, 2023). Numerous studies have linked mutations in these genes to growth traits and meat yield in livestock species (De la Rosa Reyna, *et al.*, 2010; Anh *et al.*, 2015; Ding *et al.*, 2022; Alex *et al.*, 2023).

Recently, the protocols to investigate a 15-base pair (bp) insertion of *HIAT1* and an A>T (179170) mutation of *IGF1R* genes were designed by Gao *et al.* (2020) and Alex

et al. (2023), respectively. Although these protocols seem easy to apply and cost-efficient, they have not been utilized to genotype native Turkish goat populations. In this context, this study aims i) to identify polymorphisms in the *HIAT1* and *IGF1R* genes, which have been reported to be associated with growth traits, and ii) to evaluate their potential use in (MAS) programs in HAI, HNM, and KBK goats to improve meat traits.

Material and Methods

Sample Collection and DNA Isolation

A total of 222 animals belonging to KBK (n = 70), HAI (n = 76), and HNM (n = 76) goats were sampled from at least three different farms located in Antalya province based on oral interviews with farmers to minimize kinship. A representative image of the studied goat populations is illustrated in Figure 1. Blood samples were subjected to a salting-out method described by Miller *et al.* (1988) to isolate genomic DNA. The success of DNA isolation was assessed via 1% agarose gel electrophoresis (Figure 2), while the quality and quantity measurements of the extracted DNA were evaluated using a spectrophotometer (Allsheng Nano-400A). DNA samples were then diluted to a final concentration of 50 ng/μL before polymerase chain reaction (PCR) was performed.

Detection of Polymorphisms in *HIAT1* and *IGF1R* Genes

In this study, the primer sets and PCR protocols reported by Gao *et al.* (2020) and Alex *et al.* (2023) were followed to genotype three native Turkish goat populations in terms of the polymorphisms of *HIAT1* and *IGF1R* genes, respectively. Since the polymorphism in the *HIAT1* gene occurs due to a 15 bp insertion, a standard PCR procedure was employed. In contrast, as the polymorphism in the *IGF1R* gene is a



Figure 1. The representative image of a) HNM, b) KBK, and c) HAI goats

single-nucleotide polymorphism (SNP; 179170 A>T), the tetra-primer ARMS-PCR (T-ARMS-PCR) method was utilized. The primer sets and the expected PCR product sizes for the *HIAT1* and *IGF1R* polymorphisms are given in Table 1.

The PCR reaction used to detect the *HIAT1* gene polymorphism in this study was carried out in a total reaction volume of 50 μ L [H_2O : 32,5, $MgCl_2$: 5, 10X buffer 4, dNTPs: 4 (2.5 mM), each primer: 0.5 (10 pmol), Taq: 0.5 (5U) and, DNA: 3 (50 ng)]. PCR amplification was carried out as follows: the first denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 60 °C for 45 seconds, extension at 72°C for 30 seconds, and the last extension at 72°C for 10 minutes. T-ARMS PCR used to determine *IGF1R* gene polymorphism was performed in a total volume of 50 μ L [H_2O : 30,5, $MgCl_2$: 5, 10X buffer 4, dNTPs: 5 (2.5 mM), each primer 0.5 (10 pmol), Taq: 0.5 (5U) and, DNA: 3 (50 ng)]. Finally, the PCR products of the *HIAT1* and *IGF1R* genes were separated on 3.5% and 2.5% agarose gel electrophoresis, respectively, at 75 V for 120 minutes to genotype each animal.

Statistical Analyses

Popgene v.1.32 software (Yeh *et al.*, 1997) was utilized i) to calculate allele and genotype frequencies, ii) to estimate observed (H_o) and expected (H_e) heterozygosity, and iii) to assess Hardy-Weinberg equilibrium (HWE) via chi-square test. This software was also used to estimate genetic distance values among studied goat populations, which were further processed in MEGA 11 software (Tamura *et al.*, 2021) to draw an unweighted pair-group with arithmetic average (UPGMA) dendrogram.

Results

Agarose gel electrophoresis-based genotyping revealed that three native Turkish goat populations showed polymorphisms regarding *HIAT1* (Figure 2) and *IGF1R* (Figure 3) variations.

The allele and genotype frequencies, genetic diversity parameters, and HWE test for the studied polymorphisms across three goat populations are summarised in Table 2. Regarding the *HIAT1* gene

Table 1. Some descriptive information for the studied gene regions.

