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Determination of *Sac*II and *Mbo*II polymorphisms in the Nerve Growth Factor (NGF) gene in four native Turkish goat populations

Türkiye'de yetiştirilen dört keçi populasyonunda Sinir Büyüme Faktörü (NGF) geninde *Sac*II ve *Mbo*II polimorfizmlerinin belirlenmesi

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ABSTRACT

Nerve Growth Factor Gene (*NGF*) has important functions in the maintenance of viability and proliferation of ovarian cells. Recent studies on caprine *NGF* gene revealed two SNPs (determined by *Sac*II/C291A and *Mbo*II/A705G polymorphisms) related with body length and litter size in goats. This study aimed to determine *Sac*II and *Mbo*II polymorphisms of *NGF* in native Turkish goat populations including Turkish Hair (HAI), Kabakulak (KBK), Honamlı (HNM) and Norduz (NRD) by using PCR-RFLP. Amplificated PCR fragments of 808 were digested with *Sac*II, while amplificated PCR fragments of 319 bp were digested with *Mbo*II restriction enzyme in order to detect different genotypes of *NGF* gene. The "A" allele frequency ranged from 0.500 (HAI, HNM and KBK) to 0.950 (NRD), while the "C" allele frequency ranged from 0.025 (NRD) to 0.500 (HAI, HNM and KBK) in *NGF*/*Sac*II polymorphism. The "A" allele frequency ranged from 0.213 (HAI) to 1.000 (NRD), while the "G" allele frequency ranged from 0.000 (NRD) to 0.787 (HAI) in *NGF*/*Mbo*II polymorphism. Deviation from HW equilibrium was significant in HNM goat population ($P < 0.05$). In this study, polymorphisms of caprine *NGF* gene in native Turkish goat populations were revealed for the first time. The results obtained from this study showed that *NGF/Sac*II polymorphisms could be used for body length in NRD population while *NGF/Mbo*II polymorphisms could be used for litter size in HAI, HNM and KBK populations in MAS studies.

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ÖZ

Sinir Büyüme Faktörü *(NGF)* geni yumurtalık hücrelerinin yaşamını devam ettirmesi ve çoğalmasında önemli fonksiyonlara sahiptir. Keçi *NGF* geni üzerinde yapılan son çalışmalar, iki SNP'in (*Sac*II/C291A ve *Mbo*II/A705G polimorfizmi) vücut uzunluğu ve bir batında doğan yavru sayısı ile ilişkili olduğunu ortaya çıkarmıştır. Bu çalışmada, Türkiye'de yetiştirilen Kıl (HAI), Kabakulak (KBK), Honamlı (HNM) ve Norduz (NRD) keçi populasyonlarının *NGF* geninde *Sac*II ve *Mbo*II polimorfizminin PCR-RFLP yöntemiyle belirlenmesi amaçlanmıştır. *NGF* geninde farklı genotiplerin belirlenmesi için çoğaltılan 808 bç uzunluğundaki PCR ürünleri *Sac*II, 319 bç uzunluğundaki PCR ürünleri ise *Mbo*II restriksiyon enzimi ile kesilmiştir. *NGF/Sac*II polimorfizminde A allel frekansı 0.500 (Kıl, Honamlı ve Kabakulak) ile 0.950 (Norduz) aralığında değişirken, C allel frekansı 0.025 (Norduz) ile 0.500 (Kıl, Honamlı ve Kabakulak) aralığında değişmiştir. *NGF/Mbo*II polimorfizminde A allel frekansı 0.213 (Kıl) ile 1.000 (Norduz) aralığında değişirken, G allel frekansı 0.000 (Norduz) ile 0.787 (Kıl) aralığında değişmiştir. Honamlı populasyonunda HW dengesinden sapma önemli bulunmuştur ($P < 0.05$). Bu çalışmada, Türkiye'nin yerli keçi populasyonlarında keçi *NGF* gen polimorfizmi ilk defa ortaya çıkarılmıştır. Bu çalışmandan elde edilen sonuçlar, *NGF/Sac*II polimorfizminin Norduz populayonunda vücut uzunluğu, için, *NGF/Mbo*II polimorfizminin ise Kıl, Honamlı ve Kabakulak populasyonlarında bir batında doğan yavru sayısı için markör destekli seleksiyon (MAS) çalışmalarında kullanılabileceğini göstermiştir.

