AGRICULTURE AND NATURAL RESOURCES

Journal homepage: http://anres.kasetsart.org

Research article

HMGA2 gene polymorphisms and their effects on main growth traits indices in Awassi and Karakul sheep

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Article Info

Article history:
Received 17 December 2019
Revised 22 May 2020
Accepted 30 May 2020
Available online 30 December 2020

Keywords:
Awassi,
HMGA2 gene,
Karakul,
Production traits

Abstract

Associations were identified between the genetic polymorphism of the High Mobility Group AT-hook 2 (*HMGA2*) gene and growth traits in Awassi and Karakul breeds. Four polymerase chain reaction amplicons were designed to cover the coding regions of this gene. Several growth traits were analyzed every 3 mth until age 1 yr, namely body weight and length, wither and rump heights, chest and abdominal circumferences and average daily gains. The repeated measure analysis approach was used to analyze the association between *HMGA2* polymorphism and growth traits. P amplicons were genotyped using single strand conformation polymorphism. Two genotypes were detected in exon 2 (TT and TA) and exon 3 also had two genotypes (AA and AB). The sequencing results showed one novel nucleic acid substitution in the TA genotype (intron1:45 A > T) and one novel deletion in the AB genotype (intron2:42 A del). Individuals with the TA genotype had significantly ($p < 0.001$) higher values of the majority of growth rate indices than individuals with the TT genotype, except for birth chest circumference, birth abdominal circumference, and average daily 6–9 mth gain. Individuals with the AB genotype showed significant ($p < 0.05$) associations with 6 mth and 12 mth weights, 6 mth wither height, rump height and abdominal circumference compared to individuals with the BB genotype. In conclusion, there was a tight association between intron1:45 A > T and better growth trait performance, supporting a link for this nucleic acid substitution with the improved growth traits. This is the first report suggesting ovine *HMGA2* as a possible candidate marker for improving growth traits in Awassi and Karakul sheep.

Introduction

Production traits assessments are extremely important to the sheep industry; however, the improvement of these traits using classical breeding approaches has proven to be hard to control due to many limiting factors, such as being labor- and time-consuming and the low accuracy of traditional selection (Blair and Garrick, 2007). Instead, another approach has increasingly been reported in the literature with rapid and successful outcomes, based on the genotyping of several genetic loci to resolve their possible association with production traits (Nassiry et al., 2006). Within these genes, the High Mobility Group A2 (*HMGA2*) gene has recently been suggested to play this crucial role in genetic polymorphism (Aguiar et al., 2018). This gene is a member of a nuclear family that encodes for non-histone architectural transcription factors involved in the binding with the minor groove of AT-rich DNA sequences to induce 3D changes in the chromatin structure. This major change has been conducted via three short basic repeats called "AT-hooks", located in a highly conserved

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https://doi.org/10.34044/j.anres.2020.54.6.03

DNA binding domain of the HMGA2 protein (Cattaruzzi et al., 2007). These proteins can alter the chromatin structure by interacting with the transcription machinery and thus, they can effectively control the transcriptional activity of several genes involved in the cell proliferation (Gao et al., 2017). Consequently, HMGA2 proteins have been implicated in a variety of metabolic pathways, such as cell cycle regulation, DNA damage repair, apoptosis, senescence and telomere restoration (Zhang et al., 2019). Furthermore, it has been reported that mutations in the *HMGA2* gene affect fetal and testis development and the size of animals (Zorrilla and Yatsenko, 2013). Thus, the HMGA2 protein has been considered as a key regulator for proper growth and development (West et al., 2019), and its deficiency may lead to dwarfism in pigs, rabbits and mice (Federico et al., 2014; Carneiro et al., 2017;Chung et al., 2018). These data suggested the *HMGA2* gene as an interesting target for genetic polymorphism studies as its alteration can be associated with size alteration in pigs and other mammalian species such as mice and horses (Makvandi-Nejad et al., 2012; Federico et al., 2014; Chung et al., 2018). In addition, the genetic polymorphism of this gene has also been associated with body height indices in humans and dogs (Jones et al., 2008; Fusco et al., 2016). According to these studies, it is may be that the genetic polymorphism in the *HMGA2* gene has a noticeable association with growth traits in sheep. Furthermore, as many genes are regulated by the HMGA2 protein, any genetic substitution in the *HMGA2* gene may be associated with several alterations of production traits. Therefore, the study of the *HMGA2* genetic polymorphism in proliferative sheep can be informative when it is associated with the main growth traits indices. To date, no polymorphism study has been reported for this gene in any ovine breed. Therefore, this study was conducted to screen the *HMGA2* coding sequences and their flanking regions to assess whether or not there is a possible association with several growth indices in two proliferative breeds of sheep (Awassi and Karakul).