Gene	Method	Primer Sequences (5'-3')	Genotype Sizes (bp)	References
<i>HIAT1</i> (Insertion 15 bp)	PCR	F: AGAGCCTCAGTTTCGCTTATT	II = 198	Gao <i>et al.</i> (2020)
		R: GAGTTTATGAATCCAGCAGTTGT	DD = 183 ID = 198, 183	
<i>IGF1R</i> (179170 A>T)	T-ARMS-PCR	IR: TGTCCACATCTTACCTAAGGCTGCTG		Alex <i>et al.</i> (2023)
		OF: TAGGTGGTTAGATGGTCGGAATGAGC	AA= 323, 200	
		OR: GCCTCATTCAGGTGTCTGGAACCTCTT	AT = 323, 200, 176	
		IF: TGGTACCGCAACTGTTCTGACTGGT	TT = 323, 176	

Table 2. Gene, genotype frequencies, genetic diversity, and chi-square test results for *HIAT1* and *IGF1R* gene regions across the studied goat populations.

			Gene Frequencies		Genotype Frequencies			Genetic Diversity Parameters			HWE
Gene	Breed	n	I	D	II	ID	DD	H_o	H_e	N_e	χ^2
<i>HIAT1</i>	HNM	73	0.35	0.65	0.16(12)	0.37 (27)	0.47 (34)	0.63	0.54	1.83	2,54 ^a
	HAI	76	0.18	0.82	0.01(1)	0.34 (26)	0.65 (49)	0.66	0.70	1.42	1.45 ^a
	KBK	70	0.06	0.94	0.00	0.13(9)	0.87 (61)	0.87	0.88	1.13	0.33 ^a
Gene	Breed	n	A	T	AA	AT	TT	H_o	H_e	N_e	χ^2
<i>IGF1R</i>	HNM	73	0.92	0.08	0.85(62)	0.15 (11)	0.00 (0)	0.74	0.70	1.16	0.49 ^a
	HAI	76	0.72	0.28	0.51(39)	0.41 (31)	0.08 (6)	0.60	0.58	1.70	0.02 ^a
	KBK	70	0.79	0.21	0.70(49)	0.19 (13)	0.11 (8)	0.81	0.67	1.48	13,22 ^b

H_o : Observed heterozygosity, H_e : Expected heterozygosity; N_e : number of effective alleles, HWE: Hardy-Weinberg equilibrium; χ^2 : chi-square value $\chi^2_{(0.05;1)} = 3.84$); a: Non-significant deviation from HWE test, b: Significant deviation from HWE test

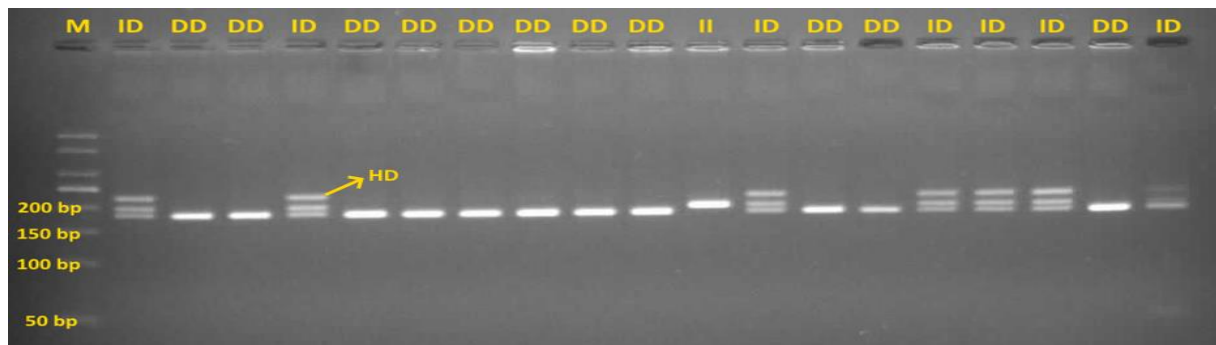


Figure 2. 3.5% agarose gel image of PCR products for *HIAT1* gene.
M: 50 bp Marker (softec, Kat. No: ZT-50BP-1); HD: heteroduplex

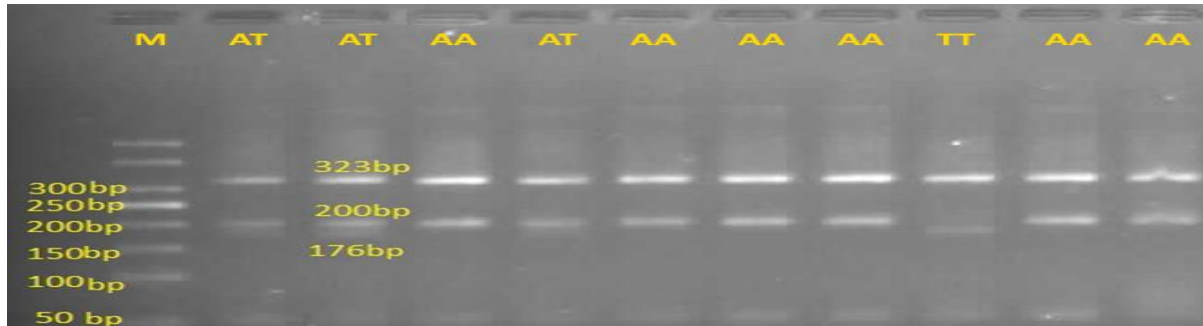


Figure 3. 3.5% agarose gel image of T-ARMS PCR products for *IGF1R* gene.
M: 50 bp Marker (softec, Kat. No: ZT-50BP-1)

