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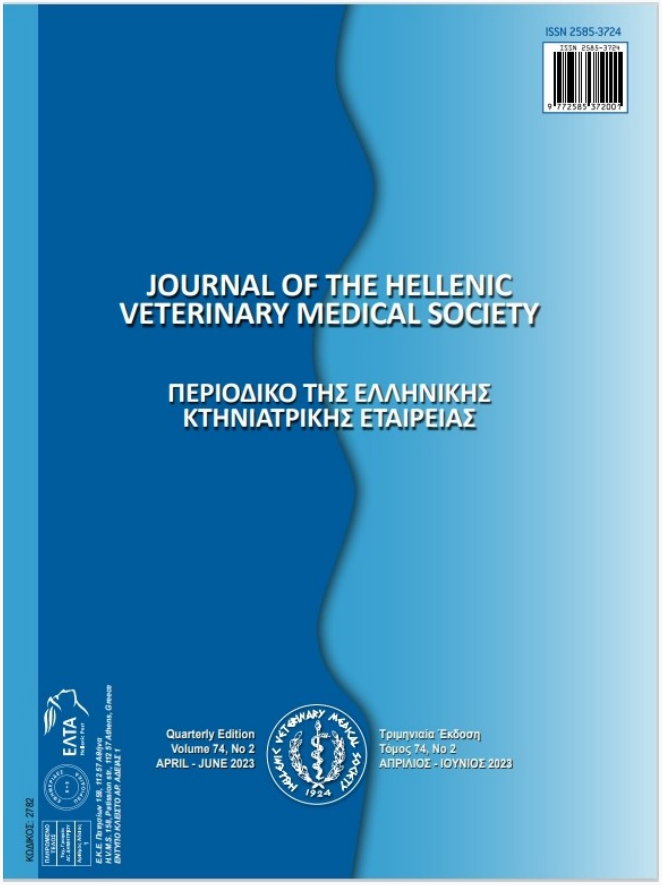

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Establishment of *AA-NAT, SCD, PROP1,* and *IGFBP3* gene frequency distributions in Karacabey Merino and Kivircik Sheep Breeds

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ABSTRACT: The aim of the current study was to investigate the genotypic dispersion of *AA-NAT, SCD, PROP1*, and *IGFBP3* genes in the target population. Primarily, the genomic DNA was isolated from blood samples by the phenol/chloroform method. The genomic analyses were performed on a total of 160 Karacabey Merino (KM) (n=80) and Kivircik sheep (n=80). The PCR-RFLP method was used to determine the genotype of the SNPs. Deviation from Hardy-Weinberg equilibrium (HWE) and population genetic assays such as observed (Ho) and expected (He) heterozygosities, effective allele numbers (Ne), and the polymorphism information content (PIC) were calculated for each gene in the population. As a result, there was no deviation from HWE was determined except *SCD* and *PROP1* gene in KM. The most frequent genotypes were found AA (66,25% and 87,50%) for *AA-NAT* gene, CC (93,75% and 91,25%) for *SCD* gene and CC genotype (70,00% and 56,25%) for *PROP1* gene in KM and Kivircik sheep. Both breeds were detected monomorphic in terms of the *IGFBP3* gene. In conclusion, the other regions of the *IGFBP3* gene which were determined as monomorphic in the studied flock could be researched. Moreover, further studies should be necessary for defining the effects of investigated genes on related traits in KM and Kivircik sheep in terms of polymorphic genes specified.

Keywords: Karacabey Merino, Kivircik, *AA-NAT, SCD, PROP1, IGFBP3*

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INTRODUCTION

The total profit of breeding is related to the productivity of small ruminants. Increased yields in sheep breeding are a result of successful optimization of reproduction of ewes moreover growth performance and survival of lambs (Koyuncu and Kara Uzun, 2009). The genomic improvement of related traits plays a vital role. In that case the structure/effect of genes on reproduction and growth performance should be understood in these populations which have larger variability for these traits.

Arylalkylamine N-acetyltransferase (*AA-NAT*) is an important enzyme called "the timezyme" that plays a critical role in melatonin (*MLT*) biosynthesis by acting as serotonin for *MLT*, regulating the seasonal animal breeding as the rate-limiting enzyme (Klein and Berg, 1970). *MLT* is called the "hormone of darkness" since its production level is controlled by the day-night cycle (circadian rhythm) (Rosa and Bryant, 2003). *AA-NAT* is a gene that encodes the enzyme alkylamine N acetyltransferase, found in all animals; it is located on chromosome 11 in sheep. It is produced in the pineal gland, which plays an essential role in adjusting various physiological ovarian and testicular changes and pathological processes in short- or long-day breeds (Lincoln, 2006; Dupre et al., 2008). Short photoperiods positively effects the level of melatonin secretion and it causes to stimulate the pituitary gland to release follicle-stimulating hormone and luteinizing hormone (Falk, 2013). Even if *MLT* displays its effect through two specific receptors, 1A and 1B, melatonin receptor 1A (*MTNR1A*) is the primary receptor mediating melatonin's reproductive and circadian effects in sheep. Therefore, the circadian oscillatory production of *MLT* synchronizes in seasonal estrus response in small ruminants (Wayne et al., 1988, Zheng et al., 2001). Ding-ping et al. (2012) investigated the associations of *AA-NAT/SmaI* polymorphism Chinese sheep breeds. They reported that the ewes with the GG genotype showed higher frequencies in the non-seasonal animals; on the contrary, the ewes with the GA genotype had higher rates in seasonal sheep breeds. Considering the critical role in *MLT* secretion, it makes sense that any polymorphism in the *AA-NAT* gene could potentially affect the seasonal estrus response in small ruminants (Ding-ping et al., 2012).

