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Polymorphism of the *callipyge* gene in an Albanian sheep breed and association with body weight

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Abstract

The *callipyge* (*CLPG*) gene is a candidate gene associated with meat production in sheep. The purpose of the study was to identify the association of polymorphisms in the 3'UTR of the *CLPG* (MEG3) gene in Albanian sheep breed with morphometric traits and body weight. A total of 87 individuals were studied regarding the morphometric traits and the polymorphisms of 3'UTR of the *CLPG* (MEG3) gene. The frequency of the mutated allele (T) was 0.339. The less frequent genotype was TT. The population was in Hardy Weinberg equilibrium. The fixation index was 10.2% indicating a moderate level of inbreeding. The results indicated no significant effect of this locus on morphometric traits and body weight. The preliminary data provided in this study suggest that the nucleotide change in the 3′UTR can be used in marker-assisted selection to increase the body weight of animals.

Keywords: Least square means, body weight, polymorphism, allelic frequencies

1. Introduction

Traditional breeding methods are widely used to improve some economically important traits. The new molecular tools offer a great possibility for the identification of preferred alleles. The spectrum of DNA markers associated with qualitative traits such as reproductive, meat, and fattening traits is increasing (Gorlov *et al.*, 2021). Marker assisted selection improves the accuracy and progress of selection in animal programs (Sutikno *et al.*, 2011). Utilization of candidate genes for selection of quantity and quality traits such as double muscle and meat tenderness is one of the primary methods that can be used by breeders to improve economic traits in sheep (Gábor *et al.*, 2009). It is very important to search and study the DNA markers associated with the productive qualities of sheep for greater breeding efficiency (Hu *et al.*, 2019).(Gorlov et al. (2020) advise the use of data on callipyge (CLPG) genotypes frequencies in selected sheep breeds to optimize and monitor the sheep breeding processes.

The CLPG gene is documented for double muscle in sheep and is a candidate gene associated with meat production (Dimitrova and Bozhilova, 2016). The CLPG gene is located in the telomeric region on ovine chromosome 18 and is inherited in a non-Mendelian pattern called polar overdominance (Cockett *et al.*, 1994). It is known that callipyge mutation causes muscle hypertrophy in pelvic limbs and loin (Cockett *et al.*, 2005). The hypertrophy condition was more pronounced in the muscles of the hind leg and loin regions (Freking et al., 2004). The CLPG is also known as one of the major genes related to growth performance and meat quality traits (Othman *et al.*, 2016). The CLPG gene is the most documented gene for double muscle in sheep (Gábor *et al.*, 2009). In the present study we intend to identify the polymorphism in the 3'UTR CLPG (MEG3) gene of Albanian sheep.

Sheep farming is very important in Albania and of great interest for the local community. Recently there has been an increased demand for lamb meat consuming. Farmers are interested not only in the quantity but also in the quality of the meat. Albania, located in Southeast Europe, is home to several indigenous sheep breeds that have adapted to the country's diverse geographic and climatic conditions. Albanian sheep breeds are known for their ability to adapt to challenging environments, resistance to diseases, and efficient utilization of natural resources (Hoda *et al.*, 2010). They have been bred over generations to thrive in the local conditions and play an important role in the livelihoods and agricultural practices of Albanian farmers. A more detailed information for the characteristics of each breed in Albania can be found elsewhere (Hysi *et al.*, 2023). In a previous study 36 SNP markers (Hoda *et al.*, 2011) are used, to estimate the genetic diversity and distances between three local sheep breeds. The current study aims to contribute to the understanding of the genetic factors influencing body measurements and body weight in Albanian sheep, which can ultimately help improve sheep breeding programs and meet the demands of the local market. Body weight is very important especially when meat production is the main objective (Cekic *et al.*, 2021). In this study, we aimed to determine the impact of the 3'UTR of CLPG (MEG3) gene polymorphism on body measurements and body weight from an Albanian local sheep breed.

The Callipyge phenotype in sheep is caused by an autosomal dominant mutation that affects muscle development, resulting in muscle hypertrophy primarily in the hindquarters. The mutation disrupts the normal gene regulation within the DLK1-DIO3 gene cluster. This cluster includes several imprinted genes, including DLK1, GTL2, and MEG3, among others.

MEG3 (Maternally Expressed Gene 3) is an imprinted long non-coding RNA (lncRNA) located within the DLK1-DIO3 gene cluster. While MEG3 is part of the same gene cluster associated with the Callipyge phenotype, it is not directly responsible for causing the phenotype itself. The MEG3 gene is part of the DLK1-DIO3 gene cluster, which is associated with the Callipyge phenotype in sheep.

