Assessment of InDel Variations in CPT1A and PRDM6 Genes Across Three Anatolian Goat Populations

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Abstract: Identifying candidate genes associated with growth traits in livestock and incorporating them into Marker Assisted Selection (MAS) studies, in addition to classical selection methods, can increase genetic progress and thus profitability. Carnitine Palmitoyl Transferase 1a (*CPT1a*) and PR domain family 6 (*PRDM6*) are among the candidate genes reported to be associated with growth traits in goats. This study aimed to identify polymorphisms in the CPT1a and PRDM6 genes related to growth traits in Kıl (HAI), Honamlı (HNM), and Kabakulak (KBK) goats, and to discuss their potential use in MAS studies. The II genotype of the *CPT1a* gene was not found in the HNM or KIL goats, whereas the KBK population included all three genotypes (II, ID, and DD). The frequency of the ID genotype ranged from 0.25 (HAI) to 0.40 (KBK), while the DD genotype frequency ranged from 0.37 (KBK) to 0.75 (HAI). For the *PRDM6* gene, the KBK population was found to be monomorphic, containing only the DD genotype. Although the II genotype was not detected in the HNM and HAI breeds, the ID genotype frequencies were calculated as 0.05 and 0.07, respectively. The study's findings point out that the *CPT1a* gene can be used in MAS studies for HNM, KBK, and HAI goats, whereas the *PRDM6* gene cannot be utilized.

Keywords: *CPT1a*, *PRDM6,* goat, indel, polymorphism

Üç Anadolu Keçisi Populasyonunda CPT1A ve PRDM6 Genlerindeki InDel Varyasyonlarının Değerlendirilmesi

Öz: Çiftlik hayvanlarında büyüme özellikleri ilişkili aday gen bölgelerinin belirlenerek klasik seleksiyon çalışmalarına ek olarak yapılacak Marker Destekli Seleksiyon (MAS) çalışmalarında kullanımı genetik ilerleme hızını ve karlılığı artırılabilir. Keçilerde büyüme özellikleri ile ilişkili olduğu bildirilen aday genler arasında Karnitin Palmitoil Transferaz 1a (*CPT1a*) ve PR domain family 6 (*PRDM6*) genleri de vardır. Gerçekleştirilen çalışmada Kıl (HAI), Honamlı (HNM) ve Kabakulak (KBK) keçilerinde büyüme özellikleri ile ilişkili *CPT1a* ve *PRDM6* genleri üzerindeki indel varyantların neden olduğu polimorfizmlerin araştırılması ve MAS çalışmalarında kullanım olanaklarının tartışılması amaçlanmıştır. *CPT1a* geni için HNM ve KIL keçilerinde II genotipi tespit edilemezken, KBK populasyonunda her üç genotip de (II, ID ve DD) tespit edilmiştir. ID genotip frekansının 0.25 (HAI) ile 0.40 (KBK) aralığında, DD genotip frekansının ise 0.37 (KBK) ile 0.75 (HAI) aralığında değiştiği tespit edilmiştir. *PRMD6* geni için KBK populasyonu monomorf (yalnızca genotip DD) bulunmuştur. HNM ve HAI ırklarında II genotipi tespit edilemezken, ID genotip frekansları sırasıyla 0.05 ve 0.07 olarak hesaplanmıştır. Çalışmadan elde edilen sonuçlar HNM, KBK ve HAI keçilerinde *CPT1a* geninin MAS çalışmalarında kullanılabileceğine ancak *PRDM6* geninin kullanılamayacağına işaret etmektedir.