In ruminants, the *SCD* gene encodes Stearoyl-CoA desaturase, an enzyme that converts monounsaturated fatty acids (MUFA) from saturated fatty acids (SFA) in dietary or de novo lipid biosynthesis via desaturation of palmitic acid (C16:0) to palmitoleic acid (C16:1) and stearic acid (C18:0) to oleic acid (C18:1) (Izadi et al., 2014). *SCD* is a protein with a mass of 37 kDa and consists of 359 amino acids (Dobrzyn and Ntambi, 2005). Therefore, this enzyme plays an essential role in lipid metabolism and maintenance of membrane fluidity based on the physiological importance of the ratio between saturated and monounsaturated fatty acids (Ntambi, 1999). The *SCD* gene in mammals has a similar gene structure consisting of 6 exons and introns and 15-24 kbp in a length. (Bernard et al., 2001). In sheep, the *SCD* gene encoding stearoyl-CoA desaturase is located in OAR22 (Kuchel et al., 2004). Multiple isoforms of *SCD* genes (*SCD1-5*) were detected in various organisms with different expression profiles according to tissues examined to date (Castro et al., 2011). The *SCD* gene has been expressed in many tissues in sheep, including mammary glands, muscles, heart, liver, lungs, kidneys, spleen, pancreas and adipose tissue (Ward et al., 1998). Quinones et al. (2017) studied to determine g.31C >A polymorphism in the promoter region of *SCD* gene in Araucano creole sheep population. They reported that the 98% allelic frequency for C allele and 2% for the A allele. In addition 93% genotypic frequency for CC genotype and 7% for CA genotypes. But there was no animal with AA genotype in their study.

The *PROP* paired-like homeobox 1 (*PROP1*) gene located on ovine chromosome 5 is vital for normal gonadotropin production (Vesper et al., 2006). A protein of *PROP1* gene is effective in the regulation of *POU1F1* gene by controlling the proliferation and differentiation of the pituitary gland (Woelfle et al., 2003). Thus, the *PROP1* gene plays an essential role in growth or reproductive metabolism by way of regulation of pituitary hormones like growth hormone (*GH*), thyroid-stimulating hormone beta (*TSH-β*), and prolactin (*PRL*) (Ekegbu et al., 2019). Ekegbu et al. (2019) investigated the effect of *PROP1* gene polymorphisms (c.109+40 T > C; c.109+207 C > T; c.45A > G) on lamb tailing, weaning weight, and growth rate to weaning; GG (c.45A > G) and CC (109+40 T > C; 109+207 C > T) had greater weaning weight and growth rate to weaning and higher lamb tailing. On the other hand, Liu et al. (2015) searched the impact of C330T polymorphism on litter size in Small Tail Han, Hu, White Suffolk, and Texel sheep breeds. The most frequent genotyped was established as CC with the litter size 1.88±0.10. Moreover, the g.2647A>G polymorphism of *PROP1* gene was determined as effective on wool traits such as fiber diameter by the study of Zeng et al. (2011).

Insulin-like growth factor-binding protein 3 (*IGFBP3*) is a member of Insulin-like growth factor binding proteins (*IGFBPs*) that bind Insulin-like growth factors (*IGFs*) to modulate biological activities (Zhang et al., 2007). The total length of the ovine *IGFBP-3* gene is approximately 8.4 kb, with five exons located on chromosome 4 (NCBI, 2021). Thus, *IGFBP3* has been reported to have an important role in growth, mammary ductal development, fertility, moreover, the immune system (Choudhary et al., 2006). So it should be defined as a genetic marker for reproduction, growth, or immunity (Saleh et al., 2019). Variation studies or nucleotide sequencing of *IGFBP-3* gene have been declared in cattle (Maciulla et al., 1997), buffalo (Padma et al., 2004), or different kinds of sheep breed such as Egyptian (Rahmani, Barki, Ossimi), Indian, Muzaffarnagar, Madgyal to now (Kumar et al., 2006; Ali et al., 2009; El-Hanafy et al., 2009; Sharma et al., 2011; Shafey et al., 2014; Mahrous et al., 2015; Rajashekhara et al., 2018; Al-Khuzai et al., 2019; Saleh et al., 2019). According to the literature, variations are observed between the flocks.