2. Materials and methods

Blood samples were collected from 87 unrelated individuals of the Albanian sheep breed. Individuals were selected from flocks that were under the care of the Center for Agricultural Technology Transfer in the Korca region. The salt out extraction method was used to extract DNA from blood samples and to determine polymorphisms of 3'UTR in CLPG (MEG3) gene as described by (Hoda *et al.*, 2011). Estimates of genetic diversity indices, genotypes and allele frequencies, mean expected and observed heterozygosities, and Hardy Weinberg equilibrium were performed using Genalex (Peakall and Smouse, 2012). The chi-square test ($\chi 2$) was used to determine the population in Hardy-Weinberg equilibrium. Eight morphometric traits were measured from these individuals: wither height (WH), rump height (RH), body length (BL), chest depth (CD), chest width (CW), rump width (RW), chest circumference (CC) and body weight (BW). The general linear model (GLM) procedure was used for the assessment of the genotype effect on the morphometric traits. The association of CLPG (MEG3) genotypes with body weight and morphometric traits was analysed with the following statistical model using the XLSTAT statistical package (Xlstat, n.d.). The p-values between 0.05 and 0.10 are accepted as trends in this study.

Yij= u + αi + €ij

Description:

Yij = mean value of body weight

u = general mean

αi = effect of genotypes CLPG gene

€ = random error

The normality of the trait distribution was analyzed by the Shapiro Wilk test. The Kruskal-Wallis test was used to determine the effect of the genotype on the sheep body traits.

3. Results

A SNP located at the 3'UTR of the CLPG (MEG3) gene (AY017222 g379G>T) was analysed for the polymorphisms. Table 1 shows the genotype and allele frequencies in the Albanian sheep breed as well as other genetic parameters. Three genotypes were identified GG, GT and TT. The frequency of the mutated allele was lower than that of the wild type. The most frequent were wild homozygous GG. The heterozygotes constituted 40.3% of the population and the lowest frequency was observed for TT homozygotes, 13.8%. The fixation index was 10.2%, indicating a moderate level of inbreeding. This level of inbreeding can explain the moderate level of observed heterozygosity. The observed heterogeneity is lower than the expected heterogeneity. The expected and observed genotypes of CLPG (3'UTR MEG3) were found in Hardy Weinberg equilibrium. The effective number of alleles (Ne) and Shannon's information index are 1.81 and 0.64, respectively.

Table 1. Genetic parameters in the Albanian sheep breed

Genotype	Frequencies	Alleles	Frequencies	Ho	He	Ne	I	F
TT	0.138	T	0.339	0.402	0.448	1.81	0.640	0.102
GT	0.403	G	0.661					
GG	0.460							

Na = Number of Different Alleles; Ne = Number of Effective Alleles; I = Shannon's Information Index; Ho = Observed Heterozygosity; He = Expected Heterozygosity; F = Fixation Index

Least squares means and standard deviations as well as the minimum and maximum values for the body measurements and body weight are shown in Table 2.

Table 2. Summary statistics for body measurements and body weight data

Variable	Minimum	Maximum	Mean	Standard deviation	Variation coefficient	Standard error of the mean
Wither height	58.000	79.000	68.517	5.237	0.076	0.565
Rump Height	62.000	89.000	68.782	4.532	0.066	0.489
Body length	70.000	124.000	87.644	16.675	0.190	1.798
Chest depth	26.000	42.000	32.874	2.916	0.089	0.314
Chest width	15.000	24.000	20.023	2.514	0.126	0.271
Rump width	16.000	30.000	22.391	2.714	0.121	0.293
Chest circumference	70.000	99.000	84.391	5.559	0.066	0.599
Cannon bone circumference	6.000	9.500	7.894	0.842	0.107	0.091
Body Weight	26.000	63.000	49.343	8.516	0.173	0.918

The polymorphism occurring at this site of the CLPG gene does not have an impact on morphometric traits (Table 3). The genotype groups were not significantly different, but there were numerical differences. The individuals with the TT genotype have a superior performance in most of morphometric traits. Based on the p values for this table, we can only conclude that the GG genotype has a tendency towards greater chest depth.

Table 3. Relationship between the CLPG polymorphism and body measurement and body weight in Albanian sheep breed.

Morphometric traits	**Symbol**	**Genotypes**			**P Values**
		GG	TG	TT	
Wither height	WH	67.675±0.833	69.086±0.891	69.667±1.521	0.372
Rump Height	RH	68±0.72	69.372±0.77	69.667±1.315	0.334
Body length	BL	89.675±2.639	87.82.822	80.4174.818	0.247
Chest depth	CG	33.475±0.456	32.6860.488	31.4170.833	0.09
Chest width	CW	19.525±0.398	20.3430.425	20.750.726	0.214
Rump width	RW	22.5±0.437	22.2580.467	22.4170.797	0.930
Chest circumference	CC	84.2±0.886	85.1720.947	82.751.617	0.419
Cannon bone circumference	CBC	7.953±0.135	7.7660.144	8.0760.246	0.467
Body Weight	BW	48.255±1.357	50.81.451	48.7172.477	0.428

