IJAS Iranian Journal of Applied Animal Science

Single Nucleotide Polymorphisms of *Mc4R* Gene and Their Associations with Growth Traits in Karacabey Merino Lambs

Research Article

S. Sevim[1*] and O. Karaca[2]

[1] Ministry of Agriculture and Forestry Aydın Provincial Directorate, Aydın, Türkiye
[2] Department of Animal Science, Faculty of Agriculture, University of Adnan Menderes, Aydın, Türkiye

Received on: 13 Jul 2023
Revised on: 16 Oct 2023
Accepted on: 6 Nov 2023
Online Published on: Dec 2023

*Correspondence E-mail: semih_sevim@yahoo.com

Online version is available on: www.ijas.ir

ABSTRACT

Mc4R gene regulates feed intake, live weight, and energy balance in animals. It is one of growth and development candidate genes, as indicated by its role in energy balance and feed intake. The goal of the research was to analyze the polymorphisms of the *Mc4R* gene and their relations with growth characteristics in Karacabey Merino lambs. 300 Karacabey Merino lambs reared at the Bandırma Sheep Breeding Research Institute in Balıkesir, Turkiye, served as the research's animal subjects. The total of 1291 bp long exon and 3'UTR regions of Mc4R gene was amplified. PCR products were analysed by DNA sequencing. A total of 6 SNP of Mc4R (g.9.T>C, g.12.G>C, g.93.G>A, g.381. G>A, g.681.G>C and g.1016. G>A) gene were identified. These SNP's have not been inducing changes sequence of amino acids. The single nucleotide polymorphisms (SNPs) of *Mc4R* gene were found significant associations between muscle depth, skin thickness, back fat thickness, average daily gain and live weight. The *Mc4R* gene was shown to be a potential genetic marker in selection programs on sheep to improve growth traits of lambs.

KEY WORDS DNA sequencing, growth traits, Karacabey Merino, Mc4R.

INTRODUCTION

Sheep breeding played an important role in humanity's transition to settled life. In Turkish society, sheep were crucial for food, shelter and even the economics. Türkiye is the largest sheep producer in Europe, with 89 million sheep heads. While 91 % of Türkiye's sheep population is composed of indigenous breeeds, the remaining is composed of cross breeds (TUIK, 2023). Sheep breeding has a substantial cultural relevance due to its proximity to the regions where it was domesticated. Since the world textile industry generally prefers Merino wool, it has been considered a necessary step to increase the number and breeding of the Merino breed in order to supply the quality fine fleece needed by the textile industry in Turkey and to develop the weaving industry (Polatoğlu, 2019). In 1933, the Ministry of Agriculture, Forestry, and Rural Affairs crossed the German fleece/meat merino in Karacabey agricultural enterprises with Kıvırcık sheep. As a result of these backcrossing studies, the Karacabey Merino-type was developed. They have a Merino genotype of approximately 90–95% (Atav *et al*. 2023). Although the main purpose of development was quality fleece production, changing demand in recent years and the meat type characteristics of the breed have led to its use as a sire line in slaughter lamb production. It has been shown that Karacabey Merino rams can be used for the production of lean lamb meat in commercial crossbred sheep breeding. For lamb production, rapid development of the breed and carcass quality good properties are advantageous (Dönmez, 2008). In recent years, technological advances in molecular genetics tools and analysis have permitted the investigation of genes ef-

fective on quantitative characteristics, particularly in farm animals, at the molecular level (Akyüz *et al*. 2017). As a result of advances in molecular technology, it has become easier to identify genetic variations and to determine the relationship between genes and growth traits. Marker Assisted Selection (MAS) aims to utilise the genetic information in markers and select improved populations for important traits. Marker-assisted selection (MAS) has shown great effectiveness in genetically improving quantitative traits with significant economic returns, such as growth traits of sheep (Bayraktar and Shoshin, 2021; Cheng *et al*. 2022; Zhai *et al*. 2022; Li *et al*. 2023). For selection on yield traits to be successful, traits must be highly heritable. In sheep, one of the most substantial economical traits is growth rate, which is under moderate genetic control. As a result, they respond directly to selection (Safari *et al*. 2005). The *Mc4R* gene is found on sheep chromosome 23. This gene, which comprises a single 999 base coding sequence, encodes a protein of 332 amino acids (Zuo *et al*. 2014). Mc4R gene regulates feed intake, live weight, and energy balance in animals (Cone, 2005). It is one of growth and development candidate genes, as indicated by its role in energy balance and feed intake (Zuo *et al*. 2014). In cattle, there are many studies reported that the gene Mc4R is associated with marbleization score, carcass characteristics, back fat thickness and body weight (Liu *et al*. 2010; Seong *et al*. 2012; Lee *et al*. 2013; Cai *et al*. 2015; Feng *et al*. 2017; Prihandini and Maharani, 2019). Latifah *et al*. (2018) reported that *Mc4R* gene polymorphism was associated with average daily gain, chest circumference and body length in goats. In addition, a substantial relationship was found between *Mc4R* gene, milk yield and percentage of fat in buffaloes (Deng *et al*. 2016). Previous studies in sheep found that the g.1538. A>G, g.1477. G>A, g.1453. T>C, g.1267. G>A, g.1229. G>A, and g.706. C>A have significant correlation with meat quality, body size, and body weight traits (Song *et al*. 2012; Zuo *et al*. 2014; Wang *et al*. 2015). The purpose of this research was to demonstrate the polymorphisms for the gene *Mc4R* by DNA sequencing and to find associations between SNP-genotypes and growth traits of Karacabey Merino lambs.