polymorphism, the frequency of the I allele ranged from 0.06 (KBK) to 0.35 (HNM), while the D allele frequency ranged from 0.65 (HNM) to 0.94 (KBK). The II genotype frequency ranged from 0.00 (HNM) to 0.16 (KBK), the ID genotype frequency ranged from 0.13 (HNM) to 0.37 (KBK), and the DD genotype frequency ranged from 0.47 (HNM) to 0.87 (KBK). No significant deviation from HWE was detected in the *HIAT1* gene polymorphism across the studied goat populations.

The result of the T-ARMS PCR analysis indicated that all populations were polymorphic in terms of *IGF1R* gene polymorphism. The frequency of the A allele for the *IGF1R* gene in the studied breeds ranged from 0.72 (HAI) to 0.92 (HNM), while the T allele frequency varied between 0.08 (HNM) and 0.28 (HAI). The AA genotype frequency ranged from 0.51 (HAI) to 0.85 (HNM), the AT genotype frequency ranged from 0.19 (HAI) to 0.41 (KBK), and the TT genotype

frequency ranged from 0.00 (HNM) to 0.11 (KBK). A significant deviation from HWE was detected only in the KBK population regarding the *IGF1R* gene polymorphism.

Among the studied populations, the lowest and highest *Ho* value for the *HIAT1* gene were estimated in the HNM (0.63) and KBK (0.87) goats, respectively. The effective allele numbers (*Ne*) for the *HIAT1* gene were determined as 1.83, 1.42, and 1.13 for the HNM, HAI, and KBK goats, respectively. The lowest observed heterozygosity for the *IGF1R* gene was found in the HAI breed (0.58), while the highest was observed in the KBK breed (0.81). The *Ne* for the *IGF1R* gene were determined as 1.16, 0.05, and 1.48 for the HNM, HAI, and KBK breeds, respectively.

The UPGMA dendrogram based on the *HIAT1* and *IGF1* genes is shown in Figure 4. According to the UPGMA dendrogram constructed from the

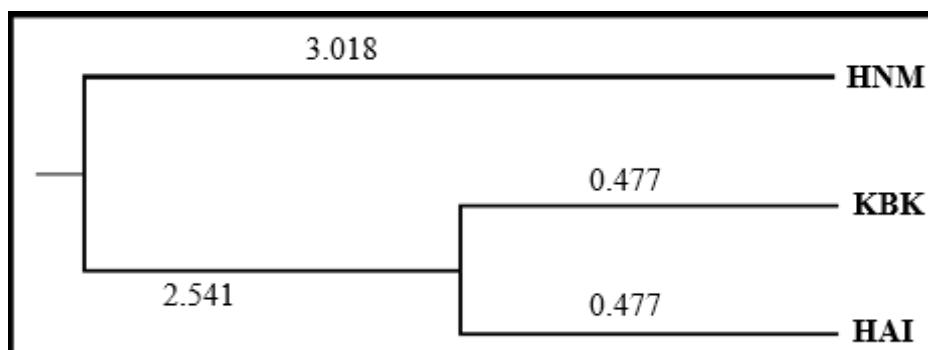


Figure 4. UPGMA cluster analysis based on *HIAT1* and *IGF1R* gene polymorphisms.

genetic distance values for both genes, the KBK and HAI populations formed a cluster, while the KBK goats were placed in a separate branch.

Discussion

Recently, several molecular-based studies have been conducted to identify candidate genes associated with meat traits such as body weight and growth in various goat breeds (Gao *et al.*, 2020; Wei *et al.*, 2021; Moaen-ud-Din *et al.* 2022; Alex *et al.*, 2023; Dai *et al.*, 2024; Xu *et al.*, 2024). Among these studies, Gao *et al.* (2020) examined the association between a 15-bp indel polymorphism in the *HIAT1* gene and some growth traits in Shaanbei White Cashmere goats. Researchers reported that the frequency of II, ID, and DD genotypes was 0.044, 0.317, and 0.639, respectively, while animals with II genotypes showed superior meat traits compared to animals with ID and DD genotypes. In the present study, the II genotype was not detected in KBK goats, whereas its frequency was 0.01 and 0.16 in HAI and HNM goats, respectively. Since the absence of II genotype in KBK could be explained by the small sample size. The frequency of the II genotype observed in the HNM goats was higher than that found in HAI goats and the value reported by Gao *et al.* (2020) in the Shaanbei White Cashmere goats. The differences in genotype frequencies across native Turkish goats and Shaanbei White Cashmere may arise from variations in ecological, demographic, and genetic background.