Anahtar Kelimeler: *CPT1a*, *PRDM6,* keçi, indel, polimorfizm

1. Introduction

Being famous for their higher adaptability to extreme environmental conditions, goats have been an indispensable part of humankind due to their direct and indirect benefits such as providing valuable animal-derived products (meat and milk) and utilizing grasslands efficiently (Karslı & Demir 2024). In Türkiye, goat breeding is mainly practiced with local populations such as Hair (HAI), Honamlı (HNM), and Kabakulak (KBK) across mountainous and forestry regions by making a significant contribution to the incomes of smallholder farmers (Günlü & Alaşahan 2010). Of these goats, HAI, which has the highest population size (nearly 90% of the total goat population) among native breeds and developed adaptation to the harsh climate of Anatolia, is raised in villages near

mountains and forests for a dual purpose (Güngör et al. 2021). As highlighted by Demir (2024), unlike the HAI breed, the rest of the local Anatolian goat hold a lower population size. KBK, which is reared in Muğla and Antalya provinces, is believed to be a variety of HAI (Karsli et al. 2020) while it shows differences in terms of several phenotypic traits such as body size, fertility, and economically important yields (Demir 2024). HNM, on the other hand, is bred by smallholder farmers living across the Taurus Mountains of the Mediterranean region covering Antalya, Isparta, and Konya provinces (Daskiran et al. 2018).

The agricultural sector has been negatively affected by numerous factors such as increasing human populations, declining animal populations, and climate change (Demir et al. 2021) whereas farmers exert great efforts to design selection studies to increase the quantity and quality of milk and meat production to meet the demand of the growing societies. Like many other economically important traits, meat yield is affected by the combination of numerous environmental factors and genes due to the nature of quantitative inheritance (Núñez-Torres & Almeida-Secaira 2022). In theory, improving both environmental factors and considering candidate genes in selection studies seems promising to increase meat production in livestock. However, as highlighted by Atay et al. (2023), enhancement of environmental factors may not be practical or affordable by the farmers since it is not inherited by the next generations. Moreover, the improvement of environmental factors should be repeated for the following production seasons. In contrast, selection studies based on candidate genes, called marker-assisted selection (MAS), are an efficient approach to improve quantitative traits including meat yield for the current and future generations. In this

approach, animals are genotyped via accurate molecular tools such as polymerase chain reaction (PCR) and subjected to mating programs to create a population with desired genotype combinations regarding multiple genes affecting the trait of interest. Several genes and their receptor regions including insulin-like growth factor (Naicy et al. 2017; Lazar et al. 2018; Alex et al. 2023), growth hormone (Sharma et al. 2013; Yan et al. 2022; Rashijane et al. 2022), and leptin (Sarmah et al. 2020) were previously reported to have direct effects on growth traits in goats. Recently, Carnitine palmitoyltransferase 1A (*CPT1A)* and PR/SET domain family 6 (*PRDM6*) genes were reported to be not only associated with growth traits in goats but also the insertion-deletion (InDel) variations on these genes could be integrated into MAS studies to improve meat yield (Wang et al. 2019: Li et al. 2021). Although no studies monitoring variations of *CPT1A* and *PRDM6* genes in native Turkish goat breeds are available in the literature, these genes could be utilized in MAS programs to improve genetic gain regarding meat yield in local Anatolian goats. Hence, this study aims i) to investigate genetic variations in *CPT1A* and *PRDM6* in HAI, HNM, and KBK goats via PCR technique and) to assess their usefulness in MAS studies to enhance meat yield.

2. Material and Methods
2.1. Animal sampling and DNA extraction

A total of 204 animals from HAI (n=56), HNM (n=78), and KBK (n=70) were sampled in different regions of Antalya province (Figure 1). This study was approved by Eskisehir Osmangazi University Animal Experiments Local Ethic Committee (Protocol No: HAYDEK-968/2023).

Figure 1. The goat breeds used study (a: HNM, b: KBK, and c: HAI goats)
Şekil 1. Çalışmada kullanılan keçi ırkları (a: HNM, b: KBK, ve c: HAI keçileri)

2.2. PCR amplification and genotyping

The PCR protocols (Table 1) reported by Li et al.

(2021) and Manjutha et al. (2023) to investigate 12 bp length of InDel variations were followed to amplify

CPT1A and *PRDM6* genes, respectively, in three Anatolian goats.

A 25 µl PCR reaction was prepared by mixing 50 ng/µl template DNA, 12.50 µl EcoTech 2X Master Mix, 10 pmol/µl of each primer, and 6.50 µl ddH2O. The thermal cycler was initiated at 95 ºC for 5 mins followed by 35 cycles of 45 sec at 95 ºC for denaturation, 45 sec at 60 ºC for annealing, and 45 sec at 72 ºC for extension.