MATERIALS AND METHODS

Animal material

Three hundred lambs (150 male and 150 female lambs) with breeding value for growth performance were selected from approximately 1200 head of lambs that were reared in Sheep Breeding Research Institute, Bandirma, Balıkesir, Türkiye. The animal care and handling procedures were reviewed and approved by the Ethical Committee of Adnan Menderes University (Approval number: 64583101/2016/183).

Live weights and ultrasound measurements

A digital scale with a sensitivity of 50 g was also used to assess the lambs' live weights at the birth, and after 45, 90, and 180 × days. The lambs average daily live weight gains were calculated using measurement age and weight data. Measurements of MLD were taken between 12 and 13 ribs using an ultrasound device (Mindray DP-20vet) equipped with a linear probe. Muscle depth (MD), skin thickness (S), back fat thickness (BFT) at the age of 90^{th} and 180^{th} days were measured as MLD characteristics.

Blood collection and DNA isolation

A total 300 blood samples were taken in vacuum tubes with K3-EDTA as an anticoagulant. A commercial kit (Exgene TM Blood SV micro kit, GeneAll® Biotechnology) was used to isolate the DNA. DNA isolation procedure was performed according to the manufacturer's recommended technique.

PCR and DNA sequencing analysis

Primers were designed using Mc4R gene sequence information. The 1291 base pair regions were amplified by PCR with the two sets of primer pairs used. The sequence of designed primers was:

Mc4RF1: TTTCCAAGTGATGCCGACCA;
Mc4RR1: TGAGAGCCAGCATGGTGAAG;
Mc4RF2: GCGATCACCATCAGTGCCATGT;
Mc4RR2: AGCTGTGGCTCATACAGACTGTTCA.

1U of Taq DNA polymerase, 1.5 mM × $MgCl_2$, 250 µM × dNTP mix, 1 × PCR buffer, 2.5 µM of each primers, and 100 ng genomic DNA were all included in the PCR volume of 25 µL. A preliminary denaturation stage at 95 ˚C for 5min, 35 cycles of a denaturation step at 95 ˚C for 40s, an annealing step at 62 ˚C for 45s, an elongation step at 72 ˚C for 45 s, and a final extension step at 72 ˚C for 10min were carried out in accordance with the PCR cycling protocol. PCR products were treated with Exo-Sap during the initial phase of purification. BigDyeTM Terminatoriv 3.1 (Applied Biosystems, USA) was utilized for cycle sequencing. Samples were put via capillary electrophoresis on an ABI3500 sequencer platform after a second purification step that involved ethanol precipitation in order to get sequencing data.

Statistical analysis

The results of the obtained sequence analysis were first opened with the FINCH TV (http://www.geospiza.com/) program, the obtained peaks were checked one by one and the base changes in the peaks were noted. With the help of the MEGA 7.0 (http://www.megasoftware.net/) program,

all samples were aligned and compared by direct observation with the reference sequence obtained from NCBI (ncbi.nlm.nih.gov NM_001126370). PopGene software was used to compute the Hardy-Weinberg equilibrium of the populations (Yeh *et al*. 1999). General Linear Model option of SPSS 20.0 (SPSS, 2011) statistical software was used to analyse correlations between SNPs genotypes and growth traits of lambs. The general linear model is expressed as follows:

Where:

$Y_{ijk} = \mu + \alpha_i + b_j + c_k + e_{ijk}$

Y_{ijk}: traits measured.
μ: overall mean for each trait.
α_i: sex effect.
b_j: genotypes effect.
c_k: dam age effect.
e_{ijk}: random error.

