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Association between Insulin–Like Growth Factor I Polymorphism and Early Growth Traits in Iranian Zandi Sheep, Found Polymerase Chain Reaction Restriction Fragment Length Polymorphism (PCR-RFLP)

Research Article

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ABSTRACT

Early growth traits in sheep are economically important. Breeding for these traits has been shown to be problematic using quantitative genetic methods, particularly in native sheep herds. Molecular genetics is useful for sheep breeding. The gene for insulin-like growth factor 1 (*IGF1*) is one of the important candidate genes known for ovine early growth traits. The aim of this study was to investigate *IGF1* polymorphism in exon 1 and its association with some early growth traits in Iranian Zandi sheep. For this purpose, 120 male and female lambs were selected randomly at up to 5 months old and blood samples were taken individually. Also, phenotypic records were collected from related farms. Genomic DNA was extracted by a salting-out method. A 265 bp region in exon 1 of *IGF1* was amplified by polymerase chain reaction (PCR), then genotyped using the restriction endonuclease HaeII. Two alleles, here called A and B, were found. Examination using PopGene32 showed allele frequencies of 0.47 A and 0.53 B. Genotype % frequencies were 28.33 AA, 37.50 AB and 34.17 BB. Chi-squared and G-squared tests showed that the population was not at Hardy-Weinberg equilibrium ($P<0.01$). An association study in SAS 9.2 found no significant effect of *IGF1* genotype on any of these early growth traits: birth weight, average daily gain up to age five months and weight at age five months. However the AA genotype was linked to the highest values of birth weight. *IGF1* polymorphism should be investigated as a molecular marker along with other polygene effects for growth traits in native sheep breeding programs.

KEY WORDS allele frequency, early growth traits, genotype frequency, *IGF1*, Zandi sheep.

INTRODUCTION

New strategies such as marker-gene assisted selection (MAS or GAS) provide high accuracy in animal breeding. This is necessary as demand for animal protein increases (Ranjbari *et al*. 2012; Gholibeikifard *et al*. 2013). Sheep of native breeds are a suitable source of protein in many countries. But there have been many problems, such as inaccuracy in pedigrees and farm records, leading to inefficient selection programs. To improve genetic gain and production, molecular markers associated with economic traits may be useful. The gene for insulin-like growth factor 1 (*IGF1*) may play important roles in the growth of multiple tissues, including mammalian muscle. Therefore it has been proposed as a candidate gene for growth traits in farm animals. It could be used in marker-assisted-selection (Honarvar *et al*. 2012). IGF is important in mammalian growth regulation, development and metabolism. It is made in the liver (Gholibeikifard *et al*. 2013; Munoz *et al*. 2011). Its two forms, *IGF1* and *IGF2* are found in most tissues. They are similar in structure and function but *IGF2* is less effective than *IGF1*. Growth hormone (GH) cannot control

IGF2 secretion. Also, the liver is the largest source of IGF found in the circulation. It has been suggested that alongside regulation by GH, nutrition might very strongly affect IGF. Nutrition has been shown to have direct and indirect effects on the IGF system (Cianfarani *et al*. 2005). Thus IGF-regulated physical growth is related to nutrition. IGF plays a key role in lactation, reproduction, fetal development and growth (Adam *et al*. 2000; Shen *et al*. 2003). Polymorphism in *IGF1* is associated with ovine fertility traits (He *et al*. 2012).

In cattle, *IGF1* is associated with reproduction and early and late growth traits, suggesting that this gene encodes a hormone like polypeptide (Grossi Ddo *et al*. 2015). Salvetti *et al*. (2007) suggested an important role for *IGF1* in the regulation of folliculogenesis and also in the pathogenesis of bovine cystic ovarian disease.

IGF1 is a major gene associated with growth traits. Its product IGF1 stimulates myogenesis, participates in the activation of cell cycle genes, inhibits apoptosis, increases glucose absorbency, increases lipid synthesis, stimulates the production of progesterone in granular cells, participates in the synthesis of DNA, RNA and proteins and in cell proliferation (Chelongar *et al*. 2014; Ge *et al*. 2001).

In Iran, sheep are raised mainly for meat production but around the world, sheep are raised for different causes (Ghafouri-Kesbi *et al*. 2008). The Zandi is native to Iran, a medium sized dual purpose breed (meat and pelt) kept in the central region.

