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Analysis of Polymorphisms on GH-MspI and IGF1-SnaBI Loci in Five Turkish Native Cattle Breeds

Yasemin Öner[1*], Onur Yılmaz[2], Candan Eriş[1], Nezih Ata[2], Cihan Ünal[3], Seyrani Koncagül[4]

[1]*Department of Animal Science, Biometry and Genetics, Faculty of Agriculture, University of Uludağ, 16059 Bursa, Turkey*
[2]*Department of Animal Science, Biometry and Genetics, Faculty of Agriculture, Adnan Menderes University, 09100 Aydın, Turkey*
[3]*Department of Zootechnics, Faculty of Veterinary Medicine, Uludağ University 16059 Nilufer/Bursa, Turkey*
[4]*Department of Animal Science, Faculty of Agriculture, Ankara University, 06110 Ankara, Turkey*

A R T I C L E I N F O

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*Corresponding Author:
E-mail: aseminoner@yahoo.com

A B S T R A C T

Growth Hormone (GH) and Insulin like Growth Factor-I (IGF1) are members of somototrophine axis pathway. They play a role in key on several mechanisms such as postnatal growth, cell differentiation and metabolism. Due to their vital importance, polymorphisms on the genes coding are worth to be understood. In this study five native cattle breeds (Native Southern Yellow (NSY), South Anatolian Red (SAR), Anatolian Grey (AG), Native Black (NB), East Anatolian Red (EAR) were investigated by PCR-RFLP method for GH-MspI and IGF1-SnaBI loci. 198 and 194 samples were analyzed for GH-MspI and IGF1-SnaBI loci, respectively. In both two loci two alleles and three genotypes were observed. Predominant alleles were A and B for GH-MspI locus IGF1-SnaBI loci, respectively. Frequencies of A and B alleles were calculated between 0.400-0.875 and 0.846-0.903 for GH-MspI locus IGF1-SnaBI loci, respectively. While among investigated population only EAR population was at Hardy-Weinberg equilibrium for IGF1-SnaBI locus, for GH-MspI only, in SAR population no deviation from Hardy-Weinberg equilibrium.

Türk Tarım – Gıda Bilim ve Teknoloji Dergisi, 6(10): 1353-1356, 2018

Beş Yerli Türk Sığır Irkında GH-MspI and IGF1-SnaBI Lokuslarındaki Polimorfizmlerin Analizi

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*Sorumlu Yazar:
E-mail: aseminoner@yahoo.com

Ö Z

Büyüme Hormonu (GH) ve İnsülin Benzeri Büyüme Faktörü-I (IGF-I) somatotropin axis yolağının üyeleridirler. Doğum sonrası gelişim, hücre farklılaşması ve metabolizmada gibi mekanizmalarda anahtar rol oynarlar. Hayati önemlerinden dolayı bunları kodlayan genlerin anlaşılmaları da gereklidir. Bu çalışmada beş yerli sığır ırkı (Yerli Güney Sarısı (YGS), Güneydoğu Anadolu Kırmızısı (GAK), Boz Irk (BI), Yerli Kara (YK) ve Doğu Anadolu Kırmızısı (DAK) GH-MspI ve IGF1-SnaBI locusları bakımından PCR-RFLP yöntemi ile incelenmiştir. GH-MspI lokusu için 198, IGF1-SnaBI lokusu için 194 örnek analiz edilmiştir. Her iki lokusta iki allel ve üç genotip gözlenmştir. GH-MspI ve IGF1-SnaBI lokusları için predominant alleler sırasıyla A ve B olarak bulunmuştur. GH-MspI ve IGF1-SnaBI locusları için A ve B allelerinin frekansları sırasıyla 0,400-0,875 ve 0,846-0,903 arasında hesaplanmıştır. İncelenen populasyonlardan sadece DAK IGF1-SnaBI bakımından Hardy-Weinberg dengesindeyken, GH-MspI lokusu için sadece GAK populasyonunda Hardy-Weinberg dengesinden sapma gözlenmiştir.

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Introduction

Developments in molecular genetics and software techniques have allowed investigating and understanding genomic regions related to production traits and breed differentiation (Plath-Gabler et al., 2001). As well known genetic improvement for a particular trait is quite complex and expensive process. Using molecular genetic markers gives promises to high selection accuracy and shorter generation interval which can reduce time and money consumed to profitability in animal production. With regard to breed differentiation these markers are accepted as estimation past, current and future population structure. Thus, it is possible to obtain data to use for designing breeding and conservation schemes.