RESULTS AND DISCUSSION

The gene *Mc4R* of sheep, which is found on chromosome × 23, encodes two exons and 332 amino acids. In the region of 1291 bp studied (Figure 1), a total of 6 SNP variants were detected, 5 of which were in the exon of 999 bp and 1 of which was in 3'UTR.

Figure 1 PCR product of MC4R gene

The detected SNPs were g.9. T>C, g.12. G>C, g.93. G>A, g.381. G>A, g.681. G>C and g.1016. G>A, respectively (Figure 2). The amino acids substation of the SNPs detected in the study's exon region were found to be unchanged. It was determined by χ 2 test results that 6 SNPs discovered in Mc4R reached Hardy Weinberg equilibrium.

Polymorphism information content (PIC) is employed to analyze the genetic wealth of a genetic marker. The following are the PIC values: PIC bigger than 0.5 remarks significant polymorphism, 0.25 < PIC < 0.5, remarks moderate polymorphism, and PIC values less than 0.25, remarks low polymorphism (Botstein *et al*. 1980; Shan *et al*. 2020).

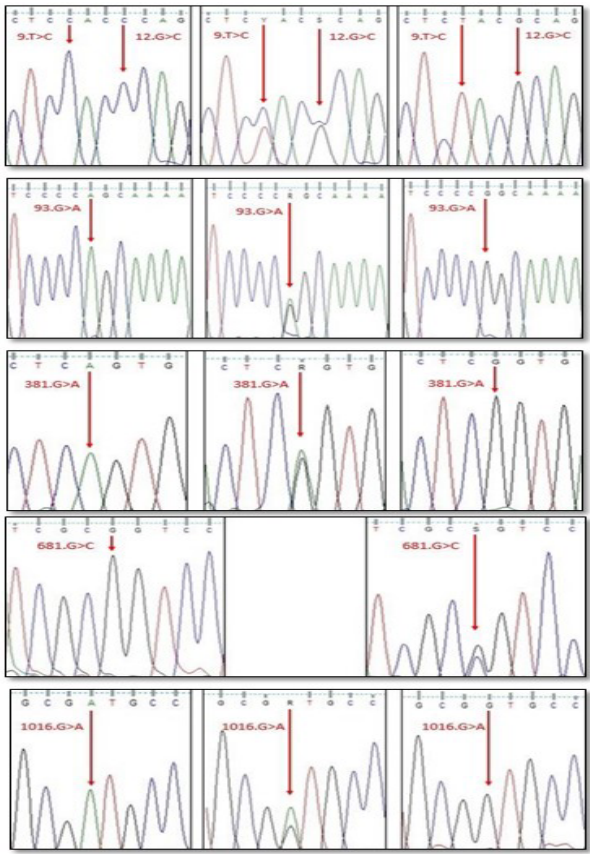

Figure 2 The electropherograms of Mc4R snp's

PIC values less than 0.25 were found at four loci in this study: g.93 G>A, g.381 G>A, g.681 G>C, and g.1016 G>A. PIC values for the other two SNPs (g.9 T>C and g.12 G>C) ranged from 0.25 to 0.5. Those 4 loci referred as moderately polymorphic and compared to the rest, those loci offer greater promise (Table1).

Least square means for growth traits and ultrasonic measurements according to SNP genotypes were given Table 2 and 3. The discovered SNPs had no effect on birth weight or 45th day live weight in Karacabey Merino lambs. The 90th day live weight and 0-90 ADG of g.12 G>C homozygous (CC) was considerably greater than mutant animals that are both homozygous (CG) and heterozygous (GG) ($P<0.05$). Live weight and the SNP g.1016G>C discovered in the 3'UTR were both substantially correlated at 180th day, ADG at 0-90th and 0-180th days ($P<0.05$). An extremely important association was found between g.1016 G > C SNP and average daily live weight gain after weaning ($P<0.01$). Furthermore, the SNPs of g.381. G>A and g.681.G>C were highly associated with 90th day back fat thickness ($P<0.01$), moreover the SNP of g.1016G>C was associated with 90th day ultrasonic skin thickness and 180th day ultrasonic muscle depth ($P<0.05$), respectively.