Its origin can be traced to the Karakul sheep of Shiraz (Khojasteh *et al*. 2006). Due to its fat tail it is suitable to lowland areas. It is reared under a migratory system. Its population is approximately two million. There is considerable interest in this breed for genetic improvement (Ghafouri-Kesbi *et al*. 2008). The aim of this study was to investigate *IGF1* polymorphism, seeking association with early growth traits.

MATERIALS AND METHODS

Sampling strategy

120 male and female native pure Zandi lambs were selected randomly at a breeding station in Qom province. Lambing of this station occurred during late January to early February.

The average weaning age was three months. Whole blood was collected from the jugular vein into vacuum tubes containing 7.5 mg ethylenediaminetetraacetic acid (EDTA), then stored at 4 ˚C.

Genomic DNA was extracted from 500 µL of blood using a modified salting-out method, then stored at -20 ˚C. DNA quality and quantity were investigated on 0.8% agarose gels, comparing with a DNA ladder.

PCR amplification

A 265 bp fragment of the ovine *IGF1* gene was amplified. The *IGF1* gene was amplified in a final volume of 15 µL including 50-100 ng of genomic DNA, 0.375 µL of each primer (0.25 µM), 200 µM of dNTPs, 5 mM of $MgCl_2$ and 1 U Taq polymerase. Primers used were those designed by Ge *et al*. (1997). The sequences of primers were IGF-F: 5'-ATTACAAAGCTGCCTGCCCC-3' and IGF-R: 5'-CACATCTGCTAATACACCTTACCCG-3' for forward and reverse primers respectively. Thermal cycle for performing PCR was accordance with Table 1. A step 2 to 4 was repeated 31 times.

Table 1 Thermal cycle for PCR

NO	Step	Temperature (˚C)	Time
1	Initial denaturation	94 ˚C	2 min
2	Denaturation	94 ˚C	45 s
3	Anealing	66 ˚C	30 s
4	Extention	72 ˚C	45 s
5	Final extention	72 ˚C	5 min
6	Hold	4 ˚C	10 min

Polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP)

In order to genotyping for all samples and PCR-RFLP analysis, the PCR products were digested in a total volume of 10 µl that included: 4 µL of PCR product, 4 µL of distilled water, 1 µL of enzyme buffer and 1 µL of HaeII enzyme incubated at 37 ˚C for 12 hour according to Yilmaz *et al*. (2005). Digested PCR products were mixed with 10x loading buffer and subjected to 2 percent agarose gel electrophoresis that contained Safe stain in 0.5x TBE. The genotyping of samples were done by comparison of digested PCR products and 50 bp DNA ladder.

Statistical analysis

Population genetic analysis was done using PopGene32. Effects with ($P \leq 0.05$) were considered significant. Associations were sought between *IGF1* genotypes and lambs' birth weight (BW), five-month weight (BW5) and average daily gain up to age five months (ADG). Least squared means (LSM) were used for multiple comparisons among genotypes using the general linear model (GLM) procedure in a fixed effect model in SAS (2004). Birth weight (BW,Y_{ijk}) was described thus:

$$Y_{ijk} = \mu + Sex_i + T_j + G_k + e_{ijk}$$

Where:
µ: overall LSM.
Sex_i: effect of sex (i=1,2).
T_j: effect of the lambing year.
G_k: effect of *IGF1* genotype (i=1,2,3).
e_{ijk}: residual random effect.

Five-month weight (BW5) and average daily gain up to age five months old (ADG) (Y_{ijklm}) were described thus:

$$Y_{ijklm} = \mu + Sex_i + b_1BW_j + b_2Dim_k + T_l + G_m + e_{ijklm}$$

Where:
b_1: regression coefficient for BW on the dependent variable. BW_j: effect of BW as a covariate.
b_2: regression coefficient for the weaning period.
Dim_k: effect of weaning period as a covariate.

Statistical interactions were not permitted between parameters.

RESULTS AND DISCUSSION

PCR-RFLP and population genetics

A 265 bp fragment was amplified from within exon 1 of ovine *IGF1* (Figure 1). Digestion of that PCR product using HaeII revealed two alleles, hence three genotypes (Figure 2): AA (179, 86 bp), BB (265 bp) and AB (265, 179, 86 bp). All three genotypes were found in the present study, showing polymorphism.

Alleles A and B were found with % frequencies 47.08 and 52.92. Genotypes AA, AB and BB were obtained with % frequencies 28.33, 37.50 and 34.17. Chi-squared and G-squared tests showed that the locus was not in Hardy-Weinberg equilibrium ($P<0.01$; Table 2).