Materials and Methods

Animals

The study involved 230 newborn lambs (*Ovis aries*), consisting of 118 (53 females and 65 males) Awassi and 112 (49 females and 63 males) Karakul breeds. The Awassi and Karakul breeds prevail in Iraq and Iran, respectively, and are proliferative breeds raised for wool and meat production (Kafi et al., 2004; Galal et al., 2008). Both breeds were raised in the Barakat Abu al Fadhl Al-Abbas Station for raising sheep (Al-Khafeel Co.; Karbala, Iraq). All animal experimental protocols that involved both breeds were conducted in the same breeding station. All the involved lambs were kept under the same raising conditions. The traits of body weight and length, wither and rump heights, chest and abdominal circumferences, and average daily gain were measured with 3 mth intervals from birth to age 12 mth. These growth traits were collected using the standards described in Zhao et al. (2017). Sheep experiments were conducted in accordance with guidelines for the care and use of agricultural animals (Vaughn, 2012). The experimental procedures of this study were approved by the Animal Committee of Al-Qasim Green University, Babil, Iraq

Sample collections and DNA extraction

Fresh blood samples were collected from sheep and stored in anti-coagulation tubes. The genomic DNA was isolated using a method described by Al-Shuhaib (Al-Shuhaib, 2017). The integrity of the extracted DNA was assessed using agarose gel electrophoresis, while the purity (260/280 optical density) and quantity (in micrograms per milliliter) were validated using a Nanodrop spectrophotometer (Biodrop; UK).

Polymerase chain reaction primers design and polymerase chain reaction

Based on the referring sequences of the ovine *HMGA2* gene (GenBank acc. no. NC_019459.2), four pairs of polymerase chain reaction (PCR) primers were designed in this study to cover the coding portions of exon 1 to exon 4 and their flanking regions using the NCBI primer BLAST (Ye et al., 2012; Fig. 1A). The details of the primer sequences are described in Table 1. The lyophilized PCR primers were purchased from Bioneer (Korea). The PCR experiments were conducted using a lyophilized PCR PreMix (Cat # K-2012; Bioneer; Korea). Both forward and reverse primers were added, 10 pmol each, to the PCR pre-mix. Then, 20 ng of the genomic DNA template was added. The annealing temperatures were determined empirically using a gradient PCR thermocycler (Mastercycler-nexus; Eppendorf, Germany). The PCR program was: 1 cycle of initial denaturation at 95°C for 5 min, 30 cycles of denaturation at 94°C for 30 s, annealing for 30 s, elongation at 72°C for 30 s and a final extension at 72°C for 5 min. PCR amplicons were separated using electrophoresis on a 1.5% (weight per volume) agarose gel in parallel with a DNA marker before being exposed to downstream genotyping reactions.

Genotyping and sequencing

Genotyping experiments were conducted using PCR single strand conformation polymorphism (PCR-SSCP) according to the instructions in Al-Shuhaib et al. (2018). The PCR products were heat-denatured at 95°C for 7 min and then chilled on ice for 10 min. Subsequently, the polyacrylamide gels were run under the constant conditions described in Table 2. The SSCP gels were stained using a rapid silver staining protocol (Byun et al., 2009). The observed heterogeneous PCR-SSCP banding patterns for polymorphic PCR products were submitted for sequencing from both forward and reverse termini according to the instructions of the manufacturers (Macrogen Inc.; Korea). Only clear chromatograms of Applied Biosystem sequence files were considered for further annotations. The sequences of the PCR-SSCP patterns were aligned alongside the referring sequences retrieved from NCBI (GenBank accession number NC_019460.2) using the BioEdit tool, version 7.1 (DNASTAR; USA).

