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METİN ERDOĞAN

MUSTAFA TEKERLİ

KORAY ÇELİKELOĞLU

ÖZLEM HACAN

SERDAR KOÇAK

See next page for additional authors

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Authors
METİN ERDOĞAN, MUSTAFA TEKERLİ, KORAY ÇELİKELOĞLU, ÖZLEM HACAN, SERDAR KOÇAK, ZEHRA BOZKURT, SAMET ÇİNKAYA, and MUSTAFA DEMİRTAŞ

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Associations of SNPs in GHR gene with growth and milk yield of Anatolian buffaloes

Metin ERDOĞAN[1,*], Mustafa TEKERLİ[2], Koray ÇELİKELOĞLU[2], Özlem HACAN[2], Serdar KOÇAK[2], Zehra BOZKURT[2], Samet ÇİNKAYA[2], Mustafa DEMİRTAŞ[2]

[1]Department of Veterinary Biology and Genetics, Faculty of Veterinary Medicine, Afyonkarahisar, Turkey

[2]Department of Animal Science, Faculty of Veterinary Medicine, Afyonkarahisar, Turkey

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Abstract: This study was carried out to investigate the associations between single nucleotide polymorphisms (SNP) in the growth hormone receptor (GHR) gene and growth and milk yields of Anatolian buffaloes. Growth records of genotyped 3012 Anatolian buffaloes and 467 lactations of 329 cows from them were used. The overall means of weights at birth, six and 12 months of ages and lactation milk yield were 29.377 ± 0.342 kg, 96.15 ± 1.48 kg, 165.54 ± 2.18 kg, and 976.3 ± 39.9 kg, respectively. Three mutations (g.31601787 G > A, g.31601784 G > A, and g.31601783 G > del) in GHR were detected. A least-squares analysis was carried out to determine the fixed effects of some environmental factors and these SNPs. The g.31601784 G > A mutation had a significant ($p < 0.05$) effect on weights at 12 months of age. The cows with GG genotype in the location of g.31601783 were superior ($p < 0.05$) in milk production. These results indicate that the SNP markers in the GHR gene could be associated with economically important traits and provide some advantages to breeders for the selection of buffaloes in early age.

Key words: Buffalo, growth hormone receptor (GHR) gene, growth, lactation, single nucleotide polymorphisms (SNP), selection

1. Introduction

The world's buffalo population has been growing due to increased demand for alternative food products. Today, approximately 207 million buffaloes are reared in the world and the number of water buffaloes (178397) in Turkey has doubled in the last decade after the launch of a Community-Based Buffalo Improvement Program conducted by the Ministry of Agriculture and Forestry[1]. The Anatolian buffaloes are in the sub-group of riverine buffalo (Bubalus bubalis bubalis) with the chromosome number of 50 (2n). Buffalo milk and meat produced in Turkey are utilized in manufacturing of deli products such as kaymak, yogurt, cheese, and sujuk. Genetic improvement in farm animals is essential to enhance the productivity of dairy and meat industry. The average milk yield of Anatolian buffalo is ranging between 763 and 1223 kg [1–4].

Selection is a powerful tool for animal breeders, and considering the nongenetic factors affecting milk yield and growth is important for efficient selection program [1, 2, 5–7]. Environmental factors affecting growth and milk production of Anatolian buffaloes were studied by different researchers in Turkey [1–3, 5, 8–11]. These traits are also genetically controlled by many genes and some molecular methods including DNA and RNA technologies have come into use in animal husbandry to aid the selection. One of the most prominent gene affecting growth is growth hormone receptor (GHR) gene. The GHR, a member of the type 1 cytokine / hematopoietin receptor family, serves as a mediator for the growth hormone (GH) to carry out its effect on post-natal growth and milk production [12, 13]. Therefore, GHR gene can be used in marker assisted selection. A lot of studies [13–21] have been conducted to associate polymorphisms in the growth hormone receptor gene with growth and milk yield in cattle.

Nevertheless, studies searching associations between mutations of the GHR gene and economic traits in buffaloes are very scarce. Çelikeloğlu et al. [9] conducted a study in a small sample of Anatolian buffaloes and notified three mutations (G3676A, G3679A and G3680del) for growth characteristics. Additionally, two novel mutations (c.380 G > A and c.836 T > A) related with milk production in Egyptian Buffaloes were also reported by El-Komy et al. [12].