Although the impact or variation analyses have been performed, the knowledge about polymorphisms of target genes are insufficient and limited according to races such as KM or Kivircik sheep breed. Generally, the genetic structure of target animals is unknown for investigated genes. To the best of our knowledge, no studies are yet, available on the comparisons in *AA-NAT, SCD, PROP1,* or *IGFBP3* polymorphisms genes among Turkish native sheep breeds. Thus, the purpose of the study is the establishment of *AA-NAT, SCD, PROP1,* and *IGFBP3* gene frequency distributions in KM and Kivircik sheep for the literature.

MATERIALS AND METHODS

Animal Sources

The study was conducted in KM (n=80) and Kivircik (n= 80) sheep breeding at the Marmara Region of Turkey for evaluating the genotypic frequency distribution of *AA-NAT, SCD, PROP1,* and *IGFBP3* genes. The animals which have identical feeding and breeding management protocol were selected randomly inside the herd.

DNA isolation and genotypic analyses

Blood samples were collected by complying with ethical considerations (Approval Number:2020-02/08). The nucleic acid extraction from samples of whole blood was carried out by phenol-chloroform, according to Green and Sambrook (2012) (A_{260}/A_{280} ratio was used for determining the purification of the genomic material). The PCR-RFLP method was chosen to evaluate the genetic variation of investigated flocks. The information about PCR steps such as primers, PCR conditions and fragment lengts (bp) were given in Table 1.

The 5 µl of PCR products were treated with 5 U of *SmaI* restriction enzyme for *AA-NAT* gene, 10 U of *MnL1*restriction enzyme for *SCD gene*, 20 U of *HhaI* restriction enzyme for *PROP1* gene, 10 U of *HaeIII* restriction enzyme for *IGFBP3* gene in 1X rCutSmart™ Buffer to determine the allelic polymor-

Table-1. The specific primers for genotyping of *AA-NAT, SCD, PROP1,* and *IGFBP3* gene polymorphism

Gene	Primers (5'→3')	PCR conditions	Fragment lengths (bp)	*References*
AA-NAT	F: AGCGTCCACTGCCTGAAAC R: GGGATGGAAGCCAAACCTC	• 95°C (5 min) • [95°C (40 sec), 60°C (40 sec), 72 °C (40 sec)] x 34 cycle, • 72°C (10 min)	1142	*Ding-ping etal., 2012*
SCD	F: AAATTCCCTTCGGCCAATGAC R: TCTCACCTCCTCTTGCAGCA	• 94°C (3 min), • [94°C (50 sec), 58°C (50 sec), 72 °C (50 sec)] x 35 cycle, • 72°C (10 min)	517	*Garcia-Fernandez et al., 2009*
PROP1	F: TCACTGAGGCCCTGCCCTGAGAGCG R: GCCCCCTACTCTGCTCAGATCCAAAG	• 95°C (5 min), • [95°C (30 sec), 60°C (30 sec), 72 °C (45 sec)] x 35 cycle, • 72°C (10 min)	145	*Liu et al., 2015*
IGFBP3	F: CCAAGCGTGAGACAGAATAC R: AGGAGGGATAGGAGCAAGAT	• 94°C (5 min), • [94°C (1 min), 60°C (1 min), 72°C (1 min)] x 35 cycle, • 72 °C (2 min)	654	*Maciulla et al., 1997; El-Hanafy et al., 2009*

phisms of *AA-NAT* (G,A), *SCD* (C,A), *PROP1* (C,T), and *IGFBP3* (A,B). The expected genotypes according to the RFLP fragments were presented in Table 2. The RFLP mixtures were incubated at 37°C overnight. Results of PCR-RFLP steps were controlled by agarose gel electrophoresis in 2 - 3% due to the product size containing ethidium bromide. Followed by, the amplified products were visualized in the DNr Minilumi imaging system (λ=312 nm).

Statistical analysis

Estimation of observed (Ho) and expected (He) heterozygosity values, an effective allele number (Ne) and a polymorphism information content (PIC) were directly calculated according to Botstein et al. (1980). The allele and genotype frequencies as well as Hardy-Wienberg equilibrium (HWE), if the population is at genetic equilibrum or not were tested with chi square analysis (χ2), were calculated by using PopGene32 program (Yeh et al. 2000).

RESULTS

AA-NAT

The PCR amplified *AA-NAT* gene with 1142 bp sequences includes the sequences of concluded part of exon1 (152 bp), intron 1 (290 bp), whole exon 2 (155 bp), intron 2 (338 bp), and part of exon 3 (207 bp) in ovine chromosome 11. *SmaI* restriction endonuclease was used to digesting to detect the novel NM_001009461:c.486A > G mutation. The digested PCR products were distinguished by the alleles of A and G for the *AA-NAT* gene. Based on the genotyping results, there are three genotypes with GG, GA, and AA determined by four bands (183 bp, 255 bp, 333 bp and 371 bp), five bands (183 bp, 255 bp, 333 bp, 371 bp and 516 bp), and three bands (255 bp, 371 bp and 516 bp), respectively given in Table 2 and Figure 1.

