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Polymorphism of IGF-1 gene in Makoei Sheep using PCR-SSCP

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ABSTRACT

The native breeds, because of their natural selection against harsh environment and adaptation to regional conditions are important to resource-poor farmers and pastoralists. The IGF1 gene (insulin-like growth factor 1) is a candidate gene for marker-assisted selection strategies.IGF-1 gene that has located on chromosome 3 in sheep is a marker for growth rate and meets production and has an important role in mammary glands cell differentiation and proliferation. Genomic DNA was isolated from the blood of 100 sheep. Gel monitoring and spectrophotometer methods were used to determine the quality and quantity of DNA. A 265bp IGF-1 exon 1 segment was amplified by standard PCR, using the locus specific primers. Polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) analysis of the 5' flanking region (Exon1) of the ovine IGF-I gene revealed three banding patterns (genotypes) named as A/A, A/G and G/G. The frequencies of the observed genotypes were 0/52,0/42,0/06, respectively. Allele frequencies were 0/73,0/27 for A,G. The most frequent allele and genotype in the 'Makoei' sheep breed were 0/73 and 0/52 for allele A and genotype AA, respectively. Observed heterozygosity (Hobs) value was 0/3942. The observed distribution of genotypes was not different than the distribution expected under the assumption of Hardy-Weinberg equilibrium. These results confirmed the potential usefulness of IGF-1 gene in marker-assisted selection programs for sheep breeding.

Key words: IGF-1 gene, polymerase chain reaction (PCR), single strand conformation polymorphism technique (SSCP), Makoei Sheep

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INTRODUCTION

Makoei is a breed of sheep classified as fat-tailed, similar to Turkish White Karaman and represents an important multi-purpose sheep for production in the East and West Azerbaijan provinces of Iran. There are more than 35 million sheep in this region of which 5 million is reported to be Makoei breed. They are multi colored: black, white with black spots on face and feet. A live female sheep weight about 47 kg. Birth weight of the lambs is 3.7–3.5 (male/females) kg. Maximum milk yield per lactation is 100 kg. (6%fat) and maximum of yearly fleece weight reaches to 3 kg per sheep [1]. Indigenous genetic resources of the world are at the risk of extinction due to absorbent crossing with commercial breeds [2].Even though mature IGF-I is a relatively small peptide, its gene is surprisingly large in mammals, comprising 80 to 100 kb of genomic DNA [3-4]. In humans, pigs, goats, rats, and chickens, the IGF1 nucleotide sequence is about 70-90 kb [4-5]. Exon numbers differ between species; for example, goats, pigs and sheep have 1-6 exons [6]and humans and rats 1-5 [3-4]. The IGF-1 gene that is located on chromosome 3 in sheep is a marker for growth rate and meat production and has an important role in mammary gland cell differentiation and prolife ratio [7]. The IGF family is made up of the following three related ligands: insulin, IGF-I, and IGF-II. Both IGFI and II are found in circulation and extracellular fluids conjugated to any one of six different IGF binding proteins (IGFBP) [8]. Insulin-like growth factors one and two (somatomedins- IGF-I and IGF-2) are structurally related proteins that have a key role in cell differentiation, embryogenesis, growth and regulation of

metabolism [9]. IGF1 is a mediator of many biological effects; for example, it increases the absorption of glucose, stimulates myogenesis, inhibits apoptosis, participates in the activation of cell cycle genes, increases the synthesis of lipids, stimulates the production of progesterone in granular cells, and intervenes in the synthesis of DNA, protein, RNA , and in cell proliferation [10]. IGF-I has been reported to be necessary for progression of cells through both the G1 and the G2/M phases of the cell cycle [11].

Polymorphism in exon I of ovine IGF-1 gene identified using single-strand conformational polymorphism analysis and revealed that two allelic variant in a 265bp in mixed and Baluchi sheep breeds[12-13]. The objective of this study was to characterize potential variation at the ovine IGF-1 gene in 'Makoei' sheep breeds using polymerase chain reaction and single-strand conformational polymorphism (PCR–SSCP) analysis.