Final elongation was optimized at t 72 ºC for 10 mins. Amplified PCR fragments were visualized via 3.5% agarose gel electrophoresis to genotype each animal. In this step, DNA fragments with 179 and 191 bp lengths were coded as I and D alleles, respectively, for the *CPT1A* gene. Similarly, the PCR bands at 275 and 287 bp length were considered as I and D alleles, respectively, for the *PRDM6* gene.

Table 1. An overview of primers to amplify *CPT1A* and *PRDM6* genes and expected band sizes
Tablo 1. *CPT1A* ve *PRDM6* genlerini çoğaltmak için kullanılan primerler ve beklenen bant büyüklükleri

Gene	Primer sequence (5'-3')	Expected band sizes (bp)	Kaynak
CPT1a	F: ACAGTCACTCCTGCTGCCAATA R: CGCTGCTCTGCGTGTCATTAT	DD = 179 ID = 191, 179 II = 191	Li vd. (2021)
PRDM6	F: GGATACAGGACAGTGTGGGC R: CAACTCACTGAGCAAGGGGT	DD = 275 ID = 275, 287 II = 287	Manjutha vd. (2023)

2.3. Statistical analysis

Popgene V.1.32 (Yeh et al. 1997) program was utilized to calculate allele frequency, genotype frequency, observed heterozygosity (H_O), expected heterozygosity (H_E), and chi-square (χ^2) test to assess deviation from Hary-Weinberg equilibrium (HWE) in three Anatolian goats in terms of *CPT1A* and *PRDM6* genes. Besides, genetic distance values among populations were estimated by the Popgene V.1.32 (Yeh et al. 1997) program and processed into MEGA 11

(Tamura et al. 2021) software to construct the UPGMA dendrogram.

3. Results

In this study, InDel variations of *CPT1A* and *PRDM6* genes were studied in three Anatolian goats by traditional PCR method in which the agarose gel images are given in Figures 2 and 3 while the genetic diversity parameters are summarized in Table 2.

Figure 2. Agarose gel image for 19 randomly chosen animals for the *CPT1A* gene variation
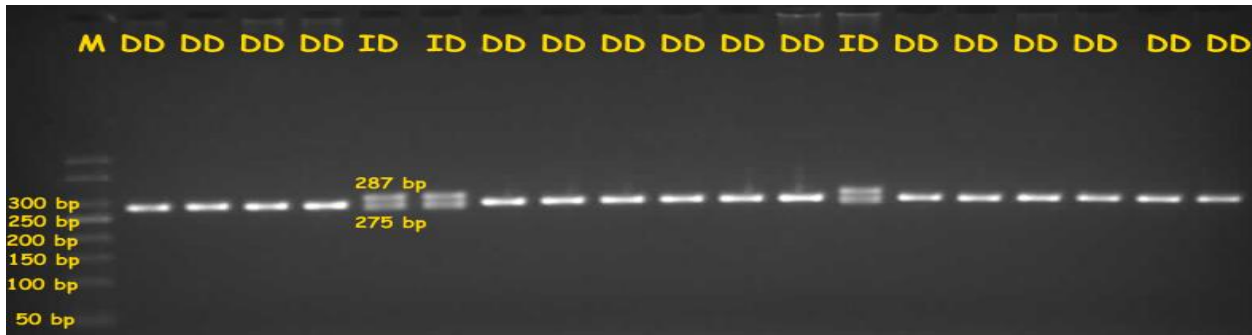**Şekil 2.** *CPT1A* geni için rastgele 19 hayvandan seçilen agaroz jel görüntüsü

Figure 3. Agarose gel image for 19 randomly chosen animals for the *PRDM6* gene variation
Şekil 3. *PRDM6* geni için rastgele 19 hayvandan seçilen agaroz jel görüntüsü

Table 2. Gene, genotype frequencies and genetic diversity parameters for studied genes
Tablo 2. Çalışılan gen bölgeleri için gen, genotip frekansları ve genetik çeşitlilik parametreleri