Table 1 Genotype and allele frequencies with chi-square analysis results for single nucleotide polymorphisms (SNPs) determined in *Mc4R* gene

Locus	Allele Freq.		Genotype Freq.				Heterozygosity		
g.9.T>C	T	C	TT	TC	CC	χ^2	Ho	He	PIC
	0.183	0.817	0.043	0.280	0.676	1.265^{ns}	0.280	0.299	0.254
g.12. G>C	G	C	GG	GC	CC	χ^2			
	0.183	0.817	0.046	0.273	0.680	2.281^{ns}	0.273	0.299	0.254
g.93.G>A	G	A	AA	AG	GG	χ^2			
	0.822	0.178	0.670	0.303	0.027	0.369^{ns}	0.303	0.293	0.249
g.381.G>A	A	G	AA	AG	GG	χ^2			
	0.042	0.958	0.920	0.076	0.003	0.480^{ns}	0.077	0.080	0.077
g.681. G>C	G	C	GG	GC	CC	χ^2			
	0.958	0.042	0.917	0.083	0.000	0.567^{ns}	0.083	0.080	0.077
g.1016.G>A	A	G	AA	AG	GG	χ^2			
	0.177	0.823	0.670	0.303	0.027	0.880^{ns}	0.307	0.291	0.248

PIC: polymorphism information content.

Table 2 Least squares mean and standard errors of growth characteristics of Karacabey Merino lambs in terms of single nucleotide polymorphism (SNP) genotypes

Locus	Allel	Birth weight	45th day live weight (kg)	90th day live weight (kg)	0-90th day ADG (kg)	180th day live weight (kg)	0-180th day ADG (kg)	90-180th day ADG (kg)
g.9.T>C	CC (203)	4.81±0.266	22.23±1.561	33.66±1.721	0.306±0.018	52.42±2.434	0.256±0.013	0.203±0.023
	TC (84)	4.51±0.269	23.58±1.585	35.16±1.747	0.321±0.019	52.72±2.471	0.258±0.013	0.191±0.023
	TT (13)	4.29±0.300	20.73±1.773	31.73±1.953	0.285±0.021	47.78±2.763	0.231±0.015	0.180±0.026
	P-value	P=0.100	P=0.171	P=0.131	P=0.138	P=0.138	P=0.144	P=0.561
g.12.G>C	CC (204)	4.38±0.253	21.45±1.495	35.09±1.650	0.322±0.018	52.09±2.334	0.255±0.013	0.178±0.022
	GC (82)	4.66±0.310	21.53±1.822	31.06±2.007	0.277±0.022	48.33±2.839	0.234±0.015	0.194±0.027
	GG (14)	4.57±0.319	23.56±1.877	34.40±2.068	0.312±0.022	52.50±2.925	0.256±0.016	0.202±0.028
	P-value	P=0.644	P=0.392	P=0.050	P=0.047	P=0.163	P=0.153	P=0.705
g.93.G>A	GG (201)	4.36±0.353	23.06±2.089	32.91±2.301	0.297±0.025	50.46±3.253	0.246±0.018	0.212±0.031
	AG (91)	4.44±0.257	20.06±1.519	33.70±1.675	0.306±0.018	50.26±2.370	0.244±0.013	0.175±0.022
	AA (8)	4.80±0.359	23.42±2.114	33.93±2.328	0.309±0.025	52.20±3.294	0.255±0.018	0.188±0.031
	P-value	P=0.633	P=0.065	P=0.886	P=0.874	P=0.876	P=0.867	P=0.240
g.381. G>A	GG (276)	4.55±0.172	22.68±1.014	34.31±1.117	0.315±0.012	53.72±1.580	0.264±0.009	0.214±0.015
	AG (23)	4.81±0.205	20.92±1.208	36.21±1.333	0.337±0.014	50.18±1.884	0.246±0.010	0.161±0.018
	AA (1)	4.25±0.661	22.94±3.889	30.03±4.287	0.261±0.046	49.02±6.065	0.235±0.033	0.199±0.057
	P-value	P=0.655	P=0.638	P=0.401	P=0.306	P=0.315	P=0.321	P=0.143
g.681. G>C	GG (275)	4.69±0.242	20.58±1.425	34.28±1.570	0.313±0.017	49.66±2.222	0.242±0.012	0.167±0.021
	GC (25)	4.38±0.317	23.78±1.872	32.75±2.062	0.295±0.022	52.28±2.917	0.255±0.016	0.216±0.028
	P-value	P=0.294	P=0.074	P=0.437	P=0.381	P=0.347	P=0.407	P=0.061
g.1016. G>A	GG (201)	4.72±0.246	22.23±1.449	32.04±1.595	0.287±0.017	48.80±2.257	0.237±0.012	0.175±0.021
	AG (92)	4.66±0.244	23.16±1.435	33.41±1.581	0.304±0.017	51.43±2.236	0.252±0.012	0.206±0.021
	AA (7)	4.22±0.318	21.14±1.882	35.09±2.071	0.321±0.022	52.69±2.929	0.257±0.016	0.193±0.028
	P-value	P=0.159	P=0.223	P=0.063	P=0.039	P=0.021	P=0.014	P=0.005

ADG: average daily gain.

