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Lack of Association between Somatotropin Receptor Gene Polymorphism and Birth Weight of Iranian Indigenous Sistani Cattle

Research Article

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ABSTRACT

The objective of the present study was to determine polymorphism within the promoter region of somatotropin receptor genes in indigenous Sistani cattle (*Bos indicus*) and associations between this polymorphism and breeding value of birth weight. The pedigree structure was included by considering 1173 animals with 600 progeny birth weight data obtained from a Zhark breeding station in Sistan and Baluchistan. Heritability was estimated for birth weight using different univariate models with the derivative-free approach of restricted maximum likelihood algorithm (DFREML). The average weight of each birth was 23.9 ± 3.16 kg. The effects of non-genetic factors were significant (P<0.01) for birth weight. Direct heritabilities (h^2) in single trait analyses were 0.31 ± 0.06. Genomic DNA was isolated from blood and semen using conventional methods. Polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) assays were used to genotype this candidate gene in 72 individuals with the birth weight record. The observed allele size was similar to that reported in the literature. The Sistani cattle showed higher frequency of alleles *ALuI* (+) than *ALuI* (-) in population. Statistical analysis was conducted to test the association of this polymorphism with the breeding value of birth weight data. There was not significant association between producing genotypes and birth weight trait. Future research on another candidate gene and growth trait strongly is encouraged to deep in the understanding of growth pattern in this breed.

KEY WORDS candidate gene, indigenous Sistani cattle, PCR-RFLP, somatotropin receptor genes.

INTRODUCTION

Sistani breed is one of the most important meat types of cattle breed in Iran. The breeding system of these cattle is non-controlled and also no selection criterion is used for the improvement of this breed. Evaluations of the performance using quantitative and molecular genetic methods are required for the design of any breeding strategy in this breed (Tavakolian, 2009). The birth weight is an economical trait in the cattle industry and both genetic and environmental factors are contributing to the variation of this trait. Environmental factors such as dam age, intrauterine malnutrition and placental functioning may lead to low birth weight (Raja *et al.* 2010). Birth weight is a reliable indicator for subsequent growth and development of animals as the high correlations existing between this trait and post-weaning traits have been reported in literature (Unalan, 2009; Shahzad *et al.* 2010). The consideration of birth weight in animal model for improvement of growth trait is necessary (Assan *et al.* 2011) since, high birth weight increases the

chance of dystocia and low rate of birth weight deceases survival rate and health of calves (Kaygisiz *et al.* 2010). To obtain an optimal breeding program to improve growth traits, a population with known non-genetic and genetic parameters, is necessary to understand the level of variability in candidate genes and the quantitative trait loci (QTL) that controls the growth variance on chromosomes. Methods simultaneously using phenotype and DNA marker information in the model can be considered of preference. Needless, to say that genetic variation of growth is very complex and knowledge about is still scarce (Van der Werf *et al.* 2007).

To date, molecular genetic techniques have already resulted in the discovery of several genes that have a major effect on some quantitative traits of interest and of genetic markers that are linked to QTL. Genes with known functions in biochemical and metabolic pathways that determine the expression of economic traits are candidates for QTL.

The somatotropin receptor gene is one of the candidate genes for beef and growth trait in livestock as well as cattle. This gene is composed of 10 exons and 9 introns and the bovine somatotropin receptor gene has been localized into the chromosome. Genetic variation in somatotropin receptor gene has been shown to be responsible for variation of milk and meat production (Harvey, 1994).

The objective of the present study is the verification of reported polymorphism in somatotropin receptor gene and test the association between this polymorphism and breeding value of birth weight in Sistani cattle.

MATERIALS AND METHODS

Animals and management

The pedigrees structure was included by considering 1173 animals with 600 birth weight data of progeny obtained during 1980-2001 from a Zhark breeding station in Sistan and Baluchistan. In this station, bulls and cows were grouped and housed separately except during mating time. The animals were routinely bred through controlled natural mating. Some bulls had been used extensively for breeding due to management reasons. The method for identification of animals was using ear tags. The experimental animals were subjected to the same feeding program on the farm. Bulls and cows were fed twice daily and water was available *ad libitum*. Regular vaccination and deworming was undertaken according to the standard.