Growth Hormone (GH) and Insulin like Growth Factor-I (IGF1) are two of the most important members of somototrophine axis pathway and assume important for growth, cell differentiation, milk production and energy metabolisms. Polymorphisms may alter quality and quantity of gene's products therefore, mutations occurred in these two genes have been investigated intensively. Growth Hormone (GH) and Insulin like Growth Factor-I (IGF1) genes are located on 19th (NCBI, 2018a) and 5th chromosomes (NCBI, 2018b) of bovine genome, respectively. A C→G transvertion in 3th intron of GH gene leads to a MspI recognizing site (Hoj et al., 1993) and a T→C transition at 5' flanking region of IGF1 gene causes a SnaBI recognizing site (Ge et al., 1997). These two SNPs have been focused because of potential effects on productive traits and suggested relation of different alleles with Bos taurus and Bos indicus originated cattle breeds.

In this study investigation of all GH-MspI and IGF1-SnaBI allelic distribution among five native cattle breeds is aimed.

Material and Methods

Sampling and DNA Isolation

The study was approved by the Ethics Committee of Uludag University (UUHADYEK), (approval date: 01/09/2015; no: 2015-10/10). Blood samples were collected from original regions of five native cattle breeds.

Genomic DNA was extracted with a genomic DNA extraction kit (NucleoSpin Blood, Macherey-Nagel GmbH & Co. KG) according to the instructions provided in the manual. Afterward, quantification and qualification of DNA were controlled using NanoDrop 2000 (Thermo Scientific, USA).

PCR – RFLP Analysis

PCR-RFLP analyses were preformed according to Ge et al. (1997) and Dybus (2002) (Table 1)

Statistical Analysis

Gene and genotype frequencies expected and observed heterozygoties (He and Ho), and Chi-square ($\chi 2$) values were calculated by POPGENE32 software program (Yeh 2000). Dendrogram was drawn by MEGA 7.18 according to Kumar et al. (2016).

Results and Discussion

Amplification of target region from GH and IGF1 genes 329 bp and 249 bp PCR products were obtained, respectively. Two alleles named A (224 bp and 105 bp) and B (329 bp) were observed for GH- MspI locus and A (223 bp and 26 bp) and B (249 bp) (Figure 1).

Gene and genotype frequencies expected and observed heterozygotes (He and Ho) were given in Table 2.

Table 1 Primer sequences, temperature for annealing step, PCR product sizes (bp) and restriction enzymes were used

Loci	Primer sequencing (5′→3′)	Annealing (°C)	PCR products	RE	Literature
IGF1	ATTACAAAGCTGCCTGCCCC ACCTTACCCGTATGAAAGGAATATACGT	58	249 bp	SnaBI	Ge et al. (1997)
GH	CCCACGGGCAAGAATGAGGC TGAGAACTGCAGGGGCCCA	52	329 bp	MspI	Dybus (2002)

Table 2 Population parametres for GH-MspI and IGF1-SnaBI loci

Locus	n	Allele frequencies		Genotype frequencies (%)			He	Ho	$\chi 2$
		A	B	AA	AB	BB			
Native Southern Yellow (NSY)									
GH	40	0.625	0.375	40	45	15	0.450	0.469	0.064^{ns}
IGF1	39	0.154	0.846	7.70	15.40	76.90	0.154	0.260	6.526*
Native Black (NB)									
GH	40	0.400	0.600	17.50	45	37.50	0.450	0.480	0.156^{ns}
IGF1	39	0.154	0.846	15	0	85	0.000	0.260	39.000***
Anatolian Grey (AG)									
GH	40	0.638	0.363	42.50	42.50	15	0.425	0.462	0.259^{ns}
IGF1	36	0.097	0.903	8.33	2.78	88.89	0.028	0.176	25.508***
East Anatolian Red (EAR)									
GH	40	0.875	0.125	77.50	20	2.50	0.200	0.219	0.294^{ns}
IGF1	40	0.113	0.888	2.5	17.5	80	0.175	0.200	0.611^{ns}
South Anatolian Red (SAR)									
GH	38	0.645	0.355	34.20	60.50	5.30	0.605	0.428	3.921*
IGF1	40	0.113	0.888	5.13	12.82	82.05	0.125	0.200	5.596*

ns=not significant, * P<0.05, ** P<0.01, *** P<0.001

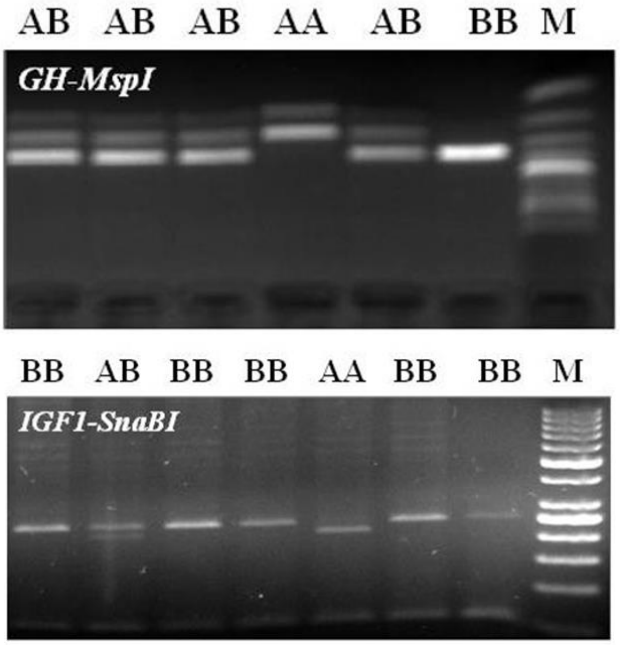

Figure 1 Electrophoretic illustration of GH-MspI and IGF1-SnaBI loci. (M: Marker, for GH-MspI 100bp, for IGF1-SnaBI 50 bp)