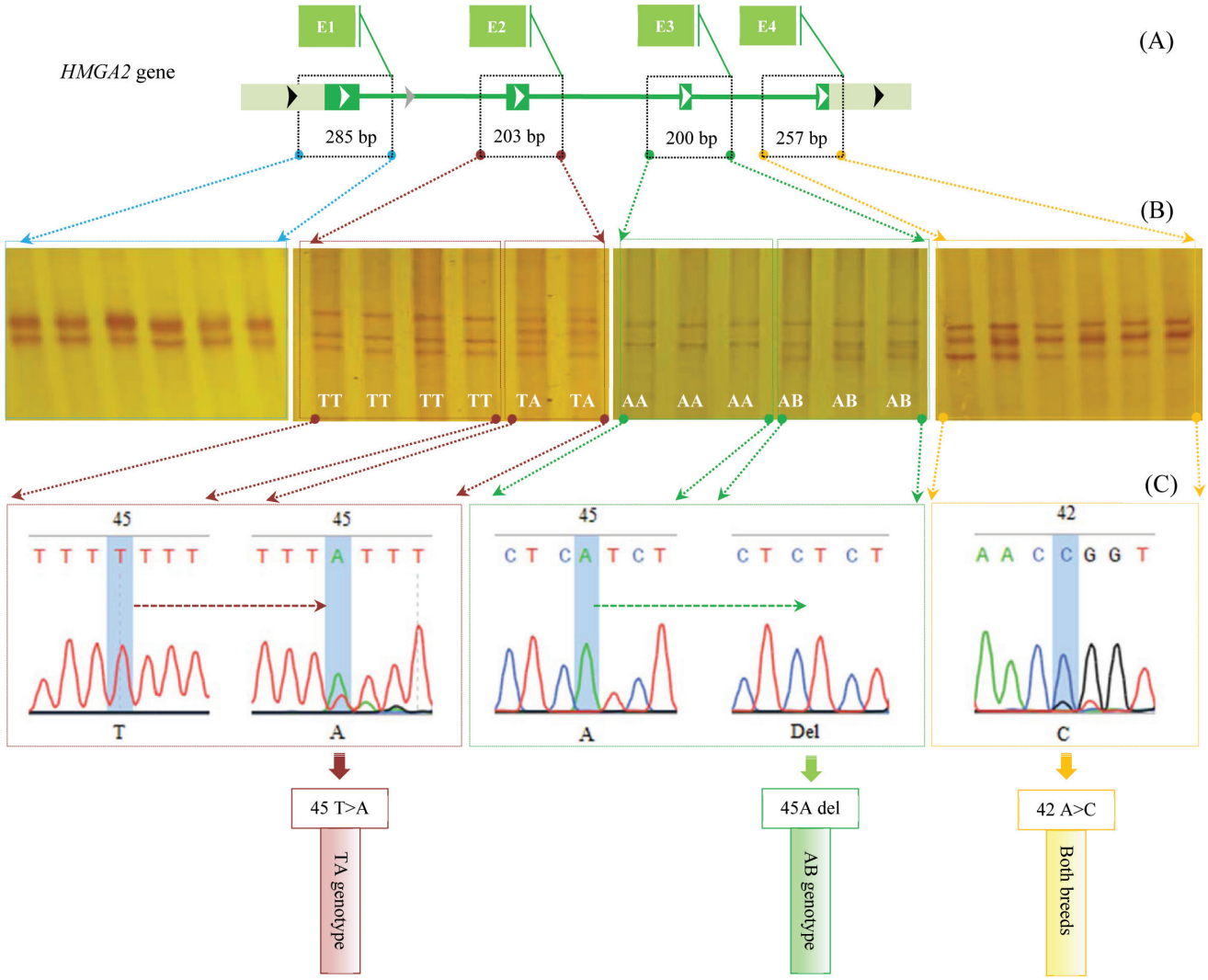

Fig. 1 Polymerase chain reaction single strand conformation polymorphism (PCR-SSCP) sequencing diagram of *HMGA2* polymorphism in two sheep breeds: (A) specific PCR primers designed for amplification of four PCR amplicons (E1–E4), scanning exon 1 to exon 4 and their flanking regions; (B**)** detected PCR-SSCP banding patterns of E1–E4 amplicons; **(**C) sequencing chromatograms of detected polymorphisms, where schematic structure of *HMGA2* gene is illustrated according to GenBank accession number NC_019460.2

Table 1 Designed primers of ovine *HMGA2* gene using NCBI primer BLAST tool based on GenBank acc. no. NC_019460.2

Exon number	Primer code	Sequence (5′3′)	Length	Annealing temperature (°C)
Exon 1	E1-F	GTTAGCGAGGTGGGCTACTG	285 bp	61.1
	E1-R	GCCTCATTCTCCAGCCCTAC		
Exon 2	E2-F	AGGAGGTTCCTATGGGTGTTG	203 bp	60.8
	E2-R	GTCAGGGTCAATTTCTTTCAGACA		
Exon 3	E3-F	TCACCCATTTCCTAGGTCTGC	200 bp	61.4
	E3-R	AGCCTTCAAGAAGACTGAGATT		
Exon 4	E4-F	GGAAAGACCATGGCGACAGA	257 bp	61.8
	E4-R	CCGCATCCTCTTTTGAAGCC		

Table 2 Electrophoresis conditions for separation of four *HMGA2* amplicons in Karakul and Awassi breeds

Amplicon	Gel concentration (%)	Running temperature (°C)	Running time (min)	Electric potential (V)	Current (A)
Exon 1	8	20	200	200	100 m
Exon 2	9	20	230	210	100 m
Exon 3	8	20	240	200	110 m
Exon 4	10%	25	270 min	200 V	110 mA

Statistical analysis

Genotype frequency, allele frequency and the χ^2 tests for Hardy-Weinberg equilibrium were calculated using the PopGene32 software, version 1.31 (Yeh et al., 1999). Based on 3 mth intervals, several growth traits were analyzed in both the Awassi and Karakul breeds: body weight, body length, wither height, rump height, chest circumference and abdominal circumference. The following general linear model was employed using JMP, ver. 13.2.1 (SAS Institute Inc., USA), for analysis of growth traits in both analyzed breeds: $Y_{ijklm} = \mu+S_i+B_j+E2_k+E3_l+E2_kE3_l+e_{ijklm}$, where Y_{ijklm} is the phenotypic value of the trait, μ is the overall mean effects of the traits, S_i is the fixed effect of the sex, B_j is the fixed effect of the sheep breed, $E2_k$ is the fixed effect of the Exon 2 genotype, $E3_l$ is the fixed effect of the Exon 3 genotype, $E2_kE3_l$ is the haplotype effect and e_{ijklm} is the residual effect of each observation. Multiple comparisons of the means were performed using the Turkey's honestly significant difference test. Any *p*-values less than 0.05 were considered statistically significant for all analyses. The effect associated with the age of rams was not matched in the linear model, as the preliminary statistical analyses indicated that this effect did not have a significant influence on the variability of traits in the analyzed populations.