Table 3 Least squares mean and standard errors of ultrasonic musculus longissimus dorsi (MLD) measurements of Karacabey Merino lambs in terms of single nucleotide polymorphisms (SNP) genotypes

Locus	Allel	90th day			180th day		
		Muscle depth	Back fat thickness	Skin thickness	Muscle depth	Back fat thickness	Skin thickness
g.9. T>C	CC (203)	2.48±0.114	0.27±0.024	0.15±0.011	2.74±0.113	0.27±0.023	0.17±0.010
	TC (84)	2.50±0.115	0.26±0.024	0.15±0.011	2.74±0.114	0.27±0.024	0.18±0.010
	TT (13)	2.36±0.129	0.25±0.027	0.13±0.013	2.52±0.128	0.30±0.026	0.16±0.012
	P-value	P=0.480	P=0.547	P=0.373	P=0.149	P=0.452	P=0.250
g.12. G>C	CC (204)	2.45±0.108	0.23±0.023	0.15±0.011	2.70±0.107	0.26±0.022	0.17±0.01
	GC (82)	2.34±0.133	0.26±0.028	0.14±0.013	2.59±0.132	0.28±0.027	0.17±0.012
	GG (14)	2.55±0.136	0.29±0.029	0.14±0.013	2.71±0.135	0.29±0.028	0.17±0.012
		P=0.170	P=0.197	P=0.909	P=0.517	P=0.557	P=0.834
g.93. G>A	GG (201)	2.51±0.151	0.26±0.032	0.14±0.015	2.71±0.150	0.30±0.031	0.17±0.014
	AG (91)	2.51±0.110	0.25±0.023	0.15±0.011	2.69±0.109	0.30±0.023	0.16±0.010
	AA (8)	2.32±0.153	0.27±0.033	0.14±0.015	2.60±0.152	0.23±0.032	0.18±0.014
	P-value	P=0.550	P=0.681	P=0.427	P=0.868	P=0.176	P=0.397
g.381. G>A	GG (276)	2.50±0.073	0.29±0.016	0.15±0.007	2.52±0.073	0.27±0.015	0.16±0.007
	AG (23)	2.39±0.088	0.19±0.019	0.13±0.009	2.63±0.087	0.27±0.018	0.17±0.008
	AA (1)	2.44±0.283	0.31±0.060	0.14±0.028	2.85±0.281	0.29±0.058	0.18±0.025
	P-value	P=0.715	P=0.002	P=0.287	P=0.299	P=0.939	P=0.457
g.681. G>C	GG (275)	2.42±0.104	0.21±0.022	0.13±0.010	2.74±0.103	0.28±0.021	0.17±0.009
	GC (25)	2.47±0.136	0.31±0.029	0.16±0.013	2.60±0.135	0.28±0.028	0.16±0.012
	P-value	P=0.725	P=0.000	P=0.070	P=0.269	P=0.894	P=0.518
g.1016. G>A	GG (201)	2.36±0.106	0.27±0.022	0.14±0.01	2.58±0.105	0.27±0.022	0.17±0.01
	AG (92)	2.44±0.104	0.27±0.022	0.13±0.01	2.71±0.103	0.27±0.021	0.17±0.009
	AA (7)	2.54±0.136	0.24±0.029	0.16±0.013	2.71±0.135	0.30±0.028	0.17±0.012
	P-value	P=0.099	P=0.394	P=0.021	P=0.027	P=0.489	P=0.733

Melanocortin-4 receptor (*Mc4R*) gene control of leptin signal and energy balance is crucial (Markison and Foster, 2007; Klimenko *et al*. 2014). The function of Mc4R in controlling energy balance and feed intake in a variety of livestock species implies that it might be a significant genetic marker for growth features. By affecting the expression and function of the genes, genetic diversity may have an effect on the phenotypic traits of animals.

In this study, we detected genetic polymorphisms in *Mc4R* gene in Karacabey Merino lambs and investigated their effects on growth traits. Our results showed a strong correlation between the *Mc4R* gene and growth performance. This observation offered new information on SNP markers, that may be useful for MAS in breeding schemes for the "National Merino Development Project".

Six SNP variants in the *Mc4R* gene were proven to have no significant impact on body weight at 45 days after delivery and birth weight. Whereas, Song *et al*. (2012) observed four SNP variants (g.1016. G>A, g.1240. T>C, g.1264. G>A and g.1325. A>G) in the sheep *Mc4R* gene in Hu sheep.

