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Polymorphism in intron-I of *Myostatin* gene and its association with estimated breeding values of growth traits in Baluchi sheep (*Ovis aries*)

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ABSTRACT

GDF8 gene is associated with skeletal muscle mass in farm animals. The association of *GDF8* gene polymorphism with growth traits including birth weight, weaning weight, 6 months weight, 9 months weight and yearling weight in 112 individuals of super-abundant sheep in Iran (Baluchi) was investigated. Polymerase chain reaction - single strand conformation polymorphism (PCR-SSCP) method was employed in screening for genetic variation. Three unique SSCP patterns (P1, P2 and P3) for intron1 region of *GDF8* gene were observed. Breeding values of growth traits were estimated by using the best linear unbiased prediction based on an animal model with a relationship matrix. Studied growth traits were examined for association analysis. Our findings suggested that the P1 genotype has the highest body weight and the highest additive estimated breeding value for the weaning weight trait. Hence, the intron1 region of *GDF8* variants could serve as a genetic marker for Baluchi sheep breeding and genetics. The other traits showed no relationship to the genotypes examined.

Key words: Association analysis, Baluchi sheep, Breeding value, *GDF8* gene

GDF8 (also known as growth and differentiation factor 8, *myostatin*) is the major regulator of myogenesis and it functions as a negative regulator of muscle growth in mammals. The *GDF8* gene is associated with increased skeletal muscle mass (double muscling) in mice (Mendias *et al.* 2008), dogs (Mosher *et al.* 2007), cattle (Marchitelli *et al.* 2003).

In sheep, intensive association studies indicated that marker-assisted selection using *GDF8* single nucleotide polymorphisms (SNPs) would be beneficial (Hickford *et al.* 2009). QTL studies showed that *GDF8* had a major effect on muscling and fat depth in New Zealand Texel sires (Johnson *et al.* 2005) and UK Texel (Walling *et al.* 2004) and Charollais (McRae *et al.* 2005) sheep.

As part of the MAS program aimed at improving growth traits in Baluchi sheep we have characterized potential variation in the ovine *GDF8* gene. In this study, we used polymerase chain reaction single-stranded conformational polymorphism (PCR-SSCP) analysis to investigate allelic variation in intron-1 of ovine *GDF8* gene. Further, we report associations of some of these alleles with production traits in Iranian Baluchi sheep.

MATERIALS AND METHODS

Baluchi lambs (112) of known pedigree were randomly selected from breeding station of Baluchi sheep (in Mashhad, Khorasan Province, Iran) to investigate with genomic screening. The included traits were: birth weight (BW), weaning weight (WW), 6 months weight (6MW), 9 months weight (9MW), and yearling weight (YW).

Amplification primer pair was designed based on the ovine *GDF8* sequence (DQ530260) to amplify 291 bp fragment of intron1 region (Intron1F: 5σ- CAC ATT TTT CCC CCA GAA GAG TGA A -3σ and Intron1R: 5σ- TTA ACA GGA GTT AAC TTA GGT AAT GTC-3σ). Amplification reactions for each fragment were done by using the following constituents: in a final volume of 25 µl containing 75 mM Tris–HCl (pH 8.8), 1 unit of Platinum *Taq* DNA polymerase, 0.1 mg/ml BSA, 0.2 mM each of dNTPs, 2 mM $MgCl_2$, 25 pMol of primers and 100 ng of purified DNA isolated from whole blood with a DNA Prep kit as recommended by the manufacturer. Amplification was performed in a thermal cycler T-Personal with the following program; after an initial denaturation step at 95 °C for 4 min, 35 cycles were programmed as follows: 94 °C for 55s, 64°C for 60s, 72 °C for 60s and final extension at 72 °C for 10 min. The DNA

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fragments were visualized on an agarose gel by ethidium bromide staining and exposure to ultraviolet light. Amplicons were subject to SSCP analysis to screen for polymorphisms using 8% polyacrylamide gels at 250 V and 6°C for 8 h in 0.5 × TBE buffer then visualized with silver staining.

Breeding values for growth traits (BW, WW, 6MW, 9MW and YW) were estimated using the best linear unbiased prediction (BLUP) based on an animal model with a relationship matrix. The analysis was conducted using restricted maximum likelihood (REML) using a derivative-free (DF) algorithm procedure. Maternal genetic or permanent environmental effects were taken into account by including appropriate random effects in the model. The fixed effects, considered in the analytical model after testing of significance, included year (5 classes), herd (2 classes), age of dam in years (8 classes), sex (2 classes) and birth type (3 classes). The interactions between fixed effects were not significant and therefore excluded. Least square analysis was accomplished using the general linear model (GLM) procedure by the SAS software package (SAS Institute 1989). Finally the model included animal effect as random effect and age of lamb as covariate factor. Following general representation of the animal model was used:

$$Y=Xb+Za+Wm+Spe+e$$

where, Y, n×1 vector of records; b, vector of fixed effects in the model with association matrix X; a, vector of direct genetic effects with association matrix Z; m, vector of maternal genetic effects with association matrix W; pe, vector of maternal permanent environmental effects with association matrix S; and e, vector of residual (temporary environment) effects.

It is assumed that direct additive genetic effects, maternal additive genetic effects, maternal permanent environmental effects and residual effects are normally distributed with the mean of 0 and variances, $A\sigma_a^2$ $A\sigma_m^2$, $Ic\sigma_{pe}^2$ and $Ie\sigma_e^2$, respectively. Also, σ_a^2, σ_m^2, σ_{pe}^2 and σ_e^2 are direct additive genetic variance, maternal additive genetic variance, maternal permanent environmental variance and residual variance, respectively. *A* is the additive numerator relationship matrix; *Ic* and *Ie* are identity matrices (square and symmetric) that have order equal to the number of dams and number of records for each trait, respectively. Also, σ_{am} denotes the covariance between direct additive genetic and maternal additive genetic effects.