MATERIALS AND METHODS

Sheep, blood sample collection and genomic DNA extraction

The Makoei breed of sheep were examined in this study, they are fat-tailed sheep with medium body size, white in color with black spots on face and feet. They are farmed in the east and west Azerbaijan provinces of Iran for meat and wool[14].Blood samples were collected into a 5 ml EDTA vacutainer tube and transferred to the laboratory within 2 hours for DNA extraction. Total DNA extractions were made with a modified salting out method [15]from whole fresh blood. Quality and quantity of extracted DNA was measured on 0.8% agaroze gel prepared in 0.5× TBE buffer (45 Mm Tris base, 45 Mm boric acid, 1mM EDTA pH 8.0) and visualized with ethidium bromide (1.0 µgml-1) and photographed under UV light.

Amplification of the exon 1 of IGF-1 gene

Two polymerase chain reaction (PCR) primers, IGF-1-up (5′-ATTACAG CTGCCTGCCCCTT-3′) and IGF-1-down(5'-CACATCTGCTAATACACCTTACCCG-3') targeting a fragment of 265bp were employed in DNA amplifications as described by Yilmaz et al.,[12].that Based on the sequence of the ovine IGF-I gene from Dickson et al.,[16](1991).PCR contained 25-50 ng genomic DNA, 10 pmoL of each primer, 2 µL 10X PCR buffer, 1.5 mM MgCl2, 200 µMdNTP and 1 unit Taq-polymerase, in a total volume of 20 µL . DNA amplifications were performed using Master cycler (Eppendorf, Germany) programmed for a preliminary step of 2 min at 95°C, followed by 31cycles of 45 s at94°C, 30 s at 58°C and 30 s at 72°C, with a final extension of 3minat 72°C. Amplification was verified by electrophoresis on 1.5% (w/v) agarose gel in 1 x TBE buffer (2 mM of EDTA, 90 mM of Tris-Borate, pH 8.3), using a 100bp ladder as a molecular weight marker for confirmation of the length of the PCR products. Gels were stained with ethidium bromide (1 µg/mL).

Single strand confirmation polymorphism (SSCP)

PCR products were mixed with 8 µl of denaturing loading dye (95% (w/v) deionized formamide, 0.05% (w/v) xylene cyanol, 0.05% (w/v) bromophenol blue and 0.02M EDTA) in a total volume of 15 µl. The mixture was denatured at 95°C for 5 min and was snap chilled on ice. The total volume was run in a 15% polyacrylamide gel, as described by Herring et al.

The electrophoresis was performed in 0.5 × TBE buffer (Tris 100 mM, boric acid 9 mM, EDTA 1mM) at room temperature (18°C) and constant 200 V for 3 h. Polyacrylamide gels were stained with silver according to the protocol described by Herring et al.

Statistical analysis

The allelic and genotypic frequencies, observed and expected Nei'sheterozygosities (HE=1-Σpi2, where Pi is the frequency of allele i) were estimated using Pop Gene32 program (ver 1.31, Canada)[17]Hardy-Weinberg equilibrium.

RESULTS

The amplification of a 265bp fragment of the IGF-1 exon 1 gene was successful in our first attempt. All extracted DNAs from rams blood samples yielded a specific single band PCR product without any nonspecific band (Figure 1).

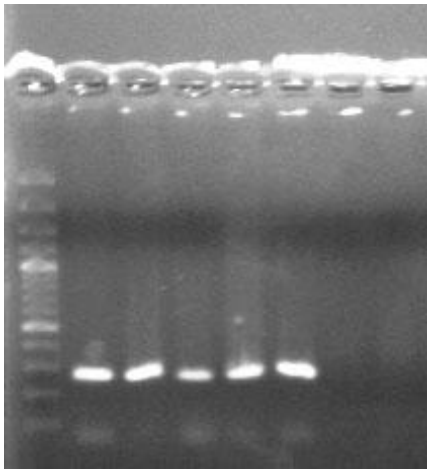

Figure 1. PCR products analyzed by Electrophoresis in a 1.5% agarose gel with ethidium bromide staining

Therefore, the PCR products were directly used for SSCP analysis. The allelic variation in the IGF-1 gene was examined by PCR-SSCP. The non-denaturing gel electrophoresis enabled visualization of single-stranded DNA (ssDNA) and SSCP band patterns. In this study, a total of three SSCP patterns were observed in the examined sheep (Figure 2).