4. Discussion

The application of DNA technology is becoming a tremendous challenge for the farm breeding of domestic animals (Caro Petrović *et al.*, 2021). The traditional methods of selection and genotype selection can increase the efficacy of improving both the livestock of an individual farm and the breed as a whole (Gorlov et al., 2021). Morphometric traits and growth intensity were also analyzed previously for different local sheep breeds reared in a small area of Albania (Hoda *et al.*, 2022; Hysi *et al.*, 2023). DNA analysis has great practical application in farm management (Caro Petrović *et al.*, 2021). Selection based on genetic markers for productivity traits such as weight gain and meat quality consist of working with animals with high genetic potential (Bozhilova *et al.*, 2020). The existence of major genes associated with productive performance can accelerate the rate of genetic improvement in meat quality and quantity through marker-assisted selection (Mahrous *et al.*, 2016). The CLPG gene was studied as a potential candidate gene for the meat production.

In this study, we aimed to determine tpolymorphisms in the 3'UTR of the CLPG (MEG3) gene in an Albanian sheep breed. The population was in Hardy Weinberg equilibrium. The level of inbreeding was 10.2%, which can be explained by the small effective population size and flock management by the farmer where one or two rams are used for breeding (Hoda *et al.*, 2009a, b). The frequency of heterozygous individuals was 0.403, indicating a moderate genetic variability within the CLPG (MEG3) gene. This can be explained by the fact that animals originated from the flocks of Center for Agricultural Technology Transfer with rams rotating every two or three years.

Also, we aimed to associate the relationship of the detected polymorphism with body measurements and body weight. These preliminary results indicated no significant effect of this locus on morphometric traits. Analysis of least-square means of body weight showed that this variable presented no significant differences among the three different genotypes. Additionally, the Kruskal-Wallis test showed no significant effect of genotype on morphometric traits. The TT was the least common genotype (0.138), whereas the frequencies of GT and GG were similar (0.403 and 0.460 respectively). Animal carriers of the TT genotype had higher values of WH, RH, CW, and CBC, while individuals with the GG genotype had higher values of BL, CG and RW. The heterozygous genotype GT has superior performance for body weight. The findings suggest that this mutation of the 3' UTR CLPG (MEG3) gene can be used in breeding programs and selection of heterozygous individuals due to the increased performance in body weight.

The research performed by (Jawasreh *et al.*, 2019) suggests that introducing the callipyge mutation in Awassi lambs allows for improvements in growth and meat characteristics without negatively affecting the desirable trait of fat tail weight, providing potential implications for enhancing meat production in local flocks of Awassi sheep, ultimately benefiting farmers in terms of improved meat quality and profitability. The Callipyge (CLPG) mutation was analysed in three Pakistani sheep breeds: Thalli, Kajli, and Lohi (Shah *et al.*, 2018) and the researchers concluded that the CLPG Thalli breed could serve as a valuable source of mutton. The results of the study performed by (Yu et al., 2018) provide additional evidence supporting the role of DLK1 as the primary effector gene in the development of muscle hypertrophy in Callipyge sheep. The study also highlights the involvement of DNTTIP1 and PDE4D as secondary effectors that respond to DLK1 signaling, contributing to the observed muscle fiber switching and hypertrophy phenotype. Esen *et al.* (2022) found that all meat-type sheep breeds, except Ramlıç, were polymorphic for the CLPG gene. Therefore, incorporating heterozygous individuals that inherited the CLPG mutation from the sire in selection programs could be beneficial for improving carcass traits. The study of (Kader-Esen et al., 2022) suggests that the CLPG gene has implications for body indexes and morphometric characterization in meat-type sheep breeds. The findings support the feasibility of incorporating these body indexes, BCI and IBF, into breeding schemes, especially when utilizing marker-assisted selection approaches. Further research and validation are necessary to fully understand the genetic and phenotypic relationships associated with the CLPG gene and its practical implications in genomic selection programs.

Studies on the CLPG gene in Albanian local sheep breeds do not exist. This research is the first effort to find candidate genes for the selection of performance traits. These preliminary results help to estimate the genetic structure of Albanian sheep at the molecular level. The study must be extended to other populations to better explain the relationship between the polymorphism of the 3'UTR CLPG (MEG3) gene and the morphological traits. The results can be used for future selection programs for local sheep.

Conclusions

The molecular analysis of 3'UTR of the CLPG (MEG3) gene of the Albanian local sheep breed showed polymorphism and a trend of association with body weight and body measurements. Two alleles were detected and the G allele was predominant over the T allele. The most common genotype was GG followed by GT. This work needs to be extended with more animals as well as with other sheep breeds to confirm the findings. The CLPG (MEG3) gene can be used for marker-assisted selection in future breeding programs for sheep breeding.

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Conflict of interests

The authors have no conflict of interest to declare.

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