Gene	Population	n	Gene frequency		Genotype frequency			Genetic diversity			HWE
			I	D	II	ID	DD	Ho	He	Ne	χ²
CPT1A	HNM	74	0.13	0.87	0.00	0.26	0.74	0.74	0.77	1.28	1.61
	HAI	56	0.12	0.88	0.00	0.25	0.75	0.75	0.77	1.28	1.14
	KBK	70	0.43	0.57	0.23	0.40	0.37	0.60	0.50	1.96	2.35
PRDM6	HNM	78	0.03	0.97	0.00	0.05	0.95	0.95	0.95	1.05	0.06
	HAI	56	0.03	0.97	0.00	0.07	0.93	0.92	0.92	1.07	0.07
	KBK	70	0.00	1.00	0.00	0.00	1.00	1.00	1.00	1.00	-

$\chi^2_{0.05;1}$: 3.84; a: significant deviation from HWE.

All populations showed polymorphism in terms of the *CPT1A* gene variation in which I allele frequency was estimated at 0.13, 0.12, and 0.43 in HNM, HAI, and KBK goats, respectively (Table 2). D allele frequency turned out to be higher than the I allele across all studied populations. No animals with II genotype were detected in HNM and HAI breeds, while all possible genotypes (II, ID, and DD) were observed in KBK goats (Table 2). 16 animals (0.23) from the KBK population carried the II genotype. ID genotype frequencies ranged from 0.25 in HAI to 0.40 in KBK goats whereas DD genotype frequencies varied between 0.37 (KBK) and 0.75 (HAI) (Table 2). The observed value of heterozygosity was higher than the expected one in only KBK goat. Ne values ranged from 1.28 (HNM and HAI) to 1.96 (KBK) across three Anatolian goats in terms of the *CPT1A* gene variation (Table 2).
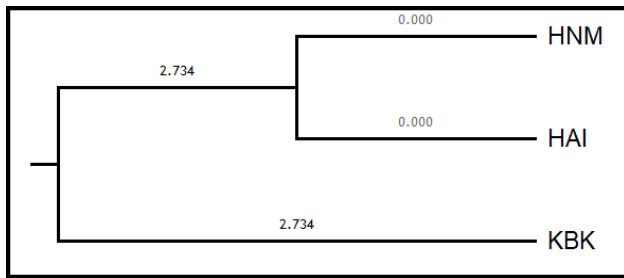

Figure 3. A genetic distance-based UPGMA dendrogram across three Anatolian goat breeds.
Şekil 3. Üç Anadolu keçi ırkı arasında genetik mesafeye dayalı bir UPGMA dendrogramı.

When it comes to the *PRDM6* variations, no polymorphisms were detected for the KBK goat in which all animals turned out to carry the DD genotype. Besides no II genotype was detected across three studied goat populations for the *PRDM6* gene. ID genotype frequencies were estimated at 0.05 and 0.07 in HNM and HAI goats, respectively (Table 2). I and D allele frequency was calculated as 0.03 and 0.97 in HNM and HAI goats, respectively. Similar expected and observed heterozygosity values were detected in all goat populations regarding the *PRDM6* gene. No significant deviation from HWE was detected in HNM and HAI goat in terms of the *PRDM6* variations (Table 2).

The UPGMA dendrogram drawn based on pairwise genetic values among populations is given in Figure 3 in which HNM and HAI clustered together while KBK constituted a separate branch.

4. Discussion

No studies focusing on revealing genetic variations of *CPT1A* and *PRDM6* genes in native Turkish goat breeds were detected in the literature. On the other hand, the number of studies from different countries is still scarce. For example, Li et al. (2021) investigated associations between 12 bp length InDel variations of the *CPT1A* gene and growth traits in Guanzhong dairy (GZ), Hainan black (HNB), Fuqing (FQ) ve Nubian (NB) goats. The authors highlighted those animals with ID and II genotypes showed superior values of growth traits compared to animals carrying DD genotype (Li et al. 2021). Additionally, II genotype frequencies were reported to be 0.21, 0.70, 0.43, and 0.28 for GZ, HNB, FQ, and NB breeds, respectively (Li et al. 2021). ID genotype frequencies were reported to range from 0.29 (HNB) to 0.58 (GZ) in Chinese goats (Li et al. 2021). The current study on three native Turkish goats showed consistent results with the findings reported for II and ID genotype frequencies in four Chinese goat breeds (GZ, HNB, FQ, and NB). Indeed, ID genotype frequency varied between 0.25 (HAI) and 0.40 (KBK) across three Anatolian goats. Although no animals with the II genotype were detected regarding the *CPT1A* genes, the frequency of the ID genotype turned out to be sufficient in HNM and HAI goats. That no animals with the II genotype were detected in the HNM breed was surprising since it is famous for its superior growth traits and live weight. Similarly, the detection of the II genotype in KBK goats is surprising. However, it is noteworthy that possession of a higher body weight could make KBK genetically different from the HAI

