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Identification of 21-bp indel polymorphism in the *LLGL1* gene in several Turkish native goat populations*

Taki KARSLI, Veli ATMACA

Eskisehir Osmangazi University, Faculty of Agriculture, Department of Animal Science, 26160, Eskisehir, Türkiye

Corresponding author: T. Karsli, e-mail: takikarsli@ogu.edu.tr
Author(s) e-mail: atmacav7@gmail.com

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ABSTRACT

Being one of the fundamental economically important traits, reproduction efficiency such as litter size plays a key role in determining profitability for livestock enterprises focusing on meat production. Numerous candidate genes identified to be associated with litter size have been subjected to Marker-Assisted Selection (MAS) studies to improve classical breeding approaches. Of these candidate genes, Scribble Cell Polarity Complex Component (*LLGL1*) was confirmed to carry 21 base pair (bp) insertion-deletion (InDel) variation which was directly related to multiple births in goats. This study aims to determine the polymorphisms caused by the 21 bp indel variation in the *LLGL1* gene in Hair (HAI), Honamlı (HNM), and Kabakulak (KBK) goats reared in Türkiye. At the end of the PCR process, all populations were found to be polymorphic. I allele frequency was higher than D allele frequency across three populations while the highest frequency of ID (0.50) II genotypes (0.47) was observed in the HAI breed. The observed heterozygosity (*Ho*) values for HNM, HAI, and KBK populations were 0.63, 0.50, and 0.63, respectively, while the expected heterozygosity (*He*) values were 0.50, 0.59, and 0.51, respectively. All populations were found to be in Hardy-Weinberg equilibrium. This study confirms that the *LLGL1* gene is promising to increase reproduction traits in three Anatolian goat populations due to conserving sufficient genetic variability. However, before proceeding with MAS studies, it is crucial to analyze the relationship between phenotypic data and the genotypes obtained for this gene region.

1. Introduction

Goats, one of the first domesticated species, have been an important farm animal for humankind throughout history (Karslı and Demir 2024). Particularly in underdeveloped and developing countries, they significantly contribute to employment and income levels in rural areas. Their higher adaptability to diverse environmental conditions such as climbing in mountainous and hilly terrains as well as a lower requirement for shelter and feeding costs are key advantages of goat farming (Ahlawat et al. 2015; Aslan et al. 2022; Demir 2024). These superior advantages have facilitated goat farming, not only in forests, but also in the mountainous and hilly lands of Türkiye including the Mediterranean region (Şirin et al. 2020).

Almost all goat breeding efforts in Türkiye are carried out with native breeds. According to official data of the Turkish Statistical Institution (TSI), the goat population in Türkiye is approximately 10.3 million (TSI 2023). The main native goat breeds in Türkiye include Hair (HAI), Honamlı (HNM), Norduz, Kilis, and Angora goats while HAI comprises 90% of the total population. HAI goats are bred throughout Türkiye, with varieties or eco-types such as Candir, Kabakulak (KBK), and Pavga, which have adapted to different geographical regions and climatic zones. It is believed that these varieties have differentiated from HAI goat in terms of certain morphological and yield characteristics. Among these, the KBK variety is reared in a region between the Muğla and Antalya provinces, and its population size is estimated to be around 30000 (Karsli et al. 2020; Aslan et al. 2022). HNM goats, which are known for their high body size and weight among domestic goat breeds, are mainly reared in Antalya, Isparta, and Burdur provinces in the Mediterranean region (Elmaz et al. 2016).

Reproductive traits are extremely important in small ruminant breeding because a moderate increase in litter size can lead to significant profits. Litter size is a key reproductive trait, especially for enterprises producing meat stock (Karslı et al. 2012; de Lima et al. 2020). In traditional selection methods aimed at increasing litter size in goats, genetic progress is slow due to the low heritability (0.05-0.10) (Ahlawat et al. 2015; de Lima et al. 2020). However, Marker-Assisted Selection (MAS) studies can accelerate genetic progress and improve selection success (Karslı and Balcıoğlu 2010; Ahlawat et al. 2015).