Results and Discussion

Polymorphism of HMGA2 gene

Two PCR-SSCP banding patterns were detected in both the E2 and E3 amplicons, while only one pattern of PCR-SSCP was observed in both the E1 and E4 amplicons (Fig. 1B). For the E2 amplicons, the sequencing results confirmed the observed two electrophoretic SSCP patterns. One substitution single nucleotide polymorphism (SNP) was detected in the heterozygous AT genotype, in which A was substituted by the T nucleotide at the 54th position in intron 1, namely intron1:45 A > T. For the E3 amplicons, one intronic single nucleotide deletion was detected in the heterozygous AB genotype, namely intron 2:45 A del. The sequencing experiments confirmed the homologous PCR-SSCP banding patterns observed in both the E1 and E4 amplicons. However, only one intronic nucleotide substitution was detected in E4 amplicons, in the third intron, intron 3:42 A > C (Fig. 1C). The representative sequences of the observed genotypes were deposited into the NCBI GenBank database under the accession numbers MN684351–MN684355.

HMGA2 genetic diversity

For the E2 amplicons, the most dominant genotype was TT, in the studied Awassi and Karakul breeds with total frequencies of 0.89 (*n*= 105) and 0.58 (*n*= 65), respectively. The analysis of the E2 loci showed a high prevalence of the T allele (0.9449 and 0.7902), followed by the A allele (0.0551 and 0.2098), for the Awassi and Karakul breeds, respectively. For the E3 amplicons, a high prevalence for AA genotype was found as it exhibited higher frequencies, 0.68 (*n* = 82) and 0.70 (*n* = 78), than AB genotype frequencies (0.32 and 0.30) in the Awassi and Karakul breeds, respectively. Higher frequencies of the A allele (0.8475 and 0.8482) compared to the B allele (0.1525 and 0.1518) were observed the Awassi and Karakul breeds, respectively. The results of the χ^2 tests indicated that both the E2 and E3 amplicons did not fit well with the Hardy-Weinberg equilibrium ($p < 0.05$). Both loci were characterized by observed heterozygosity (*Ho*) and expected heterozygosity (*He*). The values of *Ho* for both loci were higher than their *He* values, signifying a high level of genetic variation in both breeds (Table 3).

Association of HMGA2 polymorphism with growth traits

There was a noticeable association between the two observed genotypes of the E2 polymorphism and the majority of growth traits. This association was initiated by the presence of highly significant ($p < 0.001$) differences in body weight and length, and in the wither and rump height of newborn lambs between the TT and TA genotypes. Multiple comparison results indicated that individuals with the TA genotype were characterized by highly significant ($p < 0.001$) growth trait values than individuals with the TT genotype for the majority of the analyzed traits. Although birth chest circumference, birth abdominal circumference and 9 mth daily weight gain differences did not reach the significant thresholds, there was a clear tendency of individuals with TA genotypes to be superior in these measured traits. The superiority of individuals with the TA genotype over individuals with the TT genotype was increasingly observed at 3 mth, 6 mth, 9 mth and 12 mth to include almost all measured growth traits (Table 4). However, the results showed little association of the E3 polymorphism with growth traits measurements. Nonetheless, individuals with the AB genotype were characterized with significantly higher values of body weight, wither and rump heights and abdominal circumference than individuals with the AA genotype only at age 6 mth and in body weight at age 12 mth (Table 4). Thus, E3 amplicons were not involved in the other measured growth traits in other months. Both breed and sex had intermittent effects on some growth traits since only birth weight, and age 3 mth wither and rump heights were affected by breed, while only 3 mth and 6 mth body weights and 3 mth wither and rump heights were affected by sex. The Awassi breed was highly significantly ($p < 0.001$) superior in body weight at birth, while Karakul breed was highly significantly ($p < 0.001$) superior in wither height at age 3 mth. Likewise, the body weight, wither height and rump height of males was significantly ($p < 0.05$) higher than for females at age 3 mth. Furthermore, males had a highly significant ($p < 0.001$) body weight at age 6 mth. However, such significant effects of both sex and breed were not apparent for the growth traits in the other months (Supplementary Table 1). Haplotype analysis indicated there was significant co-inheritance between both detected genotypes in the E2 and E3 amplicons in correlation with the birth abdominal circumference. Individuals with the TA-AA haplotype had significantly higher values of abdominal circumference than individuals with the TA-AB, TT-AB and TT-AA haplotypes, respectively. However, no other co-inheritance associations were identified in all the analyzed traits at birth or in other months (Supplementary Table 2).