According to the results, The A and G alleles were established with the frequencies of 0.8063 and 0.1938 in KM; 0.9375 and 0.0625 in Kivircik sheep, respectively. Three different genotypes (AA, GA and GG) were observed in the investigated flock. The frequencies of AA, GA and GG were observed as 66.25%, 28.75% and 5.00% in KM, respectively. However, the frequencies of AA and AG genotypes were determined 87.50% and 12,50% with the frequency of homozygote GG genotype was 0.00% in Kivircik sheep (Table 3 and 4). The population genetic assays such as

Table-2. *AA-NAT, SCD, PROP1,* and *IGFBP3* genes.

Gene	Restriction enzyme	RFLP fragments (bp)		
AA-NAT	*SmaI*	GG (371, 333, 255, 183bp)	GA (516, 371, 333, 255, 183bp)	AA (516, 371, 255bp)
SCD	*Mnl1*	CC (269, 94, 64bp)	CA (269, 94, 76, 64bp)	AA (269, 76, 64bp)
PROP1	*HhaI*	CC (120, 25bp)	CT (145, 120, 25bp)	TT (145bp)
IGFBP3	*HaeIII*	AA (201, 201, 87, 67, 57, 18, 16, 7bp) *(Monomorphic)*		

Table 3. Genotypic frequencies for *AA-NAT*, *SCD, PROP1* and *IGFBP3* gene polymorphisms for KM.

Breed	Karacabey Merino sheep									
Gene	*AA-NAT*			*SCD*			*PROP1*			*IGFBP3*
Genotype	**AA**	**GA**	**GG**	**CC**	**CA**	**AA**	**CC**	**CT**	**TT**	**AA**
N	53	23	4	75	4	1	56	17	7	100
%	66,25	28,75	5,00	93,75	5,00	1,25	70,00	21,25	8,75	100
Allel frequencies	**A**	**G**		**C**	**A**		**C**	**T**		**A**
	0.8063	0.1938		0.9625	0.0375		0.8063	0.1938		1.000
He	0.3124			0.0722			0.3124			0.000
Ho	0.7125			0.9500			0.7875			1.000
Ne	1.4544			1.0778			1.4544			1.000
PIC	0.2636			0.0696			0.2636			-
χ2(HWE)	0.6017			9.2699			8.6408			-
P-value	0.4379			0.002			0.003			-

χ2(HWE) - Hardy-Weinberg equilibrium χ2 value; N - number of sheep; He - an expected heterozygosity; Ho - an obserced heterozygosity; Ne - an effective allele number; PIC -polymorphism information content. *P < 0.05: not consistent with HWE.

expected He, Ho, Ne or the polymorphism information contents (PIC) for KM were presented in Table 3. The expected and observed heterozygosity values (0.3124 and 0.7125), Ne (1.4544), PIC (0.2636) and the Hardy-Weinberg equilibrium (HWE) χ2 test value (0.6017) were calculated in KM. Morever, the He (0.1172), Ho (0.8750), Ne (1.1327), PIC (0.1103), HWE χ2 value (0.3181) values were detected for Kivircik sheep in Table 4. Both population genetic analyses of the genotypic data showed *SmaI* locus has not significantly deviated from HWE with comparatively less heterozygosity.

SCD

Detection of the genetic polymorphism of the *SCD* gene was performed by digestion of the PCR products of 517 bp with *MnlI* endonuclease, which was used to detect the novel g.31C>A SNP mutation in the promoter region in ovine chromosome 22. Based on the genotyping results, there are three genotypes

Table 4. Genotypic frequencies for *AA-NAT*, *SCD, PROP1* and *IGFBP3* gene polymorphisms for Kivircik sheep.

Breed	**Kivircik sheep**									
Gene	*AA-NAT*			*SCD*			*PROP1*			*IGFBP3*
Genotype	**AA**	**GA**	**GG**	**CC**	**CA**	**AA**	**CC**	**CT**	**TT**	**AA**
N	70	10	0	73	7	0	45	26	9	100
%	87,50	12,50	0	91,25	8,75	0	56,25	32,50	11,25	100
Allel	**A**	**G**		**C**	**A**		**C**	**T**		**A**
frequencies	0.9375	0.0625		0.9563	0.0437		0.7250	0.2750		1.000
He	0.1172			0.0837			0.3987			1.000
Ho	0.8750			0.9125			0.6750			0.000
Ne	1.1327			1.0913			1.6632			1.000
PIC	0.1103			0.0801			0.3102			1.000
χ2(HWE)	0.3181			0.1426			2.9445			-
P-value	0.5727			0.7056			0.0861			-

χ2(HWE) - Hardy-Weinberg equilibrium χ2 value; N - number of sheep; He - an expected heterozygosity; Ho - an obserced heterozygosity; Ne - an effective allele number; PIC -polymorphism information content. *P < 0.05: not consistent with HWE.