In recent years, many candidate genes (*BMPR1B, GDF9, BMP15, FSHβ, FSHR, POU1F1, PRLR, KiSS-1, GPR54, GH, INH, CART, CMTM2 GnRH, GnRHR, LHβ, BMP4, LLGL1, CSNS1, KDM6A, KITLG, MT2, CYP21, and AA-NAT*) have been reported to be associated with the litter size in goats which could be further integrated into MAS studies (Ahlawat et al. 2015; Wang et al. 2018; Kang et al. 2019; de Lima et al. 2020; Liu et al. 2021; Abuzahra et al. 2023). The Scribble Cell Polarity

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Complex Component (*LLGL1)* homolog protein interacts with the membrane protein syntaxin 4, which is uniquely localized in yeast, drosophila, and humans, to control Golgi vesicle trafficking and thereby regulate cell polarity. Besides the *LLGL1* protein is involved in multiple signal transduction pathways (Liu et al. 2021). Liu et al. (2021) reported that the 21 bp InDel polymorphism in the *LLGL1* gene was associated with reproduction in goats in which animals with the II genotype had a larger litter size. In this context, this study aims to explore the presence of genotype II, the preferred genotype for multiple births in terms of the LLGL1 gene, in HNM, HAI, and KBK goats, and to discuss the potential for its use in MAS studies.

2.Material and Methods

2.1. Ethic statement

This study was approved by the Local Ethics Committee of Animal Experiments of the Eskişehir Osmangazi University (Protocol No: HAYDEK-1025/2024).

2.2. Sampling strategy and DNA isolation

The blood samples used in this study were collected from various districts of Antalya between 2019 and 2021. HAI goats were sampled from four different farms in the Akseki, Manavgat, and Korkuteli districts, KBK goats were sampled from three farms in the Elmalı and Kaş district, and HNM goats were sampled from three farms in the Elmalı and Korkuteli districts. Genomic DNA was isolated from a total of 180 blood samples obtained from HAI (60 samples), HNM (60 samples), and KBK (60 samples) goats using the salting-out method described by Miller et al. (1988). The success of DNA isolation was verified using 1% agarose gel electrophoresis, and DNA quantity and quality were assessed using a spectrophotometer (Allsheng Nano-400A). A visual representation of the goats raised in Antalya is shown in Figure 1.

2.3. Determination of a 21-bp indel polymorphism within the LLGL1 gene

In this study, the PCR process was performed using the forward primer 5'-ATTCTTAGGCGCACCACGAG-3' and reverse primer 5'-CGAGGGGGTGCAACTTTGTT-3' as reported by Liu et al. (2021) to determine the 21-bp indel polymorphism within the LLGL1 gene. The PCR reaction mixture contained 4 µl of template DNA (50 ng μl^{-1}), 0.60 µl of each primer (10 pmol μl^{-1}), 8.0 µl of EcoTech 2X Master Mix, and 11.80 µl of ddH2O. The PCR program included an initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 45 s, annealing at 64°C for 45 s, and extension at 72°C for 60 s, with a final extension at 72°C for 10 min. PCR products were separated on a 4% agarose gel under 100 V and 150 min. After electrophoresis of the PCR products, the II genotype (163 bp) and DD genotype (142 bp) showed a single band, while the ID genotype (142 bp and 163 bp) showed two bands.

2.4. Statistical analyses

Allele frequencies, genotype frequencies, observed heterozygosity (*Ho*), expected heterozygosity (H_E), and Hardy-Weinberg equilibrium for the LLGL1 gene in the studied goat breeds were determined using the Popgene ver. 1.32 (Yeh et al. 1997) software package. Genetic distance values across populations were calculated by the Popgene vers.1.32 (Yeh et al. 1997) software while the MEGA 11 program (Tamura et al. 2021) was preferred to construct the UPGMA dendrogram for better visualization. Minitab software was used to compare genotype frequencies within populations (two dependent proportions) and between populations (two independent proportions) using Z-tests.

3. Results and Discussion

Upon completion of the PCR process, it was found that all studied goat breeds/varieties were polymorphic in terms of 21bp indel variants in the *LLGL1* gene. The genotypes obtained for 10 samples of the HNM breed are shown in Figure 2 while the frequencies of gene and genotype together with genetic diversity parameters across all populations are summarized in Table 1.