Table 3 Genotype, allele frequencies and genetic diversity calculated for *HMGA2* gene in two sheep breeds

Amplicon	Breed	Observed genotype		Genotype frequency		Allele frequency		*Ho*	*He*	χ^2	probability
		TA	TT	TA	TT	T	A				
E2	Awassi	13	105	0.11	0.89	0.9449	0.0551	0.1102	0.1045	0.368683	0.543723
E2	Karakul	47	65	0.42	0.58	0.7902	0.2098	0.4196	0.3331	7.703582	0.005511
		AB	AA	AB	AA	A	B				
E3	Awassi	36	82	0.32	0.68	0.8475	0.1525	0.3051	0.2596	3.704020	0.054282
E3	Karakul	34	78	0.30	0.70	0.8482	0.1518	0.3036	0.2586	3.468170	0.062560

Ho = observed heterozygosity; *He* = expected heterozygosity; *n* = number of individuals.
All χ^2 tests have one degree of freedom and significance was tested at $p < 0.05$

Table 4 Effect of different genotypes (least square mean ± SE) of exon 2 and exon 3 polymorphisms of *HMGA2* gene on growth traits in sheep

Age	Measured parameter	Exon 2			Exon 3		
		TA (n=60)	TT (*n*=170)	Significance	AB (*n*=70)	AA (*n*=160)	Significance
At birth	Body weight (kg)	4.64±0.08	4.06±0.04	**	4.32±0.08	4.38±0.05	ns
	Body length (cm)	31.14±0.28	30.04±0.15	**	30.46±0.27	30.72±0.16	ns
	Wither height (cm)	40.76±0.24	39.64±0.13	**	40.10±0.23	40.30±0.14	ns
	Rump height (cm)	41.19±0.25	39.96±0.14	**	40.55±0.23	40.61±0.14	ns
	Chest circumference (cm)	41.81±0.23	41.28±0.18	ns	41.32±0.31	41.77±0.19	ns
	Abdominal circumference (cm)	43.33±0.35	42.94±0.19	ns	42.85±0.33	43.41±0.20	ns
3 mth	Body weight (kg)	26.02±0.43	20.32±0.23	**	23.40±0.41	22.94±0.25	ns
	Body length (cm)	59.14±0.63	56.66±0.34	**	57.97±0.59	57.83±0.36	ns
	Wither height (cm)	62.54±0.49	60.47±0.27	**	61.28±0.46	61.73±0.28	ns
	Rump height (cm)	63.12±0.47	61.13±0.26	**	62.01±0.45	62.25±0.27	ns
	Chest circumference (cm)	79.67±0.86	75.09±0.47	**	78.28±0.81	76.48±0.49	ns
	Abdominal circumference (cm)	88.41±0.88	82.95±0.48	**	86.60±0.83	84.76±0.50	ns
	Average daily gain (g)	237.50±4.63	180.63±2.53	**	211.99±4.38	206.14±2.65	ns
6mth	Body weight (kg)	35.25±0.51	27.00±0.27	**	29.55±0.48	28.97±0.29	**
	Body length (cm)	69.60±0.64	65.48±0.34	**	67.10±0.60	66.32±0.36	ns
	Wither height (cm)	69.56±0.47	66.72±0.25	**	68.05±0.45	67.20±0.27	*
	Rump height (cm)	70.16±0.47	67.33±0.26	**	68.61±0.45	67.83±0.27	*
	Chest circumference (cm)	92.23±1.13	85.49±0.62	**	87.65±1.07	87.07±0.65	ns
	Abdominal circumference (cm)	102.61±1.06	95.30±0.58	**	98.52±1.00	96.63±0.60	**
	Average daily gain (g)	107.71±4.43	76.93±2.42	**	97.46±4.18	87.18±2.53	*
9 mth	Body weight (kg)	41.28±0.54	32.62±0.29	**	34.95±0.51	34.85±0.31	ns
	Body length (cm)	73.90±0.42	70.25±0.23	**	71.25±0.40	71.18±0.24	ns
	Wither height (cm)	72.46±0.36	70.04±0.19	**	70.65±0.34	70.68±0.20	ns
	Rump height (cm)	72.80±0.36	70.44±0.19	**	70.92±0.34	71.11±0.20	ns
	Chest circumference (cm)	102.20±1.54	95.09±0.84	**	96.62±1.46	97.08±0.88	ns
	Abdominal circumference (cm)	113.18±1.07	103.60±0.58	**	106.55±1.01	105.90±0.61	ns
	Average daily gain (g)	65.82±4.44	61.15±2.42	ns	61.10±4.19	65.86±2.54	ns
12 mth	Body weight (kg)	46.38±0.54	36.66±0.29	**	39.47±0.51	39.08±0.30	*
	Body length (cm)	75.80±0.37	72.25±0.20	**	73.14±0.35	73.20±0.21	ns
	Wither height (cm)	74.65±0.32	71.94±0.17	**	72.67±0.30	72.64±0.18	ns
	Rump height (cm)	74.93±0.32	72.32±0.17	**	73.00±0.30	73.01±0.18	ns
	Chest circumference (cm)	110.51±1.44	101.77±0.78	**	105.05±1.36	103.61±0.82	ns
	Abdominal circumference (cm)	123.26±1.01	111.05±0.55	**	114.40±0.95	114.17±0.57	ns
	Average daily gain (g)	55.90±2.94	46.36±1.60	**	52.62±2.78	49.64±1.68	ns