The impact of polymorphisms on sheep's EBVs was analyzed by ANOVA, followed by the Tukey test using a statistical model including different genotype on the *GH* gene as fix effect. Differences with±<0.05 were considered statistically significant.

RESULTS AND DISCUSSION

The Baluchi sheep was selected as the superabundant sheep in Iran that is typically kept in small numbers by resource-poor households in a widely tropic area. In such situations, genotype-assisted selection appears most feasible for small ruminants (Notter *et al.* 2007). Tahmoorespur *et al.* (2011) suggested that polymorphisms on growth traits candidate genes might be one of the important genetic factors that influence growth traits and maybe explain partial source of genetic variation on Baluchi sheep. The most important traits that provide optimal returns to the farmers and consumers were included in this study.

PCR-SSCP analysis on the 112 randomly selected sheep from Iranian pure breed Baluchi sheep revealed 3 unique SSCP patterns for intron1 region of *GDF8* gene. Till now intron1 region of *GDF*8 gene was subjected to several polymorphism investigations and association studies in sheep (Hickford *et al.* 2009).

Estimated heritabilities for all traits are presented in Table 1. Direct heritability estimates with appropriate models for body weight of lambs were at the highest magnitude at birth.

The direct additive heritability estimate (0.14) of BW in present study is within the range reported by other authors in the recent researches. In a review, Safari *et al.* (2005) reported weighted mean of maternal heritability estimated for BW of 0.18 to 0.24 in dual-purpose, wool and meat sheep breeds, which are similar to those reported in this study. Maternal heritability estimates for body weight of lambs decreased with age from 0.20 at birth to 0.008 at yearling age. The maternal additive genetic variances were also low, as expected for BW and YW. Direct and maternal heritability estimates for WW, obtained from literature by Safari *et al.* (2005), were higher than our estimates in the present study. The estimated value of direct heritability declined from birth to 6 months of age, reached to a value of 0.02 for 6MW and increased afterwards until yearling age. Consistent with our

Table 1. Parameter estimated of analyzed traits

Traits	σ_a^2	σ_m^2	σ_e^2	σ_p^2	h^2	m^2
Birth weight (BW)	0.05	0.07	0.23	0.35	0.14	0.20
Weaning weight (WW)	0.22	0.79	9.10	10.13	0.02	0.07
Weight at 6 months (6MW)	0.58	1.60	18.36	20.55	0.02	0.07
Weight at 9 months (9MW)	0.16	1.12	17.47	18.75	0.08	0.05
Yearling weight (YW)	2.30	0.01	17.88	20.28	0.11	0.008

Table 2. Growth traits of Baluchi sheep according to the different *GDF8* patterns

GDF8 patterns	Growth traits				
	BW	WW	6MW	9MW	YW
P1 (n=28)	4.60±0.13	25.42^{a} ±0.72	36.18±0.95	32.11±1.09	41.91±1.33
P2 (n=63)	4.42±0.08	23.52^{ab}±0.52	33.78±0.62	34.62±0.71	43.12±0.87
P3 (n=21)	4.30±0.15	22.23^{b} ±0.93	33.49±1.13	34.28±1.29	43.15±1.57
P Value	0.34	0.03*	0.08	0.15	0.72

Table 3. Estimated breeding value of growth traits of Baluchi sheep according to the *GDF8* genotypes

GH Pattern (frequency)	Weight estimated breeding values (means ± SE, kg)						
	BW		WW		6MW	9MW	YW
	(additive)	(maternal)	(additive)	(maternal)	(additive)	(additive)	(additive)
G1 (n=108)	0.03±0.02	-0.013±0.01	0.05^{ab}±0.02	-0.13±0.05	0.04±0.05	0.05±0.01	0.35±0.11
G2 (n=46)	0.04±0.01	0.025±0.01	0.05^{a}±0.01	-0.10±0.03	0.05±0.03	0.07±0.01	0.17±0.07
G3 (n=36)	0.05±0.02	0.009±0.02	-0.02^{b}±0.02	-0.20±0.06	0.15±0.05	0.06±002	0.21±0.13
P Value	0.82	0.085	0.046*	0.37	0.29	0.77	0.40

study, Mohammadi *et al.* (2010) reported such fluctuations for direct heritability estimate of Sanjabi sheep from birth to 9 months of age.

The relatively low heritability estimates for the studied traits were in accordance with such studies on other Iranian indigenous sheep breeds (Jafaroghli *et al.* 2010, Mohammadi *et al*. 2010) and can be perhaps explained by the low nutritional management, low quality of pastures and harsh climatic conditions, which result in a high environmental variance. These results indicated that selection for growth traits will result in slow genetic improvement.

In cattle, a number of *myostatin* variants of differing phenotypic consequence have been described across a variety of breeds. This supports the notion that further investigation of *GDF8* variation in different sheep breeds is valuable.

Our results showed that the *GDF*8 genotypes are associated with estimated breeding values of growth traits (Table 2). We observed a significant effect of this polymorphism on WW yield ($P<0.05$). The P1 genotype has the highest body weight and the highest additive estimated breeding value for the WW trait (Table 3). We did not observe any another association with other growth traits.

However, as the polymorphism is in non-coding DNA, it is difficult to conclude how this genetic variation might be affecting *myostatin* activity. Possibilities include that it may affect mRNA splicing, or is linked to variation elsewhere in the coding sequence that subsequently affects the amino acid sequence. It may also be linked to nucleotide variation in critical gene control regions.

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