The aim of this paper is to reveal the effects of previously reported [9] SNPs in GHR gene on growth and milk yield

[1] www.fao.org.

* Correspondence: erdogan@aku.edu.tr

of Anatolian buffaloes registered in community-based animal breeding project.

2. Materials and methods

This study was conducted under the project (TAGEM / 18 / AR – GE / 12) of Turkish Ministry of Agriculture and Forestry, General Directorate of Agricultural Research and Policies (TAGEM) and approved by the experimental animal ethics committee of the Afyon Kocatepe University (49533702 / 59).

This study was carried out in Afyonkarahisar province, Turkey. Growth and milk yield records of Anatolian buffaloes registered in the National Community-Based Buffalo Improvement Project conducted by the TAGEM. The data were retrieved from the database software named Manda Yıldızı[2] programmed for the project. Individual records of 3012 buffalo calves born in 11 growing sites of Afyonkarahisar between 2013–2017 were processed. Growth records of all calves included weights at birth, 6 and 12 months of ages, and a total of 467 lactations recorded from 2017 to 2019 belonging to 329 animals from this group were analyzed. The live weights at 6th and 12th months were estimated by the interpolation method according to Gürtan [22].

Blood samples of 3012 animals were collected from *V. jugularis* to vacuum tubes, including anticoagulant (EDTA). Genomic DNAs were extracted from blood samples using a DNA extraction kit (Thermo Fisher Scientific, K0722). Primers reported by Çelikeloğlu et al. [9] were used. The PCR mixture has consisted of 20 ng DNA, 3 mM of each primer, 1XPCR buffer with 2 mM $MgCl_2$ (Thermo), 2 mM dNTPs, and 1 U of Phusion Taq DNA Polymerase (Thermo) in a total volume of 25 µL. The thermal cycler (Veriti, Applied Biosystems) was programmed for 1 cycle at 98 °C for 10 min;,35 cycles at 98 °C for 20 s, at 64 °C for 30 s, and at 72 °C for 30 s, 1 cycle at 72 °C for 5 min. PCR products were electrophoresed on 2% agarose gel and were visualized with RedSafe (iNtRON, 21141) nucleic acid staining solution and VisionCapt (Bio-Vision, Vilber Louramat) imaging system. The PCR products were cleaned by Exonuclease I (Thermo Fisher Scientific, EN0582) and FastAP Thermosensitive Alkaline Phosphatase (Thermo Fisher Scientific, EF0652). The sequencing PCR and protocol of ethanol - EDTA cleaning were applied according to manufacturer's manual (Applied Biosystems). An ABI 3500 DNA sequencer (Applied Biosystems, Foster City, CA, USA) was used for analysing the sequencing products. DNA sequences were edited in Sequencher 4.5 (Gene Codes Corporation, Ann Arbor, MI, USA) and aligned in BioEdit 7.2.0 [23]. Genotype and allele frequencies and heterozygosity were calculated by one of the R packages named genetics by Warnes et al.[3] A microRNA database (miRBase)[4] were used to detect microRNAs binding to the region of SNPs.

Associations between each SNP and performance were analyzed by using the GLM procedure of Minitab 18.1[5] with the following models.

Model 1 for growth traits: $Y = \mu + V + BY + BS + S + DA + BW + SNP1 + SNP2 + SNP3 + e$,

where Y = observation value, µ = overall mean, V = effect of growing site (1–11), BY = effect of birth year (2013–2017), BS = effect of birth season (Winter, Spring, Summer, Autumn), S = effect of sex (male and female), DA = effect of dam age (≤ 1095, > 1095 and 2555 < , ≥ 2555), BW = effect of birth weight (≤ 25 and > 25), SNP1 = effect of g.31601787 G > A, SNP2 = effect of g.31601784 G > A, SNP3 = effect of g.31601783 G > del and e = random error N (0, σ^2) related with each observation.

Model 2 for milk yield: $Y = \mu + V + Y + M + P + LL + SNP1 + SNP2 + SNP3 + e$,

where Y = observation value, µ = overall mean, V = effect of growing site (1–10), Y = effect of calving year (2017–2019), M = effect of calving month (1–12), P = effect of parity (1, 2 and 3), LL= effect of lactation length (< 230 and ≥ 230 day), SNP1 = effect of g.31601787 G > A, SNP2 = effect of g.31601784 G > A, SNP3 = effect of g.31601783 G > del and e = random error N (0, σ^2) related with each observation.