IGF1 genotypes and early growth traits

No significant associations were found between *IGF1* genotypes and BW, BW5 or ADG (Table 3). However results approaching significance suggested that AA might have produced a 0.04 kg gain in BW and BB a 0.41 kg reduction in BW5. No effects on ADG were apparent.

Detection of quantitative trait loci (QTL) has become a 'hot' topic since the late 1980s. Most studies have investigated the contribution of marker information into existing breeding programmes. As with most new technologies, it will probably be necessary to modify breeding programmes to exploit marker-assisted selection (Weller, 2009). Marker-assisted selection has so far been successful in commercial animals (Yilmaz *et al*. 2000). *IGF1* has a remarkable diversity of biological effects on embryonic and postnatal growth.

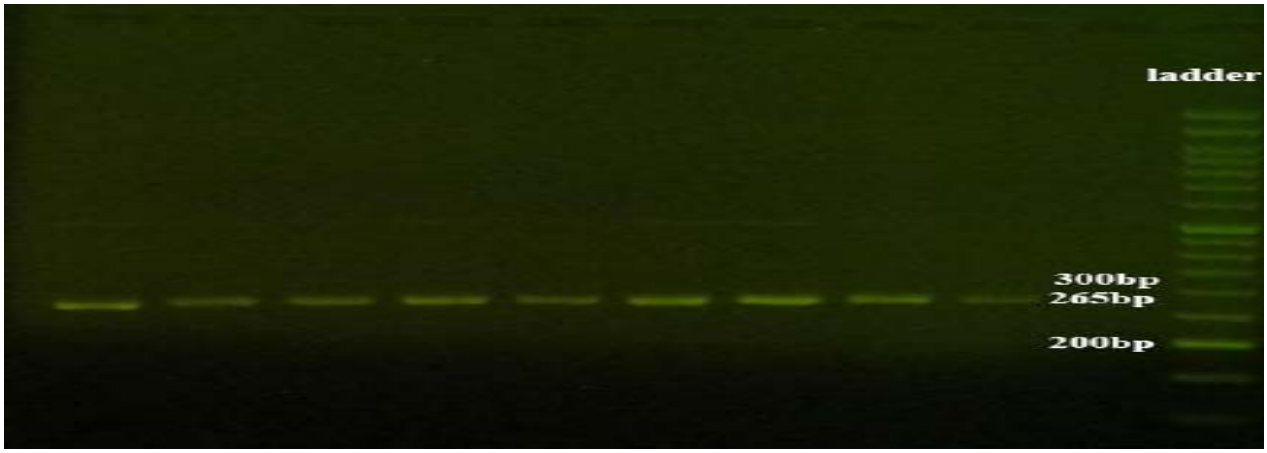

Figure 1 PCR products using IGF-F and IGF-R primers analyzed by electrophoresis in a 1.5% agarose gel with safe stain

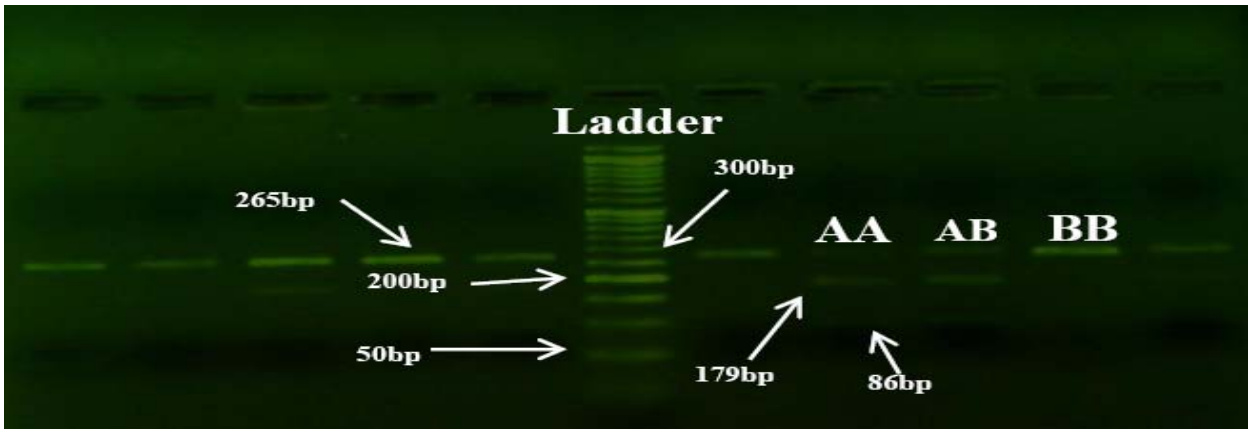

Figure 2 HaeII digestion of PCR products from animals those were genotyped.
Agarose gel (2%)
AA= 179 and 86 bp; AB= 265, 179 and 86 bp and BB= 265 bp