Non genetic and genetic parameters

The effect of fixed effects (year, season, sex and parity) on birth weight was tested by the least square analyses of variance using the GLM procedure of (SAS, 2009). The Duncan's was used as a multiple range test for testing the differences of non-genetic factors on birth weight. Effects were found to be significant ($P<0.05$) in these analyses and were retained for subsequent analyses. The covariance components for each trait were analyzed by fitting single and bivariate trait animal model using the DFREML program (Meyer *et al.* 2002). Univariate animal models via different arrangements of direct additive genetic and some of maternal additive genetic, maternal permanent and common environmental effects due to dam with adding or ignoring covariance between direct and maternal additive genetics were considered as random effects. The used models were, as follows:

1. $Y= Xb + Z_a + e$
2. $Y= Xb + Z_a + W_{pe} + e$
3. $Y= Xb + Z_a + Z_m + e$ (cova, m=0)
4. $Y= Xb + Z_a + Z_m + W_{pe} + e$ (cova, m=0)

Where:

Y, b, a, m, pe, c and e: are vectors of observations and fixed, direct additive genetic, maternal additive genetic, maternal permanent environmental, common environmental and residual effects, respectively.

X, Z_a, Z_m and W_{pe}: are the incidence matrices relating observations to the respective fixed and random effects.

Molecular study

Blood samples were randomly collected from 72 Sistani calves with a measured record of birth weight in during the 2000-2001 year and then were stored temporarily at -20 ^{o}C. DNA was extracted using a routine commercial kit. DNA quality and concentration were calculated by spectrophotometry taking the optical density (OD) at a wavelength between 260 and 280 nm. Based on the published nucleotide sequence of the promoter region and exon 1 of the bovine GHR gene (Heap *et al.* 1995) two pairs of oligonucleotide primer were synthesized according to the protocol described Aggrey *et al.* (1999) with some modification. The PCR fragment had 458 bp in length containing a polymorphic *AluI* site. The sequences of the forward and reverse primers were, as follows:

5'-TGCGTGCACAGCAGCTCAACC-3'
5'-AGCAACCCCACTGCTGGGCAT3'

The PCR was performed in a 25 µL reaction using PCR master kit (Cinna Gen Company, Iran) in a thermocycler (T-Personal Model, Biometera Company, Germany). The PCR mixture contained: 50-100 ng of the extracted DNA, 2.5 µL of 10X PCR buffer (200 m*M* $(NH_4)_2SO4$, 0.1 m*M* Tween 20% and 750 m*M* Tris-HCl (pH 8.8)), 2.5 m*M* $MgCl_2$, 200 µ*M* dNTPs and 3 µL mix of oligonucleotides

10 pmol from each primer), 1 U *Taq* DNA polymerase (Fermentas Company) and 11 µL ddH_2O.

The PCR profile included a touchdown program with the following details: an initial denaturation step at 94 °C for 4 min, 10 cycles of 94 °C (60 s), 66 °C (decrease 1 °C/cycle for 60 s) and 72 °C (60 s) followed by 25 cycles of 94 °C (60 s), 56 °C (120 s) and 72 °C (60 s) and a final extension step of 10 min at 72 °C.

PCR product for each sample was digested with 10 units *AluI* at 37 °C for at least 12 h. The digested products were separated in a 2% and 3% agaros gel for 1 h at 73 V. The gels were stained with ethidium bromide. Model of power supply for electrophoresis was PAC1000 (Bio-Rad Company, USA). The sizes of alleles were determined in relation to the DNA size standard using the computer software BIO 1D^{++}.

Genotype and allele frequencies were calculated using the computer software package Pop-Gene version 1.3 (Yeh *et al.* 1997). These genotype classes were removed from the dataset for final association analysis. Data were subjected to the least-squares by ANOVA using the GLM procedure of the SAS software package (SAS, 2009).

RESULTS AND DISCUSSION

Non genetic factors and genetic parameters of birth weight

The sex of calves, year of birth and parity had a significant (P<0.05) effect on birth weights of Sistani cattle. However, season had not significant effect on birth weight. Table 1 shows the least squares means and standard error of birth weight in different levels for non-genetic factors (year, parity, sex and season). Males were significantly (P<0.05) heavier than females for birth weight. The calves at higher parity had significantly (P<0.05) birth weight than calves in first and second parity (Table 1). A comparison of the different models of the studied traits is presented in Table 2. The models included maternal genetic effects as well as direct additive genetic effects, without considering covariance between them where the model 4 was determined as the most appropriate model for birth weight. The genotype and allele frequencies for investigated locus were reported in Table 3.