Another candidate gene reported to be associated with growth traits in goats is the *IGF1R* gene (Luo *et al.*, 2019; Lestari *et al.*, 2020; Alex *et al.*, 2023). Alex *et al.* (2023) reported that, among the genotypes (AA, AT, and TT) resulting from a point mutation (179170 A>T) in the second intron of this gene, goats with the AT genotype had significantly higher body weights ($p < 0.05$) compared to those with the AA and TT genotypes. Alex *et al.* (2023) reported the frequencies of the AA, AT, and TT genotypes of the *IGF1R* gene as 0.40, 0.44, and 0.16, respectively, in the Malabari goat breed, and 0.32, 0.40, and 0.28, in the Attappady Black goat breed. In the current study, the frequencies of the AT genotype in HNM, HAI, and KBK goats were 0.15, 0.41, and 0.19, respectively. While the AT genotype frequency observed in the HAI breed was comparable to the values reported by Alex *et al.* (2023) for the Attappady Black and Malabari breeds, the frequencies in the KBK and HNM breeds were lower.

Regarding the *IGF1R* gene, the presence of all genotypes with varying frequencies across the three goat populations (particularly the favorable

AT genotype associated with growth) and the observed heterozygosity of higher than expected heterozygosity values suggests that this gene region holds potential for MAS studies. However, II, which is the desired genotype for growth in the *HIAT1* gene, was detected at higher frequency in the HNM goats compared to other populations. The presence of high genetic variation for this gene region in the HNM goats and the presence of the HWE indicate that this gene region can be used in MAS studies in HNM goats. Türkiye contributes to global animal genetic resources with five officially registered native goat breeds (Angora, HNM, HAI, Kilis, and Norduz). Among these, HAI goats are the most commonly reared, accounting for approximately 90% of the country's total goat population (Demir, 2024). KBK goats, still classified as a variety of Hair goats, exhibit notable phenotypic differences (such as longer ear length and a larger body size) (Gezer, 2018; Karslı *et al.*, 2020). Despite these distinctions, morphological and molecular studies on this variety remain limited. In some phylogenetic analyses performed at the molecular level in recent years, it has been reported that HAI and KBK breeds are clustered differently (Karslı *et al.*, 2020; Karslı and Demir, 2024; Karslı and Atmaca 2025), while other studies assigned HAI and KBK into the same genetic cluster (Aslan *et al.*, 2022; Aktaş *et al.*, 2024). In the present study, HAI and KBK goats were found to be genetically close to each other. Since the analysis was conducted using two gene regions (*HIAT1* and *IGF1R*), the results seem preliminary, which should be further confirmed by various studies. Among the studies reviewed, the most comprehensive appears to be that of Karslı *et al.* (2020), which utilized 20 microsatellite loci. However, to more accurately differentiate between HAI and KBK goats, further research at the whole-genome level (such as studies employing SNP chips or whole-genome sequencing) is required.

Conclusions

In this study, polymorphisms in the *HIAT1* and *IGF1R* genes associated with growth traits were evaluated for the first time via cost-efficient molecular genotyping methods in Turkish indigenous goat populations. No animals with the II genotype for the *HIAT1* gene polymorphism were identified in KBK goats, while one animal carried this genotype in HAI goats. It is noteworthy that the absence of II genotype in KBK may occur due to the small sample size, which requires further studies focusing on a better sampling strategy. Still, the current findings indicate that the *HIAT1* gene polymorphism could be utilized in MAS studies only for HNM goats. On the other hand, the desired genotypes (AT) for meat traits regarding the *IGF1R* gene polymorphism were detected at sufficient frequencies among all goat populations. This finding

implies that the *IGF1R* gene is promising to improve meat production in native Turkish goat populations via future MAS programs. However, it should be noted that this study was limited to the identification of polymorphisms, and further association analyses with phenotypic data are necessary before implementing MAS. Additionally, KBK goats, which are considered a subtype of the HAI breed, were found to be genetically similar to HAI goats based on the two examined gene regions.

Author Contributions

First Author: Laboratory analyses, funding acquisition, manuscript drafting; Second Author: Laboratory analyses, manuscript drafting; Third Author: Laboratory analyses, manuscript drafting; Fourth Author: Laboratory analyses, manuscript drafting; Five Author: Laboratory analyses, manuscript drafting; Six Author: Manuscript drafting, critical review; Seven Author: Supervision, Methodology, funding acquisition, manuscript drafting, critical review.

Conflict of Interest

The author(s) declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

Ethical Statement:

This study was approved by Eskisehir Osmangazi University Animal Experiments Local Ethics Committee (Protokol No: HAYDEK-967/2023).

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