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

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ABSTRACT

Growth Hormone (GH) and Insulin like Growth Factor-I (IGF1) are members of somatomotrophine 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.

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Ö Z

Büyüme Hormonu (GH) ve İnsülin Benzeri Büyüme Faktörü-I (IGF-I) somatotropin axis yolasının yetenidirler. Doğum sonrası gelişim, hücre farklılaşma ve metabolizmada gibi mekanizmalarda anahtar rol oynarlar. Hayati önemlerinden dolayı bunları kodlayan genlerin anlaşılmasını da gerektirir. 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 bakımdan PCR-RFLP yöntemi ile incelenmiştir. GH-MspI locusu için 198, IGF1-SnaBI locusu için 194 örnek analiz edilmiştir. Her iki locusta iki allele ve üç genotip gözlemmiştir. GH-MspI ve IGF1-SnaBI locusları için prevalan allel srasıyla A ve B olarak bulunmuştur. GH-MspI ve IGF1-SnaBI locusları için prevalan allel srasıyla A ve B alleleinin frekansları srasıyla 0,400-0,875 ve 0,846-0,903 arasında hesaplanmıştır. Incelenen popülasyonlardan sadece DAK IGF1-SnaBI bakımdan Hardy-Weinberg dengesindeyken, GH-MspI locusu için sadece GAK popülasyonunda Hardy-Weinberg dengesinden sapma gözlemmiştir.

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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 transversion in 3rd 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 heterozygoties (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’) | Annaling (°C) | PCR products | RE | Literature |
|-------|---------------------------|---------------|--------------|-----|------------|
| GH    | ATTACAAAGCTGCTGGCCC       | 58            | 249 bp       | SnaBI | Ge et al. (1997) |
|       | ACCTTACCCGTATGAAGGAAATATACGT |               |              |     |            |
| IGF1  | CCCCAGGGGAAAGATGAGGC      | 52            | 329 bp       | MspI | Dybus (2002) |
|       | TGAGAACTGCGAGGGCCCA        |               |              |     |            |

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

| Locus            | n  | Allele frequencies | Genotype frequencies (%) | He   | Ho   | $\chi^2$ |
|------------------|----|-------------------|-------------------------|------|------|----------|
|                  |    | A                 | B                        | A    | AB   | BB       | ns        |
| Native Southern Yellow (NSY) |    |                   |                         |      |      |          |
| GH               | 40 | 0.625             | 0.375                    | 40   | 45   | 15       | 0.450     | 0.469     | 0.064*    |
| 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*    |
| 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*    |
| 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*    |
| IGF1             | 40 | 0.113             | 0.888                    | 2.5  | 17.5 | 80       | 0.175     | 0.200     | 0.611*    |
| 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
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_I, F_S, F_T) 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; Özkan et al., 2009; Akış et al., 2010; Reyna et al., 2010).

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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