1. Introduction

A member of neurotrophin family, *NFG* is synthesized and released from ovarian cells (Dissen et al. 2001). *NGF* and its receptors have important functions in the maintenance of viability and proliferation of ovarian cells (Chaves et al. 2013). Lower primary and secondary follicles were reported in *NGF* null mutant mice which indicates role of *NGF* in follicles development (Dissen et al. 2001). In addition, overexpression of *NGF* in epidermis caused hypertrophy of the peripheral nervous system in transgenic mice (Albers et al. 1994). Expression of *NGF* and its receptors in the goat oviduct may indicate their functions in oviductal transport, fertilization, capacitation of spermatozoa and early embryonic development in the oviduct (Ren et al. 2005).

As well as in all the other countries, in Turkey, studies have being conducted to improve economically important traits such as body length and litter size in small ruminant industry. Body length is mainly calculated for phenotypic characterization of goat breeds. Litter size, determining value of animals, is one of the most important reproductive and economic traits in goat industry (Yan et al. 2018). Breeders can gain more profit by increasing reproductive traits such as litter size. Until today, many genes including *IGF-I* (Deng et al. 2010), *ATBF1* (Zhang et al. 2015), *MSTN* (Zhang et al. 2013), *STAT5A* (Wu et al. 2014), *KiSS-1* (Cao et al. 2010), *GPR54* (Cao et al. 2011), *GDF9* (Wang et al. 2019) and *KDM6A* (Cui et al. 2018) were reported to be related to body length or litter size in goat populations. Recent studies in caprine *NGF* gene revealed two SNPs related with reproductive and economic traits in goat populations (An et al. 2013; Naicy et al. 2018). An et al. (2013) reported A705G SNP in coding region of caprine *NGF* gene in which the does with GG genotype had higher litter size than those with GA and AA genotypes. Additionally, Naicy et al. (2018) reported C291A SNP in coding region of caprine *NGF* gene in which the goats with CA genotypes showed superior values for body length and body length index than those with CC genotypes. Due to absence of AA genotype, they could not analyze the relationship between AA genotype and body length trait.

Although, traditional selection methods are insufficient to rapidly increase quantitative characters due to their low inheritance and controlling by multiple genes, traditional selection methods can be supplemented Marker Assisted Selection (MAS) to increase the reproductive and economic traits (Wang et al. 2018). By using *NGF* polymorphisms in MAS studies, the frequency of desired genotypes for litter size and body length might be increased in goat populations. Unfortunately, no study has been carried out so far to reveal *NGF* polymorphisms in native Turkish goat populations. Hence, this is the first study aimed to detect polymorphisms in the caprine *NGF* gene in four native Turkish goat populations.

2. Materials and Method

2.1. Blood samples collection and DNA extraction

In this study, a total of 121 goat belonging to HAI (n= 40), KBK (n= 30), HNM (n= 31) and NOR (n= 20) were used for polymorphism analysis. HAI, KBK and HNM populations were selected from different representative herds reared in Antalya province (Turkey), whereas NOR were selected from representative herds reared in Van province (Turkey). Blood samples were collected from the jugular vein of animals into vacutainer tubes containing EDTA as an anticoagulant and stored at -20℃ until extraction. The genomic DNA was extracted from blood samples using a salting out method reported by Miller et al. (1988). Agarose gel electrophoresis was applied to check the quality of extracted DNA.

2.2. PCR-RFLP analysis

Two set of primers reported by An et al. (2013) were used to amplify 808 and 319 bp fragments of coding region of the caprine *NGF* gene (Table 1). PCR was performed in 50 µl reaction volume with 50 ng template DNA, 5 µl 10X reaction buffer, 0.6 mM dNTP, 25 mM $MgCl_2$, 10 pM of each primers, 1.5 U of *Taq* DNA polymerase and 31.25 µl nuclease free water. The cycling protocol followed with initial denaturation at 95℃ for 5 min followed by 35 cycles of denaturation at 94℃ for 30 s, annealing at 58℃ for 30 s, extension at 72℃ for 30 s with a final extension at 72℃ for 5 min. Fragments of 808 and 319 bp lengths amplified for *NGF* gene are given in Figure 1 and Figure 2, respectively. Amplified 808 and 319 bp of PCR products were digested separately with *Sac*II and *Mbo*II restriction enzymes respectively. For this purpose, 5 µl of amplified PCR products were mixed with 2.5 U restriction enzymes (*Sac*II or *Mbo*II), 2 µl 10X buffer and 5 µl nuclease free water, and then incubated for 4 h at 37℃. In order to genotype the individuals, digested products were visualized on agarose gel electrophoresis.

2.3. Statistical analysis

Popgene V. 1.32. (Yeh et al. 1997) package program was used to calculate the allele and genotype frequencies in the *NGF* gene and to test the HW equilibrium.