Table 2 Results of the χ^2 and G^2 test and probability levels in Hardy-Weinberg equilibrium

Genotype	observed number (O)	expected number (E)	$(O–E)^2/E$	$2 \times O \times Ln (O/E)$	(χ^2_T)	Probability (χ^2_T)	(χ^2_G)	Probability (χ^2_G)
AA	34	26.4770	2.1375	17.0058				
AB	45	60.0460	3.7702	-25.9604	7.598285	0.005842	7.667885	0.005621
BB	41	33.4770	1.6906	16.6225				

Table 3 Least square means and standard errors for early growth traits of Zandi sheep according to the different *IGF1* genotypes

IGF1 genotype	Birth weight (kg)	Five-month weight (kg)	Daily gain up to five month old (kg)
AA	$4.35±0.09^{ns}$	$44.16±0.59^{ns}$	$0.289±0.004^{ns}$
AB	$4.31±0.07^{ns}$	$44.16±0.46^{ns}$	$0.289±0.003^{ns}$
BB	$4.31±0.081^{ns}$	$44.02±0.51^{ns}$	$0.289±0.003^{ns}$

Circulating *IGF1* concentrations correlate with fetal and neonatal size in several species. *IGF1* promotes the growth of fetal organs, endocrine glands and skeletal maturation in fetal sheep, in part by enhancing fetal amino acid and glucose uptake. In postnatal life, *IGF1* is a key determinant of animals' linear growth, as a result of its effect on longitudinal bone growth (promoting osteoblast division and proliferation), muscle growth (enhancing myocyte differentiation and multiplication) and cartilage growth (increasing chondrocyte colony formation) (Honarvar *et al*. 2012). Mehmannavaz *et al*. (2010) found a significant effect of *IGF1* polymorphism in Holstein bulls on estimated breeding values (EBV) for milk production traits. In Baluchi sheep Tahmoorespur *et al*. (2009) found a significant effect of *IGF1* polymorphism in exon 1 on daily gain from birth to weaning but not for birth weight, weaning weight, weaning to six month and six month to yearling age. A study of Zel (tailed) and Lori-Bakhtiari (fat-tailed) sheep found no effect of polymorphisms in the 5' flanking region of *IGF1* with carcass traits (Honarvar *et al*. 2012). In Baluchi sheep Gholibeikifard *et al*. (2013) found no association between polymorphisms in exon 3 of *IGF1* and birth weight, weaning weight, six-month weight, nine-month weight and yearling weight. Purbayramian *et al*. (2013) in Moghani sheep found no effect of *IGF1* polymorphism on birth or yearling weight.

The present study's results hinted at genotypic effects on in early-growth traits. Allele A might have produced relatively high birth weight and five-month weight, however the differences were negligible and non significant. Those results might have arisen from small population size and the polygenicity of the traits. Investigation of particular alleles suggested that one allele, here called A, had a nearly-significant effect on birth weight and five month old weight. The genotypic distribution in the present study was not in Hardy-Weinberg equilibrium. Up to date little is known about molecular characteristics of Zandi sheep in Iran but migration and non random mating might contribute to the observed disequilibrium. In this study we used native breed sheep in a rural flock and we cannot expect that the suitable allele of *IGF1* would have a significant effect.

Variation in quantitative traits is controlled by multiple genes with limited effect and current results showed that *IGF1* could not capture remarkable variation of early-growth traits in Zandi sheep. Therefore, study of sheep populations under selection programs would be useful.

CONCLUSION

The present study is the first research of *IGF1* in Zandi sheep. The results presented here indicate that there are three genotypes in exon 1 of *IGF1* in this population, but we found no significant association between this gene and early growth. However, we can confirm polymorphism of this gene in the population and it could be used as a tool in breeding programs. In some studies *IGF1* polymorphism has significant association with growth traits in animals. Therefore, more research is recommended on other regions of *IGF1* in larger populations.

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