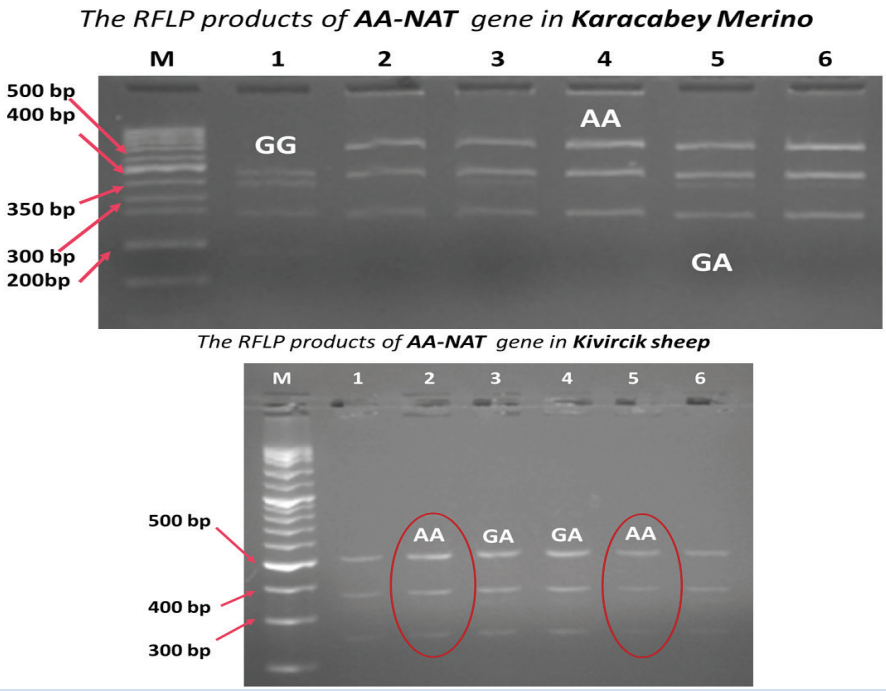

Figure 1. Agarose gel electrophoresis results of *AA-NAT* gene. The M symbolizes the 100 bp ladder molecular weight markers. Line 1 of KM belongs to GG genotype with the 371, 333, and 285 bp fragments. The Line 4 of KM; Lines 2 and 5 of Kivircik sheep exhibit AA genotype (516, 371, and 285 bp). Moreover, Line 2, 3, 5, and 6 refer to the heterozygote GA genotype (516, 371, 333, and 285 bp) for KM. (The 183 bp fragment is not visible).

with CC, CA, and AA determined by three bands (269 bp, 94 bp and 64 bp), four bands (269 bp, 94 bp, 376 bp and 64 bp), and three bands (269 bp, 76 bp and 64 bp), respectively given in Table 2 and Figure 1. In this study, two alleles were detected as the C and A alleles with the frequencies of 0.9625 and 0.0375 in KM; 0.9563 and 0.0437 in Kivircik sheep for this locus, respectively. In addition, three genotypes were identified for the *SCD* gene as CC, CA, and AA in the examined flock of KM. Only two genotypes, CC and CA were detected in Kivircik. CC, CA, and AA frequencies were calculated as 93.75%, 5.00%, and 1.25% in KM, and 91.25%, 8.75%, and 0.00% in Kivircik, respectively (Tables 3 and 4). The population genetic parameters were given as the He (0.0722), Ho (0.9500), Ne (1.0778), PIC (0.0696), HWE $\chi 2$ value (9.2699) for KM in Table 3. On the other hand, He as 0.0837, Ho as 0.9125, Ne as 1.0913, PIC as 0.0801, and HWE $\chi 2$ value as 0.1426 were found in the Kivircik flock (Table 4). The deviation from HWE was detected in the KM breed, while the Kivircik breed was in HWE for the *SCD* gene.

PROP1

The electrophoretic DNA patterns obtained from *PROP1- C330T* mutation in KM and Kivircik sheep after PCR amplification and digestion with *HhaI*. Also the band patterns of *PROP1* gene genotypes were given in Figure 3. According to the results, The C and T alleles were established with the frequencies of 0.8063 and 0.1938 in KM; 0.7250 and 0.2750 in Kivircik sheep, respectively. Three diffrerent genotypes (CC, CT and TT) were observed in the investigated flock. The frequencies of CC, CT and TT were found 70,00%, 21,25% and 8,75% in KM, respectively. The homozygote genotypes CC and TT were determined 56,25% and 32,50% with the heterozygote genotype frequency 11,25% in Kivircik sheep as well. The population genetic assays such as expected He, Ho, Ne or the polymorphism information contents for KM were presented in Table 3. The observed and expected heterozygosity values (0.7875 and 0.3124), Ne (1.4544) and $\chi 2$ value (8.6408) were calculated in KM. Moreover, the He (0.3987), Ho (0.6750), Ne (1.6632), PIC (0.3102), HWE $\chi 2$ (2.9445) value for Kivircik sheep in Table 4.