The GG genotype of g.1016. G>A showed higher weaning weight in comparison to the others genotypes (P<0.01). It was determined that only g.12 G>C SNP was effective on 90th day live weight, and other SNP mutations had no effect. Lambs carrying CC genotype were heavier in live weight at 90th day compared to GC genotype. Also, Zuo *et al*. (2014) stated that g.1229G>A SNP was associated with 4th month live weight, 6th month live weight, average daily weight gain, back fat thickness, and eye muscle area in German Merino sheep. Similar to Zuo *et al*. (2014) the SNP g.1016G>A in our study was shown to be significant on 6th month body weight, 0-90th day ADG, 0-180th day ADG, skin thickness muscle depth (P<0.05) and 90-180th day ADG (P<0.01).

In addition, Wang *et al*. (2015) discovered that SNPs g.1267. G>A in the 3'-UTR and g.706. C>A in the coding region were associated with body weight on the 180th day (P<0.01). Shishay *et al*. (2019) reported that –g.1026. G>A, g.-943.G>T and -206. G>A SNPs detected in the 5'UTR region were significantly associated with live weight (P<0.01).

Zhao *et al*. (2018) reported that g.880.G>A SNP was associated with live weight in their study in Tibetan sheep (P<0.01).

On ultrasound measurements, fat thickness of g.381. G>A (P<0.05) and g.681. G>C (P<0.01) SNPs on 90th day ultrasound measurements and skin thickness of g.1016. G>A (P<0.05) SNP was discovered to have a significant effect. According to Yılmaz *et al*. (2014) ultrasound measurements taken on the 90th day the average of the muscle depth was 2.47 ± 0.017 cm and the average of the fat thickness was 0.35 ± 0.006 cm.

Additionally, it was observed that the g.1016.G>A. SNP significantly affected the muscle depth on day on the 180th day (P<0.05). The average muscle depth of individuals with AA genotype of g.1016. G>A SNP was 2.71 ± 0.135 cm; The average of those with the GG genotype was found to be 2.58 ± 0.105 cm.

The 3'-UTR is important in the regulation of nucleocytoplasmic transport, sub cellular localization and post- transcriptional gene expression. The sequence of the molecular regulator mRNA is found more frequently in the 3'-UTR (Grillo *et al*. 2010; Wang *et al*. 2015). Because of their interactions with target mRNAs' 3'-UTRs, mRNAs seem to be generally negative regulators of gene transcripts. Therefore, it has been demonstrated by research that sequence variations in this 3'-UTR affect the appropriate expression of genes (Shibayama *et al*. 2004; Reamon-Buettner *et al*. 2007; Yuan *et al*. 2012).

Therefore, it is thought that the g.1016.G>A SNP discovered in the 3'-UTR of the study may affect the binding sequence of mRNA and may also affect sheep growth characteristics. After the detection of this SNP, it will be useful to determine the mRNA expression level of this gene and to reveal its effects on yield characteristics. It also emerges from the study that the SNPs in association with the weight of the animal and the thickness of the fat are generally made after weaning the animals, which shows the importance of the influence of maternal milk on these zootechnical parameters.

CONCLUSION

In conclusion, with the help of molecular genetic methods developed in recent years, the identification of highly productive breeding qualified animals and the effective use of these animals can be done both in a short time and at molecular costs. This increases the importance of molecular genetic methods and their integration into breeding programs day by day. With the genotyping studies to be carried out, the identification of breeding qualified animals, especially at an earlier age, will provide both economic and more genetic progress. Mc4R may be a potential candidate gene for the marker-assisted selection of production traits due to its important roles growth and development in lambs. Especially, to identify any potential indirect influences on the genes that might alter the quantitative features, it would be helpful to determine the *Mc4R* gene's mRNA expression level. As in our Karacabey Merino sheep breed, it will be useful to determine the genetic structures of the populations of our breeds by conducting similar studies especially in other sheep breeds that constitute the majority of the sheep existence of Türkiye.

ACKNOWLEDGEMENT

This research has been funded by the Ministry of Agriculture and Forestry, General Directorate of Agricultural Research and Policies of Republic of Turkiye (Project number: TAGEM/HAYSÜD/17/A07/P-01/01). The PhD thesis of the first author was produced from this project.

REFERENCES

Akyüz B., Arslan K. and İlgar E.G. (2017). Türkiye'de yetiştirilen anadolu mandalarında butirofilin (*BTN1A1*) gen polimorfizminin *HaeIII* restriksiyon enzimi ile araştırılması. *Erciyes Üniv. Vet. Fak. Derg.* **14(1),** 11-16.