For the 21 bp indel variant in the *LLGL1* gene, all goat populations studied were found to be polymorphic, and three genotypes (II, ID, and DD) were detected in all populations. The frequency of the I allele ranged from 0.57 (HNM) to 0.72 (HAI), while the frequency of the D allele was 0.43, 0.28, and 0.42 in the HNM, HAI, and KBK populations, respectively. The frequency of the II genotype ranged from 0.38 (HNM) to 0.47 (HAI), the frequency of the ID genotype ranged from 0.37 (HAI and KBK) to 0.50, and the frequency of the DD genotype ranged from 0.03 (HAI) to 0.25 (HNM). The observed heterozygosity values (0.63) were higher than the expected heterozygosity values (0.51 and 0.50, respectively) in the HNM and KBK populations while the H_E value (0.59) was higher than the H_O value (0.50) in the HAI population. The number of effective alleles was calculated as 1.96, 1.68, and 1.94 for the HNM, HAI, and KBK goats, respectively, and all populations were found to be in Hardy-Weinberg equilibrium.

According to the UPGMA dendrogram constructed based on genetic distance values between populations for the 21 bp indel variant in the LLGL1 gene, the KBK and HNM breeds were clustered together while the HAI breed formed a separate branch (Figure 3).

Figure 1. An image of representative animals from A) HNM, B) HAI, C-D) KBK goats.

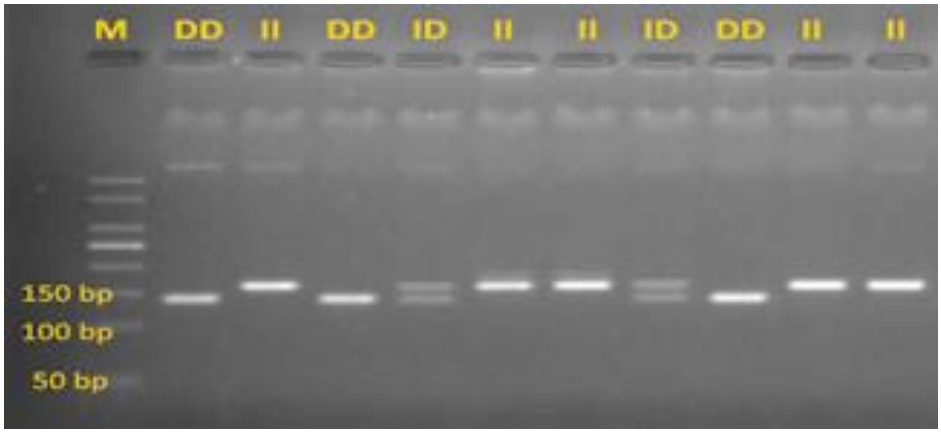

Figure 2. An agarose gel image for the detection of indel variants in the *LLGL1* gene of the HNM goat breed. M: DNA Ladder (50 bp- Thermo 100 bp; Cat.No: SM02371); II genotype (163 bp); DD genotype (142 bp); ID genotype (142 bp and 163 bp).

Table 1. Gene, genotype frequencies, and genetic diversity parameters for *LLGL1 gene*

Breed	n	Allele frequencies		Genotype frequencies			Genetic diversity parameters			HWE
		I	D	II	ID	DD	H_O	H_E	Ne	χ^2
HNM	60	0.57	0.43	0.38 (23)^{Aa}	0.37 (22)^{Aa}	0.25 (15)^{ABb}	0.63	0.50	1.96	3.83^{d}
HAI	60	0.72	0.28	0.47 (28)^{Aa}	0.50 (30)^{Aa}	0.03 (2)^{Bb}	0.50	0.59	1.68	3.27^{d}
KBK	60	0.58	0.42	0.40 (24)^{Aa}	0.37 (22)^{Aa}	0.23 (14)^{ABb}	0.63	0.51	1.94	3.62^{d}

H_O: Observed heterozygosity; H_E: Expected heterozygosity, Ne: Number of effective alleles; HWE: Hardy-Weinberg Equilibrium. Comparison of genotype rates between and within populations are given as lower- and upper-case letters, respectively (P<0.05), $\chi^2_{0.05;1}$: 3.84; d: The deviation from HWE was not statistically significant.

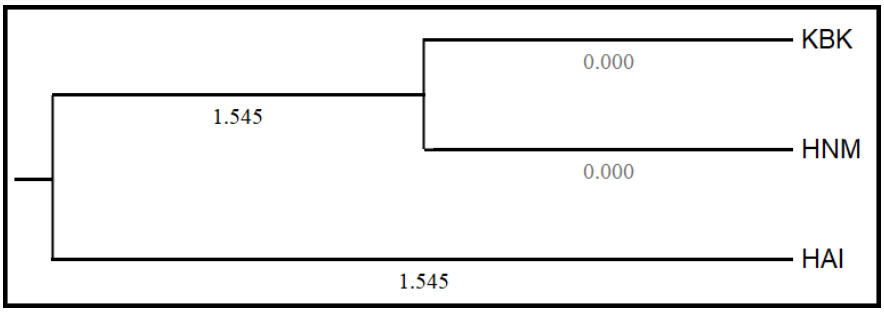

Figure 3. A genetic distance-based UPGMA dendrogram based on indel variation in the *LLGL1* genes.