3. Results and discussions

Genotype and allele frequencies and heterozygosities for SNP1, SNP2, and SNP3 mutations in studied buffalo calves are presented in Table 1. Sanger sequencing results of these three mutations in the 3'UTR region of GHR gene are also given in Figure 1.

Least squares means for the factors affecting live weights at birth, 6 and 12 months of age are shown in Table 2. The effect of growing site, birth year, and sex were significant ($p < 0.05$) in all growth traits. Overall mean for birth weight was in the ranges of the results of different researchers in Anatolian [3, 8, 9] and Murrah [24–26] buffaloes. Pandya et al. [27] reported that slightly lower birth weight (24.60 kg) than that of present study. Age of dams significantly contributed to the variation in birth weight of calves. The least squares means showed that the older cows freshened heavier calves are in agreement

[2] Tekerli M (2019). Manda Yıldızı: Data recording and processing software for buffaloes.

[3] https://cran.r-project.org/web/packages/genetics/index.html

[4] www.mirbase.org/

[5] https://www.minitab.com/en-us/products/minitab/

Table 1. SNP position, allele and genotype frequencies and heterozygosity for three SNPs used in the association study for Anatolian buffaloes.**SNP**

	Position numbers in Chromosome 19 (NC_037563.1)	**Allele Frequencies (%)**		**Genotype Frequencies (%)**			**Heterozygosity (He)**	**Hardy Weinberg p-Value**
SNP1	31601787	A (0.17)	G (0.83)	AA (0.07)	AG (0.21)	GG (0.72)	0.283	0.0001
SNP2	31601784	A (0.11)	G (0.89)	AA (0.06)	AG (0.11)	GG (0.83)	0.202	0.0001
SNP3	31601783	del (0.52)	G (0.48)	deldel (0.31)	delG (0.43)	GG (0.26)	0.498	0.0001

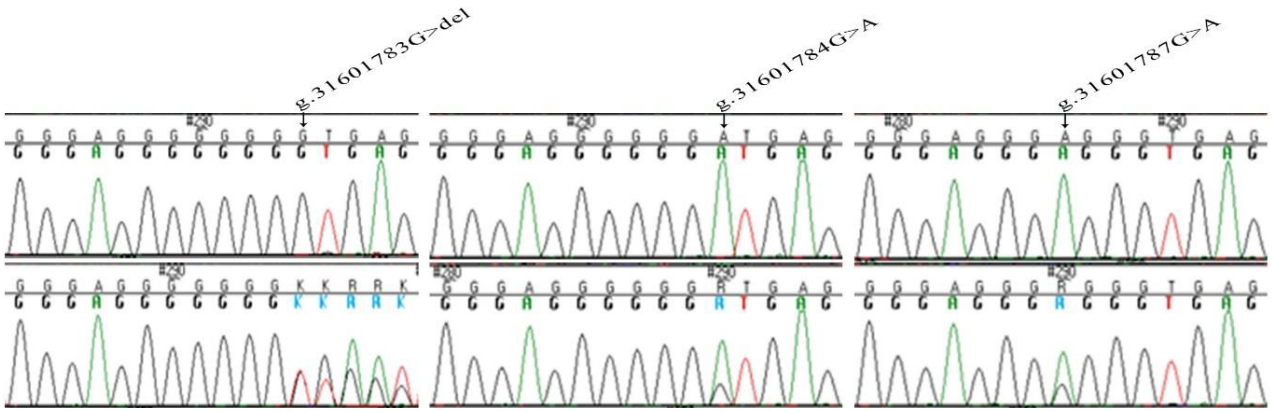

Figure 1. Illustrations of Sanger Sequencer results of the SNPs in GHR gene in studied buffalo calves.