IGFBP3

According to the PCR-RFLP results of *IGFBP3* gene, four DNA fragment were occurred after being digested with *HaeIII* restriction enzyme (201, 87, 67, 57 bp) both in Karacabey and Kivircik Merino (Figure 4). Thus, only AA genotype for *IGFBP3,* which was accepted as the null of polymorphism for target

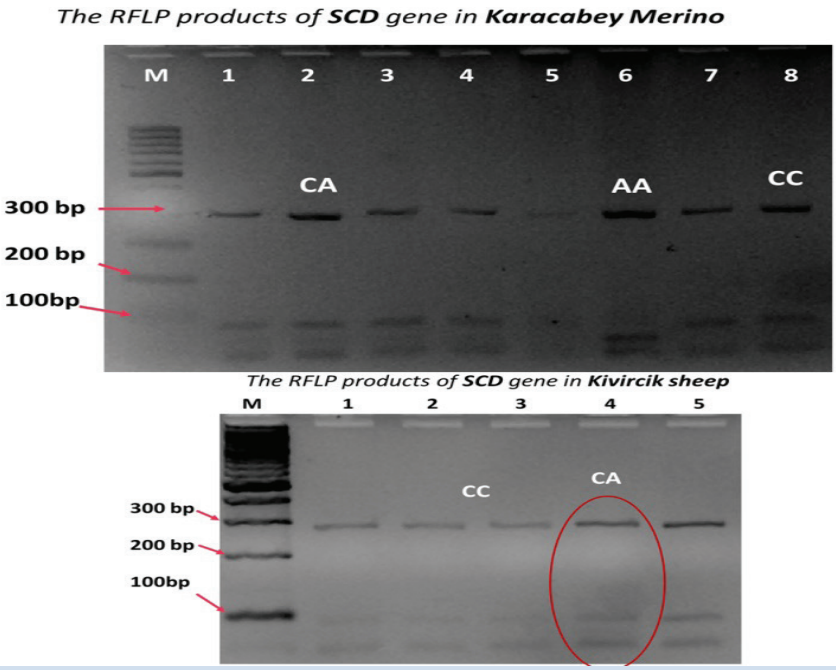

Figure 2. Agarose gel electrophoresis results of RFLP products of *SCD* gene. Line 1 to 5 and 7 of KM and Line 4 of Kivircik belong to CA genotype individuals (269, 94, 76, 64 bp products). Line 8 of KM; Line 1 to 3, and Line 5 of Kivircik belong to CC genotype with the RFLP patterns of 269, 94, and 64 bp. Also, line 6 of KM belongs to the homozygote AA genotype for the *SCD* gene in the figure (296, 76, 64 bp).

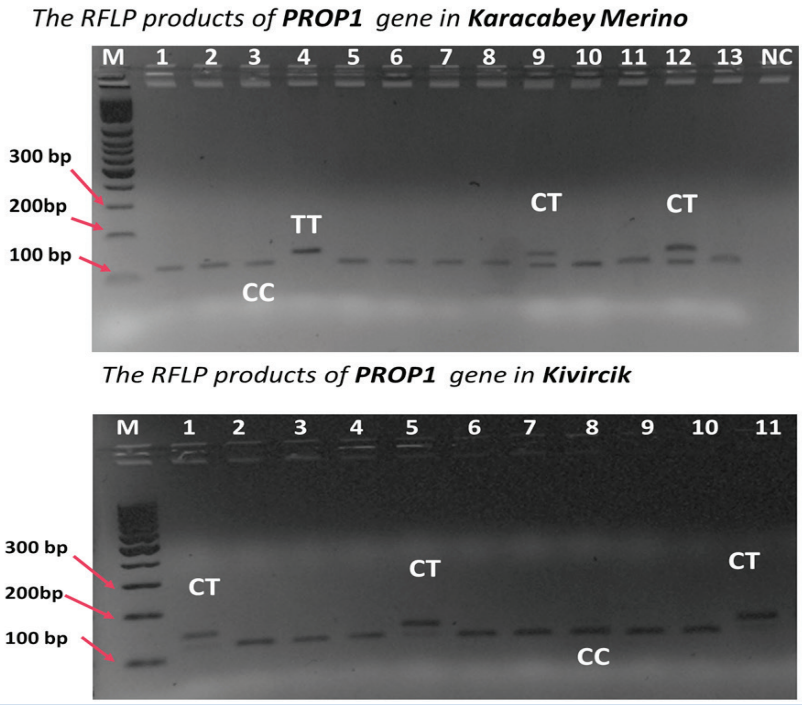

Figure 3. Agarose gel electrophoresis showing RFLP products of restricted fragments pf *PROP1* gene using restriction endonucleases: *HhaI*. The M symbolizes the 100 bp ladder molecular weight markers, and NC symbolizes the negative control of the samples. Line 9 and 12 belong to CT genotyped (145, 120 bp - 25 bp is not visible); Line 4 belongs to TT (one undigested pattern with 145 bp); moreover, the other lines belong to CC genotyped individual with 125 bp fragment in KM. Lines 1, 5, and 11 belong to heterozygote Kivircik sheep with CT genotype for *PROP1* gene in the figure. The other individuals of Kivircik sheep have owned the homozygote CC genotype.