** = significant at $p < 0.001$; * = significant at $p < 0.05$; ns = not significant

Supplementary Table 1 Effects of sex and breed (least square means ± SE) on growth traits in sheep

Age	Measured parameter	Sex			Breed		
		Female (*n*=102)	Male (*n*=128)	Significance	Awassi (*n*=118)	Karakul (*n*=112)	Significance
At birth	Body weight (kg)	4.16±0.05	4.29±0.05	ns	4.25±0.06	4.19±0.05	**
	Body length (cm)	30.38±0.19	30.41±0.20	ns	30.38±0.21	30.41±0.18	ns
	Wither height (cm)	40.01±0.16	39.96±0.17	ns	39.82±0.18	40.16±0.16	ns
	Rump height (cm)	40.30±0.17	40.32±0.17	ns	40.19±0.19	40.43±0.16	ns
	Chest circumference (cm)	41.45±0.22	41.50±0.23	ns	41.50±0.25	41.44±0.21	ns
	Abdominal circumference (cm)	42.87±0.24	43.31±0.25	ns	42.83±0.27	43.31±0.23	ns
3 mth	Body weight (kg)	21.03±0.29	22.43±0.30	*	20.55±0.32	22.81±0.28	ns
	Body length (cm)	56.80±0.43	57.68±0.44	ns	56.58±0.48	57.83±0.41	ns
	Wither height (cm)	60.54±0.34	61.62±0.34	*	60.16±0.37	61.92±0.32	**
	Rump height (cm)	61.11±0.33	62.25±0.33	*	60.83±0.36	62.44±0.31	*
	Chest circumference (cm)	76.16±0.59	75.87±0.61	ns	75.71±0.65	76.37±0.57	ns
	Abdominal circumference (cm)	83.82±0.61	84.36±0.62	ns	83.42±0.67	84.73±0.58	ns
	Average daily gain (g)	187.50±3.23	201.49±3.30	ns	181.21±3.56	206.87±3.09	ns
6mth	Body weight (kg)	28.22±0.35	30.31±0.36	**	27.99±0.39	30.37±0.34	ns
	Body length (cm)	66.30±0.44	66.88±0.45	ns	65.61±0.49	67.55±0.42	ns
	Wither height (cm)	67.10±0.33	67.91±0.33	ns	66.83±0.36	68.12±0.31	ns
	Rump height (cm)	67.67±0.33	68.57±0.34	ns	67.50±0.36	68.67±0.31	ns
	Chest circumference (cm)	86.70±0.79	87.94±0.81	ns	86.99±0.87	87.52±0.75	ns
	Abdominal circumference (cm)	97.06±0.74	97.40±0.75	ns	96.53±0.81	97.92±0.71	ns
	Average daily gain (g)	79.86±3.08	87.58±3.15	ns	82.58±3.39	84.03±2.95	ns
9 mth	Body weight (kg)	34.21±0.38	35.72±0.38	ns	33.59±0.41	36.24±0.36	ns
	Body length (cm)	71.04±0.29	71.40±0.30	ns	70.36±0.32	72.08±0.28	ns
	Wither height (cm)	70.54±0.25	70.83±0.25	ns	70.27±0.27	71.09±0.24	ns
	Rump height (cm)	70.87±0.25	71.29±0.26	ns	70.75±0.28	71.38±0.24	ns
	Chest circumference (cm)	97.17±1.07	96.66±1.10	ns	96.67±1.18	97.23±1.03	ns
	Abdominal circumference (cm)	106.00±0.74	106.22±0.76	ns	105.13±0.82	107.12±0.71	ns
	Average daily gain (g)	66.49±3.09	60.13±3.16	ns	62.24±3.41	65.18±2.96	ns
12 mth	Body weight (kg)	38.37±0.37	40.23±0.38	ns	37.61±0.41	40.86±0.36	ns
	Body length (cm)	72.80±0.26	73.65±0.27	ns	72.22±0.29	74.18±0.25	ns
	Wither height (cm)	72.53±0.22	72.79±0.22	ns	72.11±0.24	73.21±0.21	ns
	Rump height (cm)	72.94±0.22	73.08±0.22	ns	72.47±0.24	73.57±0.21	ns
	Chest circumference (cm)	103.62±1.00	104.58±1.02	ns	102.61±1.10	105.57±0.96	ns
	Abdominal circumference (cm)	113.640.70	114.99±0.72	ns	112.62±0.77	115.94±0.67	ns
	Average daily gain (g)	46.27±2.05	50.11±2.09	ns	44.73±2.25	51.39±1.96	ns