with the findings of Yilmaz et al. [3] who studied with Anatolian breed in Bitlis province. The average live weight at six months of age was among the findings of varying studies in Anatolian [3, 9, 10], Murrah [25] and Surti [27] buffaloes. The average live weight at 12 months of age in the current study was lower than the findings of [8, 9] but higher than the results of Yilmaz et al. [3] in Anatolian and Pandya et al. [27] in Surti buffaloes. There was a gradual increase in yearling weights throughout the years. Developments in both birth weights and yearling weights of calves may be a consequence of selection program in community-based improvement project. The calves born in autumn had the highest live weights at 12 months of age. Spring and summer born calves did not differ from each other but significantly ($p < 0.05$) lower than the others. The fact that the calves keeping indoors and, the autumn and winter calves to be highly suppressive on the later-born calves due to farm management could be accounted for this situation. In the current study, the birth weight had significant ($p < 0.05$) effect on both live weight at six and 12 months of age. Calves heavier than 25 kg at birth were heavier in yearling weights. Anatolians can be thought as in size of moderate among buffalo breeds, but the findings of present and other studies showed that their growth can be affected from different environmental factors including climatic conditions and feeding and management systems.

Least squares means for lactation milk yield in studied Anatolian buffaloes are presented in Table 3. The effects of calving year, calving month, parity, and lactation length were found to be significant ($p < 0.05$). The milk yield fluctuated throughout the years due to environmental factors. The highest milk yield was obtained in February calvers. The overall mean of lactation milk yield determined in this investigation (976.3 kg) was between the values reported by Koçak et al. [1], Tekerli et al. [2] and Yilmaz et al. [3] in Anatolian (763.99–1087.49 kg) and Karoly et al. [28] in Greece and Bulgaria (700–1800 kg), and were lower than that of Verma et al. [29] and Kumar et al. [30] in Murrah buffaloes (1365.00–2253.88 kg).

The effects of three SNP mutations in the GHR gene were analyzed together and it was observed that the SNP3 tended to be significant ($p < 0.10$) in birth weight. The data were not enough to detect the significance of the differences in this trait. There were no significant effects of the other two mutations (SNP1 and SNP2) on this character. All three SNPs on live weight at 6th month were insignificant. While the effect of the SNP2 mutation was significant ($p < 0.05$) on the live weight at 12th month, the effects of the SNP1 and SNP3 mutations were found to be insignificant. Calves carrying AA genotype were heavier in live weight at 12 months compared to GG genotype. The effect of the SNP3 on milk yield was significant ($p < 0.05$).

Table 2. Least squares means for Growth traits in Anatolian buffaloes.

Factors	***n***	**Birth Weight (kg)**	**6th Month Weight (kg)**	**12th Month Weight (kg)**
μ	3012	29.377 ± 0.342	96.15 ± 1.48	165.54 ± 2.18
SNP1				
A / A	201	29.432 ± 0.490	95.42 ± 2.10	165.76 ± 3.08
A / G	628	29.368 ± 0.377	96.47 ± 1.63	164.95 ± 2.39
G / G	2183	29.331 ± 0.288	96.55 ± 1.27	165.91 ± 1.86
SNP2				*
A / A	177	29.779 ± 0.495	97.31 ± 2.12	168.60 ± 3.12^{a}
A / G	334	29.157 ± 0.411	95.72 ± 1.77	165.40 ± 2.59^{ab}
G / G	2501	29.194 ± 0.283	95.41 ± 1.24	162.63 ± 1.83^{b}
SNP3		†		
del / del	923	29.039 ± 0.314	95.98 ± 1.36	164.89 ± 2.00
del / G	1309	29.546 ± 0.360	95.82 ± 1.57	165.56 ± 2.30
G / G	780	29.546 ± 0.426	96.64 ± 1.83	166.18 ± 2.69
Birth Year		**	***	***
2013	29	28.239 ± 0.904^{c}	94.98 ± 3.83^{b}	162.84 ± 5.62^{bcd}
2014	741	29.141 ± 0.331^{c}	100.42 ± 1.46^{ab}	153.42 ± 2.14^{d}
2015	887	29.506 ± 0.327^{bc}	84.01 ± 1.44^{c}	156.26 ± 2.11^{c}
2016	798	30.024 ± 0.333^{a}	98.70 ± 1.45^{b}	170.16 ± 2.13^{b}
2017	557	29.974 ± 0.335^{ab}	102.62 ± 1.46^{a}	185.04 ± 2.15^{a}
Birth Season			**	***
Winter	318	29.405 ± 0.411	94.17 ± 1.77^{b}	167.38 ± 2.61^{b}
Spring	1393	29.388 ± 0.344	97.46 ± 1.49^{a}	160.87 ± 2.18^{c}
Summer	1092	29.472 ± 0.358	95.09 ± 1.55^{b}	161.19 ± 2.28^{c}
Autumn	209	29.242 ± 0.453	97.86 ± 1.94^{a}	172.73 ± 2.86^{a}
Sex		***	**	***
Male	1507	30.108 ± 0.352^{a}	97.25 ± 1.53^{a}	168.10 ± 2.25^{a}
Female	1505	28.645 ± 0.351^{b}	95.04 ± 1.52^{b}	162.99 ± 2.23^{b}
Maternal Age (day)		***		
≤ 1095	243	28.482 ± 0.439^{c}	95.06 ± 1.88	165.93 ± 2.75
> 1095 and < 2555	1393	29.564 ± 0.344^{b}	96.67 ± 1.50	164.74 ± 2.20
≥ 2555	1376	30.085 ± 0.345^{a}	96.71 ± 1.51	165.96 ± 2.21
Birth Weight (kg)			***	*
≤ 25	327	-	93.65 ± 1.72^{b}	163.45 ± 2.53^{b}
> 25	2685	-	98.64 ± 1.46^{a}	167.64 ± 2.15^{a}