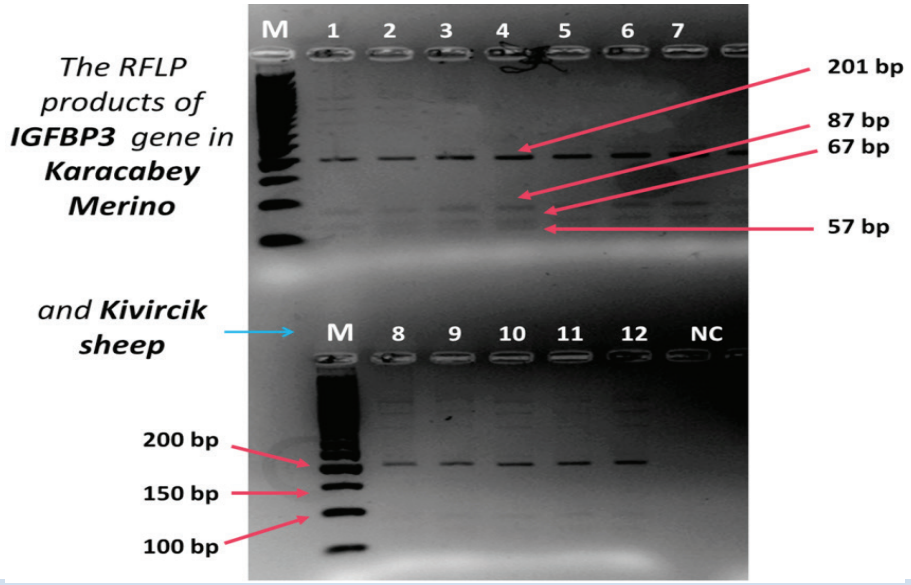

Figure 4. The results of RFLP products of *IGFBP3* after digestion by the *HaeIII* enzyme of KM and Kivircik, respectively. The M symbolizes the 100 bp ladder molecular weight markers, and NC symbolizes the negative control of the samples. Line 1 to 12 belongs to AA genotype with the 201, 87, 67, and 57 bp bands.

substitution, was observed in the whole of the studied animals (Table 3 and 4). Also, the allele frequency of A was calculated as 1.000, and the other population genetics, such as chi-square for HWE or PIC could not be calculated.

DISCUSSION

Even though *AA-NAT* is a vital candidate gene for animal reproduction; there were limited studies conducted to detect the effect of the *AA-NAT* gene on the seasonal reproductive patterns in livestock species

until the last decade. Specifically, the studies were conducted to detect the effect of *AA-NAT* on the reproductive seasonality in the Chinese Jining Grey goat (Ming-xing et al., 2013), Indian goat breeds (Sharma et al., 2015), Chinese sheep breeds (Ding-ping et al., 2012), Turkish native sheep breed (Öner et al., 2014), Turkish Awassi breed (Addin et al., 2016), Egyptian sheep breeds (Fathy et al., 2018), Bulgarian sheep breeds (Bozhilova-Sakova et al., 2019). Mainly, Ding-ping et al. (2012) reported that the GG genotype displayed higher frequencies in the reproduction of non-seasonal sheep; but the heterozygous genotype had more frequent in the reproduction of seasonal sheep breeds. In the current study, animals with AA and GA genotypes were much higher than animals with GG genotypes in seasonally reproductive KM and Kivircik breeds. The A to G transition in exon three, which caused the changes in amino acid (Arg>Gly) structures, had significant differences between the genotypic frequencies of seasonal and non-seasonal sheep breeds. Therefore, the novel 486A>G mutation of the *AA-NAT* gene may potentially affect the seasonal oestrus cycle in the sheep population due to the apparent modification of the *AA-NAT* protein structure, which exerts better biological function and might affect the seasonality of reproduction of sheep.