Molecular study

The observed number of alleles at the somatotropin receptor loci was similar to that previously reported by Aggrey *et al.* (1999). PCR-RFLP in the promoter of the bovine somatotropin receptor loci was detected. There was one polymorphic *AluI* site in 458 bp fragment (Figure 1). The digested *AluI* (+/+) PCR product exhibited two fragments of 296 and 162 bp (invisible in gel) revealing a mutation at position -1182. The mutation was an A to transversion.

Table 1 Least squares means and standard error of birth weight

	Body weights (kg)		
Factors	N	BW	Significant
Year			
1980	20	24.30±0.61	a
1990	50	22.50±0.31	b
1991	32	22.24±0.31	b
1992	40	22.14±0.36	b
1993	15	24.05±0.49	a
1994	57	25.15±0.31	a
1995	38	23.90±0.21	ab
1996	46	21.14±0.31	c
1997	53	23.02±0.61	ab
1998	70	24.60±0.30	a
1999	45	25.07±0.40	a
2000	24	22.80±0.30	b
2001	70	25.07±0.40	a
Season			
Spring	240	23.28±0.44	ns
Summer	120	23.21±0.53	ns
Autumn	180	23.55±0.21	ns
Winter	60	23.32±0.35	ns
Parity			
1	270	21.19±1.20	c
2	126	23.19±0.45	b
3	90	24.76±0.68	ab
4	70	25.82±0.68	a
5	30	25.21±1.10	a
6	14	25.71±1.20	a
Sex			
Female	420	25.12±0.42	b
Male	180	23.21±0.21	a

Means are caring at least one similar alphabet in superscripts for a particular variable are not significantly different.

Association test

There was not significant association between genotypes of somatotropin receptor gene and birth weight trait (Table 4).

Non genetic factors and genetic parameters

The significant effect of environmental factors on birth weight in this study may be due in part to the differences in the male and female endocrine system, maternal effects, year and inadequate availability of nutrition during pregnancy or limited uterine space and maternal ability of the dam in different ages (Olawumi and Salako, 2010; Topal *et al.* 2010).The reasons for the significant influence of cow age may be related to differences in maternal effects, nursing and maternal behavior of the cows with different ages on per weaning traits of calves (Kaygisiz *et al.* 2010). Therefore, it can be stated that the birth weight of calves is an important factor in determining the growth performance of Sistani cattle in the next growth period of life (Bayram and Aksakal, 2009; Habib *et al.* 2009).

Table 2 Genetic parameters and heritability value for body weight

Traits	Models	σ^2_a	σ^2_m	σ^2_{pe}	σ^2_e	σ^2_p	h^2_a	h^2_m	h^2_C	AIC
BW	Model 1	2.86	-	-	6.62	9.45	0.30±0.01	-	-	-228.31
	Model 2	0.16	-	2.7	4.25	9.45	0.16±0.12	-	0.28±0.08	-218.04
	Model 3	0.18	2.37	-	4.20	9.45	0.19±0.09	0.25±0.00	-	-213.31
	Model 4	0.13	1.37	1.66	3.20	9.45	0.13±0.10	0.14±0.00	0.17±0.00	-210.07

σ^2_a: direct additive genetic variance; σ^2_m: maternal additive genetic variance; σ^2_{pe}: maternal permanent environmental variance; σ^2_e: residual variance; σ^2_p: phenotypic variance; h^2_a: direct heritability; h^2_m: maternal heritability; h^2_c: maternal permanent environmental effect and AIC: mean best fitted model, Akaike's Information Criterion.

The differences between the obtained results of heritability estimation are mainly due to a data structure and heritability estimation of most of the traits with equal or higher representation in the good environment (Kaygisiz *et al.* 2010).

The differences in reported heritability estimates may also be due to the choice of the estimation method (regression as REML or the choice of the model as sire or animal model, single or multi trait, gene combinations over one or several generations, etc.).