3. Results and Discussion

In this study, two genotypes including AA (808 bp) and CA (301, 507 and 808) were detected in *NGF*/*Sac*II polymorphism (Figure 3). All individuals of HAI, HNM, and KBK showed CA genotype which is reported to be related with higher body length. Except one, all individuals of NRD population were with AA genotype. The frequencies of AA and CA genotypes were 0.95 and 0.05 in NRD population, while the frequencies of

Table 1: Primer sequences and restriction enzymes to detect SNPs on caprine *NGF* gene

SNP	Primer Sequence	PCR Products (bp)	Enzyme	Genotypes	References
C291A	F: 5-ATAGCGTAATGTCCATGTTG-3 R: 5- ATTTACAGGTTGAGGTAGGG-3	808	*Sac*II	AA: 808 CA: 301-507-808 CC:301-507	Naicy et al. 2018
A705G	F: 5-CTGGGAGAGGTGAACATC-3 R: 5-ACAGGTTGAGGTAGGGAG-3	319	*Mbo*II	AA: 319 GA: 79-240-319 GG: 79-240	An et al. 2013

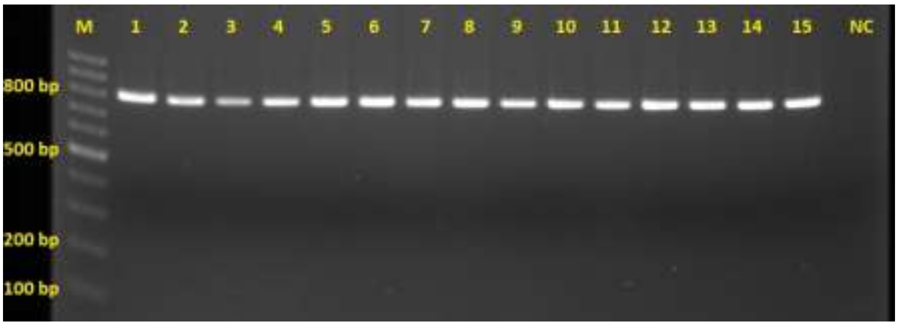

Figure 1. PCR products with 808 bp length for the *NGF* gene in goat populations, M: Thermo 100 bp ladder Cat. No: SM0241: PCR amplicons of caprine NGF gene; NC: Negative control.

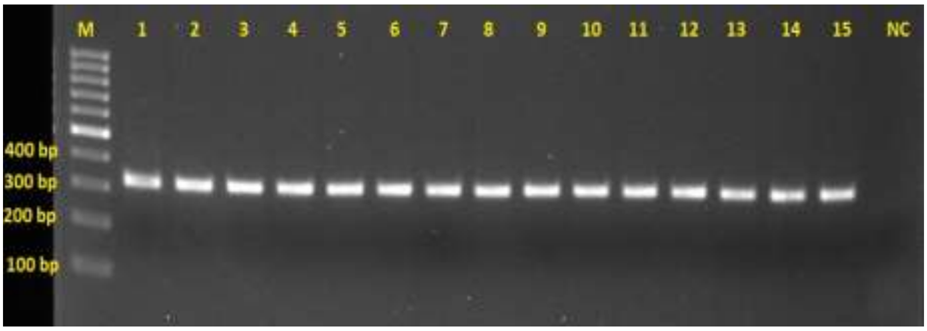

Figure 2. PCR products with 319 bp length for the *NGF* gene in goat populations, M: Thermo 100 bp ladder Cat. No: SM0241; 1-15: PCR amplicons of caprine NGF gene; NC: Negative control.

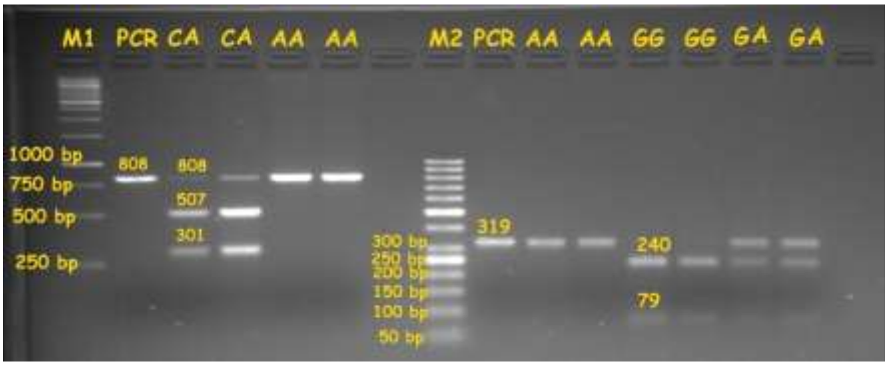

Figure 3. Digestion image of caprine *NGF* gene using *Sac*II and *Mbo*II restriction enzymes, M1: Thermo 1 kb DNA ladder; Cat. No: SM0311; M2: Thermo 50 bp DNA ladder; Cat. No: SM0371; PCR: Undigested PCR product; 2.5% agarose gel.