Liu et al. (2021) investigated the relationship between the 21 bp indel variant in the ***LLGL1*** gene and litter size in Shaanbei White Cashmere Goats (SBWC), identifying two genotypes II and ID. The frequencies of these genotypes in SBWC goats were reported as 0.959 for II and 0.041 for ID. The researchers found that goats carrying the II genotype had a higher litter size. In contrast to Liu et al. (2021), this study identified all three genotypes (II, ID, and DD) in this study. The frequencies of the II genotype, which is favorable for litter size, ranged from 0.38 to 0.47 in three Turkish goats, which are lower than those reported for SBWC goats. This difference probably occurred due to sampling strategy, as samples were randomly selected from herds without multiple birth records. Another possible reason is that the multiple birth rates are generally lower in Turkish native goat breeds.

While numerous studies have investigated polymorphisms in major or candidate genes associated with multiple births in Turkish native sheep breeds (Karslı et al. 2011; Gedik 2021; Çelikeloğlu et al. 2021; Atay et al. 2023; Kirikçi 2023), research on goat breeds is still scarce (Demir et al. 2020; Karslı and Demir 2024). No studies on the *LLGL1* gene linked to multiple births in Turkish native goat breeds have been found in the literature. However, Karslı and Demir (2024) who investigated variations in the *CMTM2* and *CSN1S1* genes which are also related to litter size,reported that the frequency of the desired II genotype in these gene regions varied between 0.09 and 0.29 in HNM, KBK, and HAI goats. For the 21 bp indel polymorphism in the *LLGL1* gene, the frequency of the desired II genotype for litter size (ranging between 0.38 and 0.47) was higher than that observed in the *CMTM2* and *CSN1S1* genes. Although these gene regions differ, the higher frequency of the II genotype, which is favorable for multiple births in the *LLGL1* gene, suggests that it could be prioritized in MAS studies.

In HNM, KBK, and HAI goats, there was no statistical difference in the frequencies of the II genotype, which is desirable for multiple births in the *LLGL1* gene. In HAI goats, which had the highest II genotype frequency, the H_O value (0.50) was lower than the H_E value (0.59), unlike in the other breeds. Although the H_O values obtained in the studied breeds were lower than those reported by Liu et al. (2021) for SBWC goats, the effective allele numbers (Ne) were higher. Additionally, all populations studied were found to be in Hardy-Weinberg equilibrium. Overall, when considering the genetic diversity parameters and Hardy-Weinberg equilibrium values from the

current study, it can be concluded that the populations have sufficient genetic diversity, with low levels of inbreeding due to excess heterozygosity. This is a promising result for the sustainability of these populations.

The KBK goats used in this study are considered a variety of HAI goats. However, the UPGMA dendrogram, based on genetic distance values calculated from the *LLGL1* gene variations, showed that KBK goats were genetically closer to HNM goats than HAI goats. Similar results were observed in other studies using microsatellite markers (Karsli et al. 2020) and PCR (Karslı and Demir 2024). To determine whether KBK goats are truly a variety of HAI goats, it would be useful to conduct further studies using molecular methods, such as Next Generation Sequencing or SNP chips, on a whole genome basis.

4. Conclusion

In this study, the 21 bp indel variation in the *LLGL1* gene was investigated for the first time in HNM, KBK, and HAI goats. The II genotype, which results from the 21 bp indel in the *LLGL1* gene and has been associated with multiple births, was observed at varying frequencies in Turkish native goat breeds. The high frequencies of the II genotype across the three populations, along with the genetic diversity parameters, suggest that this gene region could be useful in MAS studies. However, before MAS studies are initiated, it is essential to analyze the relationship between phenotypic data and the genotypes obtained for this gene region. Additionally, it should be noted that, as with other farm animals, multiple births in goats are a quantitative trait that follows polygenic inheritance. Therefore, working with as many genes as possible, rather than focusing on a single gene, will likely increase the success of breeding programs aimed at enhancing litter size.

Authors' contributions

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

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