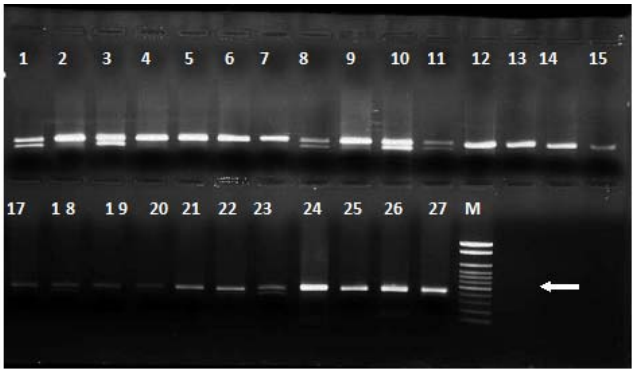

Figure 1 PCR-RFLP of the GHR PCR product separated on agarose gel
The genotype of *AluI* (-/-) exhibited one fragment of 458 bp
The genotype of *AluI* (+/+) was cleaved into two fragments 296 and 162 bp (invisible in gel)

Association study

The approach for testing candidate alleles looks for a relationship between the phenotype and a genetic variance that may or may not alter gene structure and function of the expression in the product. The use of genetic markers could create a more reliable mechanism to develop breeding programs selective for cattle growth rate. In the present study, the allele frequencies were expected to be significantly different between the Sistani and other cattle breeds because the different genetic makeup and selection programs.

The results of the present study exhibited a lack of association between somatotropin receptor gene and birth weight in the Sistani cattle breed. A review of similar works carried out by Moody *et al.* (1995) showed a polymorphism in the bovine growth hormone receptor (GHR) gene by digesting polymerase chain reaction (PCR) products with the restriction enzyme *AluI*. Falaki *et al.* (1996) reported allelic variation in the structural or regulatory sequences of growth hormone and its receptor gene directly or indirectly affected milk traits. Misio *et al.* (1998) sequenced 273 bp of the 3' flanking region in the bovine GHR and they found three length variants and one base substitution polymorphism in this region. In contrast of our findings, Aggrey *et al.* (1999) reported that *AluI* (+/+) bulls had a higher estimated breeding value (EBV) for more fat than *AluI* (-/-) bulls. As a justification of such differences between results, it is a discussable breed genome structure of Holstein breed as an exotic high milk productive cattle breed and on another side Sistani cattle as an indigenous breed of the country.

Table 3 Genotype and allele frequencies for investigated locus

Year of birth	N	Genotype number			Allele frequency	
2000	32	5	20	7	0.54	0.46
2001	40	8	32	0	0.40	0.60

Year of birth for each group was different.

Table 4 Association between genotype of GHR and BV in birth weight

Genotype	N	(Mean±SE)
(+/+)	13	0.17±0.01
(+/-)	55	0.18±0.06
(-/-)	7	0.18±0.02

Ge *et al.* (1999) identified three single nucleotide polymorphisms in the promoter region of GHR gene, one in the promoter gene and four in the exon 10 of the GHR gene. Lucy *et al.* (1998) reported a shorter allele with 11 consecutive (TG) in common on *Bos indicus* cattle, whereas longer 16-to (TG) 20 repeat alleles predominated in *Bos taurus* breeds.

Some studies reported the presence of (TG) 11 motif repeat on growth hormone receptor allele in Angus steers raised under commercial conditions due to a decrease in their growth of approximately on average 17 kg at weaning and approximately 23 kg at slaughter. The further justification for presenting these results could be the amplification of different regions of sompatotropin receptor genes that are promoter regions and also the restriction of enzymes used for PCR-RFLP method was different than other similar studies of sompatotropin receptor genes. Evaluation of the association between genotypes and other growth and body conformation traits or carcass quality were suggested using high number of animals and different candidate genes

in Sistani cattle. The critical step of the association study was the identification of polymorphisms in populations which it was achieved in this present study.

CONCLUSION

Association study between candidate gene polymorphisms and important traits in livestock is the first step to investigate on favorable allele verification for marker assisted selection. Quantitative traits such birth weight are regulated by many genes and are affected by interactions among them, and thus, a candidate gene associated with a trait in one population may have a different effect, or show no effect at all, in another population due to the negative effects of other genes and epistatic interactions of the candidate gene with other genes in the population. This theory is supported by many association studies, in which a polymorphism was significantly associated with performance traits in one family or breed, but not in another family or breed.

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