†: p < 0.10, *: p < 0.05, **: p < 0.01, ***: p < 0.001, and μ: Overall mean.

The highest milk yield was reached in buffalo cows with GG genotype in this SNP. Al-Husseini et al. [31] reported that miRNAs that regulate the expression of mRNA post-transcriptionally in most biological pathways, mutations in the region of detected SNPs in this study may cause changes in a similar manner. According to Vidal-Gómez et al. [32], miR-6750-3p miRNA is associated with the development and functionality of the reproductive system, organ development and disorders in humans. Therefore, the SNP3 may also have a potential in buffaloes due to miR-6750-3p miRNA can only bind to the region in the presence of G allele.

In conclusion, significant environmental factors found in this study must be considered when evaluating

Table 3. Least squares means for milk yield in studied buffaloes.

Factors	***n***	**Milk Yield (kg)**
μ	467	976.3 ± 39.9
SNP1		
A / A	53	1007.5 ± 56.2
A / G	68	974.9 ± 50.5
G / G	346	946.4 ± 31.6
SNP2		†
A / A	42	989.8 ± 56.9^{ab}
A / G	36	1016.8 ± 59.3^{a}
G / G	389	922.3 ± 29.8^{b}
SNP3		*
del / del	143	942.9 ± 35.5^{b}
del / G	214	954.3 ± 42.9^{b}
G / G	110	1031.6 ± 53.9^{a}
Calving Year		*
2017	90	926.6 ± 51.1^{b}
2018	171	1022.5 ± 41.8^{a}
2019	206	979.7 ± 38.8^{ab}
Calving Month		*
1	13	943.1 ± 81.9^{b}
2	26	1156.3 ± 63.6^{a}
3	73	985.5 ± 49.4^{b}
4	55	961.6 ± 50.1^{b}
5	59	990.2 ± 50.1^{b}
6	58	912.0 ± 50.9^{b}
7	45	1005.1 ± 52.7^{b}
8	48	1008.9 ± 53.8^{b}
9	37	1010.4 ± 55.8^{b}
10	22	902.5 ± 64.5^{b}
11	13	960.9 ± 77.0^{b}
12	18	878.8 ± 68.5^{b}
Parity		**
1	282	922.2 ± 37.1^{b}
2	145	1006.6 ± 43.1^{a}
3	40	1000.0 ± 56.9^{ab}
Lactation Length (day)		***
< 230	234	838.8 ± 40.5^{b}
≥ 230	233	1113.7 ± 43.1^{a}

†: $p < 0.10$, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$ and μ: Overall mean.

the animals for their growth and lactation yield. Due to influential effects of SNP2 on yearling live weight and SNP3 on milk yield, it can be suggested that these two SNPs could be useful genetic markers for commercial traits in buffaloes. Further research is needed to characterize more genomic regions of the same gene.

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Conflict of interest
All authors declare there is no conflict of interest among them and with any other people or corporations.

Ethical statement
This study was approved by the Afyon Kocatepe University Animal Experiments Local Ethics Committee (49533702/59).

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