Several studies were conducted to detect the genetic polymorphism in *SCD* genes of sheep raised with different selection purposes, including meat and dairy, and various fat metabolisms, like fat-tailed and thin-tailed (Aali et al., 2014, Aali et al., 2016, Calvo et al., 2018). García-Fernández et al. (2010) studied to determine QTL, which harbors the *SCD* gene for the milk fatty acid composition on OAR22 in the Spanish Churra breed and reported a priori effect of the *SCD* gene on milk fat percentage. On the other hand, the other studies showed that the frequency of the A allele was 0.02 in Chilean sheep (Quinones et al., 2017) and 0.014 in Lori-Bakhtiari sheep (Aali et al., 2014). Similarly, the allelic frequency was 0.04 for both KM and Kivircik breeds in the present study. Based on the results, despite the low frequency observed in the A allele in beef breeds such as KM and Kivircik in the *SCD/Mnl1* SNP marker, it may be that the A allele frequency is much higher in dairy breeds. As the reason for this, we can say that there is a severe narrowing in the A allele frequency in KM and Kivircik populations, depending on the selection of animals based on carcass and meat characteristics over time. Sequence analyses in beef sheep populations revealed a high frequency of the C allele in a region associated with transcription factors such as WT1, NF-1, and AP-2α. These factors bind to the promoter regions of lipid metabolism genes, including *SCD*, related to fat deposition and fatty acid composition (Reardon et al., 2010, Hirwa et al., 2011, Bakhtiarizadeh et al., 2014).

The other gene that has insufficent knowledge about genotype structure and effects of polymorphisms or mutations in various sheep breed, *PROP1* gene, were studied in KM and Kivircik sheep in current study. Ekegbu et al. (2019) reported that *PROP1* gene polymorphims (c.109+40T>C, c.109+207C>T and c.45A>G) had significant effects on lamb tailing, weaning weight and growth rate to weaning in Romney sheep breed. Similarly, Zeng et al. (2011) investigated the effect of *PROP1* gene on growth traits in Chinese Merino and the significant impact on growth trait such as rump width or chest girth was adjusted for A2660G→Thr181 Ala polymorphism. On the other side, the effect on wool traits was studied by Zeng et al. (2011), according to the result the greatest fiber diameter was observed in NN genotype for g.2647A>G polymorphism that located on partial exon 3 and partial 3'UTR region. Not only the growth or wool traits but also the fertility traits were studied for *PROP1* gene in various sheep breed (Liu et al., 2015). Liu et al. (2015) declared the remarkable effect of C330T mutation on litter size. The most frequent genoype was noted as CC for Small Tail Han, Hu, White Suffolk and Texel sheep in the study. Although, the most frequent allele for C330T pattern was observed as CC for *PROP1*, the higher value for litter size was observed in individuals with TT genotype (2.71±0.22). Our results showed that, as demostrated in previous studies performed in Small Tail Han, Hu, White Suffolk and Texel sheep, the most frequent genotype was CC in investigated flocks. Compared with Kivircik sheep, KM was found polymorphic for C330T of *PROP1* too and the most frequent genotype was detected as CC (70.00%). To our knowledge, this is the first study to investigate the genetic variation of *PROP1* gene in KM and Kivircik sheep breed.

The results obtained in the present study for *IGFBP3* gene are in agreement with the findings reported by Ali et al. (2009), El-Hanafy et al. (2009), Shafey et al., (2014), and Mahrous et al. (2015), Saleh et al. (2019). They obtained eight fragments (201, 201, 87, 67, 57, 19, 16 and 7 bp) for the *HaeIII* restriction site (GG↓CC), which symbolizes the absence of polymorphism in Egyptian sheep breeds such as Rahmani, Awassi, Ossimi, and Barki. Similar results were

indicated in Indian sheep breed (Marwari, Mandya, Madras Red, and Banur) by Kumar et al. (2006) that no polymorphism were exhibited for *IGFBP3* gene of these breeds. Moreover, the Muzaffarnagari and Madgyal sheep declared as monomorphic for *IGFBP3* polymorphism with the AA genotype by Sharma et al. (2011) and Rajashekhara et al. (2018) in aggrement with the current study.

Regarding the population genetic parameters, the high Ho values were observed for all genes for both investigated sheep breeds, which resulted in low genetic variabilities of He, Ne, and PIC values, except *IGFBP3* due to monomorphic structure. According to Botstein (1980), PIC values for the SNP marker are closely related to determining how informative the observed diversity among animals in a population is. In this respect, *AA-NAT* and *PROP1* with 0.2636 might be considered moderately informative in the KM breed and *PROP1* with 0.3102 in Kivircik. However, *SCD* with 0.0696 for KM and *AA-NAT* with 0.1103, and *SCD* with 0.0801 for Kivircik sheep seemed low informative markers.

CONCLUSIONS

The PCR-RFLP method was used to determine the genotype of the KM and Kivircik sheep in current study. According to the result the most frequent genotypes were found AA for *AA-NAT* gene, CC for *SCD* gene and CC genotype for *PROP1 gene* in KM and Kivircik sheep, respectively. All breeds were found monomorphic in terms of *IGFBP3* gene. Thus, the other regions of *IGFBP3* gene should be researched in the further studies. In addition, the novel researches could be necessary for defining the effects of determined polymorphisms on related traits in target breeds.

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