Atav R., Bugdaycı B., Şen A., Ergünay U., Ünal G.P., Ünal Ö.E., Karagöz G., Işık R., Soysal İ.M., Özder M., Arat S. and Eroğlu B. (2023). Creating a high-quality wool-oriented Turkish merino herd and investigation of mechanical and dyeability properties of fabrics produced from Turkish merino in comparison with Australian merino. *Color Technol.* **10,** 1-14.

Bayraktar M. and Shoshin, O. (2021). Estimate of the association of *IGF-I* and *IGFALS* genes with growth traits in Hamdani sheep. *Brazilian Arch. Biol. Technol.* **64,** 1-9.

Botstein D., White R.L., Skolnick M. and Davis R.W. (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *Am. J. Hum. Genet.* **32,** 314-331.

Cai X., Mipam T.D., Zhao F.F. and Sun L. (2015). SNPs detected in the yak *MC4R* gene and their association with growth traits. *Animal.* **9(7),** 1097-1103.

Cheng J., Xin Li X., Wang J., Zhou B., Lin C., Yang X. and Zhang X. (2022). Identification of polymorphic loci in *OSMR* and *GHR* genes and analysis of their association with growth traits in sheep. *Anim. Biotechnol.* **34(7),** 2546-2553.

Cone R.D. (2005). Anatomy and regulation of the central melanocortin system. *Nat. Neurosci.* **8,** 571-578.

Deng T.X., Pang C.Y., Liu M.Q., Zhang C. and Liang X.W. (2016). Synonymous single nucleotide polymorphisms in the *MC4R* gene that are significantly associated with milk production traits in water buffaloes. *Genet. Mol. Res.* **15,** 1-8.

Dönmez O. (2008). Bursa İli koyunculuk işletmelerinin yetiştiricilik açısından yapısı. MS. Thesis. Tekirdağ, Turkey.

Feng J., Li H. and Yan C.G. (2017). The correlation analysis between the SNPs of *MC4R* gene and meat quality traits in Yanbian yellow cattle. *China Anim. Husband. Vet. Med.* **44,** 1406-1413.

Grillo G., Turi A., Licciulli F., Mignone F., Liuni S., Banfi S., Gennarino V.A., Horner D., Pavesi G., Picardi E. and Pesole G. (2010). UTRdb and UTRsite (RELEASE 2010): A collection of sequences and regulatorymotifs of the untranslated regions of eukaryotic mRNAs. *Nucleic Acids Res.* **38,** 75-80.

Klimenko A., Usatov A., Getmantseva L., Kolosov Y., Tretyakova O., Bakoev S., Kostjunina O. and Zinovieva N. (2014). Effects of melanocortin-4 receptor gene on growth and meat traits in pigs raised in Russia. *Am. J. Agric. Biol. Sci.* **9,** 232-237.

Latifah L., Maharani D., Kustantinah A., and Hartatik T. (2018). Association of melanocortin 4 receptor gene polymorphism with growth traits in Bligon goat. *J. Indonesian Trop. Anim. Agric.* **43(4),** 343-351.

Lee Y.S., Park S., Kim H., Lee S.K., Kim J.W., Lee H.K., Jeong D.K. and Lee S.J. (2013). A C1069G SNP of the *MC4R* gene and its association with economic traits in Korean native cattle (brown, brindle, and black). *Elect. J. Biol.* **16(5),** 14-21.

Li W., Wang X., Zhang X., Li F., Zhang D., Li X., Zhang Y., Zhao Y., Zhao L., Xu D., Cheng J., Wang J., Zhou B., Lin C. and Wang W. (2023). Polymorphism of sheep *PRKAA2* gene and its association with growth traits. *Anim. Biotechnol.* **34(4),** 1324-1330.

Liu H., Tian W., Zan L., Wang H. and Cui H. (2010). Mutations of *MC4R* gene and its association with economic traits in Qinchuan cattle. *Mol. Biol. Rep.* **37(1),** 535-540.

Markison S. and Foster A.C. (2007). Targeting melanocortin receptors for the treatment of obesity. *Drug Discov. Today*. **3,** 569-576.

Polatoğlu M.G. (2019). Cumhuriyet döneminde hayvancılığın sanayiye tatbikine bir örnek: Merinos yetiştiriciliği. *Atatürk Araşt. Merk. Derg.* **100,** 585-620.

Prihandini P.W. and Maharani D. (2019). Melanocortin‐4 receptor (*MC4R*) gene as the main gene for rapid growth selection in beef cattle. *Agric. Food Sci.* **29(2),** 85-95.