** and * = significant different at $p < 0.001$and $p < 0.05$, respectively; ns = not significant

The current study investigated the association between *HMGA2* genetic variation and growth traits measurements in two breeds of sheep. This association was based on the genotyping of four loci (exon 1 to exon 4) within the *HMGA2* genetic sequences in two types of sheep breeds. No genetic polymorphism was found in both E1 and E4, while both the E2 and E3 amplicons showed two genotypes in both studied breeds. However, these genotypes only differed in intronic sequences, with no genetic polymorphism detected in the investigated coding portions of the *HMGA2* gene. However, it is well-known that SNPs occur in non-coding regions more frequently than in coding regions (Castle, 2011). Furthermore, it has been demonstrated that the Awassi breed is characterized by high intronic variations rather than exonic sequences (Al-Shuhaib et al., 2019). Thus, the intronic heterogeneity of E2 polymorphism was associated with the majority of growth traits with highly significant ($p = 0.001$) superiority of individuals with the TA genotype over individuals with the wild type TT genotype. However, it seems to be rational that sheep having the TA genotype were more favored than the sheep with the TT genotype due to their significant superiority in terms of almost all measured growth traits. In addition, the breed effect was involved in the current study, since Karakul breed individuals exerted significantly higher values of some of the growth traits than individuals from the Awassi breed. Likewise, the sex of lambs had a relative contribution in this association as males had higher values in some of the measured growth traits at birth and at three-monthly intervals. However, faster growth and larger weight have been usually reported in male lambs (Mirderikvandi et al., 2016). In contrast to E2, no clear association with growth traits was observed for E3, signifying little involvement of this locus in the measured growth traits in the investigated populations. Though individuals with AB genotypes had higher values for the majority of growth traits at age 6 mth than individuals with AA genotypes, this superiority was not apparent at other months of measurement with the one exception being for body weight which re-appeared at age 12 mth. However, it seems that the intron 2:45 A del SNP of the AB genotype was not continuously associated with growth traits of sheep for almost all measured age intervals. Unlike the intron 2:45 A del SNP, it can be stated that the observed intron 1:45 T > A SNP was strongly linked with better growth traits measurements. This may indicate that the intron 1:45 T > A SNP may have a more effective role than the intron 2:45 A del SNP in modulating the binding activity

Supplementary Table 2 Effects of haplotype (least square means ± SE) on growth traits in sheep