CA genotype was 1.00 in HAI, HNM and KBK populations (Table 2). This finding is accordant with results of Naicy et al. (2018). In a study on Malabari and Attappady Black goat breeds, Naicy et al. (2018) reported two genotypes including CC (301 and 507 bp) and CA (301, 507 and 808 bp) in caprine *NGF* gene after *Sac*II digestion. On the contrary, is it reported that the frequency of CC (0.66) was higher than the frequency of CA (0.34) in Indian goat breeds. It is also emphasized that the goats with CA genotypes had superior values for body length and body length index than those with CC genotypes.

Among the goat breeds raising in Turkey, HNM and NRD have the highest and the lowest body length trait, respectively (Yılmaz et al. 2012). KBK goat is a subtype which differs from HAI goats due to some morphological traits including ear length, live weight, etc. It is known that live weight of KBK, rearing in limited regions of Turkey including Kaş, Elmalı and Fethiye provinces, is higher than HAI goats. In this respect, it is not surprising that frequency of CA genotype, which is related to higher body length, in HAI, HNM and KBK was higher than in NRD population in this study. The results obtained in this study showed that HNM, HAI and KBK populations were monomorphic for *NGF* gene (all individuals are CA genotype). Therefore *NGF*/*Sac*II polymorphism can not be used in HNM, HAI and KBK populations for MAS studies.

In this study, AA (319 bp), GA (79, 240 and 319 bp) and GG (79 and 240 bp) genotypes were generated in *NGF*/*Mbo*II polymorphism (Figure 3). The frequency of A allele ranged from 0.213 (HAI) to 1.000 (NRD), while the frequency of G allele ranged from 0.000 (NRD) to 0.787 (HAI) (Table 2). While no individual with AA genotype was detected in HNM population, all individuals of NRD population were with AA genotype. The higher G allele frequency was detected in HAI, HNM and KBK populations. Similarly, it is reported that G allele frequencies were higher than A allele frequency in Xinong Saanen, Guanzhong and Boer goat populations (An et al. 2013). It is also reported that the does with GG genotype had higher litter size than those with GA and AA genotypes.

Table 2. Allele and genotype frequencies for *NGF* gene in four goat populations reared in Turkey.

Gen	**Populations**	**n**	**Allele Frequencies**		**Genotype Frequencies**			χ^2
			A	C	AA	CA	CC	
SacII	**NRD**	20	0.975	0.025	0.950 (19)	0.050 (1)	0.000	0.013^a
	HAI	40	0.500	0.500	0.000	1.000 (40)	0.000	-
	HNM	31	0.500	0.500	0.000	1.000 (31)	0.000	-
	KBK	30	0.500	0.500	0.000	1.000 (30)	0.000	-
	Populations	**n**	A	G	AA	GA	GG	χ^2
MboII	**NRD**	20	1.000	0.000	1.000 (20)	0.000	0.000	-
	HAI	40	0.213	0.787	0.025 (1)	0.375 (15)	0.600 (24)	0.580^a
	HNM	31	0.338	0.662	0.000 (0)	0.677 (21)	0.323 (10)	8.133^b
	KBK	30	0.233	0.767	0.067 (2)	0.333 (10)	0.600 (18)	0.140^a

$\chi^2_{0.05;1}$: 3.84; a: Deviation from H-W equilibrium is not significant, b: Deviation from H-W equilibrium is significant ($P < 0.05$).

In this study, GG genotype, which is related to higher litter size in goats, was not detected in NRD population, while variable values were detected in HAI, HNM and KBK populations. It is known that litter size ranges from 1 to 1.5 in studied goat populations (Yilmaz et al. 2012). *NGF* is not a major gene on litter size and it is possible that there are other genes affecting litter size in goats. Although HAI, KBK and HNM are not prolific populations, GG genotypes were detected in these populations. Detecting of GG genotype in HAI, HNM and KBK populations shows that *NGF/Mbo*II polymorphisms could be integrated in MAS studies in terms of litter size. Additionally, it is necessary to research other genes affecting litter size in goat populations. The use of *NGF* gene together with other genes affecting litter size will increase the success rate in MAS studies.

4. Conclusion

In this study, polymorphisms of *NGF/Sac*II related to body length and *NGF/Mbo*II related to litter size were revealed in four goat populations raising in Turkey for the first time. Desired genotypes for body length (CA) and litter size (GG) were detected in variable frequencies in native Turkish goat populations. The results of this study showed that NGF/*Mbo*II polymorphisms could be used in MAS studies for litter size in HAI, HNM and KBK populations.

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