Reamon-Buettner S.M., Cho S.H. and Borlak J. (2007). Mutations in the 30-untranslated region of *GATA4* as molecular hotspots for congenital heart disease (CHD). *BMC Med. Genet.* **8,** 38-45.

Safari E., Fagorty N.M. and Gilmour A.R. (2005). A review of genetic parameter estimates for wool, growth, meat and reproduction traits in sheep. *Livest. Prod. Sci.* **92(3),** 271-289.

Seong J., Suh D.S., Do Park K., Lee H.K. and Kong H.S. (2012). Identification and analysis of *MC4R* polymorphisms and their association with economic traits of Korean cattle (Hanwoo). *Mol. Biol. Rep.* **39(4),** 3597-3601.

Shan H., Song X., Cao Y., Xiong P., Wu J., Jiang J. and Jiang Y. (2020). Association of the melanocortin 4 receptor (*MC4R*) gene polymorphism with growth traits of Hu sheep. *Small Rumin. Res.* **192,** 1-9.

Shibayama A., Cook E.H.J., Feng J., Glanzmann C., Yan J., Craddock N., Jones I.R., Goldman D., Heston L.L. and Sommer S.S. (2004). *MECP2* structural and *3'-UTR* variants in schizophrenia, autism and other psychiatric diseases: a possible association with autism. *Am. J. Med. Genet. B.* **128,** 50-53.

Shishay G., Liu G., Jiang X., Yu Y., Teketay W., Du D., Jing H. and Liu C. (2019). Variation in the promoter region of the *MC4R* gene elucidates the association of body measurement traits in Hu sheep. *Int. J. Mol. Sci.* **20(2),** 240-248.

Song X.M., Jiang J.F., Zhang G.Z., Shi F.X. and Jiang Y.Q. (2012). DNA polymorphisms of the Hu sheep melanocortin-4 receptor gene associated with birth weight and 45-day weaning weight. *Genet. Mol. Res.* **11,** 4432-4441.

SPSS Inc. (2011). Statistical Package for Social Sciences Study. SPSS for Windows, Version 20. Chicago SPSS Inc., USA.

Türkiye Istatistik Kurumu (TUIK). (2023). https://biruni.tuik.gov.tr/medas/?kn=101&locale=en, Accessed Sep. 2023.

Wang Y., Wang C., Zhang J., Meng C., Zhang X., Wang Z., Fang Y., Mao D. and Cao S. (2015). Three novel *MC4R* snps associated with growth traits in Hu sheep and East Friesian Hu crossbred sheep. *Small Rumin. Res.* **125,** 26-33.

Yeh F., Yang R.C. and Boyle T. (1999). Popgene (v.1.32): Microsoft Windows-based free ware for Population Genetic Analysis. Available at: http://www.ualberta.ca/~fyeh/popgene_download.html.

Yilmaz O., Sezenler T., Alarslan E., Ata N., Karaca O. and Cemal İ. (2014). Karacabey Merinosu, Karya ve Kıvırcık kuzularda sütten kesim döneminde kabuk yağı kalınlığı ve musculus longissmus dorsi thoracis et lumborum (MLD) derinliğinin ultrason ölçümleri. *Kafkas Üniv. Vet. Fak. Derg.* **20,** 829-834.

Yuan M., Zhan Q., Duan X., Song B., Zeng S., Chen X., Yang Q. and Xia J. (2012). A functional polymorphism at miR-491-5p binding site in the *3'-UTR* of *MMP-9* gene confers increased risk for atherosclerotic cerebral infarction in a Chinese population. *Atherosclerosis.* **226(2),** 447-452.

Zhai R., Wang W., Zhang D., Li X., Zhang Y., Zhao Y., Zhao L., Wang J., Xu D., Cheng J., Li W., Zhou B., Lin C., Zeng X., Yang X., Ma Z., Liu J., Cui P. and Zhang X.X. (2022). Novel polymorphism at *KLF15* gene and its association with growth traits in Hu sheep. *Anim. Biotechnol.* **2022,** 1-7.

Zhao X.L., Cao S.J. and Wang J.Q. (2018). Association analysis of *MC4R* gene polymorphisms and genotype combination with growth traits of Tibetan Sheep (*Ovis aries*). *J. Agric. Biotechnol.* **26(3),** 429-436.

Zuo B., Liu G., Peng Y., Qian H., Liu J., Jiang X. and Mara A. (2014). Melanocortin-4 receptor (*MC4R*) polymorphisms are associated with growth and meat quality traits in sheep. *Mol. Biol. Rep.* **41,** 6967-6974.