Age	Measured parameter	Haplotype			
		TA-AA	TA-AB	TT-AA	TT-AB
At birth	Body weight (kg)	4.69±0.08[a]	4.59±0.13[a]	4.06±0.05[a]	4.05±0.07[a]
	Body length (cm)	31.19±0.28[a]	31.09±0.48[a]	30.24±0.17[a]	29.83±0.24[a]
	Wither height (cm)	40.78±0.24[a]	40.73±0.40[a]	39.81±0.15[a]	39.46±0.21[a]
	Rump height (cm)	41.14±0.24[a]	41.23±0.42[a]	40.06±0.15[a]	39.85±0.21[a]
	Chest circumference (cm)	42.33±0.32[a]	41.29±0.55[a]	41.20±0.20[a]	41.34±0.28[a]
	Abdominal circumference (cm)	44.07±0.35[a]	42.58±0.59[ab]	42.75±0.21[b]	43.11±0.31[ab]
3 mth	Body weight (kg)	25.87±0.42[a]	26.16±0.72[a]	20.00±0.26[a]	20.63±0.37[a]
	Body length (cm)	59.41±0.62[a]	58.86±1.06[a]	56.23±0.39[a]	57.07±0.55[a]
	Wither height (cm)	62.84±0.48[a]	62.24±0.82[a]	60.61±0.30[a]	60.32±0.43[a]
	Rump height (cm)	63.36±0.47[a]	62.87±0.80[a]	61.13±0.29[a]	61.13±0.41[a]
	Chest circumference (cm)	78.37±0.85[a]	80.95±1.45[a]	74.57±0.53[a]	75.61±0.75[a]
	Abdominal circumference (cm)	87.19±0.87[a]	89.62±1.49[a]	82.33±0.54[a]	83.56±0.77[a]
	Average daily gain (g)	235.26±4.62[a]	239.73±7.84[a]	177.01±2.88[a]	184.25±4.07[a]
6mth	Body weight (kg)	34.75±0.51[a]	36.67±0.86[a]	26.81±0.31[a]	27.67±0.44[a]
	Body length (cm)	69.09±0.63[a]	70.24±1.08[a]	65.26±0.39[a]	66.26±0.56[a]
	Wither height (cm)	69.08±0.47[a]	70.37±0.80[a]	66.52±0.29[a]	67.46±0.41[a]
	Rump height (cm)	69.68±0.47[a]	71.07±0.81[a]	67.17±0.29[a]	67.98±0.42[a]
	Chest circumference (cm)	92.24±1.13[a]	94.11±1.92[a]	85.05±0.70[a]	85.88±0.99[a]
	Abdominal circumference (cm)	102.15±1.06[a]	105.34±1.80[a]	94.40±0.66[a]	96.61±0.93[a]
	Average daily gain (g)	98.96±4.42[a]	116.73±7.48[a]	75.67±2.75[a]	78.19±3.88[a]
9 mth	Body weight (kg)	40.88±0.54[a]	42.38±0.92[a]	32.53±0.34[a]	32.95±0.48[a]
	Body length (cm)	73.72±0.42[a]	73.66±0.72[a]	70.19±0.26[a]	70.61±0.37[a]
	Wither height (cm)	72.55±0.36[a]	72.10±0.61[a]	69.95±0.22[a]	70.26±0.31[a]
	Rump height (cm)	72.93±0.36[a]	72.49±0.61[a]	70.42±0.22[a]	70.50±0.32[a]
	Chest circumference (cm)	102.22±1.54[a]	104.63±2.61[a]	94.91±0.96[a]	94.33±1.35[a]
	Abdominal circumference (cm)	112.74±1.07[a]	116.05±1.81[a]	103.12±0.66[a]	103.89±0.94[a]
	Average daily gain (g)	68.13±4.43[a]	63.50±7.50[a]	63.59±2.76[a]	58.70±3.89[a]
12 mth	Body weight (kg)	45.85±0.53[a]	47.48±0.91[a]	36.50±0.33[a]	37.33±0.47[a]
	Body length (cm)	75.53±0.37[a]	75.34±0.64[a]	72.34±0.23[a]	72.58±0.33[a]
	Wither height (cm)	74.63±0.32[a]	74.38±0.54[a]	71.86±0.19[a]	72.20±0.28[a]
	Rump height (cm)	74.94±0.31[a]	74.57±0.54[a]	72.25±0.19[a]	72.56±0.28[a]
	Chest circumference (cm)	109.82±1.43[a]	112.33±2.43[a]	101.18±0.89[a]	103.07±1.26[a]
	Abdominal circumference (cm)	122.91±1.00[a]	124.96±1.71[a]	110.73±0.62[a]	111.50±0.88[a]
	Average daily gain (g)	55.17±2.93[a]	56.63±4.96[a]	44.11±1.82[a]	48.60±2.58[a]

Values in the same row superscripted with different lowercase letters differ significantly at $p < 0.05$

of the altered HMGA2 proteins with its corresponding sequences of proliferation genes, thereby having a more efficient effect on growth traits. However, more definitive data are needed to confirm this suggestion. The finding from the current study verified that the intron 1:45 T > A SNP could be a potential marker-assisted selection site playing an important role in growth traits. In support of this finding, *HMGA2* genetic polymorphism could affect several quantitative trait loci such as fat and reproduction in beef cattle (Bolormaa et al., 2014). However, the highly significant ($p = 0.001$) association seen between E2 polymorphism and the growth indices in the current study were observed at age 12 mth. This finding may indicate the more prevalent role of *HMGA2* genetic polymorphism with the progression of age in the two breeds of sheep studied. Furthermore, these results showed that the *HMGA2* gene had a significant effect on growth traits, which signifies the important role played by this gene in the process of growth. The current assessment of *HMGA2* variation within and between Awassi and Karakul populations could meet the needs of the current development of animal husbandry in the analyzed populations. Consequently, the investigation of the *HMGA2* gene may have direct utilization in marker-assisted selection to improve the growth traits in Awassi and Karakul breeds with promise for implementation in other breeds. Other studies have reported an apparent association between the *HMGA2* gene and adult height measurements in humans (Yang et al., 2010; Takeshita et al., 2011), body growth in mice and dogs (Zhou et al., 1995), body weight indices in dogs (Jones et al., 2008), body size variations in horses (Makvandi-Nejad et al., 2012) and body composition in pigs (Muráni et al., 2007). Unfortunately, no association was found in a published study between *HMGA2* polymorphism and growth traits in sheep or even in the closely related genus of goats. Therefore, the current study is the first to describe ovine *HMGA2* polymorphism and its association with production traits.

The genotyping experiments regarding *HMGA2* gene polymorphism showed that sheep with the TA genotype in exon 2 had superior growth traits than sheep with the AA genotype. In the TA genotype, one novel SNP (intron 1:45 T > A) exerted a tight linkage with these observed differences in growth traits. Therefore, *HMGA2* gene variation has interesting implications in agriculture by acting as a potent marker for the assessment of several growth traits in sheep and it may be utilized in future marker-assisted selection programs.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Acknowledgments

This research was partially supported by the College of Agriculture, Al-Qasim Green University. The authors thank the APS stations for their help and support in the experimental procedures.

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