breed.

A 12 bp length of genetic variations of *PRDM6* genes was previously investigated in Shaanbei White Cashmere and associated with several growth-related parameters (Wang et al. 2020). The authors reported that animals with the DD genotype showed superior values in terms of chest depth and chest width while the II genotype was advantageous for body length. The frequency of II, ID, and DD genotypes in Shaanbei White Cashmere goats was reported as 0.57, 0.37, and 0.06, respectively while the D allele frequency (0.25) was lower than the I allele (0.75) (Wang et al. 2020). Another study carried out by Manjutha et al. (2023) revealed that the D allele frequency (ranging from 0.84 to 0.96) was higher than the I allele frequency (varied between 0.01 and 0.05) in two native Indian goats (Attapady Black and Malabari). The current study showed similar findings reported for native Indian goats (Manjutha et al. 2023) while consistent results were observed compared to Shaanbei White Cashmere goats reared in China (Wang et al. 2020). The reason underlying of these differences may be explained by the origins of the studied goat breeds in which Shaanbei White Cashmere goats were derived from *Capra hircus laniger*. On the other hand, HAI, KBK, HNM, Attapady Black, and Malabari are descendants of hair goats.

Today, KBK goats are considered a variety of the HAI breed (Aslan et al. 2022) but this information has been questioned by several studies. For example, using 20 microsatellite loci, Karsli et al. (2020) highlighted that KBK is genetically closer to HNM rather than HAI in phylogenetic analyses. A similar finding was also reported by Karsli and Demir (2020) based on the variations related to *CSN1S1* and *CMTM2* genes. In contrast, Aslan et al. (2022) highlighted that KBK was genetically closer to HAI than the HNM breed according to polymorphisms of 9 microsatellite loci. Unlike the results of previous studies, this study showed that KBK was genetically from both HAI and HNM breeds. However, the current study focused on two genes which do not represent the whole genome. Therefore, future studies based on next-generation sequencing and array technologies are required for determination of whether KBK is a variety of HAI breed or not.

5. Conclusion

The increasing trend in the human population implies that meat demand will also increase in the future. Therefore, the development of selection strategies based on genes related to meat yield will play a vital role in meeting increasing demand in the agriculture sector. Considering these facts, this study aimed to investigate a 12 bp length of InDel variations of *CPT1A* and *PRDM6* genes in three Anatolian goats via PCR technique. The desired genotype for *CPT1A* (II) was detected only in KBK goats while a sufficient number of animals with ID genotype were detected across all populations. This finding indicates that MAS studies could be carried out in KBK whereas II genotypes may be obtained in HAI and HNM breeds via mating ID genotypes. On the other hand, no animals with the II genotype were detected in KBK goats for the *PRDM6* gene whereas the frequencies of the ID genotype were insufficient across three Anatolian goats. Therefore, the *PRDM6* gene was not useful for MAS studies due to a lack of genetic variations. Since meat yield shows quantitative inheritance, future studies may focus on revealing the other candidate genes which could be subjected to MAS studies in local Anatolian goats.

Author contributions

YA: Lab Analysis, Data analysis, Data collection, **VA:** Lab Analysis, Data analysis, Data collection **ED:** Methodology, Draft writing, **TK:** Supervision, Methodology, Funding acquisition, Draft writing;

Ethical Permission

This study is reviewed and approved by the Eskişehir Osmangazi University Animal Experiments Local Ethic Committee (Protocol No: HAYDEK-968/2023).

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