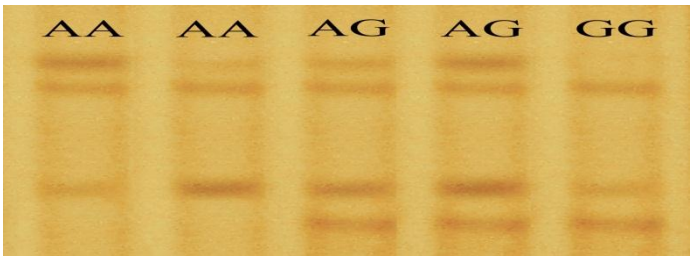

Figure 2.SSCP polymorphism of 'Makoei' sheep IGF-1gene. Three different PCR-SSCP patterns (genotype) were identified.

The frequencies of the observed genotypes were 0/52,0/42,0/06, respectively. Allele frequencies were 0/73,0/27 for A,G. Observed heterozygosity (Hobs) value was 0.3942 (table 1).

Table 1.Observed allele and genotypic frequencies for IGF-1 locus in 'Makoei' sheep

Allele frequency		Frequency genotypic		
A	G	AA	AG	GG
0.73	0.23	0.52	0.42	0.06

The observed distribution of genotypes was not different than the distribution expected under the assumption of Hardy-Weinberg equilibrium. The expected homozigosity (Homexp) value for IGF-1 was 0.6018, expected heterozygosity (Hexp) was 0.3982 and average heterozygosity was 0.3942 (Table 2).

Table 2.Summary of heterozygosity statistics for all loci

Locus	ObsHom	ObsHet	ExpHom	ExpHet	Nei	AveHet
IGF-1	0.58	0.42	0.6018	0.3982	0.3942	0.3942

Effective number of alleles was 1.65 and Shannon's Information index (I) was 0.58 (Table 3).

Table 3.Summary of genetic variation statistics for all loci.

Locus	Na	ne	I
IGF-1	2	1.65	0.58

na = observed number of alleles; ne = Effective number of alleles (Kimura and Crow, 1964); I =Shannon's Information index (Lewontin, 1972)

DISCUSSION

The PCR-SSCP method A simple and quick method was developed to score the marker genotype, which will reduce the time and cost of typing the marker genotype by approximately three-fourths. This method of scoring the marker

genotype can be applied to selection of sheep if the marker is proven to be associated with performance traits in sheep. In other words, this marker can affect phenotypic traits or be in disequilibrium linkage with polymorphisms affecting traits [18]. In earlier studies of a dinucleotide repeat polymorphism in the 5 flanking region of the IGF-I gene in cattle and swine, a possible role of this somatomediater in production traits was evident [19]. There have been studies of the allelic frequency of IGF-I gene in different cattle breeds, and beef cattle is considered to be a model for this system [18, 20, 21]. In Angus cattle, these primers produced a frequency of 0.64 for the A allele and 0.36 for the B allele [18]. The role of IGF-I and its binding proteins has been reviewed [22-23] IGF-I mediate cell proliferation and is essential for normal development of the mammary gland during puberty and pregnancy [24]. Most studies of beef cattle have revealed an association between gene polymorphism and body condition scoring and weight, particularly in early life stages. This gene in different breeds may affect different characteristics in the same species, depending on the selection program.

In the present study were find out a SNP (single nucleotide polymorphism), in the 5' flanking region of the ovine IGF-I gene in Makoei sheep. For a fragment of 265bp in the 5' flanking region of the ovine IGF-I gene, 467 to 732bp upstream from the 5' end of Exon1, three conformational patterns were observed. Found the same patterns that corresponded with the three genotypes A/A, A/B, and B/B in mixed breed sheep. In this study, variation in the exon I sequence of the sheep ovine IGF-1 gene was investigated by polymerase chain reaction–single strand conformational polymorphism (PCR–SSCP) analysis. Two alleles (A, B) and three genotypes (AA, AG, GG) were observed in IGF-1 exon 1gene of 'Makoei' sheep. The most frequent allele and genotype in the 'Makoei' sheep breed were 0/73and 0/52 for allele A and genotype AA, respectively. Results of this study partly are in accordance with the results Previous researches that two allele observed in three mixed-breed and Baluchi and 'Makoei'sheep breeds. This study may be regarded as the beginning of attempts to understand the genetic variability of native sheep breeds in the Azerbaijan region. This study confirmed the importance of molecular studies beside the morphological data in detecting genetic variation among individuals in selecting diverse parents to constructing a new population successfully. Further studies are necessary to determine whether the polymorphisms in these coding regions are intrinsically related to the quantitative traits, such as body weight, daily weight gain and growth trait.

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