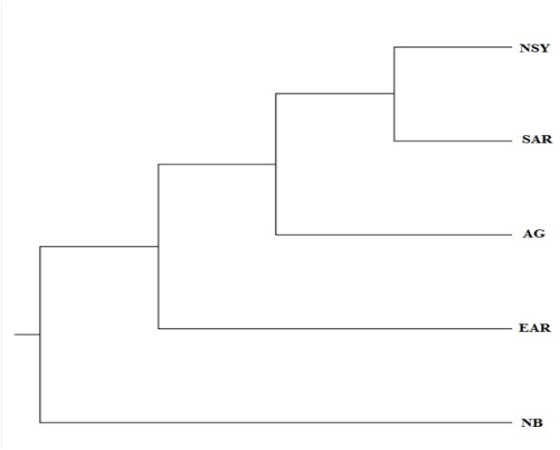

Figure 2 Dendrogram drawn by PCR-RFLP results of GH-MspI and IGF1-SnaBI loci.

Allele and genotype frequencies observed for the two loci were concordance with the other authors from different countries and studies carried out in Turkish native cattle breeds (Yao et al., 1996; Zhou et al., 2005; Curi et al., 2005; Özkan et al., 2009; Akış et al., 2010; Reyna et al., 2010).

Wright's F-statistic (F_{IT}, F_{IS}, F_{ST}) values were estimated at -0.020, 0.079 and 0.098 for GH-MspI and 0.560, 0.563 and 0.005 IGF1-SnaBI loci. Lower heterozygosity level in IGF1-SnaBI locus is indicated from these Fs values (Table 2).

Different allelic distribution for GH-MspI and IGF1-SnaBI loci among *B. indicus* and *B. taurus* related breeds has been reported by several authors (Lagziel et al. 2000; Curi et al. 2005; Sodhi et al. 2007). It is suggested that B allele was associated with *B. taurus* and *B. indicus* for GH-MspI and IGF1-SnaBI loci, respectively. According to PCR-RFLP analysis A allele was predominant at GH-MspI locus except for NB population. For IGF1-SnaBI locus B allele was found as prevalent among all breeds as concordance with the other studies previously. Although in some studies this allele was found as fixed (Curi et al., 2005; Li et al., 2006) or nearly fixed (Reyna et al., 2010; Yurnatis et al., 2017), in some *B. taurus* orjinated breed populations it has reached till 0.90 (Lirón et al., 2012).

Due to origin related allelic distribution we also drew a UPGMA dendrogram (Figure 2) by using GH-MspI and IGF1-SnaBI allele frequencies.

The dendrogram obtained was quite similar to those of the others by drawn using autosomal (Özşensoy et al., 2010) and unipaternal markers (Özdemir et al., 2009; Özşensoy et al., 2014).

According to dendrogram obtained from PCR-RFLP results while SAR and NSY breeds clustered together, AG located near to these two breeds. Native Black breed separated from the rest of the breeds.

While IGF1-SnaBI locus were found influential on carcass and growth traits (Curi et al., 2005; Reyna et al., 2010; Nicolini et al., 2013; Siadkowsca et al., 2006; Szewzuk et al., 2013), no relationship were found with milk production traits in neither in Montbeliarde and Iranian Holstein breeds (Mehmenavaz et al., 2010; Szewzuk, 2006) nor NSY and SAR breeds from Turkey (Akış et al., 2010) Associations with GH-MspI polymorphism has been reveal not only for milk (Hoj et al., 1993; Yao et al., 1996; Lagziel et al., 1999; Zhou et al., 2005; Pawar et al., 2007; Rincón et al., 2013) and meat production traits (Unanian et al., 2000; Hernández et al., 2016) but also has been revealed for fertility and reproduction (Unanian et al., 2002; Gorbani et al., 2009; Mullen et al., 2011; Arango et al., 2014; Öner et al., 2017).

These investigated two loci may be important for phenotypic traits due to their critical roles in metabolic activities. In our study due to lack of the phenotypic data it has not been possible for any association analysis. However, it is clear that at least GH-MspI locus exhibit an appropriate distribution in order to design association analysis in Turkish native cattle breeds. Further studies should be carried out to figure out functional importance of these mutations and relationships with phenotypic traits. On the other hand, these two loci might be used in diversity studies with more RFLP loci. By using more suitable RFLP loci accuracy of phylogenetic trees observed may be increased.

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